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ORIGINAL PAPER

Comparative mapping of DNA sequences in rye (*Secale cereale* L.) in relation to the rice genome

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Abstract The rice genome has proven a valuable resource for comparative approaches to address individual genomic regions in Triticeae species at the molecular level. To exploit this resource for rye genetics and breeding, an inventory was made of EST-derived markers with known genomic positions in rye, which were related with those in rice. As a first inventory set, 92 EST-SSR markers were mapped which had been drawn from a non-redundant rye EST collection representing 5,423 unigenes and 2.2 Mb of DNA. Using a BC1 mapping population which involved an exotic rye accession as donor parent, these EST-SSR markers were arranged in a linkage map together with 25 genomic SSR markers as well as 131 AFLP and four STS markers. This map comprises seven linkage groups corresponding to the seven rye chromosomes and covers 724 cM of the rye genome. For comparative studies, additional inventory sets of EST-based markers were included which originated from the rye-mapping data published by other authors. Altogether, 502 EST-based markers with known chromosomal localizations in rye were used for BlastN search and 334 of them could be in silico mapped in the rice genome. Additionally, 14 markers were included which lacked sequence information but had been genetically mapped in rice. Based on the 348 markers, each of the seven rye chromosomes could be aligned with distinct portions of the rice genome, providing improved insight into the status of the rye–rice genome relationships. Furthermore, the aligned markers provide genomic anchor points between rye and rice, enabling the identification of conserved ortholog set markers for rye. Perspectives of rice as a model for genome analysis in rye are discussed.

Dedicated to Prof. em. Dr. Dr. h.c. Günter Wricke on occasion of his 80th birthday.

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Introduction

Rye (*Secale cereale* L.) is cultivated in Europe on 5.2 million hectares (FAOSTAT 2008), with a regional focus on Eastern Europe, Germany, and Scandinavia. Compared to other important Triticeae cereals, rye is outstanding with regard to its abiotic stress tolerance, self-incompatibility, extensive heterosis, and its role as a genetic resource in wheat and triticale breeding. The molecular tools available for rye breeding and genetics, though, are considerably less

abundant than, e.g., for wheat or barley. To-date, genetic maps describing the entire rye genome are in the majority of cases based on RFLP markers that originally had been developed in other Triticeae species (e.g. Devos et al. 1993; Loarce et al. 1996; Korzun et al. 2001; Ma et al. 2001). Usually, markers were developed for individual sub-genomic regions bearing specific genes for disease resistances, restoration ability and other selected traits (Rorat et al. 1991; Wricke et al. 1996; Shimizu et al. 1997; Van Campenhout et al. 2000; Stracke et al. 2003; Miftahudin et al. 2004; Hackauf and Wehling 2005). As a consequence and despite the progress which has been achieved towards developing sequence-specific markers (Saal and Wricke 1999; Korzun et al. 2001; Hackauf and Wehling 2002, 2003; Nagy and Lelley 2003; Khlestkina et al. 2004, 2005; Varshney et al. 2007, Kofler et al. 2008), the number of markers in the rye genome has remained limited, leaving large gaps in sub-genome regions.

It has recently been demonstrated for rye that the development of genomic SSR markers is likely to be compromised by the highly repetitive nature of the rye genome (Kofler et al. 2008). In contrast, the development of EST-based markers does not suffer from genomic repetitiveness. In addition, this approach allows to directly address sub-genomic regions-of-interest in a target genome, provided that markers are at hand which are anchored in a reference genome. In this case, the anchor markers provide an orientation when searching the reference genome for neighbouring stretches of DNA sequence which potentially bear additional markers for the target genome. With regard to breeding research in rye, marker enrichment of sub-genomic regions using anchored DNA markers as starting points may prove particularly interesting in the context of introgression-line libraries (Falke et al. 2008), where donor-chromosome segments are to be delineated in their extensions and further narrowed down via recombination. In rye, the rice genome has recently been used as a reference for the targeted development of EST-derived markers to enrich sub-genomic regions on chromosomes 1RS (Mago et al. 2005), 2RL (Hackauf and Wehling 2005), 4RL (Hackauf et al. 2007) and 7RS (Miftahudin et al. 2004; Collins et al. 2008).

To address sub-genomic regions in related grass species using the rice genome data requires to know about the extent of colinearity, at least at the sub-chromosomal level. The relationships between conserved linkage blocks in Triticeae cereals and rice (Moore et al. 1995) has recently been described more precisely by large-scale comparative DNA sequence analyses of ESTs mapped in wheat (Sorrells et al. 2003; Salse et al. 2008) and barley (Stein et al. 2007) in relation to rice. For rye, an analysis of its sub-genomic relationships to rice is still lacking.

In the present study, we have made use of EST-based markers included in various genetic maps of rye established by us or other authors. The aim was to assemble an inventory of gene-derived markers with known and unequivocal chromosomal locations in rye which can be set in relation to the rice genome. Results are presented concerning (1) the establishment of novel EST-based SSR markers in rye (2) the establishment of a genetic map which comprises 92 EST-based markers and provides a tool to elaborate on a recently published introgression library of rye (Falke et al. 2008), (3) rye–rice relationships at the sub-chromosomal level deduced from the present and 26 other genetic maps of rye published in the course of the last 15 years, and (4) identification of novel, PCR-based conserved ortholog set (COS) marker candidates. Furthermore, the putative molecular nature of the investigated EST-based markers was deduced by assigning them to plant-gene ontologies.

Material and methods

Plant material

For segregation and linkage analyses, the same BC1 population *9953* was used as previously described (Hackauf and Wehling 2003; Falke et al. 2008). Briefly, this population originated in a cross of the elite inbred line L2053 from the Petkus gene pool as recurrent parent (kindly provided by Dr. H. Wortmann, Hybro GmbH & Co. KG, Kleptow, Germany) and an unadapted Iranian primitive rye population ("Altevogt 14160") as donor. A single F1 offspring plant from this cross was backcrossed with L2053 as pollen parent to produce the BC1. The male gamete from the Altevogt population which contributed to the BC1 is anonymous with respect to its genotype and was, as a consequence, not available for polymorphism survey experiments. Instead, a random sample of 12 BC1 offspring individuals was screened for polymorphism at each marker locus, which gave a probability >99% to detect at least one heterozygous individual in case of parental gamete polymorphism at a given marker locus.

SSR-marker development

About 8,122 rye ESTs were imported from the EMBL format [EST data available at the EBI (ftp://ftp.ebi.ac.uk)] and were processed using the Sputnik EST clustering pipeline (Rudd et al. 2003). Sequences were clustered using the Hashed Position Tree (HPT) method (Biomax informatics, Martinsried, Germany) using the parameters of a similarity link threshold of 0.7 and seven network iterations. The resulting sequence clusters were assembled using the CAP3 application (Huang and Madan 1999). The resulting 'unigene' sequences were used in the identification of candidate SSRs. To define a candidate SSR a minimum length threshold

for the number of repeat units was imposed as noted previously (Hackauf and Wehling 2002). Primers for selected EST-SSRs were designed using the software package Primer3 (Rozen and Skaletsky 2000). *Secale cereale* microsatellite (SCM) primer sequences are available for scientific purposes at http://www.jki.bund.de/SCMprimer/start.php3.

Marker analysis

DNA from individual plants was extracted from 2-week-old leaves using standard methods. AFLP fingerprints were generated using *Pst*I/*Mse*I primer combinations according to Vos et al. (1995) by Keygene N.V. AFLP marker nomenclature was based on the relative mobility of AFLP fragments. The suffices "P1" or "P2" were added to the marker names according to the markers' recurrent or donor–parent origin, respectively. The 27 rye genomic SSR markers described by Saal and Wricke (1999) as well as a set of 63 SSRs (Lochow Petkus rye microsatellites, lprm) kindly provided by Dr. V. Korzun, Lochow Petkus GmbH, Bergen, Germany, were used for the chromosomal localization of linkage groups. Genomic wheat SSR markers known to be suitable for cross-species amplification in rye (Saal and Wricke 1999; Korzun et al. 2001) and barley (Hernandez et al. 2002) as well as EST-derived SSRs from barley (Pillen et al. 2000; Ramsay et al. 2000) and wheat (Table 1) were included in the study. In addition to SSR markers, four previously mapped STS anchor markers were used. The STS marker *TC80147* was developed and assayed according to a previously described protocol (Hackauf and Wehling 2005) using the primers 5′-AAAGA CAGCATTGGACAGCAT-3′ and 5′-CAAAATGTCAGC TTGCTTTGAG-3′. Methods for the marker loci *TC76893*, *TC76051,* and *Xiac501* are reported elsewhere (Roux et al. 2004; Ruge et al. 2003).

SSRs were assayed on LI-COR Model 4200 automated fluorescent DNA sequencers (LI-COR Inc., Lincoln, NE) as described earlier (Hackauf and Wehling 2002). The thermal cycling conditions for barley, wheat and individual rye SSR markers were 15 min 95°C, 5× (45 s 95°C, 5 min 68°C lowered by 2°C/cycle), 1 min 72°C, 2× (45 s 95°C, 2 min 58°C lowered by 2°C/cycle, 1 min 72°C), 39× (45 s 95°C, 2 min 54°C, 1 min 72°C), 10 min 72°C, constantly 10°C).

Segregation analysis

Eighty-seven BC1 individuals were used for map construction using the software package JoinMap v.3.0 (Van Ooijen and Voorrips 2001). Linkage groups were separated using a LOD score of 4.0. The Kosambi function was used to convert recombination values to genetic distances (cM). As a criterion for the quality of the genetic map, the goodness of fit given as a chi-square value was assessed. Average chi-square values below 3.0 were considered to be acceptable goodness-of-fit values (Van Ooijen and Voorrips 2001).

Comparative sequence analysis of mapped ESTs

In addition to the ESTs mapped in the present study, the chromosomal positions of markers previously mapped in rye (Rorat et al. 1991; Devos et al. 1993; Loarce et al. 1996; Shimizu et al. 1997; Taylor et al. 1998; Ma et al. 2001; Masojć et al. 2001; Wehling et al. 2003; Roux et al. 2004; Hackauf and Wehling 2005; Mater et al. 2004; Mago et al. 2005; Simeone and Lafiandra 2005; Bolibok et al. 2007; Fontecha et al. 2007; Lapitan et al. 2007; Matos et al. 2007; Milczarski et al. 2007; Wricke and Hackauf 2007; Collins et al. 2008), barley (Stein

Table 1 EST-derived wheat SSRs for cross-species amplification in rye

Locus	Acc.-no.	Primer (5′ > 3′)	Amplicon (bp)	Repeat	Annotation
TmLr10	AF326781	**F:** GCC CAC CCG AAA GGA GAA AGA AAG **R:** AGT CGA TGC GCT ACC ACC ACA CTC	155	(AG)17	*Lr10* disease resistance locus
TaVP1	AJ400714	**F:** TTC GCA TCG TCT CTT GGT TCA **R:** GCC ATC ATT GTA CCA GCA ATT TGT	385	$(TCT)_6$	vp1D gene for VIVIPAROUS1
TaTIP	U86762	**F:** CGG CCG CGT CGA CCT CTC CAC TCC **R:** CTG ACC GGC ATT TTC GCT CAC CTC	104	$(TCT)_5$	Gamma-type tonoplast intrinsic protein
WHTUBE2	M28059	**F:** CAA ACC TAC AAG CAG GGC AAG GAG **R:** AAC CCT GAC CTT CCA CAC ACC	210	$(AGG)_4$ $(AAG)_5$	Ubiquitin carrier protein (E2)
TAATP2	X74545	**F:** TCC CCG GAC GGC TAC CTC TTC **R:** TCT TGG CAC CGA CGT ACT TGT	115	$(CCG)_6$	ATP synthase beta subunit
Y14008	Y14008	**F:** GGC ACG AGA TCC TCT CCT C **R:** GAT GAA CTG CGA CGG GAT GTC	241	$(CCT)_8$	Gibberellin 20-oxidase

et al. 2007), and rice (Harushima et al. 1998) were obtained either from the GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) and Gramene (http://www.gramene.org/) database, respectively, or from the original publications. Sequence information of cDNA and genomic DNA probes originating from wheat (KSU, WG, PSR), barley (ABC, BCD, BG, MWG and cMWG), oat (CDO), maize (CSU), sorghum (HHU) and rice (RZ) was accessed via the GrainGenes, Gramene as well as the CeResDB (http://jicbio.bbsrc.ac.uk/cereals/) databases. The origin of these probes has been described before (Devos et al. 1993; Van Deynze et al. 1995). Rye (release 3, December 22, 2003), barley (release 9, September 15, 2004) and wheat (release 10, January 14, 2004) tentative consensus (TC) sequences for publicly available EST sequence data (Quackenbush et al. 2000) as well as the publicly available rice genome sequence (release 4.0, January 12, 2006), including the genetic positions in centiMorgan (Yuan et al. 2000) and the plant-gene ontologies, were obtained from TIGR Rice Genome Annotation website (Yuan et al. 2005). Sequence-similarity searches ($E \leq 1E\text{-}10$) between the different data sets were performed using the BlastN algorithm (Altschul et al. 1990). For the searches, the output was limited to three descriptions ($v = 3$) and three alignments ($b = 3$). For the remaining parameters, default values were used. Using a stringency cut-off of $\geq$80% identity with the marker sequence over a minimum of 100 bases, we parsed out the rice gene sequences putatively orthologous to the anchor markers. All data was deposited and processed in a local relational database system. To test the significance of orthology (Passarge et al. 1999) between a rye chromosome and rice, we first identified the rice chromosome representing the largest number of significant matches to that particular rye chromosome. Subsequently, we used a binomial distribution (Linkiewicz et al. 2004) to calculate the probability of finding the observed number of matches under the null hypothesis of no orthology, i.e. random distribution. Probabilities <0.01 were considered as evidence of significant orthology, i.e. these genes in rye and rice arose from a common ancestor gene (Passarge et al. 1999). The test was performed independently each for the best and second best BlastN hit. Results were visualized using the software MapChart (Voorrips 2002).

Identification of COS primers

Intron-spanning primers from conserved regions of the cDNA probes mapped in rye were identified by integrating and processing data deposited in the PiP database of potential intron polymorphism markers (Yang et al. 2007) in the local relational database system.

Results

EST-SSR marker development

A set of publicly available EST sequences from rye was obtained from the sequence database at the EBI. These data set contained 8,122 entries representing 3.7 Mb of DNA sequence. Sequence redundancy was removed from the collection by clustering and assembling the sequences within the Sputnik application (Rudd et al. 2003). The non-redundant sequence collection was reduced to 5,423 unigenes that in turn represented over 2.2 Mbs of transcript sequence. These unigene sequences were annotated for similarity to other sequence collections, for domain content and to assign tentative structure and function where possible. Current annotations are available from the openSputnik website (Rudd 2005) at http://sputnik.btk.fi.

The clustered rye unigenes were screened for putative perfect or imperfect SSR motifs up to the penta-nucleotide repeat size. PCR primers were derived for 290 novel unigenes bearing SSRs with perfect, imperfect, compound or interrupted di-, tri-, or tetra-nucleotide repeats, respectively, and 176 (61%) primer pairs resulted in scorable PCR assays. Together with the 157 SCM established before from the same data set (Hackauf and Wehling 2002), 333 expressed rye genes could be captured via PCR assays (ESM Table 1).

Map construction

Map construction for BC1 population *9953* included EST-derived and genomic SSR markers from rye, genomic SSR markers from wheat, and EST-derived SSRs from wheat and barley. Using a skeleton map consisting of 131 AFLP markers, 92 EST-derived and 25 genomic SSR markers were mapped to a total of seven linkage groups (Fig. 1). For a total of 72 of these SSR markers chromosomal localization has been determined before (cf. footnotes in Fig. 1). As a result, a genetic map comprising 724 cM was constructed which describes the chromosomal positions of 252 PCR-based markers, i.e., 117 SSR, 131 AFLP, and four STS markers (Fig. 1). The map positions of 45 SSR markers is described for the first time.

Chromosome 1R is covered by 19 SSR, 21 AFLP, and two STS markers defining an interval of 113.4 cM. Thirty-eight markers including 18 SSRs and 20 AFLPs and encompassing 84.6 cM could be mapped on chromosome 2R. The 35 markers mapping on chromosome 3R describe a sub-genomic region of 71.3 cM. A linkage group of 14 SSRs, two STSs and 14 AFLPs spanning 107 cM mapped to chromosome 4R. The most distal marker on this chromosome is *Xiac501*, which alone adds 18 cM to the length of the chromosomal map. Chromosome 5R represents the

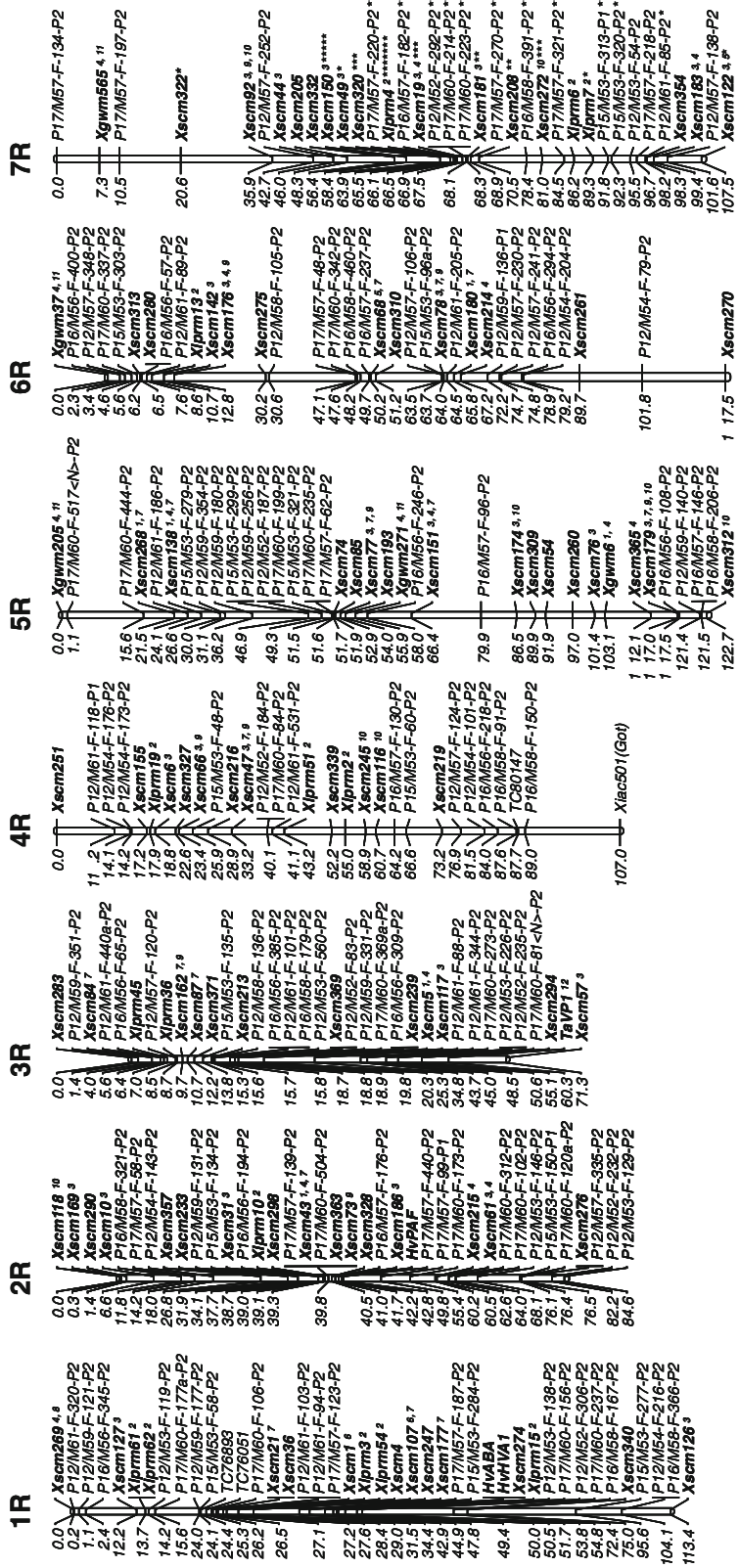

Fig. 1 A genetic linkage map of rye comprising SSR, STS, and AFLP markers. SSR and STS markers are given in *bold*. *Superscripts* indicate markers previously mapped in rye (*1* Saal and Wricke 1999, *2* V. Korzun personal Communication, *3* Hackauf and Wehling 2003, *4* Khlestkina et al. 2004 cf. Khlestkina et al. 2005 for the underlying rye ESTs, *5* Wehling et al. 2003, *6* Roux et al. 2004, *7* Bolibok et al. 2007, *8* Lapitan et al. 2007, *9* Milczarski et al. 2007, *10* Wricke and Hackauf 2007) and wheat (*11* Röder et al. 1998, *12* Bailey et al. 1999). Segregation distortions of markers are indicated by *asterisks* for significance levels of $\alpha = 0.1$ (*), 0.05 (**), 0.01 (***), 0.005 (****), 0.001 (*****), and 0.0005 (******)

largest linkage group of this map with 18 SSR and 19 AFLP markers covering a sub-genomic region of 122.7 cM. A linkage group of 14 SSR and 20 AFLP markers spanning 117.5 cM was assigned to rye chromosome 6R. The map of chromosome 7R comprises 19 SSR and 17 AFLP markers defining an interval of 107.5 cM. Among the SSR mapped

in this population 92 (87.6%) are derived from EST sequences and 25 markers (21.4%) are of genomic origin.

Genomic distribution of the SSR markers appeared to be uniform, although a number of closely linked markers were present in the distal part of chromosome 6RS as well as in the centromeric parts of chromosomes 1R, 5R and 7R, respectively. Distorted segregations were found for 24 out of 36 markers on chromosome 7R, with 22 markers deviating significantly from expectation (Fig. 1). The segregation distortions were observable across a 34 cM interval between markers *Xscm150* and *P15/M53-F-320-P2*. All distorted segregations were characterized by excess of heterozygotes, with the most pronounced deviation near marker locus *Xlprm4* where the donor-allele frequency in the segregating progeny was about 37% instead of 25%.

Comparative genome analysis of rye versus rice

We have searched a data set of cDNA and some genomic clones from different grass species against TaGI and HvGI to extend the lengths of the DNA sequences available for BlastN sequence-similarity searches with regard to the markers mapped in rye. We identified 2,653 genetic markers that were represented by a TC sequence. For 502 of these markers the chromosomal localisation in rye has been determined in different mapping studies. BlastN sequence similarity searches of these 502 ESTs mapped in rye were conducted to identify putatively orthologous genes in rice (ESM Table 2). To test reliability of BlastN-based in silico mapping of rye markers in the rice genome, 41 markers with known map positions in rye were used which had previously been genetically mapped in rice, too, by Harushima et al. (1998). These markers correspond to 31 single-copy and five duplicated gene loci. For 38 (93%) of these markers, in silico map positions in rice deduced via BlastN sequence comparison were similar or identically equal to their genetic map positions in rice (ESM Table 3).

Using the best BlastN hit in each alignment, a subset of 334 (66.5%) markers matched putatively orthologous gene sequences in the rice genome and could, thus, be mapped in silico. For 309 (92.7%) of these marker sequences, information on the tentative molecular function inferred from electronic annotation as well as assignment to plant-gene ontologies is available (ESM Table 2). Additionally, 14 markers were included which lacked sequence information but had been genetically mapped in rice by Harushima et al. (1998). The rye/rice comparison of the present study was, thus, based on 348 genetic markers (Table 2). BlastN hits for markers mapping to specific rye chromosomes were not randomly distributed over rice chromosomes. Rather, significant clustering of hits occurred, allowing to assign the seven rye chromosomes to defined regions of individual rice chromosomes (Table 2).

For marker sequences mapping on chromosome 1R of rye, significant sequence similarities were found across 119.3 cM on rice chromsome R5, i.e., across nearly the entire length of this chromosome (Fig. 2, ESM Table 2). In contrast, the remaining rice chromosomes display patchworks of regions with putative homoeology to various rye chromosomes. These subgenomic regions range from 3.8 cM in size on rice chromosome R3 up to 63.7 cM on rice chromosome R6 (Fig. 2).

The second-best BlastN hits of ESTs mapped in rye appeared to be randomly distributed among the 12 rice chromosomes, with the exception of markers mapping on rye chromosomes 1R and 3R which display pronounced clustering on rice chromosome R1 (ESM Table 5). Among the marker sequences mapping on rye chromosome 1R, 17 gave both a statistically significant best and second-best BlastN hit (ESM Table 4). Best as well as second-best hits

Table 2 Distribution of rye markers with best BlastN hit on individual rice chromosomes

Rye chromosome	Rice chromosome[a]													Probability[b]	
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	Total	First	Second
1R	10	4	1	5	***38***	2	0	5	0	*9*	3	1	78	<0.0001	0.0539
2R	4	5	5	***21***	1	1	***12***	0	1	2	1	2	54	<0.0001	0.0010
3R	***24***	2	3	2	3	5	1	3	0	0	0	2	45	<0.0001	0.1504
4R	1	8	*5*	0	0	**14**	0	3	2	1	4	2	40	<0.0001	0.0108
5R	3	1	***17***	2	2	0	2	3	*8*	1	2	*7*	48	<0.0001	0.0241
6R	4	***14***	2	1	0	3	2	3	2	1	2	0	34	<0.0001	0.1636
7R	4	2	**19**	7	2	*6*	1	*6*	0	0	0	1	48	<0.0001	0.0572
Total	50	36	52	38	46	31	18	22	13	14	12	15	348[c]		

[a] Numbers in italics indicate rye markers mapping to known colinear rice chromosomes (according to Moore et al. 1995), statistically significant numbers of hits are given in bold

[b] Calculated probabilities for the rice chromosomes with the two largest numbers of significant matches to a particular rye chromosome

[c] Including 14 markers genetically mapped by Harushima et al. (1998)

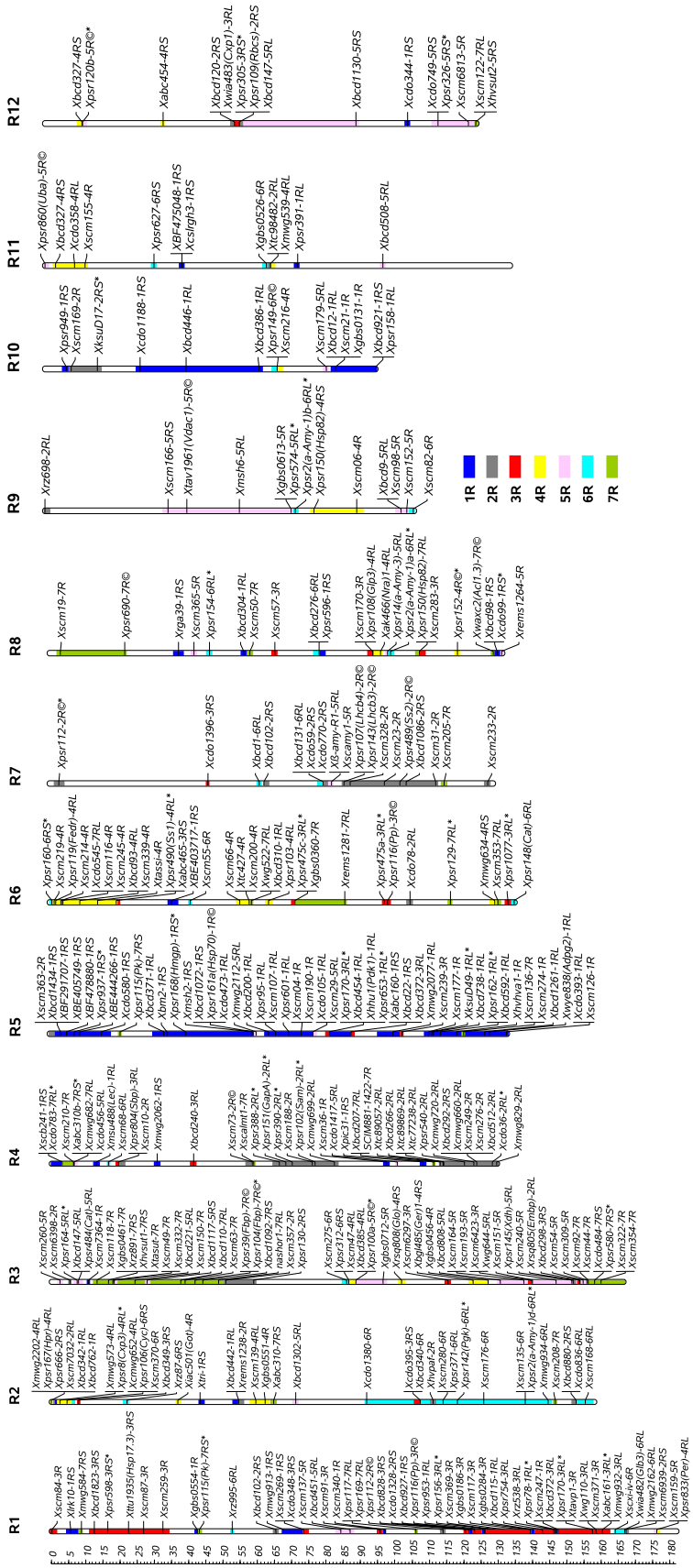

Fig. 2 Rice–rye genome relationships. Rice genome view showing the rye chromososme location for the most similar marker sequences

for three of the markers were found on rice chromosome R1, with two of these markers, namely *Xlrk10* and *Xgbs554*, resulting in identical genetic positions of their best and second-best hit, respectively. In each case, though, the best and second-best hit for a given marker identified different physical loci on rice chromosome R1 (cf. Os_locus designation in ESM Table 4). The majority ($n = 11$) of the 17 rye 1R markers resulted in best BlastN hits on rice chromosome R5 while their second-best hits matched sequences on rice chromosome R1 (ESM Table 4).

Significant clustering of second-best hits ($n = 8$) on rice chromosome R1 was also observed for seven markers mapping on rye chromosome 3R (ESM Table 5). In contrast, the best hits of these seven markers did not uniformly cluster on a specific rice chromosome but were distributed across five different rice chromosomes (ESM Table 4). Only two of the seven best BlastN hits were found on rice chromosome R1.

In order to identify regions of the rye genome colinear with sub-chromosomal regions of rice, we searched five whole-genome rye linkage maps for markers which yield significant BlastN hits with putative rice orthologs and define genetic intervals in a rye map. In total, 35 sub-chromosomal intervals were identifed on the seven rye chromosomes, with 16 intervals comprising more than two markers (ESM Table 6). Rye chromosome 6R was represented by a relatively small segment covering a genetic interval of 6.3 cM (ESM Table 6). Genetic intervals picked up from the five different rye maps were pooled for a given rye chromosome and arranged according to the map positions of the corresponding rice-gene orthologs (ESM Table 6). In 11 of the 16 intervals comprising more than two markers, there was colinearity of the EST-derived markers in rye and their putative orthologs in the rice genome. For the linkage groups mapping on rye chromosomes 1R, 2R, 3R, 5R, and 6R, colinear stretches of significant BlastN hits were found on rice chromosomes R5, R4 as well as R7, R1, R3 as well as R12, and R2, respectively (ESM Table 6), which corresponded to the known homoeology of the Triticeae group 1, 2, 3, 5, and 6 chromosomes and the respective rice sub-chromosomal segments. In the case of rye chromosomes 4R and 7R, the situation turned out to be more complex. Besides marker intervals corresponding to known homoeologies of Triticeae and rice chromosomes (i.e., rye 4R and rice R3; rye 7R and rice R6, R8), there were blocks of markers which had significant BlastN hits to colinear rice genes on other chromosomes. For instance, some linkage blocks on rye chromosome 4R significantly hit colinear blocks on rice chromosomes R2, R6, and R8, respectively. With regard to rye chromosome 7R, significant sequence similarities identified stretches of colinearity with rice chromosome R3.

In 7 of the 35 sub-chromosomal segments the order of individual markers determined for rye was not consistent with the position of their putative orthologs in rice. These exceptions apply to 9 out of 95 markers located on chromosomes 1R (*Xscm4-1R*, *Xbcd22-1RS*), 2R (*Xscm233-2R*), 3R (*Xttu1935*(*Hsp17.3*)*-3RS, Xscm371-3R*), 5R (*Xscm260-5R*), and 7R (*Xscm332-7R, Xscm150-7R, Xscm49-7R*). The majority of these nine markers had been mapped in the BC mapping family *9953*.

Identification of COS primers

The 334 probes mapped in rye and rice were analyzed to identify a set of primers addressing orthologous sequences conserved in rye and other grasses. For a total of 59 sequences (17.7%) distributed across all the seven rye chromosomes, 122 primer pairs could be identified in the PiP database (ESM Table 7) which potentially provide novel, intron-spanning STS markers in rye. The deduced primer pairs represent 39 RFLP probes and 20 ESTs, the latter of which have been used before to establish either SSR or SNP markers in rye.

Discussion

Genetic map

The genetic map presented is based on PCR-based markers and comprises the positions of 117 SSR, 131 AFLP, and four STS markers. From SSR-based maps in other grass species such as barley (Liu et al. 1996; Pillen et al. 2000), maize (Senior et al. 1996), rice (Temnykh et al. 2000) or wheat (Röder et al. 1998) it is known that SSR markers generally display a relatively uniform distribution across the genome. The BC1 mapping data on rye reported in the present study is in agreement with this general notion, although distribution of EST-SSRs in barley has been reported to follow the segmentation of the barley genome in gene-rich and gene-poor regions at the sub-megabase level (Varshney et al. 2006). A subset of the rye SSR markers had been mapped in rye before by others (cf. footnotes in Fig. 1) at positions consistent with those reported in the present study. Other SSR markers had originally been mapped in wheat. For instance, the marker *TaVP1-3R* was derived from the wheat *Vp1* genes. *Vp1*-orthologous loci were detected on the long arms of wheat chromosomes 3A, 3B, and 3D (Bailey et al. 1999) and the genomic wheat SSR markers *Xgwm271-5R* and *Xgwm205-5R* have been mapped on homoeologous group 5 chromosomes in wheat (Röder et al. 1998). For 45 EST-derived markers, their chromosomal localization in rye could be determined for the first time.

Chromosome 7R was the only chromosome in mapping population *9953* where pronounced deviations from the expected 1:1 segregation ratios of markers occured. Since the mapping population was a backcross progeny the causes of this segregation distortion cannot be unambiguously dissected, i.e., whether gametic or zygotic selection was acting or whether the distortions reflect an advantage of heterozygosity over homozygosity (heterosis). Since, the recurrent parent was a highly inbred line we assume disadvantage of recurrent-parent allele homozygosity as the most probable reason for the distorted segregations. The segregation distortion would, thus, indicate a chromosomal region bearing one or several recessive factors which pose(s) a measurable genetic load to the recurrent parent line L2053.

SSR markers are readily used in breeding practice due to their codominant inheritance, a relatively high degree of polymorphism and robustness across varying laboratory practice. The linkage map established in the present study comprises 117 SSR markers, most of them have been derived from rye ESTs. In rye, a first set of SSR markers was developed from genomic small-insert libraries and 12 markers mapped on six of the rye chromosomes (Saal and Wricke 1999). In an initial approach to exploit publicly accessible rye-EST databases for SSR-marker development, a total of 157 functional SSR primer pairs were developed and 38 polymorphic EST-SSR markers mapped in rye (Hackauf and Wehling 2002, 2003). Covering all the seven rye chromosomes, the markers mapped in the present study add to previously published data on the chromosomal localization of sequence-specific markers in rye (Shimizu et al. 1997; Saal and Wricke 1999; Van Campenhout et al. 2000; Hackauf and Wehling 2002, Nagy and Lelley 2003; Stracke et al. 2003; Miftahudin et al. 2004; Khlestkina et al. 2004, 2005; Kuleung et al. 2004; Varshney et al. 2007; Kofler et al. 2008). A particular feature of the present map is that it is based on the same cross as the introgression library reported by Falke et al. (2008). This introgression library contains donor chromosomal segments (DCS) from an exotic rye accession and is currently used to identify DCS which bear novel genes for male fertility restoration, disease resistances and other agronomically favourable traits. The molecular markers contained in our map may, thus, directly be applied to characterize valuable DCS at the molecular level, e.g., to delineate their genetic extensions, to narrow down their sizes via recombination, or to identify candidate trait genes via comparative approaches by use of markers which are anchored in reference genomes.

Comparative genome analysis

Looking at the genomic rye/rice relationships may be interesting because rye is outstanding in that it carries a number of sub-chromosomal rearrangements in relation to other Triticeae cereals such as barley or wheat. Results from comparative mapping studies in other cereals may, thus, not immediately be transferred to the case of rye. To-date, genome-wide comparative mapping efforts in relation to the rice genome have not considered rye. A reason may be the limited number of EST-markers which have been mapped in an integrated fashion in this cereal crop plant. Usually, comparative mapping requires a larger number of EST-derived markers whose genetic positions have been mapped in a target genome. In the present study, we have made an inventory of 502 EST-based markers drawn from a total of 27 genetic maps of rye and in silico mapped these markers in the rice genome. A drawback in this effort was that the 27 genetic maps were not sufficiently linked with each other by common markers to properly calculate a consensus map which would be useful for a comparative mapping of EST-derived markers. For this reason, we generally confined our comparison to displaying the chromosomal locations of rye markers instead of using map positions, and we worked out the rice/rye relationships by taking the genetic maps of the 12 rice chromosomes and indicating the tentative homoeologies of parts of these maps with sub-chromosomal regions of the seven rye chromosomes. The picture (Fig. 2) obtained with this approach considers the best significant BlastN hits for all the 348 markers investigated. For a subset of 95 markers, which were comprised in genetic intervals of two or more markers in five of the 27 maps, the rye/rice relationships could be worked out more directly, i.e., by taking the chromosomes of rye as reference (see below).

In silico mapping relies on using proper statistical parameters in sequence-similaritiy searches. We are confident about the reliability of the in silico mapping results of the present report since the majority (>90%) of markers which have been genetically mapped in rice (Harushima et al. 1998) were found at similar positions via in silico mapping. Part of the RFLP probes used in the present study to investigate the rye/rice relationships have proven to be useful for comparative mapping between wheat and rice (Van Deynze et al. 1995). For the wheat/rice (Sorrells et al. 2003) and barley/rice (Stein et al. 2007) comparisons, similar statistical parameters of sequence-similaritiy searches have been used as described in our study.

For in silico mapping EST-derived markers in the rice genome we have used the concept of the dual-synteny approach (Singh et al. 2007) which uses best and second-best BlastN hits to deduce evolutionary relationships between genomes. The best BlastN hits obtained for EST-based markers from various rye maps displayed clustering on specific rice chromosomes, suggesting homoeologous relationships between the respective subchromosomal regions in the two species.

The comparative analysis of rice versus rye as summarised in Fig. 2 is based on significant BlastN hits, which

were taken as evidence for putative orthology of the compared sequences. A similar approach has previously been followed in the case of barley versus rice (Stein et al. 2007). In total, 200 (57.5%) rye ESTs were assigned to linkage groups of rice which are presumed to be syntenic according to the circular model of grass genome colinearity (Moore et al. 1995).

Besides sequence similarities observed with single EST-derived markers, further evidence for homoeology of sub-chromosomal regions may come from BlastN alignments of two or more EST-derived markers which together form a linkage group and, thus, define a genetic interval. In the present study, 95 (27.3%) rye ESTs with significant BlastN hits were found to define genetic intervals mapping to either of the seven rye chromosomes. When comparing the order of markers in these linkage groups with the respective map positions in the rice genome (ESM Table 6), the majority of marker orders proved to be in accordance with the model of grass genome colinearity (Moore et al. 1995). For nine out of 95 markers, their orders determined in either of five rye mapping populations did not agree with the map positions of their tentative orthologs in rice. Seven of these nine markers had been determined in their order by use of mapping population BC *9953*. A possible reason that this family gave rise to most of the observed discrepancies may be its relatively small size, which together with its backcross structure caused limited statistical information content for mapping.

The status of rye–rice genome relationships at the sub-chromosomal level as depicted in Fig. 2 has not been described before. The relationships between rye chromosomes and rice generally were consistent with the data previously reported for wheat and barley (Sorrells et al. 2003; Stein et al. 2007). Some of the rye–rice genomic relationships as depicted in Fig. 2 and ESM Table 6 deserve further discussion.

Rye chromosome 1R

For the homoeologous group 1 Triticeae chromosomes colinear linkage blocks have been reported to reside on rice chromosomes R5 and R10, respectively (Moore et al. 1995). Concerning the 78 markers used in the present study and mapping on rye chromosome 1R, significant (i.e., non-random) clustering of best BlastN hits was found on rice chromosome R5 for 38 of these markers. This result is consistent with the observations reported for the homoeologous barley chromosome 1H (Stein et al. 2007) and is in agreement with the extensive colinearity between rice chromosome R5 and homoeologous group 1 chromosomes of wheat (Sorrells et al. 2003). Although there appeared to be a tendency of 1R markers ($n = 9$) matching rice sequences on chromosome R10, this was not statistically significant.

With respect to markers on rye chromosome 1R, analysis of the second-best BlastN hits revealed non-random distribution; however, with clustering on rice chromosome R1 and, thus, on a different rice chromosome as compared with the best-significant hits. This observation probably reflects the duplicated nature of R5 and R1 sub-genomic regions as suggested earlier (Kishimoto et al. 1994) and corroborated later on (Guyot and Keller 2004; Wang et al. 2005; Yu et al. 2005, Salse et al. 2008). In barley, non-random distribution of BlastN hits was reported for non-colinear markers mapping to barley chromosome 1H, with clustering on rice R1 (Stein et al. 2007). It has recently been shown that the same duplications involving segments from rice chromosomes R1 and R5, are present in wheat on chromosomes 3 and 1 (Valarik et al. 2006). These duplications, thus, appear to have preceded the divergence of the Triticeae and rice genomes (Salse et al. 2008). As has been noted earlier (Keller and Feuillet 2000), the presence of paralogous sequences on rice chromosomes R5 and R1 may complicate the use of the rice genome as a blueprint for targeted gene isolation from homoeologous group 1 chromosomes of the Triticeae. In rye, this chromosome is of particular interest because it harbours genes for a number of resistances or tolerances to biotic (Wricke et al. 1996; Forsström and Merker 2001; Mater et al. 2004; Roux et al 2004; Mago et al. 2005) and abiotic stresses (Villarea et al. 1998; Ehdaie et al. 2003), for restoration ability (Wricke et al. 1993; Miedaner et al. 2000) as well as for self-incompatibility (Wricke and Wehling 1985). The example of rye chromosome 1R mentioned above highlights the need for species-specific studies of the sub-genomic relationships among grass genomes.

Rye chromosome 3R

Concerning the BlastN analysis for EST markers mapping on rye chromosome 3R, the significant clustering of best and two-second-best hits on rice chromosome R1 is in line with the known homoeologous relationship between rice chromosome R1 and the group 3 Triticeae chromosomes (Moore et al. 1995; Van Deynze et al. 1995; Smilde et al. 2001; Sorrells et al. 2003; Stein et al. 2007). However, results obtained for best and second-best hits were not always congruent since five of seven markers with second-best hits on R1 gave rise to best hits somewhere else. Whether this observation is of biological relevance or just reflects some shortcoming in the BlastN-based in silico mapping approach needs further research.

Rye chromosome 5R

Considering markers mapping on rye chromosome 5R, a significant portion ($n = 17$) matched to sequences on rice R3. Increased albeit not statistically significant numbers of

hits were also found on rice chromosomes R9 and R12, which again is consistent with earlier reports (Moore et al. 1995) on colinearity between part of homoeologous group 5 Triticeae chromosomes and the mentioned rice chromosomes. In contrast to the best BlastN hits, second-best hits for markers on five of the seven rye chromosomes were randomly distributed across the 12 rice chromosomes.

Rye chromosomes 4R and 7R

There were two exceptional rye chromosomes. BlastN hits for markers genetically mapping on rye chromosomes 4R and 7R displayed significant clustering on rice chromosomes R6 and R3, respectively. There appeared to be a tendency of 4R markers matching rice sequences on chromosome R2 ($n = 8$) and 7R markers matching rice sequences on rice chromosomes R4 ($n = 7$), R6 ($n = 6$) and R8 ($n = 6$), respectively. However, neither of these latter relationships were statistically significant. Homoeologous group 4 chromosomes of wheat and barley had been identified as homoeologs of rice chromosome R3, while the group 7 chromosomes have been reported to be homoeologous to rice chromosomes R6 and R8 (Moore et al. 1995; Sorrells et al. 2003; Stein et al. 2007). When considering these relationships with regard to rye one has to take into account the chromosomal rearrangements which have occured between the genomes of rye and wheat (Devos et al. 1993). For instance, taking into account the 7S/4RL and the 4L/7RS translocational differences observed between wheat and rye (Devos et al. 1993), the orthology of linkage blocks on rye chromosome 4R and rice R6 as well as rye 7R and rice R3, respectively (Fig. 2), appears consistent with previous reports on the genome relationships between rice and the Triticeae species of wheat and barley (Van Deynze et al. 1995; Gale and Devos 1998; Sorrells et al. 2003; Stein et al. 2007). This applies also to the putative orthology of linkage blocks on rye chromosome 4R and rice chromosome R2, as the distal segment of chromosome 4RL has been reported to be homoeologous with the distal parts of the short arms of the wheat group 6 chromosomes (Devos et al. 1993). The 4RL/R2 relationship has been successfully used to develop novel STS markers for the restorer gene *Rfp1* in rye (Hackauf et al. 2007).

The comparative analysis of the present study between rye and rice is based on 348 EST-derived markers which have been used in rye. Although our results do not yet provide a comprehensive view of the rye–rice relationships, the present picture generates some implications for comparative genetic approaches. For instance, the marker loci *Xrz891* and *ScAMLT1*, which have recently been reported to be closely linked to the aluminium tolerance locus *Alt4* on chromosome 7RS (Collins et al. 2008), correspond to orthologous rice genes which are located on different chromosomes, namely, R3 and R4 (Fig. 2). Rearrangements like this have to be borne in mind when devising strategies which rely on the rice genome as a model, like, for instance, marker saturation of selected sub-genomic regions or positional gene cloning in rye. Our results, thus, stress the need of a detailed picture of the rye–rice relationships at the sub-chromosomal level if the rice genomic resources be exploited for rye genetics in a straightforward way.

Identification of COS marker candidates

Those EST-derived markers which displayed significant sequence similarities with rice genes provide anchor points between rye and rice and enable the targeted development of primer pairs serving to establish COS markers (Fulton et al. 2002) for rye. Based on conservation in the coding sequences of isolated grass gene sequences, a number of STS markers have been established in subgenomic regions of rye using the rice genome as a blueprint (Miftahudin et al. 2004; Mago et al. 2005; Hackauf and Wehling 2005; Hackauf et al. 2007). In the present study, we have identified a set of 122 pairs of intron-spanning primers. A majority of these primers offer the opportunity to convert hybridization-based RFLP marker assays into PCR-based marker systems. Being anchored in conserved exon sequences, the presented COS primers span the less conserved introns between adjacent exons and, hence, provide the potential of detecting inter- and intraspecific polymorphisms in grass genes more readily than could be accomplished using other types of markers (Yang et al. 2007).

In the present study, a number of novel SSR markers derived from rye ESTs have been mapped together with genomic SSR and AFLP markers in rye, and markers from this map as well as 26 other rye maps were used to depict rye/rice relationships at the sub-chromosomal level. This inventory of available mapping information on gene-derived markers in rye in relation to the rice genome may serve a starting point for future comparative genetic approaches in rye, a Triticeae species with yet quite limited genomic resources.

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