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Detection of donor effects in a rye introgression population with genome-wide prediction

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With 5 figures

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Abstract

Introgression populations are developed to make genetic resources for breeding purposes available. In the case that the number of donor segments exceeds the number of lines, genome-wide prediction (GWP) methods are suggested as promising for the analysis of such populations. Our objectives were to characterize a rye introgression population with the Rye5K SNP assay and to apply a GWP model with a modification of the restricted maximum likelihood procedure that yields heteroscedastic variances to detect significant donor effects. The introgression lines (ILs) carried on average 4.6 donor segments with a mean length of 27 cM and represented 94% of the donor genome. Two donor effects were detected that significantly increased thousand-kernel weight. We found four donor effects for protein, total pentosan and starch content that can improve baking quality. Three donor effects for protein content were observed for improving feeding purposes and one donor effect for starch content to improve ethanol production. The effects were localized to small genomic regions. Consequently, these ILs can improve rye breeding by directly employing them in breeding programmes for variety development.

Key words: *Secale cereale* L. — introgression population — introgression line — Rye5K SNP array — genome-wide prediction — heteroscedastic marker variances — RMLV

Introgression libraries or introgression populations employ the strategy of incorporating chromosome segments of mainly exotic donors into elite backgrounds by marker-assisted backcrossing. Ideally, such populations represent a set of homozygous lines each carrying a single marker-defined donor segment in the background of an elite recipient (Zamir 2001). In this way, phenotypic variation of a specific line compared to the recipient can be attributed specifically to the introgressed segment. The principle of detecting donor segments affecting specific traits with introgression populations was first established in tomato (Eshed and Zamir 1994, 1995) and has been well proven in various cereals for diverse traits (*cf. e.g.* Ishikawa et al. 2005, Pestsova et al. 2006, Szalma et al. 2007, Falke et al. 2008, Schmalenbach et al. 2009).

The development and analysis of introgression populations has so far been mainly based on a limited number of molecular markers generated from anonymous genomic regions such as restriction fragment length polymorphisms (RFLPs; *cf.* Eshed and Zamir 1994, Szalma et al. 2007), amplified fragment length polymorphisms (AFLPs; *cf.* Finkers et al. 2007, Jeuken et al. 2008) or simple sequence repeats (SSRs; *cf.* Falke et al. 2008, Schmalenbach et al. 2009). Dense genetic linkage maps, however, are essential for the precise identification of donor segments carrying the putative favourable alleles. Today, single nucleotide polymorphisms (SNPs) have become the marker system of choice for plant geneticists and breeders (Rafalski 2002, Ponting et al. 2007) due to their (i) high abundance in the genome, (ii) suitability for multiple assays and (iii) low cost per data point. Up to now, the potential of high-resolution genotyping with SNP arrays has been demonstrated for many genomic approaches, but they are rarely applied for the analysis of introgression populations (Schmalenbach et al. 2011).

In practice, introgression populations typically consist of a set of introgression lines (ILs) which contain several and/or overlapping donor segments (Liu et al. 2006, Falke et al. 2008). This hinders pairwise testing to find the specific donor segments affecting the traits of interest. For these cases, linear model analysis with fixed effects has been suggested (Falke and Frisch 2011, Mahone et al. 2013). However, if the number of donor segments exceeds the number of ILs, the donor effects are not estimable with such models. Recently, genome-wide prediction (GWP) approaches are proposed as promising to this problem (Falke et al. 2014).

Rye (*Secale cereale* L.) is an economically important and widely cultivated crop for bread, feed and as a renewable energy source in Middle and Eastern Europe. Of all small-grain cereals, it has the highest winter hardiness and is outstanding with regard to biotic and abiotic stresses. As an outcrossing species, considerable heterosis can be exploited in hybrid breeding programmes. The lack of genomic resources in rye has been solved with the recently developed Rye5K SNP array (Haseneyer et al. 2011).

We developed a BC_2S_3 introgression population based on a cross between the elite line L2053-N and the primitive rye population Altevogt 14160 by marker-assisted backcrossing using AFLP and SSR markers (Falke et al. 2008). First attempts to find the ILs differing significantly from the recipient and to detect the responsible donor segments were performed by applying a two-sided Dunnett test (Dunnett 1955, Falke et al. 2008, 2009a,b, 2010) and linear model analysis (Mahone et al. 2013).

In this study, we re-analysed a set of rye ILs with the high-resolution Rye5K SNP array to precisely characterize our rye introgression population. Subsequently a GWP model with a modification of the restricted maximum likelihood procedure that yields heteroscedastic variances (RMLV; Hofheinz and Frisch 2014) was used to detect the specific donor effects that affected the traits of interest.

Materials and Methods

Plant material and agronomic trials: A rye (*Secale cereale* L.) introgression population originating from a cross between the inbred line

L2053-N (bred by Hybro GmbH & Co KG, Schenkenberg, Germany), as recipient, and the Iranian primitive rye population Altevogt 14160 (provided by the Botanical Garden, Warsaw, Poland), as donor, was used for our analyses. A set of BC_2S_3 lines (previously mentioned as introgression library A) was derived by marker-assisted backcrossing with AFLP and SSR markers (Falke et al. 2008).

The performance *per se* was assessed in field trials (Falke et al. 2008, 2009a). Briefly, the field trials were conducted in 2 years at five sites in Germany (Bergen, Eckartsweier, Hohenheim, Oberer Lindenhof and Wulfsode). We analysed the performance of the ILs together with the recipient L2053-N (ten plots per replicate) and the donor Altevogt 14160 (three plots per replicate). The experimental design at each location was a 10 × 9 α-design (Patterson and Williams 1976) with three replicates. Data were recorded for plant height (cm), thousand-kernel weight (g), protein, total pentosan and starch content in grain (%), the latter three estimated by near-infrared reflectance spectroscopy (NIRS) from milled grain. Near-infrared spectra were recorded with a FT-NIRS instrument (Bruker MPA, reflectance mode, 850–2500 nm). The samples were scanned twice in duplicate repacking using two different petri dishes of 8.7 cm diameter and 1 cm height as sampling cups on rotating device (average of 32 scans in 10 s, two spectra per sample). Prediction models were calculated with OPUS software from Bruker (Bruker Optic GmbH, Ettlingen, Germany), version 6.5. Calculations were carried out with a modified partial least squares (PLS) procedure using a validation and a scatter correction of the spectra (SNV). Spectra were tested as original and as 1st derivatives. Two sets of samples were prepared for calibration and prediction. The samples were randomly distributed among the calibration and validation sets. Suitability of the models was controlled with the validation set. Prediction quality was calculated as determination coefficient, standard error of prediction and as RPD value, which relates the standard error of prediction to the standard deviation of the original data (SEP/SD). The relevant statistics for calibration and validation are given in Table S1.

We focused in this study on the traits plant height and the yield component thousand-kernel weight due to their importance for plant breeders as well as on the quality traits protein, total pentosan and starch content as a relevant factor for baking quality, feeding purposes and ethanol production. For baking quality, low protein content combined with high pentosan and starch content is required, while for feeding, high protein and low pentosan content are favoured. For ethanol production, starch content should be maximized.

Genotypic analysis and characterization of the introgression lines: Genotyping of the subset of 37 ILs and the recipient was performed with the Rye5K SNP array containing 5234 markers (Haseneyer et al. 2011). Out of these, the chromosomal positions of 3272 SNP markers were determined according to the rye consensus genetic linkage map as reported by Martis et al. (2013).

Estimation and test of the effects from the donor segments: The genetic effects of the donor segments on a phenotypic trait were estimated with the linear model:

$$\boldsymbol{y} = \boldsymbol{1}\beta_0 + \boldsymbol{Zu} + \boldsymbol{e}.$$

Here, $\boldsymbol{y}$ is the vector of the phenotypic values of N introgression lines, $\boldsymbol{\beta_0}$ is a fixed intercept, $\boldsymbol{Z}$ is the design matrix relating the donor segments to the introgression lines, $\mathbf{u}$ is the vector of the donor segment effects, and $\mathbf{e}$ is the vector of residuals.

To construct the design matrix $\boldsymbol{Z}$, markers for which the alleles were in complete linkage disequilibrium in the introgression population were combined to donor segments. The elements of $\boldsymbol{Z}$ are coded in the design matrix such that the number represents the donor segment zygosity, that is as 0,1,2. Details on the structure of the design matrix $\mathbf{Z}$ are described by Falke et al. (2014).

For estimation of the donor segment effects, we used the RMLV method suggested for GWP (Hofheinz and Frisch 2014). The calculations were carried out with the software SelectionTools (www.uni-giessen.de/population-genetics/downloads). Subsequently, we adopted a permutation test similar to that suggested by Churchil and Doerge (1994) for QTL mapping. For carrying out the permutation test for the effect u_i of the ith donor segment, entries of the ith column of $\boldsymbol{Z}$ were randomly permuted and u_i was estimated for the random permutations. The distribution of the u_i from r random permutations was used to approximate the distribution under the null hypothesis that 'the segment has no effect on the phenotype'. Comparison of the effect estimate obtained for the actually observed phenotypic data with the approximated distribution of effects under the null hypothesis was used to assign p-values to the donor effect estimates. The p-values from testing linear contrasts and from the permutation test were adjusted with a modified Bonferroni procedure (Hochberg 1988).

Results

High-resolution genotyping revealed that the BC_2S_3 lines represented 94% of the donor genome. No large gaps were observed on any chromosome (Figs 1–5). The ILs carried on average 4.6 donor segments with a mean length of 27 cM (Table S2). Most of the donor segments were in the homozygous state.

The results of the field trials have been reported in detail previously (Falke et al. 2008, 2009a). The performance of the donor Altevogt 14160 exceeded the recipient L2053-N for thousand-kernel weight, protein and starch content, while the recipient showed a higher total pentosan content and a considerably shorter plant height. The ILs had the tendency to be more similar to the recipient. REML estimates of the genotypic variance were significant ($P < 0.01$) for all traits indicating that there is genetic variation between the ILs.

The RMLV method detected seven donor effects that significantly ($P < 0.05$) increased the plant height (Fig. 1, Table S3). The respective donor segments were distributed over the whole genome. Almost every IL carried a donor segment significantly ($P < 0.05$) affecting the plant height.

For thousand-kernel weight, we found two donor effects that significantly ($P < 0.05$) increased and six donor effects that significantly ($P < 0.05$) reduced the performance (Fig. 2, Table S3). The favourable donor segments were located on chromosomes 5R and 7R, while the unfavourable ones on chromosomes 1R, 3R, 5R and 6R. Eight ILs carried donor segments with significant ($P < 0.05$) favourable and unfavourable effects. If these ILs carried only one favourable donor segment, the unfavourable overcame the favourable one and the performance was reduced (2124, 2128, 2135 and 2136). If the ILs (2118 and 2119) carried two favourable donor segments, the positive effect overcame the negative and the ILs showed a significant ($P < 0.05$) higher thousand-kernel weight than the recipient.

For protein content, three donor effects were detected that significantly ($P < 0.05$) increased the performance, and one that significantly ($P < 0.05$) reduced the performance (Fig. 3, Table S3). The donor segments increasing the protein content were found on chromosomes 1R, 5R and 6R, and the segment reducing the performance was found on chromosome 7R. The two ILs (2131 and 2136) carrying a significantly ($P < 0.05$) positive and negative donor segment resulted in an increased protein content.

For total pentosan content, the RMLV method found two donor effects significantly ($P < 0.05$) increasing the pentosan content (Fig. 4, Table S3). Both donor segments were detected on chromosome 3R.

For starch content, we detected one donor effect with a significant ($P < 0.05$) positive effect on the starch performance and three donor segments with a significant ($P < 0.05$) negative

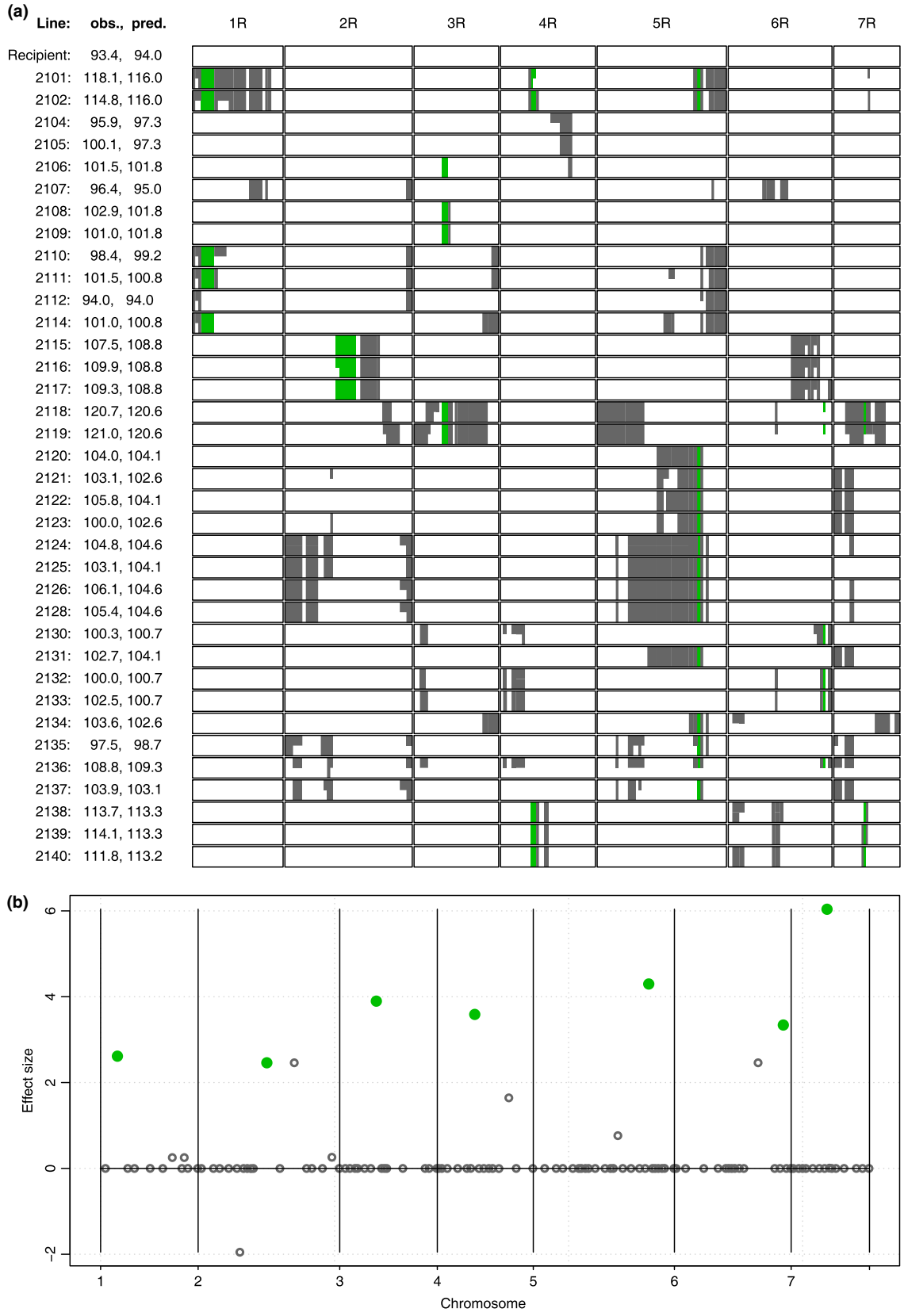

Fig. 1: Donor segment effects for plant height. (a) Observed (obs.) and predicted (pred.) plant height (cm) of the recipient and the ILs 2001–2040 of the rye introgression population. In the graphical genotypes, white colour indicates chromosome segments of the recipient and grey the introgressions from the donor. Green colour denotes donor segments that increase plant height. (b) Estimated size of the donor segment effects from the RMLV analysis plotted along the seven chromosomes of rye; grey circles denote donor segments that are not significant and green colour denotes significant ($P < 0.05$) effects increasing plant height

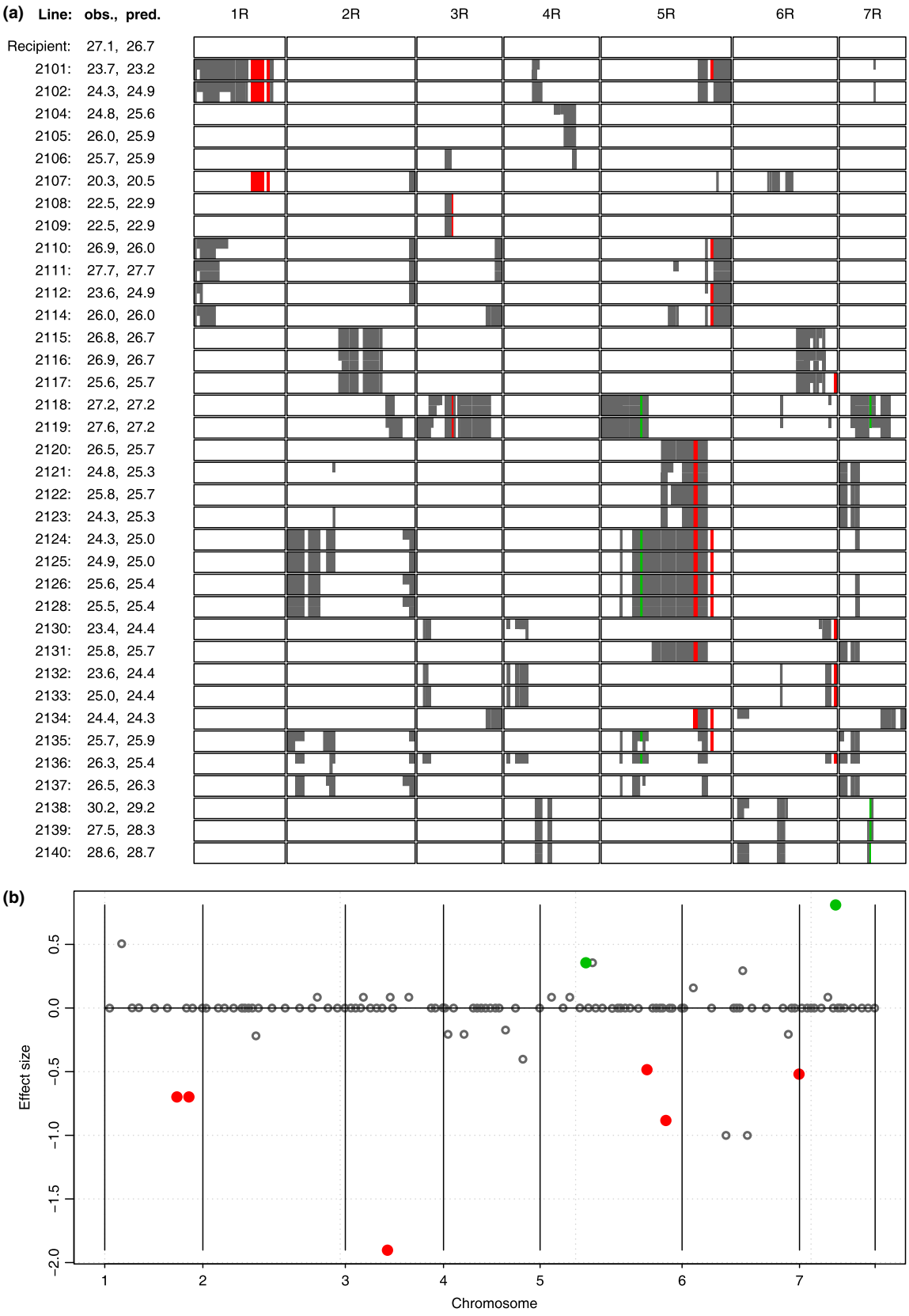

Fig. 2: Donor segment effects for thousand-kernel weight. (a) Observed (obs.) and predicted (pred.) thousand-kernel weight (g) of the recipient and the ILs 2001–2040 of the rye introgression population. In the graphical genotypes, white colour indicates chromosome segments of the recipient and grey the introgressions from the donor. Green colour denotes donor segments that increase thousand-kernel weight and red colour segments that decrease thousand-kernel weight. (b) Estimated size of the donor segment effects from the RMLV analysis plotted along the seven chromosomes of rye; grey circles denote donor segments that are not significant, green colour denotes a significant ($P < 0.05$) effect increasing and red a significant ($P < 0.05$) effect decreasing thousand-kernel weight

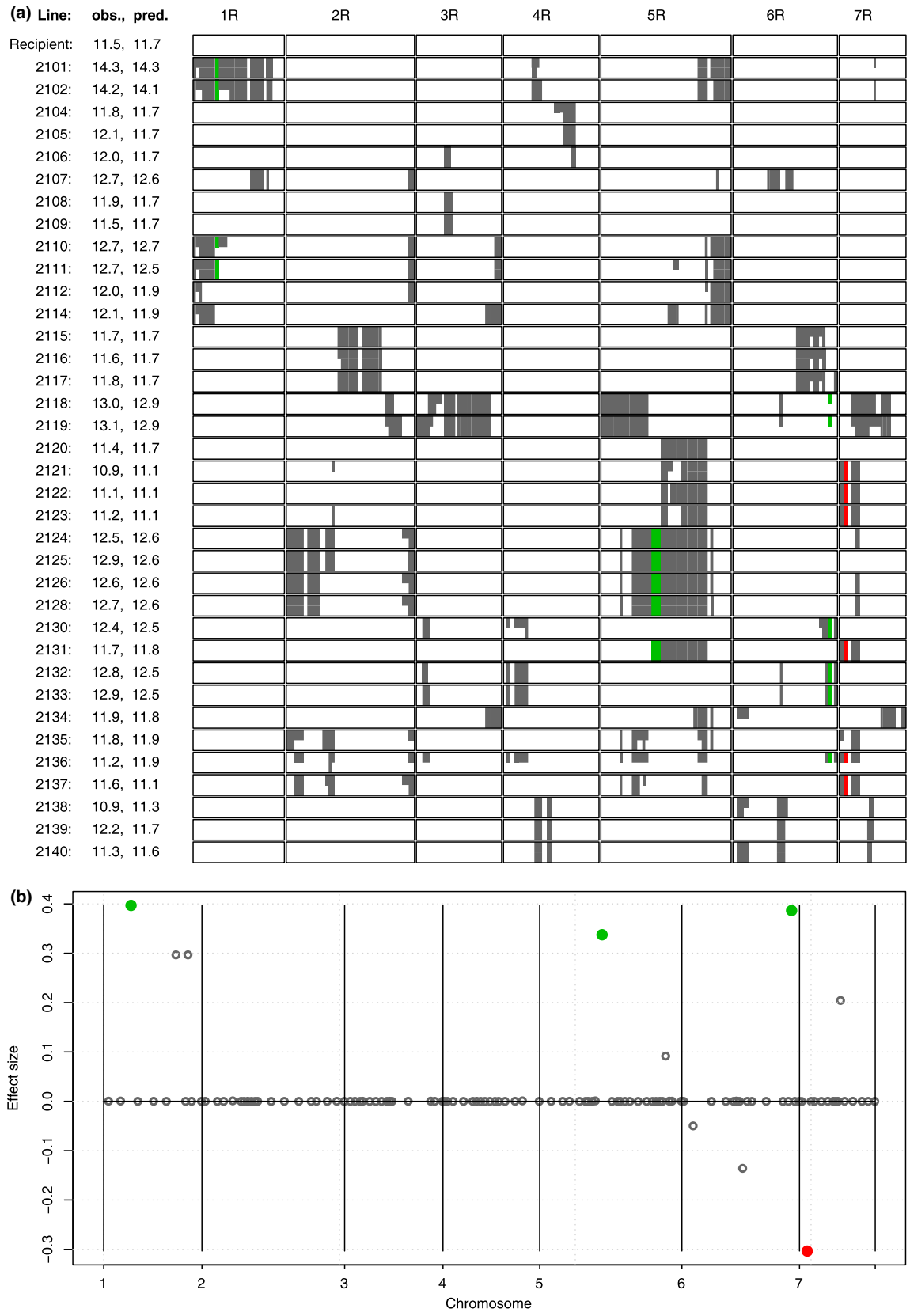

Fig. 3: Donor segment effects for protein content. (a) Observed (obs.) and predicted (pred.) protein content (%) of the recipient and the ILs 2001–2040 of the rye introgression population. In the graphical genotypes, white colour indicates chromosome segments of the recipient and grey the introgressions from the donor. Green colour denotes donor segments that increase protein content and red colour segments that decrease protein content. (b) Estimated size of the donor segment effects from the RMLV analysis plotted along the seven chromosomes of rye; grey circles denote donor segments that are not significant, green colour denotes a significant ($P < 0.05$) effect increasing and red a significant ($P < 0.05$) effect decreasing protein content

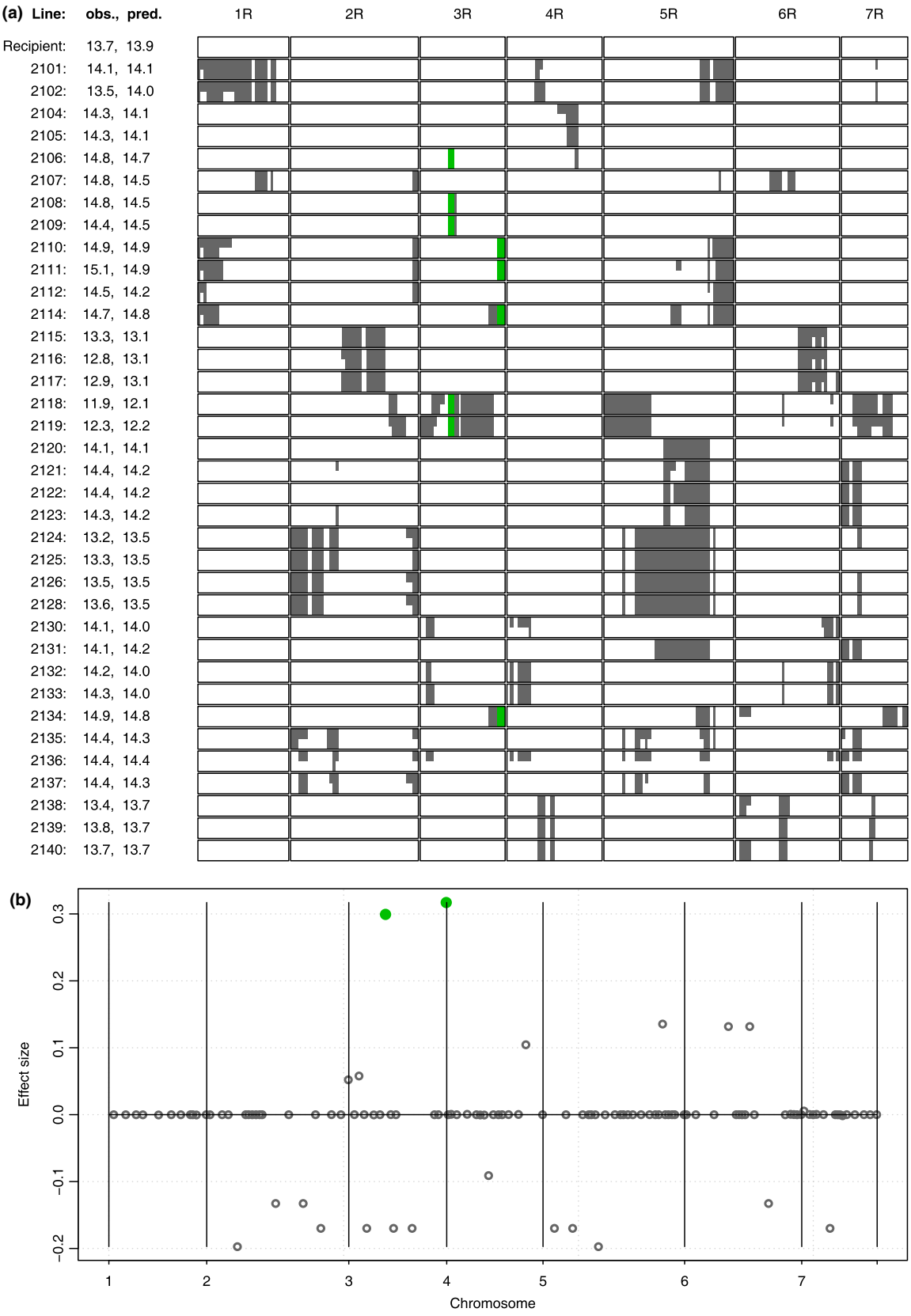

Fig. 4: Donor segment effects for total pentosan content. (a) Observed (obs.) and predicted (pred.) pentosan content (%) of the recipient and the ILs 2001–2040 of the rye introgression population. In the graphical genotypes, white colour indicates chromosome segments of the recipient and grey the introgressions from the donor. Green colour denotes donor segments that increase pentosan content and red colour segments that decrease pentosan content. (b) Estimated size of the donor segment effects from the RMLV analysis plotted along the seven chromosomes of rye; grey circles denote donor segments that are not significant, green colour denotes a significant ($P < 0.05$) effect increasing pentosan content

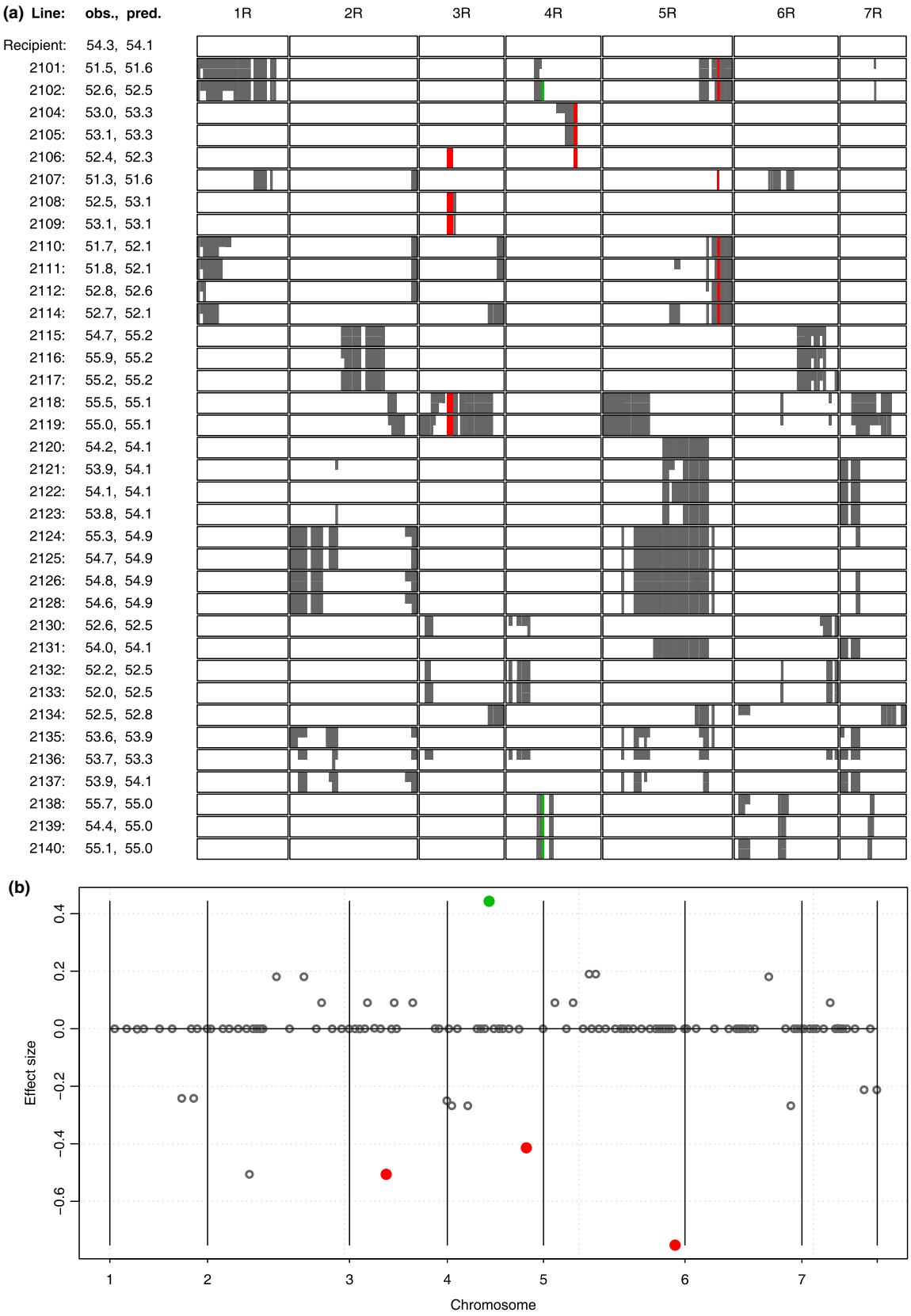

Fig. 5: Donor segment effects for starch content. (a) Observed (obs.) and predicted (pred.) starch content (%) of the recipient and the ILs 2001–2040 of the rye introgression population. In the graphical genotypes, white colour indicates chromosome segments of the recipient and grey the introgressions from the donor. Green colour denotes donor segments that increase starch content and red colour segments that decrease starch content. (b) Estimated size of the donor segment effects from the RMLV analysis plotted along the seven chromosomes of rye; grey circles denote donor segments that are not significant, green colour denotes a significant ($P < 0.05$) effect increasing and red a significant ($P < 0.05$) effect decreasing starch content

effect on the starch performance (Fig. 5, Table S3). The donor segment with a positive effect was located on chromosome 4R, while those with negative effects on chromosomes 3R, 4R and 5R. IL 2102 contained the positive and the negative donor segment from chromosome 5R. Here, the negative effect exceeded the positive which lead to a reduced starch content compared to the recipient.

Discussion

Characterization of the introgression population with high-density mapping

The rye introgression population was initially developed and characterized with up to 137 SSR markers and 14 AFLP primer combinations (Falke et al. 2008). The marker-assisted backcrossing resulted in BC_2S_3 ILs carrying on average 4.7 introgressions with a mean length of 13 cM. The total population covered 74% of the donor genome. In this study, the introgression population was re-analysed with the high-density Rye5K SNP array and the chromosomal positions of the SNP markers were determined according to a rye consensus genetic linkage map (Martis et al. 2013). In general, consensus genetic mapping is more complex than mapping based on single data sets. Therefore, limitations such as differences in recombination rate, exchange distribution along chromosomes or variation in dominance of the used markers can occur (Ronin et al. 2012). The re-analysis of our introgression population validated mainly our previous results but also revealed that the donor genome coverage is considerably higher with 94% and that additional donor segments exist. For example, new individual introgressions were found on chromosome 2R in several ILs (2107, 2110–2112, 2124–2126, 2128 and 2135–2137), on chromosome 3R (ILs 2110, 2111, 2114 and 2134) and on chromosome 4R (ILs 2130, 2132, 2133 and 2136). The detection of new additional donor segments when using high-resolution SNP arrays is in close agreement with results found for a barley introgression library (Schmalenbach et al. 2011) and can be attributed to the higher mapping accuracy of the SNP arrays. Accurately characterized introgression populations are a prerequisite for precise donor effect detection. Our results suggest that introgression populations can be better characterized with high-resolution genotyping assays than with a limited number of markers.

Detection of donor effects

The detection of donor segments with favourable effects has initially been developed in tomato by Eshed and Zamir (1994). The interest of this approach has been growing as these introgression populations allow the simultaneous detection of favourable effects and variety development in nearly one step. Thus, it facilitates the successful use of these effects in the breeding process and reduces the time required for variety development. So far, mainly pairwise testing is used to determine whether an IL differs significantly from the recipient. Here, it is advantageous that the ILs carry only single donor segments to assign the effect to the specific segment. In practice, however, the development of introgression populations is size limited by the number of concurrent backcross programmes and field space, and thus, the ILs carry mainly multiple donor segments. The situation can easily occur that the introgression population contains more donor segments than lines. In this case, the donor effects are not estimable with fixed linear models. Integrating GWP methods can overcome the drawbacks of pairwise testing and fixed linear models. In combination with permutation tests, the RMLV model (Hofheinz and Frisch 2014) is particularly recommended to detect donor effects in introgression populations with multiple or overlapping introgressions and provides the detection of positive and negative effects in individual ILs (Falke et al. 2014). Our rye introgression population contains 168 disjunct chromosome segments and therefore more donor segments than ILs. Consequently, the RMLV model seems here the appropriate tool of choice. However, the effects detected in this study are not yet validated. Due to the small sample size, cross-validation is not an option. We plan experimental validation of the effects in an independent validation experiment.

Plant height is a trait affecting the fitness in natural populations and plays an essential role in plant breeding programmes as selection criterion. Its inheritance is expected to be complex, controlled by many loci distributed over the whole genome (Schön et al. 2004, Wang et al. 2006, Miedaner et al. 2011, 2012). In this study, RMLV detected on each chromosome a significant donor effect (Fig. 1) and, thus, confirmed the results from the literature. All of the significant donor effects were associated with an increase of plant height which agrees with other studies in cereals using exotic germplasm in introgression populations (Pillen et al. 2003, Septiningsih et al. 2003, Liu et al. 2006, Von Korff et al. 2006, Falke et al. 2009a,b, Miedaner et al. 2011). The analysis of our introgression population with the Dunnett test (Dunnett 1955) showed that nearly every IL had a significantly increased plant height compared to the recipient (Falke et al. 2009a). RMLV confirmed these results, but additionally enabled the precise localization of seven donor effects which were responsible for the increased plant height (Fig. 1). In conclusion, our results support the assumption of the very complex inheritance of plant height.

Grain yield is proposed to follow the infinitesimal model of quantitative genetics (Fisher 1918), and thus, it is not expected that marker-assisted selection can be successfully employed. We therefore focused on the yield component thousand-kernel weight. Two donor segments with effects significantly increasing the thousand-kernel weight were detected on chromosomes 5R and 7R (Fig. 2). Both effects correspond well with large effect QTL found with classical QTL mapping (Miedaner et al. 2012) and major genes (Wricke 2002). The high effects of these QTL were explained as an indication of single genes. Our results strengthened this assumption. The Dunnett test (Dunnett 1955) found eight ILs with a significant decreased and one IL with a significant increased thousand-kernel weight compared to the recipient (Falke et al. 2009a). These results were confirmed by the RMLV method. However, even more ILs with significant donor effects were found with RMLV than with pairwise testing. Interestingly, many of these ILs carried both a significant favourable and an unfavourable donor effect (Fig. 2). Here, mainly the unfavourable dominated the favourable effect and a lower thousand-kernel weight was observed. We explain this by the fact that these ILs carried mostly two unfavourable and only one favourable donor segment. In conclusion, the confirmation of the major genes and the possibility to detect positive and negative donor effects in individual ILs support the high power of the used GWP model.

Plant height and yield components are among the most important traits in rye breeding. Quality traits in rye, however, vary depending on the end-use purpose of the breeding programme. We focused in our study on protein, total pentosan and starch content as they are all of crucial importance for baking quality, feeding and ethanol production.

For protein content, we detected three donor segments with a significant effect that resulted in a increased protein content compared to the recipient (Fig. 3). These segments were located on chromosomes 1R, 5R and 6R. Miedaner et al. (2012) detected QTL on chromosomes 1R and 6R with classical QTL mapping, too. However, these QTL detected in other backgrounds were located on different positions on the chromosomes. Moreover, one donor segment with a significant negative effect was found on chromosome 7R, which has not been described in the literature yet. We rate these results as an indication that we found here new alleles for protein content from the exotic donor. We therefore conclude that the donor segments with significant effects on chromosomes 1R, 5R and 6R are good starting points for improving feed quality and the donor effect on chromosome 7R for improving baking quality. For protein content, all ILs detected by the Dunnett test as significantly different from the recipient were also found with RMLV. However, here occurred the same situation as for thousand-kernel weight, if an IL carried both, positive and negative donor effects, only RMLV enabled their detection. In this situation, the positive effect dominated the negative effect and a higher protein content was observed. We explain this by the higher *per se* performance of the donor compared to the recipient (Falke et al. 2009a).

For total pentosan content, two donor effects that significantly increase the pentosan content were found by RMLV on chromosome 3R (Fig. 4). This result confirmed the results from the Dunnett test and additionally identified the two responsible segments. One of these two donor segments corresponded well with a QTL for total pentosan content detected on chromosome 3R in a segregating population with a different genetic background (Miedaner et al. 2012). The other significant donor segment might be an indication for new favourable alleles introduced through the exotic donor. Hence, the eight ILs carrying the two donor segments can directly be used for improving baking quality of elite material.

For starch content, RMLV detected one donor segment on chromosome 4R with a significant positive effect (Fig. 5). Miedaner et al. (2012) found also QTL on chromosome 4R with classical mapping but on other positions. This can indicate that our exotic germplasm contributes new favourable alleles to improve starch content. We therefore conclude that the detected donor segments in ILs 2102, 2138, 2139 and 2140 might be valuable for improving baking quality and ethanol production.

Conclusion

The analysis of our rye introgression population using RMLV confirmed many QTL described in the literature. Moreover, for the quality traits, segments with donor effects with obviously new and particularly favourable alleles were detected. It is remarkable that such results can be found in genetic resources having such an inferior *per se* performance like the applied donor Altevogt 14 160 (Falke et al. 2009a,b). These donor effects can directly be exploited in breeding programmes for improving baking and feed quality, and ethanol production. Thus, this should encourage geneticists and plant breeders to invest more time and work in genetic resources. Compared to our previous studies using pairwise testing with the very conservative Dunnett test (Falke et al. 2008, 2009a), we found more segments with significant donor effects using the RMLV method. We explain this by the fact that the GWP model allows the detection of positive and negative effects in individual ILs. These donor effects might cancel each other out if using pairwise testing, and thus, there were no significant donor effects detected. For utilizing favourable donor effects without getting the unfavourable ones, typically, further backcrosses are recommended to split the different donor segments into several sub-ILs by marker-assisted selection. An advantage of our rye introgression population here is that the significant donor segments are relatively small when further backcrossed into elite lines. Thus, linkage drag can be drastically reduced due to the sharper localization of the effects to smaller genomic regions. We therefore conclude that the application of RMLV opened a new possibility for plant breeders and geneticists when working with introgression populations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Statistics for calibration and validation.

Table S2. Donor segments.

Table S3. Donor effects.