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ORIGINAL PAPER

Testcross performance of rye introgression lines developed by marker-assisted backcrossing using an Iranian accession as donor

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Abstract Introgression libraries facilitate the identification of favorable exotic alleles or genomic regions, which can be exploited for improving elite breeding material. We evaluated the first two introgression libraries in rye (*Secale cereale* L.) on the phenotypic and molecular level. Our objectives were to detect candidate introgression lines (pre-ILs) with a better testcross performance than the recurrent parent and identify donor chromosome segments (DCS) responsible for the improved performance. We introduced DCS from the self-incompatible heterozygous exotic Iranian primitive rye accession Altevogt 14160 (donor) into the genetic background of the elite inbred line L2053-N (recurrent parent) by marker-assisted backcrossing and developed 40 BC_2S_3 lines in each introgression library. Testcross performance for three agronomic and six quality traits was evaluated in replicated field trials across two testers at five locations over 2 years. The phenotypic effect of the DCS was analyzed for all traits. The pre-ILs had on average a testcross performance comparable to that of the recurrent parent. Significant ($P < 0.05$) differences between individual pre-ILs and the recurrent parent were detected for all traits except for heading date. For more than 60% of the significant ($P < 0.05$) differences, the pre-ILs were superior to the recurrent parent. For some pre-ILs, specific DCS were identified containing presumably quantitative trait loci responsible for the superior hybrid performance. Consequently, our study revealed that the development and employment of introgression libraries offers the opportunity for a targeted increase of genetic diversity of elite rye material for hybrid performance of agronomically important traits.

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Introduction

Improvement of plant breeding material frequently leads to a severe reduction in the genetic variation of elite germplasm (Bulmer 1971; Ladizinsky 1985; Tanksley and McCouch 1997). The limited genetic variation can result in a lack of (1) adaptation to biotic and abiotic stress (Tanksley and Nelson 1996), (2) genetic variation for specific quality traits (Whitt et al. 2002), or (3) genetic variation for quantitative traits leading to a decline of long-term selection response (cf. Haussmann et al. 2004).

Natural genetic variation occurring in wild relatives of cultivated crops provides a large genetic resource for plant breeding (Tanksley and McCouch 1997; Gur and Zamir 2004) and is expected to play a key role in future breeding progress (Zamir 2001). The detection of favorable alleles in genetic resources and their use in plant breeding programs requires extensive field experiments to characterize the effects of these favorable alleles from the exotic donor on agronomic important traits.

In rye (*Secale cereale* L.), hybrid breeding has superseded the development of population varieties in West and Central Europe during the last 20 years (Geiger and Miedaner 1999). Parental lines of new hybrid varieties are developed from crosses of elite breeding materials and are selected on the basis of their per se and hybrid performance (Tomerius et al. 2008). Hybrid performance for highly heterotic and complex traits is determined from testcross evaluations. High selection pressure and low effective population size are supposed to narrow down the genetic diversity of self-fertile hybrid rye (Geiger and Miedaner 1999). In the large gene pool of self-incompatible rye from Europe and Asia a tremendous variation is available, but its usefulness for hybrid breeding is restricted due to (1) missing assignment to heterotic groups, (2) high mutational load, and (3) lacking adaptation to high-input agriculture. The usability of genetic resources as crossing parent cannot be tested per se without a severe bias due to extreme plant height, high risk of lodging, and pre-harvest sprouting that masks positive grain yield effects.

For increasing the genetic diversity of elite breeding material, Eshed and Zamir (1994) proposed the development of introgression lines (ILs). These are characterized by a systematic introgression of individual, short, and marker-defined exotic donor chromosome segments (DCS) into the genetic background of the breeding material. ILs are obtained by a cross between the exotic donor and the elite recurrent parent followed by several generations of backcrossing with the recurrent parent and with at least one generation of selfing. Introgression libraries were first developed in tomato (*Lycopersicum esculentum*; Eshed and Zamir 1994). This method was adopted for numerous grain species including barley (*Hordeum vulgare* L.; Matus et al. 2003; von Korff et al. 2004; Schmalenbach et al. 2008a), maize (*Zea mays* L.; Ribaut and Ragot 2007; Szalma et al. 2007), rice (*Oryza sativa* L.; Lin et al. 1998), and wheat (*Triticum aestivum* L.; Liu et al. 2006). In 1999, we initiated the establishment of the first two rye introgression libraries by marker-assisted backcrossing (Falke et al. 2008). The candidate introgression lines (pre-ILs) of our libraries comprised marker-defined DCS from the self-incompatible heterozygous exotic Iranian primitive rye accession Altevogt 14160 (donor) introduced into the genetic background of the elite inbred line L2053-N (recurrent parent). In each backcross and selfing generation up to BC_2S_3 the number and length of DCS as well as the proportion of the recurrent parent genome and donor genome coverage was assessed by molecular markers in each set of pre-ILs (Falke et al. 2008).

The majority of agronomically important traits are quantitatively inherited and, thus, affected by numerous genes and environmental factors. For facilitating the identification of quantitative trait loci (QTL), Tanksley et al. (1996) suggested the employment of ILs because the phenotypic variation between ILs and the recurrent parent can be directly associated with the introgressed segment. For several crops, such as tomato (Eshed and Zamir 1995; Tanksley et al. 1996; Bernacchi et al. 1998a, b; Fulton et al. 2000), barley (Pillen et al. 2003; von Korff et al. 2006; Schmalenbach et al. 2008b), wheat (Huang et al. 2003), soybean (*Glycine max* L.; Concibido et al. 2003), rice (Septiningsih et al. 2003; Tian et al. 2006; Tan et al. 2007), or rye (Falke et al. 2008) favorable exotic alleles or genomic regions for agronomic and quality traits could be identified by using ILs. These genomic regions can be employed to improve the elite breeding material. While per se performance of two baking quality traits has been evaluated in a companion study (Falke et al. 2008), the testcross performance for agronomic and quality traits has not yet been investigated.

The objectives of our study were to (1) to investigate the testcross performance of pre-ILs from two rye introgression libraries for three agronomic and six quality traits with field trials across five locations in 2 years employing two testers, (2) determine whether the testcross performance of pre-ILs outperformed significantly the testcross performance of the corresponding recurrent parent, and (3) attempt to discover DCS responsible for the superior performance.

Materials and methods

Marker-assisted development of introgression libraries

The employed selection procedure for developing the two introgression libraries A and B was described in detail by Falke et al. (2008). Briefly, the recurrent parent L2053-N was an elite inbred line from the Petkus gene pool developed by Hybro GmbH & Co KG (Schenkenberg, Germany) and the donor Altevogt 14160 was an Iranian primitive population provided by the Botanical Garden Warsaw (Poland). Altevogt 14160 was chosen as donor parent despite of its poor agronomic performance to discover favorable alleles especially for traits with a limited genetic variability in elite breeding material. L2053-N and three

single plants of the heterozygous Altevogt 14160 were crossed in 1999 to produce the F_1 base populations. The progeny of one F_1 plant was used to generate introgression library A, and the remaining two to develop introgression library B. One random kernel per library was used for individual backcrossing to L2053-N to generate BC_1. The BC_2 population was generated in 2001 followed by three selfings to generate the BC_2S_3 lines.

Progenies of each generation were selected by molecular markers. The procedures for amplified length polymorphism (AFLP) and simple sequence repeat (SSR) assays were described in detail by Falke et al. (2008). Briefly, a total of 131 and 182 AFLP loci as well as 137 and 118 SSR markers polymorphic between the parental lines were employed for genotyping of introgression libraries A and B, respectively. We used 87 (library A) and 88 (library B) random BC_1 individuals for constructing the genetic linkage maps and 40 BC_2S_3 lines per population for analyzing the introgression libraries. Genetic linkage maps of the individual populations were constructed with software JoinMap 3.0 (van Ooijen and Voorrips 2001). A LOD threshold of 3.0 was used for declaring linkage in two-point analyses and Kosambi's (1944) mapping function was employed for calculating map distances. Graphical genotyping of BC_2S_3 pre-ILs was performed with software PLABSOFT (Maurer et al. 2008), which is implemented as an extension of the statistical software R (R Development Core Team 2004).

Plant materials

39 pre-ILs of each introgression library as well as the donor and the recurrent parent were crossed to the unrelated cytoplasmatically male-sterile testers from the Petkus gene pool L2092-P × LY2130-N (T1) developed by Hybro GmbH & Co KG (Schenkenberg, Germany) and Lo55-P × Lo88-N (T2) developed by KWS LOCHOW GmbH (Bergen, Germany). Testcrossing was done in 2004 by open pollination in field plots isolated by polyethylene walls, a common procedure for producing experimental hybrids (Geiger and Miedaner 1999).

Agronomic trials

The experimental design at each location was a 10 × 9 α-design (Patterson and Williams 1976) with two replications. Each genotype was grown in plots of 1.25 m width and 4–4.5 m length, representing about 5 m^2. All experiments were machine planted and harvested as grain trials with a combine. From the harvest a representative sample (500 g) was taken for quality analyses. Agronomic treatments included application of 70–120 kg N ha^{-1}, two sprayings of plant growth regulators (1.5 l ha^{-1} CCC and 1.5 l ha^{-1} Terpal C), and one fungicide spraying (1.5 l ha^{-1} Opus Top). Testcrosses with the recurrent parent (L2053-N) were included tenfold to improve accuracy, with the donor (Altevogt 14160) as duplex entries in each experiment. Data were recorded for the agronomic traits grain yield (dt ha^{-1}), heading date (1,…,9; where 9 is early) and plant height (cm), as well as the quality traits thousand-kernel weight (g) investigated from a representative sample, test weight determined by weighing 250 ml of grain and extrapolating to 100 l (kg), falling number (sec) analyzed by using 7 g of whole meal with FN 1700 (Perten Instruments Hamburg, ICC/No. 107/1), pentosan, protein, and starch content in grain (%), the latter three estimated by near-infrared reflectance spectroscopy. The field trials were conducted in 2005 and 2006 at three sites in the South (Hohenheim, Oberer Lindenhof, and Eckartsweier) and at two in the North (Wulfsode and Bergen) of Germany. The agronomic traits for T1 could be assessed only at four locations in both years due to a lack of seeds; moreover, in 2006 only four locations for T2 could be harvested due to pre-harvest-sprouting at Oberer Lindenhof. The quality traits were only ascertained for T1 at the locations Hohenheim, Wulfsode, and Bergen in both years. Furthermore, pentosan, protein, and starch content were only determined in 2006.

Statistical analyses

Lattice analyses of variance for all agronomic traits were performed for each experiment and location using software PLABSTAT (Utz 2001). Adjusted entry means were 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 REML, using PROC MIXED of SAS (SAS Institute 2004). To take into account the variation in accuracy of the individual lattices, least square means were weighted with the reciprocal error variance of a mean (Piepho 1999). The linear model for agronomic traits (grain yield and plant height) was

$$\begin{aligned} Y = {} & \mu + G_r D + T_s + L_v + J_w + C + (GT)_{rs} D \\ & + (GL)_{rv} D + (GJ)_{rw} D + (TL)_{sv} + (TJ)_{sw} + (LJ)_{vw} \\ & + (CL)_{v} + (CJ)_{w} + (GTL)_{rsv} D + (GLJ)_{rvw} D \\ & + (GTJ)_{rsw} D + (TLJ)_{svw} + (CLJ)_{vw} + (GTLJ)_{rsvw} D + e, \end{aligned} \quad (1)$$

where G_r ($r = 1, \ldots, 78$) are the genotypes, T_s ($s = 1, 2$) the testers, L_v ($v = 1, \ldots, 5$) the locations, and J_w ($w = 1, 2$) the years. Interaction effects are denoted by combination of the corresponding main effects, and e denotes the residual error of mean. C is a classification variable and D is defined as a dummy variable; both variables have one

level for the testcross with the recurrent parent ($C = 1$; $D = 0$) and one level for the testcrosses with the pre-ILs ($C = 2$; $D = 1$). C and D were employed to (1) introduce the mean of the pre-ILs into the model and (2) ensure that the check (testcross with the recurrent parent) was not included in the estimation of the variance-covariance matrices (Piepho et al. 2006). T_s, L_v, J_w, C, and the interactions $(LJ)_{vw}$ and $(CJ)_w$ were considered as fixed effects and the remaining effects were considered as random. The quality traits were assessed only for tester T1 and, therefore, we excluded T_s and all interactions with T_s for these traits. Furthermore, for pentosan, protein, and starch content the factor J_w, and all interactions with J_w were also excluded because these three traits were assessed only in 2006. Variance components were estimated combined for both libraries because Akaike's Information Criterion (AIC) indicated that estimating separate variance components would not result in an improvement of model fit.

The heritability $\bar{H}^2$ was computed with an ad hoc measure for unbalanced test designs as

$$\bar{H}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \bar{v}/2}, \qquad (2)$$

where σ_g^2 is the genotypic variance and $\bar{v}$ is the mean variance of a difference between two adjusted treatment means (BLUE) (Holland et al. 2003; Piepho and Möhring 2007; Emrich et al. 2008).

An a priori planned t-test for $k - 1$ multiple comparisons, where k is the number of test units, and a both-sided Dunnett test (Dunnett 1955) for multiple comparisons of least square means were used to determine significant differences between the pre-ILs and the recurrent parent L2053-N, which was regarded as a control. We employed a generalization of the Dunnett procedure due to Hsu (1992), which uses a factor-analytic approximation to the variance-covariance matrix of adjusted means and has been adapted to mixed models in an implementation available in the MIXED procedure of the SAS system. A significance level of $\alpha = 0.05$ was employed. The linear model was

$$\begin{aligned} Y = \mu + G_r + T_s + L_v + J_w + (GT)_{rs} + (GL)_{rv} + (GJ)_{rw} \\ + (TL)_{sv} + (TJ)_{sw} + (LJ)_{vw} + (GTL)_{rsv} + (GLJ)_{rvw} \\ + (GTJ)_{rsw} + (TLJ)_{svw} + (GTLJ)_{rsvw} \quad . \end{aligned} \qquad (3)$$

For the agronomic traits (grain yield and plant height), G_r, T_s, L_v, J_w, and the interactions $(GT)_{rs}$ and $(LJ)_{vw}$ were considered as fixed effects and the remaining factors were considered as random. Quality traits were assessed only for tester T1, hence, we excluded T_s and all interactions with T_s from the linear model. The effect G_r of the genotypes was assumed fixed and the remaining effects were assumed random. Pentosan, protein, and starch content were measured only in 2006, therefore, the effect J_w was not included in the linear model. The t- and Dunnett tests were computed with PROC MIXED of software SAS (SAS Institute 2004).

Results

The results of the AFLP and SSR analyses have been reported in detail previously (Falke et al. 2008). The genetic linkage maps in BC_1 of populations A and B spanned a total of 738 and 636 cM with average interval lengths of 2.8 and 2.2 cM, respectively. The donor genome coverage of BC_2S_3 lines was 74% for introgression library A and 59% for introgression library B. Most of the pre-ILs contained one to three homozygous DCS, with a mean length of 13 cM (library A) and 10 cM (library B). The mean proportion of the recurrent parent genome was 92 % (library A) and 95 % (library B).

Testcross performance (least square means) of the recurrent parent L2053-N exceeded the donor Altevogt 14160 for grain yield, falling number, and pentosan content and showed a considerably shorter plant height (Table 1). In contrast, the testcross performance for thousand-kernel weight, test weight, and protein and starch content were higher for the donor than for the recurrent parent. For all traits, the testcross performance of pre-ILs of both introgression libraries had the tendency to be more similar to the recurrent parent. However, we also observed for both introgression libraries a certain variation in single cases indicated by the minimum and maximum values.

Variance components for heading date were not estimated because data were based on scorings. REML estimates of the genotypic variance σ_g^2 were significant ($P < 0.01$) for all traits (Table 2). Estimates of genotype × tester variance σ_{gt}^2 were significant ($P < 0.05$) for grain yield and plant height, estimates of genotype × year variance σ_{gj}^2 for plant height and falling number, and estimates of genotype × location variance σ_{gl}^2 for grain yield as well as pentosan and starch content. Estimates of genotype × year × location variance σ_{gjl}^2 were significant ($P < 0.05$) for grain yield, test weight, and falling number. Estimates of the residual error variance σ_{gtjl}^2 were significant ($P < 0.05$) for plant height. Estimates of $\bar{H}^2$ ranged from 0.05 for pentosan content to 0.95 for falling number and were high for all traits with high genotypic variance σ_g^2.

The t-test for multiple comparisons, which does not adjust for multiple testing, was significant ($P < 0.05$) for 113 out of 312 comparisons between pre-ILs and the recurrent parent in introgression library A (Fig. 1) and for 69 comparisons in introgression library B (Fig. 2). Out of these, 69 (library A) and 43 (library B) pre-ILs were

Table 1 Testcross performance (least square means) of the donor population Altevogt 14160 and the recurrent parent L2053-N, average, minimum, and maximum testcross performance of the candidate introgression lines (pre-ILs), as well as minimum and maximum standard error (SE) of the testcross performance for three agronomic and six quality traits averaged across two testers (T1: L2092-P × LY2130-N and T2: Lo55-P × Lo88-N), five locations, and 2 years

Trait	Tester × Altevogt 14160	Tester × L2053-N	SE		Tester × pre-ILs					
					Library A			Library B		
			Min	Max	Mean	Min	Max	Mean	Min	Max
Agronomic traits										
Grain yield (dt ha^{-1})	65.7	77.3	1.21	1.99	76.7	64.7	80.9	77.7	72.6	81.5
Plant height (cm)	142.0	118.4	2.18	2.87	119.8	108.5	131.7	120.3	114.0	132.6
Heading date (1–9)	5.4	5.0	0.13	0.22	5.1	4.8	5.3	5.1	4.8	5.5
Quality traits[a]										
Thousand-kernel weight (g)	34.2	33.2	1.24	1.55	33.9	30.5	37.7	33.4	30.3	35.7
Test weight (kg)	71.2	68.7	1.51	1.56	69.1	65.4	71.4	69.3	67.6	70.7
Falling number (s)	216.3	241.2	81.09	82.60	242.6	192.9	276.2	241.2	226.0	260.3
Pentosan content (%)[b]	8.0	8.6	0.12	0.21	8.6	7.9	9.2	8.6	8.1	8.8
Protein content (%)[b]	8.3	7.9	0.20	0.31	8.0	7.6	8.9	7.8	7.1	8.3
Starch content (%)[b]	63.0	62.2	0.16	0.41	62.1	60.6	63.2	62.4	61.8	63.8

[a] Based on one tester

[b] Analyzed only in 2006

Table 2 REML estimates of variance components and heritabilities ($\bar{H}^2$) of rye testcross progeny from introgression libraries A and B for two testers over five locations and 2 years for agronomic and quality traits

Variance component	Agronomic traits		Quality traits[a]					
	Grain yield (dt ha^{-1})	Plant height (cm)	Thousand-kernel weight (g)	Test weight (kg)	Falling number (s)	Pentosan content (%)[b]	Protein content (%)[b]	Starch content (%)[b]
σ^2_g	6.34**	15.49**	1.94**	0.75**	119.44**	0.03**	0.06**	0.17**
σ^2_{gt}	1.55**	1.37**	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_{gj}	0.09	1.88*	0.12	0.06	93.27**	–[c]	–[c]	–[c]
σ^2_{gl}	1.01*	0.11	0.08	0.00	0.00	0.01*	0.11	0.03*
σ^2_{tj}	1.41	5.37	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_{tl}	0.00	1.90	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_{cl}	0.00	0.04	0.00	0.01	0.00	0.04	0.00	0.06
σ^2_{gtj}	0.00	0.91	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_{gtl}	0.28	0.00	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_{gjl}	2.42**	0.47	0.16	0.09*	70.49**	–[c]	–[c]	–[c]
σ^2_{tjl}	7.00	20.90	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_{cjl}	0.15	0.00	0.00	0.00[c]	0.00	–[c]	–[c]	–[c]
σ^2_{gtjl}	0.76	1.04**	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
σ^2_e (min–max)[d]	2.6–26.2	1.8–10.9	0.4–1.5	0.1–0.4	57.2–435.6	0.0–0.0	0.0–0.1	0.1–0.1
$\bar{H}^2$	0.90	0.94	0.85	0.76	0.95	0.05	0.08	0.19

*,** Significant at the 0.05 and 0.01 probability level, respectively

[a] Based on one tester

[b] Analyzed only in 2006

[c] Not estimated

[d] σ^2_e of the lattice analyses of variances of individual environments; for combined analyses of variances σ^2_g was fixed to 1

significantly better than the recurrent parent. For agronomic traits, 16 (library A) and ten (library B) differences between pre-ILs and the recurrent parent were significantly negative. However, we detected five pre-ILs in each introgression library with a significantly ($P < 0.05$) higher testcross yield than L2053-N. In introgression library A these pre-ILs showed DCS on all chromosomes except chromosome 1R, while in introgression library B DCS were present on chromosomes 1R, 2R, 4R, 6R, and 7R. Three pre-ILs in introgression library A were significantly ($P < 0.05$) shorter than the recurrent parent and carried DCS on chromosomes 2R, 5R, and 7R. For quality traits, we observed that the *t*-test detected mainly for thousand-kernel weight and test weight pre-ILs with a significantly ($P < 0.05$) superior testcross performance than the recurrent parent. Eighteen (library A) and eight (library B) pre-ILs showed significantly ($P < 0.05$) higher values for thousand-kernel weight, and for test weight 11 (library A) and 17 (library B) pre-ILs were significantly ($P < 0.05$) better than L2053-N. Pre-ILs showing significantly ($P < 0.05$) higher thousand-kernel weight contained DCS on all chromosomes in introgression library A and on chromosomes 1R, 4R, 5R, 6R, and 7R in introgression library B. For test weight we detected positively significant pre-ILs carrying DCS on all chromosomes in introgression library A and on chromosomes 1R, 3R, 4R, 6R, and 7R in introgression library B. For falling number four positively significant ($P < 0.05$) pre-ILs were observed in introgression library A carrying DCS on chromosomes 2R, 3R, 4R, 5R, 6R, and 7R. Moreover, the *t*-test for multiple comparisons was positively significant ($P < 0.05$) for pentosan content for four pre-ILs in introgression library A carrying DCS on chromosomes 2R, 3R, 5R, and 7R. For protein content 18 (library A) and 3 (library B) positively significant ($P < 0.05$) pre-ILs were detected, which contained DCS on all chromosomes in introgression library A and on chromosome 1R in introgression library B. We detected six (library A) and ten (library B) pre-ILs with a significantly ($P < 0.05$) higher starch content and observed that these pre-ILs carried DCS on all chromosomes except on 3R in introgression library A and on all chromosomes except on 5R in introgression library B.

Using the Dunnett test, the number of pre-ILs showing a significantly ($P < 0.05$) superior performance compared to the recurrent parent was reduced in comparison to the *t*-test (Figs. 1, 2). For agronomic traits, only one pre-IL in introgression library A was significantly ($P < 0.05$) shorter than the parental control. For quality traits, we observed nine pre-ILs in introgression library A and two pre-ILs in introgression library B with a significantly ($P < 0.05$) higher thousand-kernel weight as well as five (library A) and seven (library B) pre-ILs with a significantly ($P < 0.05$) higher test weight than the recurrent parent. For pentosan content, one pre-IL was positively significant ($P < 0.05$) in introgression library A. We detected five (library A) and one (library B) pre-IL with a significantly ($P < 0.05$) higher protein content and one (library A) and two (library B) pre-ILs showed significantly ($P < 0.05$) higher values for starch content than L2053-N.

One and six pre-ILs carrying a single DCS as well as eight and two pre-ILs carrying two DCS of introgression library A and B, respectively, were significantly different (*t*-test, $P < 0.05$) to the recurrent parent for at least one trait (Tables 3 and 4). These pre-ILs allow a localization of QTL regions. QTL for grain yield are presumably located on chromosomes 3R and/or 4R (pre-ILs 2106, 2143, 2172), 1R (2147), and 2R and/or 6R (2179). QTL for plant height are likely located on chromosomes 2R and/or 5R (2125). QTL for thousand-kernel weight are supposed to be located on chromosome 5R and/or 2R (2125, 2126), and/or 7R (2120, 2123), and/or 6R (2178). QTL for test weight are presumably located on chromosomes 2R and/or 5R (2125), 4R (2145), and 1R and/or 3R (2168, 2169). QTL for falling number can be assumed on chromosomes 2R and/or 5R (2125, 2126). QTL for pentosan content can be assumed on chromosome 3R (pre-IL 2107) and on 5R and/or 7R (pre-IL 2134). Five pre-ILs with double DCS on chromosomes 2R, 5R, and 7R as well as pre-IL 2107 with a DCS on 3R and pre-ILs 2148 and 2149 with DCS on 1R indicate for QTL for protein content in these genomic regions. QTL for starch content are presumably located on chromosomes 4R (2144, 2145), 1R and/or 3R (2169), and 2R and/or 6R (2179).

Discussion

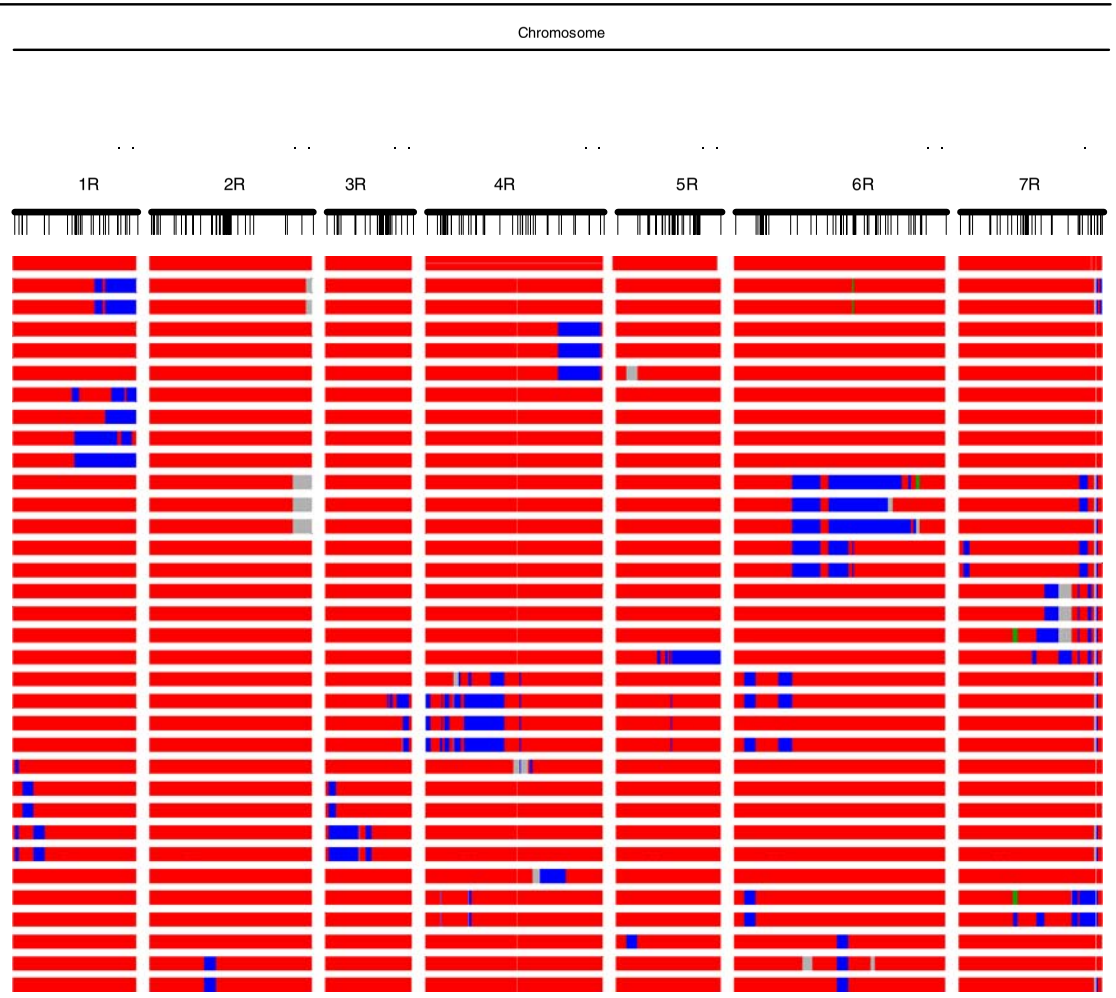
Most plant breeding procedures are expected to result in a severe reduction of the genetic variation (Bulmer 1971; Tanksley and McCouch 1997). To counteract this reduction and to increase the genetic variation, introgression of exotic germplasm provides a promising opportunity (Gur and Zamir 2004). As exotic germplasm carries agronomically undesirable alleles, several studies have shown that introgression libraries are a useful tool to identify agronomically interesting alleles and subsequently transfer these into elite breeding material (Eshed and Zamir 1995; Pillen et al. 2003; Huang et al. 2003; Concibido et al. 2003; Septiningsih et al. 2003; Schmalenbach et al. 2008b). Therefore, we established two introgression libraries in rye (Falke et al. 2008). In the present study, we employed the *t*-test and the Dunnett test for multiple comparisons to detect pre-ILs showing significant ($P < 0.05$) differences in the testcross performance compared to their corresponding recurrent parent. These analyses allowed us to identify genomic regions responsible for the differences in performance.

Tester x Genotype	Agronomic traits: Grain yield (dt / ha)	Agronomic traits: Plant height (cm)	Quality traits[a]: Thousand-kernel weight (g)	Quality traits[a]: Test weight (kg)	Quality traits[a]: Falling number (sec)	Quality traits[a]: Pentosan content (%)[b]	Quality traits[a]: Protein content (%)[b]	Quality traits[a]: Starch content (%)[b]
L2053-N	77.3	118.4	33.2	68.7	241.2	8.6	7.9	62.2
2101	+1.19	+13.28**	+1.67**	+2.06**	-12.59	-0.49**	+0.13	+0.45
2102	+0.49	+9.55**	+2.22**	+2.69**	+3.82	-0.71**	+0.05	+0.64*
2103	+0.71	+7.43**	+1.34*	+2.21**	-14.77	-0.70**	-0.10	+1.06**
2104	+2.25	+4.77**	-1.15*	+0.11	-17.88	+0.10	-0.11	-0.19
2105	+1.95	+3.83*	-0.68	+0.09	-12.33	-0.18	-0.25*	+0.19
2106	+3.04*	+4.70**	-0.89	+0.11	-3.52	-0.08	+0.08	-0.02
2107	-12.65**	-2.95	-2.63**	-3.28**	-48.12**	+0.40**	+1.02**	-1.59**
2108	-0.26	+0.76	-1.92**	-1.06**	-13.95	+0.04	-0.06	+0.03
2109	+0.56	+0.29	-1.87**	-1.26**	-9.88	+0.03	-0.04	0.00
2110	-0.77	+1.19	-0.68	+0.02	+12.86	+0.22	+0.29*	-0.31
2111	+0.30	+1.71	+1.20*	+0.84*	-2.08	+0.04	+0.24*	-0.07
2113	-1.37	-0.53	+0.95	+0.78	+11.67	+0.12	+0.27*	-0.22
2114	+1.92	+0.29	+1.02	+1.45**	+9.41	+0.09	+0.17	-0.03
2115	+3.90**	+2.74	-0.30	+0.48	+7.61	-0.12	+0.04	+0.15
2116	+3.32**	+3.85*	-0.34	-0.29	+9.13	-0.29*	+0.02	+0.68**
2117	+2.83*	+3.74*	+0.01	+0.46	+2.48	-0.34**	-0.15	+0.65**
2118	+2.14	+9.10**	+2.28**	+1.29**	+7.02	-0.36**	-0.01	+0.33
2119	+3.22*	+10.26**	+2.54**	+1.11**	+8.88	-0.25*	+0.05	+0.06
2120	-1.77	+0.47	+4.52**	+0.45	+2.69	+0.03	+0.27*	-0.49*
2121	-0.16	-0.15	+2.11**	+0.30	-2.18	+0.04	-0.05	-0.03
2122	-1.86	-0.23	+1.60**	+0.44	+2.35	+0.01	+0.25*	-0.23
2123	-1.11	-2.28	+2.67**	+0.27	-11.10	+0.08	+0.30*	-0.28
2124	-2.48	-5.53**	+1.02	+0.20	+28.19*	-0.19	+0.57**	+0.06
2125	-3.79**	-5.78**	+1.23*	+0.89*	+31.71*	-0.30*	+0.38**	+0.11
2126	-0.14	+1.00	+1.64**	+0.67	+34.94**	-0.10	+0.33**	-0.06
2127	-5.34**	+1.37	-2.65**	+0.27	-7.08	+0.01	+0.36*	-0.20
2128	-0.24	-0.54	+1.17*	+0.45	+30.82*	-0.16	+0.40**	+0.07
2130	-1.20	-0.90	+0.13	+0.80*	+6.00	-0.08	+0.11	-0.16
2131	-1.60	-2.41	+2.47**	+0.59	+13.89	-0.20	-0.12	+0.39
2132	-0.92	-1.27	-0.32	+0.57	+2.24	-0.18	+0.29*	-0.22
2133	-1.93	+0.96	-0.04	+0.24	+2.01	-0.05	+0.58**	-0.56*
2134	-11.83**	+1.26	+0.95	-0.65	-47.36**	+0.54**	+0.72**	-1.62**
2135	-3.32**	-9.83**	0.00	-0.05	+12.17	+0.27*	+0.66**	-0.67**
2136	-2.08	-1.41	+1.53**	-0.39	-10.13	+0.29*	+0.35**	-0.82**
2137	-1.60	-2.83	+0.84	+0.23	+23.93	+0.03	+0.36**	-0.29
2138	-0.65	+3.31	+1.95**	+0.18	-1.27	-0.16	+0.06	+0.04
2139	+2.26	+4.47*	+3.59**	+1.46**	+23.84	-0.28*	-0.13	+0.57*
2140	+1.88	+3.45	+3.12**	+1.33**	+4.58	-0.40**	-0.25*	+0.68**

*,** Significant at the 0.05 and 0.01 probability level, respectively

Fig. 1 Testcross performance of the recurrent parent L2035-N and differences to the testcross performance of 38 candidate introgression lines (pre-ILs) from introgression library A for agronomic and quality traits. Only pre-ILs with significant ($P < 0.05$) differences of the *t*-test in at least one trait were included. Underlined differences indicate that the trait was also significant ($P < 0.05$) with the Dunnett test. The respective chromosome and the marker position (vertical bars) are presented above the figure, red coloring indicates homozygous state of the recurrent parent, blue coloring homozygous state of the donor, green coloring heterozygous state, and gray coloring missing data. *,**Significant at the 0.05 and 0.01 probability level, respectively. [a] Based on one tester, [b] analyzed only in 2006

Tester x Genotype	Agronomic traits: Grain yield (dt / ha)	Agronomic traits: Plant height (cm)	Quality traits[a]: Thousand-kernel weight (g)	Quality traits[a]: Test weight (kg)	Quality traits[a]: Falling number (sec)	Quality traits[a]: Pentosan content (%)[b]	Quality traits[a]: Protein content (%)[b]	Quality traits[a]: Starch content (%)[b]
L2053-N	77.3	118.4	33.2	68.7	241.2	8.6	7.9	62.2
2141	-2.39	+1.31	-0.26	-0.58	-4.28	-0.20	+0.32**	+0.54*
2142	-0.96	+1.81	-0.17	-0.45	-2.56	-0.26*	+0.02	+0.60*
2143	+4.22*	+8.78**	-	-	-	-	-	-
2144	-1.85	+14.14**	-2.89**	-	+1.10	-0.56**	-0.66**	+1.65**
2145	-1.52	+12.64**	-2.55**	+1.99**	+8.22	-0.41**	-0.78**	+1.13**
2146	-1.72	+1.78	-0.18	-0.49	+9.39	-0.13	-0.15	+0.50*
2147	-2.98*	+1.01	-0.02	-1.03**	-12.66	+0.06	+0.15	+0.17
2148	-4.02**	+0.66	+0.05	-0.12	-10.60	-0.23	+0.42**	-0.02
2149	-4.70**	+1.50	-0.49	-0.98*	-15.15	-0.07	+0.36**	-0.37
2150	+1.42	+1.91	+1.62**	+1.29**	+13.22	-0.02	-0.07	+0.18
2151	+1.34	+1.60	+1.62**	+1.25**	+7.66	-0.02	-0.21	+0.15
2152	+2.84*	+3.40	+2.54**	+0.84*	+4.17	+0.04	+0.01	-0.25
2153	+1.05	+1.83	+2.08**	+0.29	+7.40	-0.05	+0.13	+0.13
2154	+2.24	+2.56	+1.74**	+0.03	-5.31	+0.09	+0.11	-0.25
2155	+0.79	-0.98	+1.91**	+1.54**	+1.66	-0.12	-0.16	+0.51*
2156	+2.90*	-0.16	+0.71	+1.14**	+2.42	-0.16	+0.03	+0.34
2157	+0.51	-0.06	+0.62	+1.62**	-4.86	-0.35**	+0.13	+0.51*
2161	+0.56	+7.57**	-	-	-	-	-	-
2163	-0.78	-2.99	-0.29	+0.87*	+4.56	+0.08	-0.03	-0.10
2164	-0.04	-0.09	-0.59	+1.42**	+4.27	+0.04	-0.17	+0.12
2165	+1.77	+1.08	-1.17*	+1.61**	+1.72	0.00	-0.06	0.00
2166	-2.03	+1.72	-1.30*	+1.48**	+0.07	-0.02	+0.11	-0.06
2167	+0.54	+1.35	+1.75**	+0.26	-6.51	+0.02	-0.16	+0.05
2168	-0.01	+2.22	+0.79	+1.35**	-1.39	+0.12	-0.06	+0.03
2169	+0.56	+0.27	-1.12*	+1.18**	+2.85	-0.13	-0.37**	+0.50*
2170	-1.99	-2.20	-0.77	+1.13**	+9.91	-0.08	-0.04	+0.06
2171	-0.17	-0.04	-0.71	+0.81*	+10.50	+0.17	-0.25*	-0.16
2172	+3.06*	+3.01	+0.41	-0.48	-2.67	+0.20	-0.08	-0.26
2176	+2.10	-0.23	+0.49	+1.07**	+2.55	-0.09	-0.17	+0.27
2177	+0.12	-0.59	+0.85	+1.48**	-8.47	-0.33**	-0.17	+0.59*
2178	+2.40	+4.83**	+1.23*	+0.35	-6.18	-0.09	-0.14	+0.26
2179	+3.84**	+4.62**	-0.55	+0.55	+11.29	-0.13	-0.16	+0.52*
2180	+1.24	+5.45**	-0.51	+0.26	+19.21	-0.15	-0.03	+0.23



*,** Significant at the 0.05 and 0.01 probability level, respectively

[a] Based on one tester

[b] Analyzed only in 2006

Fig. 2 Testcross performance of the recurrent parent L2035-N and differences to the testcross performance of 33 candidate introgression lines (pre-ILs) from introgression library B for agronomic and quality traits. Only pre-ILs with significant ($P < 0.05$) differences of the *t*-test in at least one trait were included. Underlined differences indicate that the trait was also significant ($P < 0.05$) with the Dunnett test. The respective chromosome and the marker position (vertical bars) are presented above the figure, red coloring indicates homozygous state of the recurrent parent, blue coloring homozygous state of the donor, green coloring heterozygous state, and grey coloring missing data. *,**Significant at the 0.05 and 0.01 probability level, respectively. [a]Based on one tester, [b]analyzed only in 2006

Table 3 Candidate introgression lines (pre-ILs) of library A carrying one or two donor chromosome segments (DCS) and showing significant ($P < 0.05$) differences to the recurrent parent L2053-N detected by the *t*-test, the corresponding chromosomal location of the DCS, and the detected significant traits

Tester × Genotype	DCS				Trait performance	
	Chr.[a]	Marker position (cM)	Flanking markers	Length of marker interval (cM)	Superior	Inferior
2105	3R	27.8	P12/M61-F-344-P2	14.3		PC[i]
		36.7	P12/M61-F-88-P2			
	4R	71.0	Xscm339	15.2		
		80.3	Xscm116			
2106	3R	27.8	P12/M61-F-344-P2	14.3	GY[c]	PH[d]
		36.7	P12/M61-F-88-P2			
	4R	78.2	Xscm245	3.8		
		80.3	Xscm116			
2107	3R	27.8	P12/M61-F-344-P2	14.3	PentC[h], PC	GY, TKW[e], TW[f], FN[g], SC[j]
		36.7	P12/M61-F-88-P2			
2108	3R	27.8	P12/M61-F-344-P2	14.3		TKW, TW
		36.7	P12/M61-F-88-P2			
	4R	19.7	Xscm251	22.1		
2120	5R	48.5	P17/M60-F-199-P2	46.2	TKW, PC	SC
		91.0	Xscm54			
	7R	7.3	Xgwm565	5.3		
2123	5R	55.0	Xgwm271	35.9	TKW, PC	
		89.0	Xscm309			
	7R	0.0	P17/M57-F-134-P2	47.1		
		46.0	Xscm44			
2125	2R	8.2	Aif051[b]	21.8	PH, TKW, TW, FN, PC	GY, PentC
		24.7	Xscm215			
	5R	29.1	Aif069	62.0		
		89.0	Xscm309			
2126	2R	8.2	Aif051	18.1	TKW, FN, PC	
		22.3	P17/M60-F-312-P2			
	5R	29.1	Aif069	62.0		
		89.0	Xscm309			
2134	5R	65.5	Xscm151	28.3	PC, PentC	GY, FN, SC
		89.0	Xscm309			
	7R	84.5	P17/M57-F-312-P2	24.7		
		107.5	Xscm122			

[a] Chromosomal location of the DCS

[b] Primer information is available for non-commercial use on request by B. Kusterer (kusterer@hybro.de), Hybro GmbH & Co. KG, 17291 Schenkenberg, Germany

[c] Grain yield

[d] Plant height

[e] Thousand-kernel weight

[f] Test weight

[g] Falling number

[h] Pentosan content

[i] Protein content

[j] Starch content

Table 4 Candidate introgression lines (pre-ILs) of library B carrying one or two donor chromosome segments (DCS) and showing significant ($P < 0.05$) differences to the recurrent parent L2053-N detected by the *t*-test, the corresponding chromosomal locations of the DCS, and the detected significant traits

Tester × Genotype	DCS				Trait performance	
	Chr.[a]	Marker position (cM)	Flanking markers	Length of marker interval (cM)	Superior	Inferior
2143	4R	85.0	P12/M59-F-172-P2	26.7	GY[c]	PH[d]
		109.8	P12/M53-F-217-P2			
2144	4R	85.0	P12/M59-F-172-P2	26.7	SC[i]	PH, TKW[e], PentC[g], PC[h]
		109.8	P12/M53-F-217-P2			
2145	4R	85.0	P12/M59-F-172-P2	26.7	TW[f]; SC	PH, TKW, PentC, PC
		109.8	P12/M53-F-217-P2			
2147	1R	59.6	P12/M54-F-284-P2	19.5		GY, TW
		78.1	P12/M54-F-104-P2			
2148	1R	39.4	MGL123	19.4	PC	GY
		65.4	P12/M57-F-322-P2			
	1R	70.3	Xlprm15	6.8		
		73.0	Aif038[b]			
2149		39.4	MGL123	38.9	PC	GY, TW
		78.1	P12/M54-F-104-P2			
2168	1R	7.7	Xlprm62	6.9	TW	
	3R	4.5	P12/M53-F-139-P2	4.8		
		6.6	P12/M53-F-226-P2			
2169	1R	7.7	Xlprm62	6.9	TW, SC	TKW, PC
	3R	4.5	P12/M53-F-139-P2	4.8		
		6.6	P12/M53-F-226-P2			
2172	4R	76.0	P17/M60-F-95-P2	16.6	GY	
		85.0	P12/M59-F-172-P2			
2178	5R	12.8	Aif006[b]	7.1	TKW	PH
	6R	66.8	P12/M56-F-495-P2	7.3		
		70.5	P12/M59-F-437-P2			
2179	2R	38.2	P17/M60-F-306-P2	7.5	GY, SC	PH
		41.3	P17/M60-F-409-P1			
	6R	66.8	P12/M56-F-495-P2	7.3		
		70.5	P12/M59-F-437-P2			
2180	2R	38.2	P17/M60-F-306-P2	7.5		PH
		41.3	P17/M60-F-409-P1			
	6R	66.8	P12/M56-F-495-P2	7.3		
		70.5	P12/M59-F-437-P2			

[a] Chromosomal location of the DCS

[b] Primer information is available for non-commercial use on request by B. Kusterer (kusterer@hybro.de), Hybro GmbH & Co. KG, 17291 Schenkenberg, Germany

[c] Grain yield

[d] Plant height

[e] Thousand-kernel weight

[f] Test weight

[g] Pentosan content

[h] Protein content

[i] Starch content

Identification of QTL in rye by using introgression libraries

Most important agronomic traits are quantitatively inherited and, thus, affected by many genes and environmental factors. Therefore, QTL analyses target at the detection of genomic regions carrying genes underlying a phenotypic trait. Since the development of molecular marker technologies, the analysis of complex traits has advanced. Nevertheless, only a few QTL analyses have been described in rye up to now (Börner et al. 1999, 2000; Masojc and Milczarski 2005; Masojc et al. 2007).

In plant breeding research, usually F_2, recombinant inbred line or backcross populations are used for QTL mapping studies. However, in these mapping populations the resolution power for identifying QTL with high accuracy is limited (Eshed and Zamir 1995) due to (1) insufficient sample size of the mapping population (cf. Lande and Thompson 1990; Melchinger et al. 1998; Lübberstedt et al. 1998; Schön et al. 2004) or (2) linked QTL in repulsion phase on the parents, cancelling out in the mapping population (Falke et al. 2007). Moreover, to transfer the discovered potentially valuable QTL into elite breeding material, extensive marker-assisted backcross programs are required (Tanksley and Nelson 1996). Pillen et al. (2003) reported that QTL alleles from exotic stocks frequently lost their effects after transferring into elite material probably due to epistatic interactions. Therefore, Tanksley and Nelson (1996) presented the advanced backcross QTL analysis for simultaneously detecting and transferring beneficial alleles from exotic germplasm into elite breeding material. In this study, we pursued another strategy to identify QTL (Matus et al. 2003; von Korff et al. 2004). We developed two introgression libraries solely by marker-assisted selection, targeting a high coverage of the employed exotic donor, and evaluated the testcross performance of the pre-IL progenies in the field. This allows the identification of associations between phenotypes and beneficial genomic regions by comparing each single pre-IL with the recurrent parent.

The *t*-test identified three pre-ILs of introgression library A of which the testcrosses are significantly shorter than the testcross with the recurrent parent, although the testcrosses of the donor were on average 23.4 cm taller than the testcrosses of the recurrent parent (Table 1). These pre-ILs contained DCS on chromosomes 2R, 5R, and 7R (Fig. 1). We detected five pre-ILs in each introgression library with a significantly ($P < 0.05$) higher grain yield than L2053-N, which carry DCS on almost all chromosomes (Figs. 1 and 2). Börner et al. (1999) identified a QTL for plant height on chromosome 5R explaining 61.1% of the variation. In a companion study, they detected 21 QTL of which ten mapped also on chromosome 5R. In particular, plant height as well as grain yield and its components showed QTL on chromosomes 2R and 5R (Börner et al. 2000). Hence, our result, that beneficial genomic regions for agronomic traits are located on chromosomes 2R and 5R, agrees well with the results of Börner et al. (1999, 2000). Moreover, further possible QTL regions for agronomic and quality traits distributed over the whole genome were identified with our introgression libraries (Figs. 1, 2). We attribute the newly detected QTL to uncovered new genetic variation, originating from the Iranian primitive rye population employed as donor, which is not available in conventional elite germplasm. In some cases, mostly for pre-ILs carrying only one or two DCS, the location of QTL could be assigned to specific chromosome regions (Tables 3 and 4). However, several pre-ILs were genotypically very similar but showed different phenotypic performance (Figs. 1, 2). These differences are most likely attributable to environmental variation and the residual variance in the field trials. Moreover, since the donor genome coverage was <80%, deviating phenotypic performances between similar lines could also be due to extra DCS so far not discovered or on deviating extensions of the detected DCS. We conclude that, within the limits determined by the accuracy of field trials, introgression libraries provide a valuable tool for identifying and verifying QTL in rye.

Exotic germplasm for improving agronomic traits of elite breeding material

In most cases, exotic germplasm has poor agronomic performance and, therefore, the difficulty in utilizing exotic resources consists of identifying favorable alleles and subsequently transferring these into elite breeding material. Up to now, several studies have identified advantageous exotic genes for biotic and abiotic stress tolerance (von Korff et al. 2005; Finkers et al. 2007; Siangliw et al. 2007; Jeuken et al. 2008) as well as genes improving quality traits (Matus et al. 2003; Pillen et al. 2003; Kunert et al. 2007). In contrast, the potential use of exotic germplasm for improving agronomic traits (von Korff et al. 2006) is still in discussion, due to its sizeable negative effects on the phenotypic performance. Using ILs might overcome this problem through the systematic introgression of small marker-defined exotic segments in the elite background (Eshed and Zamir 1994). Moreover, only ILs which demonstrated their superiority in the field tests, were further used for introgression into elite breeding germplasm.

In our study, we employed the Iranian primitive rye population Altevogt 14160, which is not suitable for developing parental lines of commercial hybrids. It is characterized by non-shattering ears, high susceptibility to lodging as well as low kernel weight and grain yield. Altevogt 14160 was inferior to the recurrent parent L2053-

N for the agronomic traits but also for several quality traits even when crossed to elite testers (Table 1). In both introgression libraries, estimates of the genotypic variance σ_G^2 were significant ($P < 0.01$) for all traits (Table 2) indicating that new genetic variation was generated from the exotic donor Altevogt 14160. As expected, most of the pre-ILs were similar to the recurrent parent in both introgression libraries with respect to the means (Table 1). Positively significant ($P < 0.05$) comparisons between pre-ILs and the recurrent parent were more often detected for quality than for agronomic traits (Figs. 1, 2). Nevertheless, the *t*-test detected ten and three positively significant differences for grain yield and plant height. Hence, these results indicate that the exotic donor provides, besides the linkage drag, also favorable alleles for agronomic traits that are not detectable in routine yield trials. Our findings agree well with the results of other studies, which showed that exotic materials, despite of inferior phenotypes, can carry alleles increasing the agronomic performance of elite breeding material in tomato (Eshed and Zamir 1995; Tanksley et al. 1996; Bernacchi et al. 1998a, b; Fulton et al. 2000), barley (Pillen et al. 2003; von Korff et al. 2006; Schmalenbach et al. 2008b), wheat (Huang et al. 2003), soybean (Concibido et al. 2003), and rice (Septiningsih et al. 2003; Tian et al. 2006; Tan et al. 2007). Consequently, our research highlights the potential of exotic germplasm as a resource for increasing the limited genetic diversity of hybrid rye, and, thus, providing the basis for further breeding success.

Use of ILs in plant breeding programs

The development and employment of introgression libraries offers the opportunity to identify favorable exotic QTL alleles for improving elite varieties.

The fast development of new varieties is essential for the economical success of breeding companies, therefore, breeding programs require suitable long-term strategies. The establishment as well as the molecular and phenotypic evaluation of introgression libraries is very time-consuming and expensive. The gain of these libraries lies in the transfer of QTL alleles from exotic germplasm in elite varieties. However, to successfully transfer exotic germplasm and, therefore, demonstrate its utility for practical breeding, remaining challenges for future research are: (1) Interactions between DCS and genetic background can be critical for transferring QTL alleles from one background to another (Eshed and Zamir 1995; Melchinger et al. 1998). Hence, for transferring exotic QTL alleles into parental lines of hybrid cultivars, low epistatic interactions are advantageous (von Korff et al. 2004). (2) Complex phenotypes can be affected by pleiotropy or linkage due to several DCS per IL or long DCS. Therefore, ILs with single and small DCS should be developed to dissect complex traits (Tan et al. 2007).

The pre-ILs of our introgression libraries contained mostly multiple DCS, most likely this resulted from the employed breeding scheme. This relatively large average number of DCS per pre-IL made it difficult to assign trait effects to specific DCS. Moreover, the donor genome coverage of our introgression libraries was smaller than 80%. We attribute this low coverage partly to deleterious alleles in the respective chromosome regions resulting in the extinction of entire backcross families. Nevertheless, our study revealed that exotic germplasm has the potential for improving agronomic and quality traits, because we identified genomic regions responsible for a better testcross performance of single pre-ILs compared to their elite recurrent parent. Our results illustrated that individual QTL alleles from the exotic poor-yielding population can improve many important traits, and in particular that there exist alleles of which the positive phenotypic effect is not apparent in the donor population. Thus, introgression libraries provide an approach for unmasking beneficial alleles.

An important advantage of the pre-ILs developed in our study are that the assessment of the positive effects of exotic alleles is performed in an elite genetic background, which can be used directly for variety development. Since 2007, two German rye breeding companies (Hybro GmbH &Co KG; KWS LOCHOW GmbH) started integrating promising pre-ILs into their breeding programs. However, in order to get more precise information about the localization of agronomically important QTL further research is necessary. To enable fine mapping of QTL and, thus, to increase the resolution of these QTL further backcross generations are required to shorten the DCS and isolate pre-ILs with individual DCS.

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