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ORIGINAL PAPER

Identification of quantitative trait loci in rye introgression lines carrying multiple donor chromosome segments

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Abstract Introgression libraries can be used to make favorable genetic variation of exotic donor genotypes available in the genetic background of elite breeding material. Our objective was to employ a combination of the Dunnett test and a linear model analysis to identify favorable donor alleles in introgression lines (ILs) that carry long or multiple donor chromosome segments (DCS). We reanalyzed a dataset of two rye introgression libraries that consisted of ILs carrying on average about four donor segments. After identifying ILs that had a significantly better per se or testcross performance than the recipient line with the Dunnett test, the linear model analysis was in most instances able to clearly identify the donor regions that were responsible for the superior performance. The precise localization of the favorable DCS allowed a detailed analysis of pleiotropic effects and the study of the consistency of effects for per se and testcross performance. We conclude that in many cases the linear model analysis allows the assignment of donor effects to individual DCS even for ILs with long or multiple donor segments. This may considerably increase the efficiency of producing sub-ILs, because only such segments need to be isolated that are known to have a significant effect on the phenotype.

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Introduction

Introgression libraries ideally consist of a set of homozygous lines, each of which carries a single marker-defined donor chromosome segment (DCS) in the genetic background of an elite line (Eshed et al. 1992; Eshed and Zamir 1994). These DCS are introduced into the genetic background of the recipient line by marker-assisted backcrossing and should cover the entire genome of the donor. The approach of introgression libraries was first demonstrated by Eshed et al. (1992) in tomato to broaden the restricted genetic variation of the breeding material and to exploit natural variation available in genetic resources.

Introgression libraries are an important resource for the identification of quantitative trait loci (QTL) and the discovery of genes (Zamir 2001; Kearsey 2002). From a practical point of view, introgression libraries might overcome the drawbacks of the classical QTL mapping approach, since they do not separate the process of QTL detection and their use in breeding. Thus, (1) QTL alleles will not lose their effects after being transferred into breeding material due to epistatic interactions with the genetic background and (2) the transfer of QTL alleles into breeding material does not require further extensive

marker-assisted backcrossing programs (Tanksley and Nelson 1996). Introgression libraries are, therefore, a very interesting approach for practical plant breeding as development time is a key factor in the efficacy of trait manipulation in seed companies.

Analysis of introgression libraries typically involves a series of pairwise tests between the introgression lines (ILs) and the recipient for the traits of interest. This procedure has proven to be useful for finding genomic regions that carry beneficial alleles including yield-related traits in tomato (Eshed and Zamir 1955), wheat (Pestsova et al. 2006), and barley (Schmalenbach et al. 2009), agronomic traits in barley (Matus et al. (2003, 2009, 2011), maize (Szalma et al. (2007), and rye (Falke et al. (2009a, b), quality traits in barley (Matus et al. 2003; Schmalenbach and Pillen 2009), tomato (Rosseaux et al. 2005), melon (Eduardo et al. 2007), and rye (Falke et al. (2008, 2009a, b) as well as biotic stress in tomato (Finkers et al. (2007), lettuce (Jeuken et al. (2008) and barley (Schmalenbach et al. (2008).

In practical experiments, however, the ideal introgression library with lines containing only a short single marker-defined chromosomal segment of the exotic parent is mostly not available; either multiple segments are present (e.g., Liu et al. (2006; Falke et al. 2008) and/or long segments (cf Eshed et al. 1992; Chetelat and Meglic 2000; Matus et al. 2003; Jeuken and Lindhout 2004; Eduardo et al. 2005; Keurentjes et al. 2007; Schmalenbach et al. 2011). Due to these unbalanced DCS, the following questions remain: (1) which segment carries the putative QTL and/or (2) where is the QTL on the DCS located? At present, further backcross generations and subsequent field tests are employed to answer this question. These isolate or shorten the individual DCS with the goal to locate the QTL. This is necessary because statistical procedures that are able to precisely detect the location of a QTL when an IL carries several and/or longer DCS are, to our knowledge, still lacking.

Using marker-assisted backcrossing, we developed two rye introgression libraries consisting each of 40 BC_2S_3 lines. Each line carries on average three–five DCS (Falke et al. 2008). In separate experiments, a two-sided Dunnett test (Dunnett 1955) was used to determine ILs carrying DCS with putative QTL regions for agronomic and quality traits for per se as well as for testcross performance (Falke et al. 2008, 2009a, b).

In the present study, we reanalysed these data by employing pairwise Dunnett tests for identification of ILs that differ from the recipient and subsequently a linear model to identify the precise location of QTL in the unbalanced introgression library. In particular, our objectives were to (1) develop an analysis procedure for identifying QTL more precisely in introgression libraries with unbalanced DCS, (2) apply it to our rye ILs to identify QTL for agronomic and quality traits, (3) compare the determined QTL with QTL regions found in previous analyses, (4) examine the consistency of QTL for per se and testcross performance, and (5) investigate the presence of pleiotropic QTL effects.

Materials and methods

Development of introgression lines

The development of the introgression libraries is described in detail by Falke et al. (2008). Briefly, two rye introgression libraries, A and B, consisting each of 40 BC_2S_3 lines were developed by marker-assisted backcrossing to introduce exotic DCS of the Iranian primitive rye population Altevogt 14160 (provided by the Botanical Garden Warsaw, Poland) into the genetic background of the elite line L2053-N from the Petkus gene pool (bred by Hybro GmbH & Co KG, Germany). For library A and B, 131 and 182 amplified fragment length polymorphism (AFLP), respectively, and 137 and 118 simple sequence repeat (SSR) markers, respectively, were used to characterize and select individual plants in each backcross and selfing generation from BC_1 to BC_2S_3, to produce a total of 40 lines for each introgression library.

Agronomic trials

The evaluation of the field experiments has been described in our companion articles (Falke et al. 2008, 2009a, b). Briefly, the experimental design at each location was a 10 × 9 α-design (Patterson and Williams 1976) with three replicates for assessing per se performance and two replicates for testcross performance. For evaluating the testcross performance, the ILs of both libraries were crossed with the unrelated cytoplasmatically male-sterile testers from the Petkus gene pool L2092-P × LY2130-N (T1; bred by Hybro GmbH & Co KG, Schenkenberg, Germany) and Lo55-P × Lo88-N (T2; bred by KWS LOCHOW GmbH, Bergen, Germany). Trait data were collected for the agronomic traits grain yield (per se: g m^{-2}; testcross: dt ha^{-1}) and plant height (cm). A representative sample of grain (per se: 200g; testcross: 500g) was taken for quality analyses to record thousand kernel weight (g), test weight (kg), falling number (s), pentosan, protein, and starch content in grain (%). The latter three were estimated by near-infrared reflectance spectroscopy.

The field trials were conducted in separate but adjacent experiments at five sites in Germany (Bergen, Eckartsweier, Hohenheim, Oberer Lindenhof, and Wulfsode) in 2 years. The per se performance at Oberer Lindenhof was

evaluated only for grain yield and plant height for 1 year. Testcross performance of the agronomic traits for T1 could not be recorded at Eckartsweier in both years and for T2 at Oberer Lindenhof only for 1 year. Testcross performance of the quality traits was assessed only for T1 at Bergen, Hohenheim, and Wulfsode in both years. Pentosan, protein, and starch content were measured only in 1 year.

Statistical analysis

Analyses of variance for per se and testcross performance have been reported previously by Falke et al. (2008, 2009a, b). Briefly, ordinary lattice analyses for all traits were performed for each experiment and location using software PLABSTAT (Utz 2001). Adjusted entry means were then used to compute combined analyses of variance across locations (Cochran and Cox (1957). Variance components were estimated based on adjusted entry means and effective error mean squares from the individual lattice analyses by restricted maximum likelihood estimation (REML), using PROC MIXED of SAS (SAS Institute 2004). Estimates of the genotypic variances were significant, indicating that new genetic variation was generated by the exotic donor.

Introgression lines with a significantly different performance than the recipient were detected with a two-sided Dunnett test (Dunnett 1955) employing a type I error rate of $\alpha = 0.05$. The model was fitted with PROC MIXED of the SAS system (SAS Institute 2004) as described by Falke et al. (2008, 2009a, b). Briefly, the following model was used:

$$Y = \mu + G_r + L_s + J_t + (GL)_{rs} + (GJ)_{rt} + (LJ)_{st} + (GLJ)_{rst} + e$$

where G_r ($r = 1,...,78$) are the genotypes, L_s ($s = 1,...,5$) the locations, and J_t ($t = 1, 2$) the years. In the testcross analysis, additional terms were included in the model to account for the tester and interactions effects. For the analyses, genotypes were considered fixed factors while the other factors were included as random factors in the above analyses.

In order to allocate QTL to specific DCS, a linear model was fitted employing the principle that was described in mathematical detail in the simulation study of Falke and Frisch (2011). Briefly, the chromosomes were divided into segments that correspond to the DCS present in the library. For each segment, the effect β_s of the donor genome was estimated and tested for being significantly different from zero with standard linear model methodology and a comparison-wise type I error rate of $\alpha = 0.05$. QTL were considered to be putatively pleiotropic if a QTL was found for two or more traits in close proximity. However, because QTL can only be resolved to DCS, or in some cases subsegments, putative pleiotropic QTL may be in fact separate genes located proximally in the genome.

The model used was:

$$Y = \mu + L_s + J_t + M_u + e$$

where M_u is a marker or non-segregating group of markers (introgressed segment). In the testcross analysis, an additional model factor T_w for the wth tester effect was included in the above model. The effect of each segment was estimated with the linear model using $\hat{\beta} = (X'X)^{-1}X'y$. The part of the design matrix that codes for the effects of the donor segments X_D consisted of a g by h matrix, where g was the number of phenotypes and $h = 1 + u$, the number of included markers plus the intercept. For the levels of marker factor M, donor parent genome received a 1, recipient parent marker scores received a 0, and heterozygous loci received a 0.5. This produced a vector β, consisting of the genotypic value of the recipient parent β_0 and an effect β_u for each marker segment.

Each M was then tested with the null hypothesis $H_0 : k'\beta = 0$, where $k_u = 1$ and $k_v = 0$ for all $v \neq u$ and the corresponding F statistic as $F(H_0) = Q/(\text{SSE}/\text{DFE})$ where $Q = (k'\hat{\beta})'[k'(X'X)^{-1}k]^{-1}(k'\hat{\beta})$, $\text{SSE} = y'y - \hat{\beta}'X'y$, and $\text{DFE} = N - \text{rank}(X) - stw$. N is the total number of genotypes, s the number of locations, t the number of years, and w the number of testers (when applicable).

Results

The recipient had a higher per se performance for pentosan content and a shorter plant height than the donor, whereas grain yield was nearly equal for both. The Dunnett test detected 162 pairwise comparisons between the recipient and the ILs to be significant ($P < 0.05$), and in 20 % of these, the ILs showed a superior performance. The recipient had a higher testcross performance than the donor for grain yield, falling number, and pentosan content and showed a shorter plant height. With the Dunnett test, we found 58 significant ($P < 0.05$) pairwise comparisons between testcrosses of the recipient and ILs and thereof 59 % had a superior testcross performance.

For all considered traits, we investigated the ILs that were significantly better than the recipient. In addition, we investigated ILs with significantly lower starch content than the recipient, because starch content is known to be negatively correlated with the other traits. DCS with effects on per se performance were detected by the linear model in all ILs of introgression library A that were identified by the Dunnett test as being significantly different from the recipient (Fig. 1). In library B, DCS with effects on per se

performance were found in 13 out of 15 significant ILs (Fig. 3). DCS with effects on testcross performance were detected in 20 out of 22 significant ILs of introgression library A (Fig. 2) and in 3 out of 12 significant ILs of introgression library B (Fig. 4).

With the linear model, the regions carrying putative QTL were identified precisely in many cases. QTL with p values below 0.05 are listed in Tables 1, 2, 3, 4. The effects given in Tables 1, 2, 3, 4 are 2α, or two times the allelic substitution effect, in the per se and α for the testcross. These effects therefore represent the substitution of homozygous recipient genomic segments with homozygous DCS for per se and to heterozygous DCS for testcross. For per se performance, putative QTL for thousand kernel weight were detected on chromosomes 4R, 6R, and 7R (library A), for pentosan content on chromosomes 1R, 3R, and 5R−7R in library A and on chromosomes 3R−7R in library B, for starch content on all chromosomes in library A and chromosomes 1R and 3R to 7R in library B as well as for protein content on chromosomes 1R–3R and 5R–7R in library A and on chromosomes 1R and 3R to 5R in library B. For testcross performance, the linear model found putative QTL for thousand-kernel weight on chromosomes 1R, 4R–7R (library A), for test weight on chromosomes 1R and 4R–7R in library A and on chromosome 4R in library B, for pentosan content on chromosome 7R (library A), for starch content on chromosome 1R–3R, 5R, and 7R in library A and on chromosome 4R in library B as

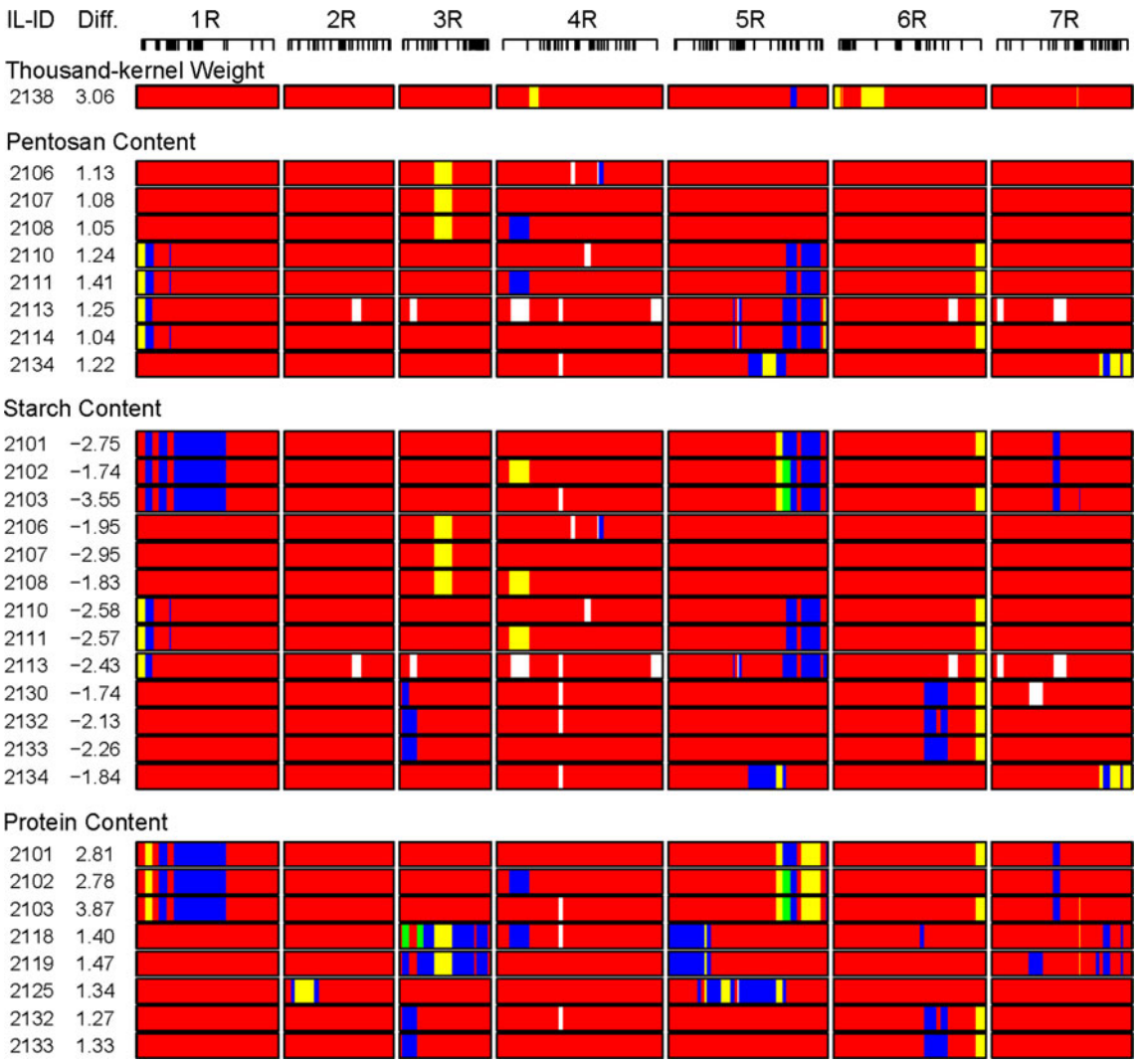

Fig. 1 Per se performance of introgression library A: differences in the performance between the recipient and introgression lines (ILs). Only ILs with significant ($P<0.05$) differences of the Dunnett test were included. The respective chromosome and marker position (*vertical bars*) are presented above the figure; *blue coloring* denotes homozygous donor introgressions, *red coloring* indicates homozygous state of the recipient, *green coloring* denotes heterozygous state, *white coloring* denotes missing data, and *yellow coloring* indicates donor introgressions found to be significant with the linear model test (color figure online)

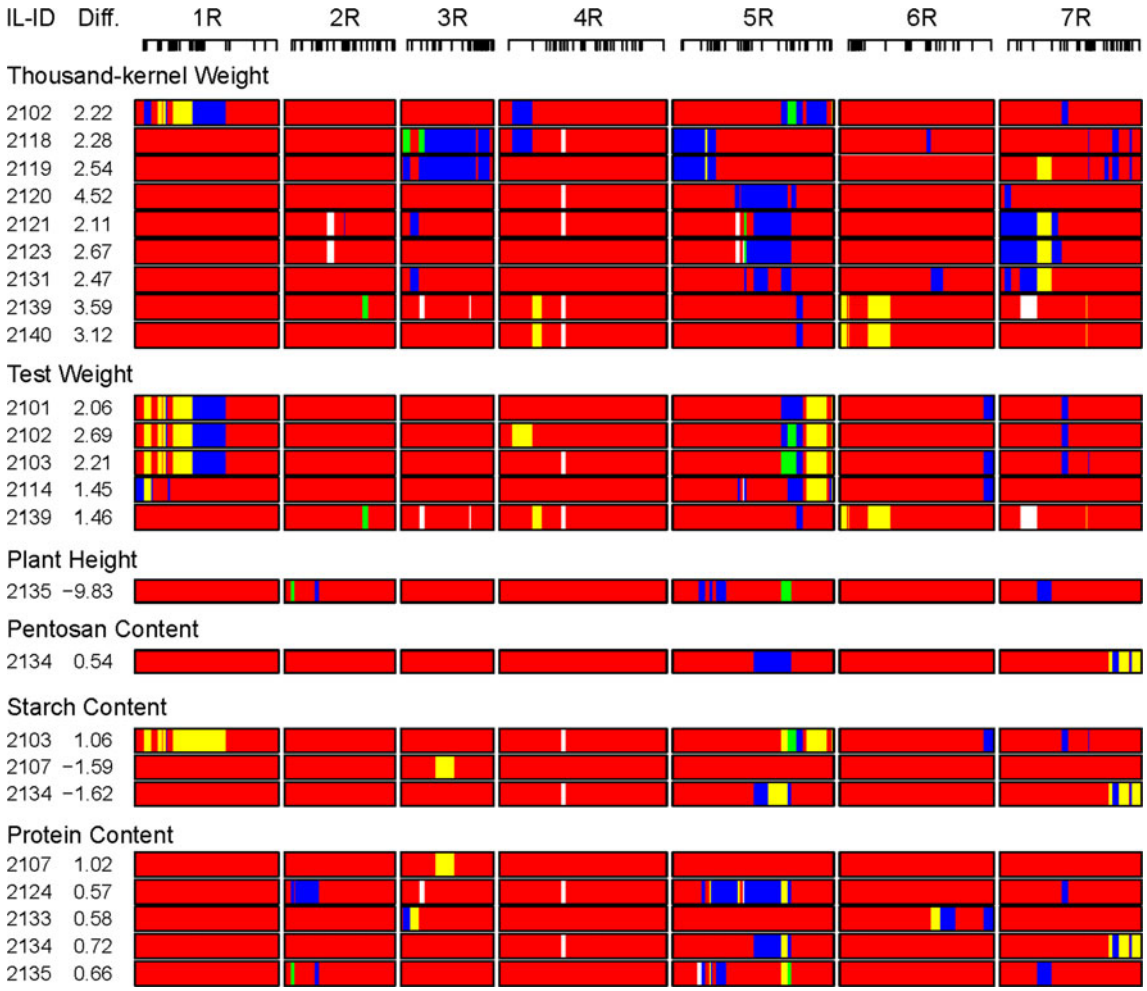

Fig. 2 Testcross performance of introgression library A: differences in the performance between the recipient and introgression lines (ILs). Only ILs with significant ($P<0.05$) differences of the Dunnett test were included. The respective chromosome and marker position (*vertical bars*) are presented above the figure; *blue coloring* denotes homozygous donor introgressions, *red coloring* indicates homozygous state of the recipient, *green coloring* denotes heterozygous state, *white coloring* denotes missing data, and *yellow coloring* indicates donor introgressions found to be significant with the linear model test (color figure online)

well as for protein content on chromosome 3R and 5R–7R (library A).

Pleiotropic QTL were identified by the linear model in many instances. Results indicate that while pleiotropy between starch, pentosan, and protein content is not the general case, there were several QTL found that indicate a level of pleiotropy. In introgression library A, QTL for per se performance for pentosan, starch, and protein content were present on chromosomes 3R, 6R, and 7R, while QTL affecting two of the three traits occur on chromosomes 1R (pentosan and starch content) and 5R (starch and protein content) (Fig. 1). QTL detected for per se performance in introgression library B showed also pleiotropic effects. Chromosomes 3R, 4R, and 5R carried QTL for pentosan, starch, and protein content and chromosome 6R for pentosan and starch content. Some contradictory results for pleiotropic QTL were also seen. Introgression line 2166, for instance, while containing putative pleiotropic QTL for starch and protein content on chromosome 4R, was not declared significantly different from the recipient parent for pentosan content in the previous analysis, along with IL 2164 and 2165 for protein content.

Consistency between QTL for per se and testcross performance was observed in both introgression libraries. In introgression library A, QTL on chromosomes 3R (starch and protein content), 5R (starch and protein content), and 7R (pentosan, starch, and protein content) show pleiotropy consistently in both the per se and testcross performance (Figs. 1, 2). Similar results were found for introgression library B. A putative QTL for starch content on chromosome 4R were detected for both per se and testcross performance.

In addition to consistency between per se and testcross performance, there were six instances where QTL were

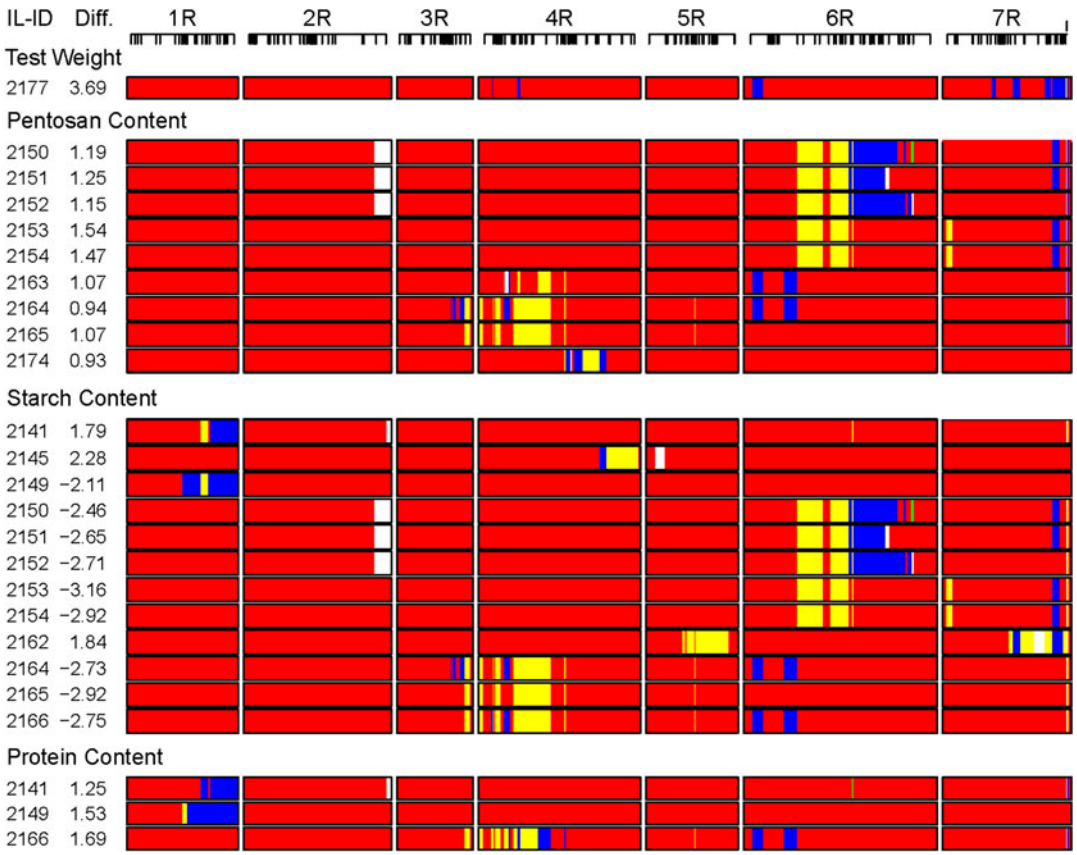

Fig. 3 Per se performance of introgression library B: differences in the performance between the recipient and introgression lines (ILs). Only ILs with significant ($P<0.05$) differences of the Dunnett test were included. The respective chromosome and marker position (*vertical bars*) are presented above the figure; *blue coloring* denotes homozygous donor introgressions, *red coloring* indicates homozygous state of the recipient, *green coloring* denotes heterozygous state, *white coloring* denotes missing data, and *yellow coloring* indicates donor introgressions found to be significant with the linear model test (color figure online)

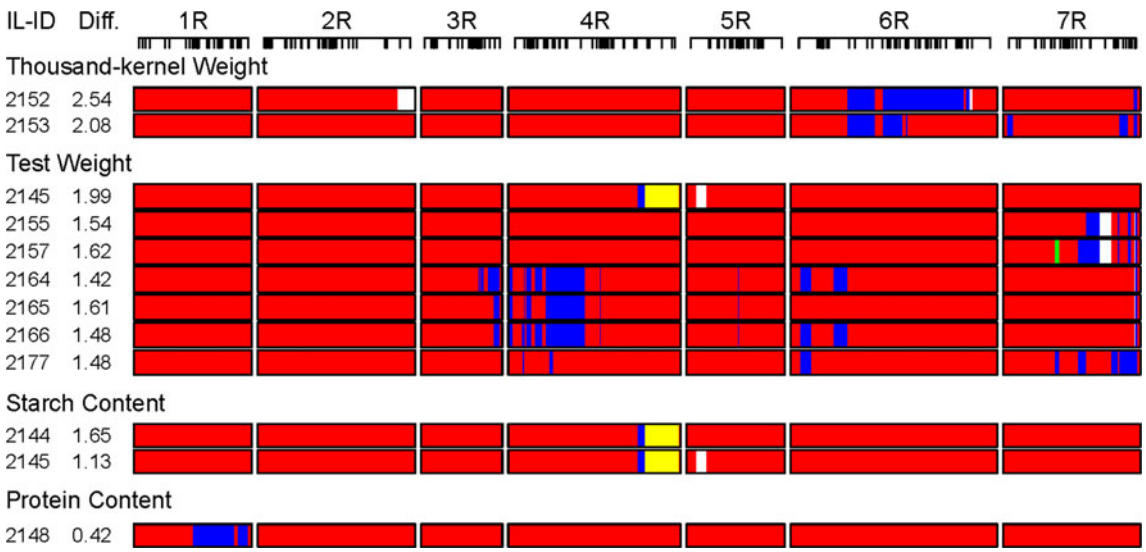

Fig. 4 Testcross performance of introgression library B: differences in the performance between the recipient and introgression lines (ILs). Only ILs with significant ($P<0.05$) differences of the Dunnett test were included. The respective chromosome and marker position (*vertical bars*) are presented above the figure; *blue coloring* denotes homozygous donor introgressions, *red coloring* indicates homozygous state of the recipient, *green coloring* denotes heterozygous state, *white coloring* denotes missing data, and *yellow coloring* indicates donor introgressions found to be significant with the linear model test (color figure online)

Table 1 QTL detected for different traits in the per se data of introgression library A

Trait	Location	QTL effect
Pentosan content	Chr.3 (27.8–36.7)	0.937
	Chr.7 (84.5–107.5)	0.669
	Chr.6 (116.7)	0.553
	Chr.1 (0.3–2.4)	0.440
	Chr.5 (120.5)	0.289
	Chr.5 (79.0)	0.282
Protein content	Chr.5 (85.6)	1.312
	Chr.3 (27.8–36.7)	1.202
	Chr.1 (0.0)	1.186
	Chr.5 (29.1)	1.178
	Chr.6 (116.7)	0.908
	Chr.7 (67.5)	0.643
	Chr.2 (45.2)	0.641
	Chr.1 (11.8), Chr.5 (102.2)	0.382
	Chr.2 (8.3–22.3), Chr.5 (46.0–46.1)	0.283
Starch content	Chr.4 (19.7)	1.011
	Chr.2 (45.2)	0.462
	Chr.1 (0.3–2.4)	−0.648
	Chr.7 (84.5–107.5)	−0.978
	Chr.1 (0.0)	−1.026
	Chr.6 (116.7)	−1.448
	Chr.5 (85.6)	−1.471
	Chr.3 (27.8–36.7)	−3.076
Thousand-kernel weight	Chr.4 (30.5), Chr.6 (2.3–6.7), Chr.6 (30.1–30.5)	1.057
	Chr.7 (66.1)	0.816

For the traits listed, the location of QTL (with approximate position or interval in cM) and their corresponding estimated effects are given. When multiple chromosomes are given for the same QTL, the segments containing these QTL are confounded

Table 2 QTL detected for different traits in the testcross data of introgression library A

Trait	Location	QTL effect
Pentosan content	Chr.7 (84.5–107.5)	0.320
Protein content	Chr.3 (27.8–36.7)	1.543
	Chr.5 (85.6)	1.020
	Chr.5 (29.1)	0.843
	Chr.3 (11.5)	0.477
	Chr.6 (71.8–74.4)	0.392
	Chr.7 (84.5–107.5)	0.358
	Chr.5 (50.7)	0.150
Starch content	Chr.2 (85.0)	1.451
	Chr.1 (20.6–43.0)	1.260
	Chr.1 (44.3–67.7)	0.635
	Chr.1 (11.8), Chr.5 (102.2–116.1), Chr. 5 (121.7)	0.476
	Chr.5 (120.6)	0.452
	Chr.5 (79.0)	−0.463
	Chr.7 (84.5–107.5)	−0.861
	Chr.5 (85.6)	−1.110
	Chr.3 (27.8–36.7)	−2.318
Thousand-kernel weight	Chr.7 (35.9)	2.682
	Chr.1 (20.6–43.0)	1.911
	Chr.4 (30.5), Chr.6 (2.3–6.7), Chr.6 (30.1–30.5)	1.542
	Chr.5 (120.6)	1.370
	Chr.7 (66.1)	1.092
	Chr.5 (26.2)	1.091
Test weight	Chr.1 (20.6–43.0)	1.782
	Chr.4 (19.7)	1.191
	Chr.5 (120.6)	1.057
	Chr.1 (11.8), Chr.5 (102.2–116.1), Chr. 5 (121.7)	0.685
	Chr.7 (66.1)	0.557
	Chr.4 (30.5), Chr.6 (2.3–6.7), Chr.6 (30.1–30.5)	0.497

For the traits listed, the location of QTL (with approximate position or interval in cM) and their corresponding estimated effects are given. When multiple chromosomes are given for the same QTL, the segments containing these QTL are confounded

present in both introgression libraries. Though the maps were created separately for each library, comparing centi-Morgan (cM) locations of QTL in both libraries enables a rough comparison to judge overlap. Putative QTL for kernel composition traits (pentosan, starch, and/or protein content) found in common between the two introgression libraries were located on chromosomes 1R, 5R, 6R, and 7R. Another potential common QTL lies on chromosome 4R, however here the cM locations did not overlap exactly.

Discussion

Introgression libraries were usually analyzed with a series of pairwise tests to detect whether the recipient and the ILs differ with respect to the investigated traits (Eshed and Zamir 1995; Matus et al. 2003; Rosseaux et al. 2005; Eduardo et al. 2007; Finkers et al. 2007; Szalma et al. 2007; Jeuken et al. 2008; Falke et al. 2008, 2009a, b; Schmalenbach et al. 2008, 2009, 2011); Schmalenbach and Pillen (2009). However, pairwise tests that detect phenotypic differences between the ILs and the recipient, such as the Dunnett test, are unable to identify the precise location of a QTL when multiple or long DCS are present in an IL.

The two introgression libraries investigated in our study consisted each of 40 ILs. 39 of the 40 ILs of introgression library A contained multiple DCS, as well as 25 of the ILs of library B. In most instances, the original Dunnett

Table 3 QTL detected for different traits in the per se data of introgression library B

Trait	Location	QTL effect
Pentosan content	Chr.6 (75.2–75.6)	0.853
	Chr.4 (76.0–83.5)	0.795
	Chr.4 (27.8)	0.740
	Chr.4 (46.0)	0.710
	Chr.6 (39.6–63.6)	0.621
	Chr.6 (66.8–70.5)	0.555
	Chr.7 (5.7–6.0)	0.435
	Chr.6 (60.3)	0.427
	Chr.3 (50.9), Chr.4 (0.0–26.3), Chr.4 (30.7–36.4), Chr.5 (35.0)	0.294
Protein content	Chr.3 (50.9), Chr.4 (0.0), Chr.4 (10.7–15.2), Chr.4 (26.3–36.4), Chr.5 (35)	0.547
	Chr.4 (20.3)	0.531
	Chr.1 (39.4–41.7)	0.492
	Chr.4 (9.4)	0.475
	Chr.4 (10.2)	0.239
Starch content	Chr.7 (87.2)	1.524
	Chr.4 (92.9–109.8)	1.344
	Chr.7 (74.6–75.4), Chr.7 (85.1)	0.925
	Chr.5 (28.0), Chr.5 (32.5), Chr.5 (34.5), Chr.5 (35.5–51.9), Chr.7 (47.6)	0.768
	Chr.5 (30), Chr.5 (32.9)	0.760
	Chr.7 (75.8)	−0.663
	Chr.7 (57.7–58.2)	−0.671
	Chr.4 (60.3)	−0.751
	Chr.7 (5.7–6.0)	−0.813
	Chr.6 (66.8–70.5)	−0.847
	Chr.1 (53.9–56.3)	−0.913
	Chr.4 (27.8)	−1.175
	Chr.6 (39.1–63.6)	−1.214
	Chr.3 (50.9), Chr.4 (0.0), Chr.4 (30.7–36.4), Chr.5 (35.0)	−1.254
	Chr.4 (46.0)	−1.339
	Chr.6 (75.2–75.6)	−1.592

For the traits listed, the location of QTL (with approximate position or interval in cM) and their corresponding estimated effects are given. When multiple chromosomes are given for the same QTL, the segments containing these QTL are confounded

analysis was unable to point towards single donor introgressions that were responsible for the detected phenotypic differences. In such situations, further experimental work can help to locate the position of QTL (Rousseaux et al. 2005). To accomplish this, the DCS of a significant IL are split up into several sub-ILs by further backcrosses. Then the sub-ILs are compared with the recipient. However this procedure is time and cost intensive.

Table 4 QTL detected for different traits in the testcross data of introgression library B

Trait	Location	QTL effect
Starch content	Chr.4 (92.9–109.8)	0.946
	Chr.4 (85.0)	0.354
Test weight	Chr.4 (92.9–109.8)	1.572

For the traits listed, the location of QTL (with approximate position or interval in cM) and their corresponding estimated effects are given. When multiple chromosomes are given for the same QTL, the segments containing these QTL are confounded

Instead of generating sub-ILs, employing a linear model analysis after having carried out the Dunnett test can help to identify QTL in ILs with multiple DCS. For example, in introgression library A, ILs 2121, 2123, and 2131 contain multiple DCS across several chromosomes. The testcross performance for thousand-kernel weight of all of these lines was detected as being significantly different from the recipient by the Dunnett test, but the location of the causative alleles could not be exactly determined. The linear model analysis pointed to the common DCS on chromosome 7R in all three ILs, thus lessening the potential length of DCS for fine-mapping from around 50 to under 20 cM. Hence, the linear model analysis allowed us to identify genomic regions carrying putative QTL much more precisely than the Dunnett test alone. We therefore conclude that the combination of the Dunnett test with a linear model analysis provides a valuable tool to identify and localize QTL, and may help to reduce the need for further splitting of the DCS in ILs with multiple segments.

The linear model analysis further allowed a much more detailed analysis of the pleiotropic effects of DCS than the Dunnett test alone. For example, the analysis revealed that putative QTL found on the DCS on chromosomes 4R and 6R which increase the per se performance for pentosan and protein content in introgression library B were also responsible for a decrease in starch content. Similar observations can be made throughout both libraries for per se and testcross performance. In practical breeding programs such results on pleiotropy might help to identify DCS that increase the performance of one of two negatively correlated traits without negative effects on the second trait. Fine-mapping and/or further sub-IL generation would help to determine if the pleiotropic QTL detected in this study are the result of single QTL or several linked QTL. For the purposes of this study, we can only localize QTL to DCS and assume that they are either a single QTL or two or more tightly linked QTL.

The more precise assignment of QTL to individual DCS with the linear model also allowed investigation of the consistency between QTL for per se and testcross performance. The rather low consistency observed in our analysis

suggests that testcross experiments are essential to assess the usefulness of introgressed DCS in hybrid rye breeding. In general, such analyses might assist the breeder in deciding on intensity of pre-selection among lines before going to the more resource demanding testcross phase. Additionally, the extensibility of this technique can allow for detection of gene interactions (epistasis) as well as model building. The utility and extensibility of regression for use in IL analysis has been demonstrated, for example, in rice (Wang et al. 2006, 2007) and maize (Coles et al. (2011).

To summarize, we conclude that employing a linear model test is a very promising method that allows the detection of favorable DCS in introgression libraries consisting of ILs that carry long or multiple DCS. It has the potential to greatly enhance the efficiency of producing sub-ILs, because only segments with a significant effect need to be isolated.

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