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ORIGINAL PAPER

Elena K. Khlestkina · Ma Hla Myint Than · Elena G. Pestsova · Marion S. Röder · Sergey V. Malyshev · Viktor Korzun · Andreas Börner

Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.) including 39 expressed sequence tags

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Abstract The genetic map of rye contains predominantly restriction fragment length polymorphism (RFLP) markers but also a limited number of microsatellite markers, which are known to be more reliable and easier to apply. We report here the saturation of the genomic map of rye with additional microsatellite-derived markers that we obtained from the rye expressed sequence tag (EST) databases and the Gatersleben collection of wheat microsatellite markers (WMS). A total of 99 loci (39 EST and 60 WMS) were mapped into the RFLP frameworks of four rye mapping populations consisting of 139, 64, 58 and 60 RFLPs, respectively. For another ten WMS loci, which amplified PCR products not polymorphic in any of the mapping populations, chromosome and chromosome arm locations were determined using wheat-rye addition lines. Chromosomes 1R, 2R, 3R, 4R, 5R, 6R and 7R were enriched with 9, 19, 9, 13, 27, 16 and 16 microsatellite loci, respectively. The microsatellite loci mapped were evenly distributed along the chromosomes, which is important for the further application of these markers for gene mapping or diversity studies in rye. Forty-four of the WMS loci mapped in rye were found to be homoeologous to those mapped in bread wheat (*Triticum aestivum* L.).

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E. K. Khlestkina · M. H. M. Than · E. G. Pestsova · M. S. Röder · S. V. Malyshev · V. Korzun · A. Börner (✉)
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK),
Corrensstrasse 3,
06466 Gatersleben, Germany
e-mail: boerner@ipk-gatersleben.de

E. K. Khlestkina
Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences,
Lavrentyeva ave. 10,
Novosibirsk, 630090, Russia

M. H. M. Than
Department of Botany, Mandalay University,
Mandalay, Myanmar

E. G. Pestsova
Max-Planck-Institut für Züchtungsforschung,
Carl-von-Linnè-Weg 10,
50829 Cologne Germany

S. V. Malyshev
Institute of Genetics and Cytology NASB,
220074 Minsk, Belarus

V. Korzun
Lochow-Petkus GmbH,
PF 1197, 29296 Bergen, Germany

Introduction

Rye (*Secale cereale* L.) is a major crop in many areas of northern and eastern Europe. Compared to other winter cereals, rye has an excellent tolerance to low temperatures and the ability to realise relatively high grain yields under soil conditions in which other crops perform poorly (Madej 1996). Rye is, however, also important as a genetic source for wheat improvement programmes, and it is a constituent of triticale, a synthesised crop. The identification and intrachromosomal mapping of genes responsible for important agronomical traits is one of the main tasks of rye genetics, and to that end, well-saturated molecular linkage maps of rye, which are reliable and easy in application, are required.

The first linkage maps of rye were based on restriction fragment length polymorphism (RFLP) markers (Devos et al. 1993; Philipp et al. 1994; Senft and Wricke 1996). By using selected anchor probes of earlier published maps both of rye (Devos et al. 1993; Philipp et al. 1994; Senft and Wricke 1996) and other cereals such as wheat (Gale et al. 1995), barley (Graner et al. 1991; Heun et al. 1991) or oats (O'Donoughue et al. 1995) and combining them, Korzun et al. (2001) published a linkage map of rye consisting of 139 RFLPs, 19 isozyme and protein markers, 13 microsatellites, ten known functional sequences and two morphological genes. This map allowed users to choose additional probes of wheat, rye or barley for saturating rye genome regions of their interest. A second extended linkage map of rye has recently been published

that contains 200 RFLPs, 179 amplified (A)FLPs, 88 random amplified polymorphic DNAs (RAPDs) and 12 protein loci (Bednarek et al. 2003).

Microsatellite [or simple sequence repeat (SSR)] markers are known to be abundant, highly polymorphic, reliable and relatively easy in application. Although already extensively used for creating maps in wheat (Röder et al. 1995, 1998) and barley (Saghai Maroof et al. 1994; Ramsay et al. 2000; Li et al. 2003), they are poorly represented in the current rye genomic maps. Thirteen microsatellites were mapped in rye by Korzun et al. (2001), whereas 20 microsatellites were genetically mapped or assigned to rye chromosomes by Saal and Wricke (1999).

There are several likely possibilities for increasing the number of rye microsatellite markers. One option is the utilisation of public sequencing resources, as demonstrated by Hackauf and Wehling (2002a, b), while another lies in the large number of mapped wheat and barley microsatellites, some of which may amplify PCR products in rye as well. The aim of the investigation reported here was the saturation of the genomic map of rye using rye expressed sequences tags (ESTs) and wheat microsatellite markers (WMS) of the Gatersleben collection.

Materials and methods

Plant materials

Four rye mapping populations developed earlier were used. One population originated from a reciprocal cross of the two rye inbred lines P87 and P105 (Korzun et al. 2001). Three other mapping populations, N6×N2, N7×N2 and N7×N6, were obtained by crossing rye lines N6 (cv. Steel), N7 (cv. Vyatka) and N2 (cv. Monstrous) (Malyshev et al. 2003). The frameworks of the four mapping populations consisted of 139, 64, 58 and 60 RFLPs, respectively. Seventy-two individuals of each mapping population were used for analysis. A set of wheat-rye (Chinese Spring/Imperial) addition lines developed by Driscoll and Sears (1971) was employed to determine the chromosome and chromosome arm locations of microsatellite loci not mapped due to lack of polymorphism .

Microsatellite markers and PCR amplification

A total of 8,930 rye EST sequences of the genebank (http://www.ncbi.him.gov) were screened to identify microsatellite sequences with di-, tri- and tetra-nucleotide repeats. Flanking primers to the microsatellites were designed and used to perform the PCR. The identification and localisation of microsatellites were performed by using MISA Perl5 script as described by Thiel et al. (2003). Identified rye EST microsatellites were designated rye expressed microsatellite sites (REMS). Flanking primers were designed using programme PRIMER 3.0 (Rozen and Skaletsky 1998). An 18-bp extension identical to the sequence of an M13 primer was added to the 5′-end of each forward primer for simplifying the PCR procedure. The amplification reaction was performed in a volume of 10 µl in Perkin-Elmer thermocyclers. The reaction mixture contained 0.5 pmol of forward primer (with M13 tail), 2.5 pmol of reverse primer, 0.15 pmol of Cy-5 modified M13 primer, 0.2 m*M* of each deoxynucleotide, 1.5 m*M* $MgCl_2$, 1 U*Taq* polymerase and 50–100 ng of template DNA. The 3-min denaturation (95°) step was followed by five cycles of 45 s at 95°C, 5 min at 68°C (−2° per cycle) and 1 min at 72°C, then by five cycles of 45 s at 95°C, 5 min at 58°C (−2° per cycle) and 1 min at 72°C, then by 27 cycles with 45 s at 95°C, 30 s at 47°C and 1 min at 72°, followed by a final 10-min step at 72°C.

In addition, a total of 651 markers from the Gatersleben collection of wheat microsatellites (Röder et al. 1998; M.S. Röder et al., unpublished data) were screened, including wheat microsatellites provided by M. Ganal (TraitGenetics, Gatersleben). The amplification reaction for these markers was performed as described in Plaschke et al. (1995a) and Röder et al. (1998).

Fragment analysis was performed using automated laser fluorescence sequencers (ALFexpress, Amersham-Pharmacia, Piscataway, N.J.). Fragment size was calculated applying the computer programme FRAGMENT ANALYSER VER. 1.02 (Amersham-Pharmacia) in a comparison with internal size standards. All sequence information can be obtained from the authors upon request.

Table 1 Survey of microsatellite markers (REMS) derived from rye ESTs and mapped on chromosomes 5R and 7R

Locus	GenBank accession no.	Repeat type and length	Expected size (bp)	Number of alleles[a]
Xrems1167-5R	BE494952	(CGG)5	247	2
Xrems1174-5R	BE495233	$(GAGT)_5$	302	4
Xrems1186-5R	BE495963	$(CAC)_5$	221	3
Xrems1205-5R	BE586813	$(ACAT)_6$	281	3
Xrems1218-5R	BE587316	$(AG)_8$	230	3
Xrems1237-5R	BE637153	$(TAGC)_5$	288	4
Xrems1264-5R	BE705252	$(CGTC)_5$	282	2
Xrems1266-5R	BE705296	$(GA)_8$	202	3
Xrems1135-7R	BE493989	$(GA)_6$	172	4
Xrems1162-7R	BE494705	$(GCC)_5$	200	3
Xrems1187-7R	BE496005	$(CAA)_5$	215	2
Xrems1188-7R	BE496047	$(TC)_7$	187	3
Xrems1197-7R	BE586481	$(CGC)_5$	192	2
Xrems1234-7R	BE637039	$(AGC)_6$	256	2
Xrems1235-7R	BE637059	$(GGC)_5$	226	2
Xrems1236-7R	BE637143	$(CGG)_5$	173	2

[a]Detected in five parental rye genotypes

Statistical analysis

The individual plants were genotyped for each microsatellite locus to build linkage maps for all seven chromosomes. The microsatellite loci were integrated into the RFLP frameworks of four mapping populations using MAPMAKER 2.0 (Lander et al. 1987). CentiMorgan distances were calculated applying the Kosambi map-unit function (Kosambi 1944).

Results

Microsatellite marker analysis

Of the 8,930 rye EST sequences screened, 207 microsatellite sequences with di-, tri- and tetra-nucleotide repeats (2.3%) were identified. Sixty-five of these 207

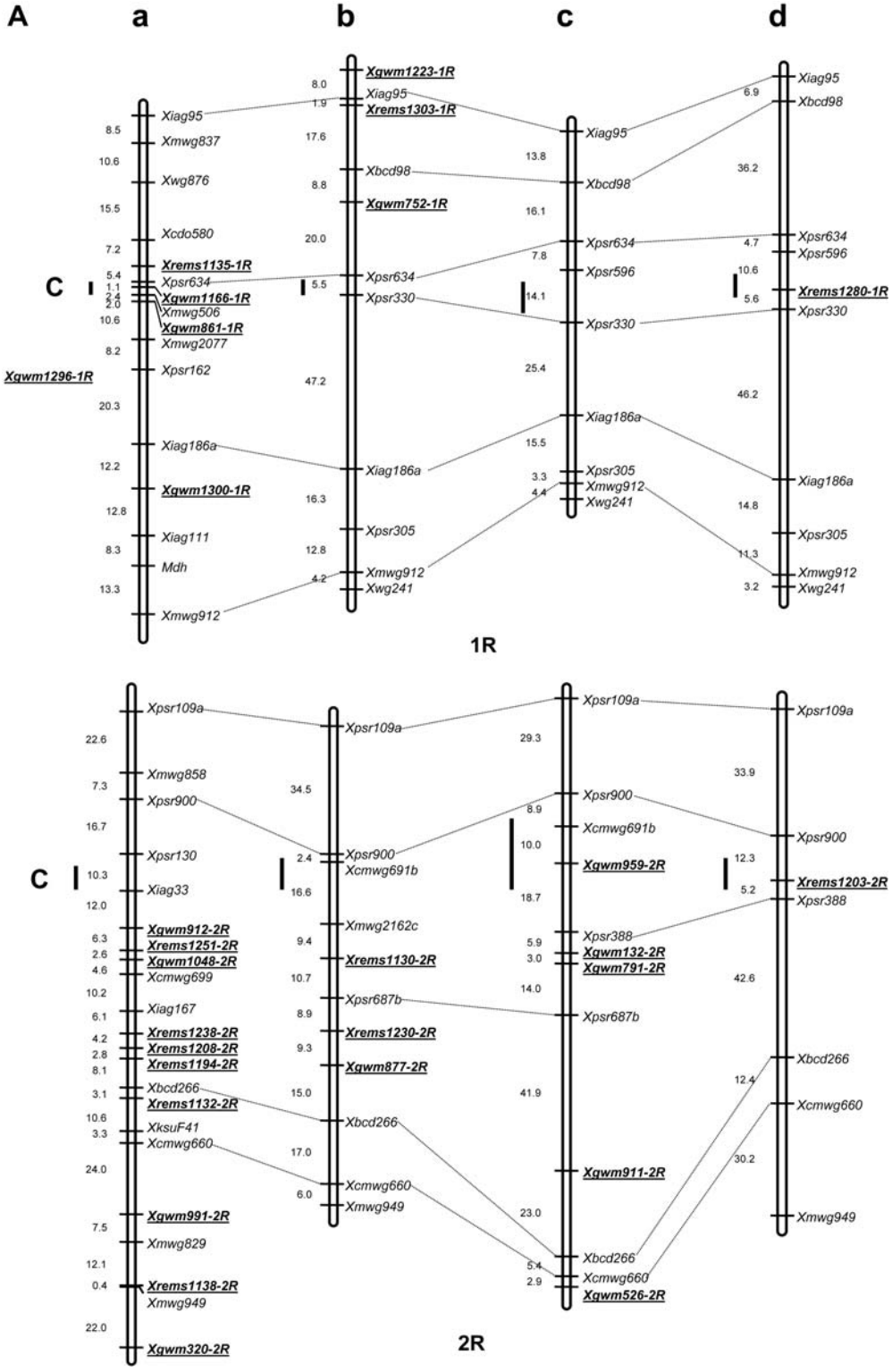
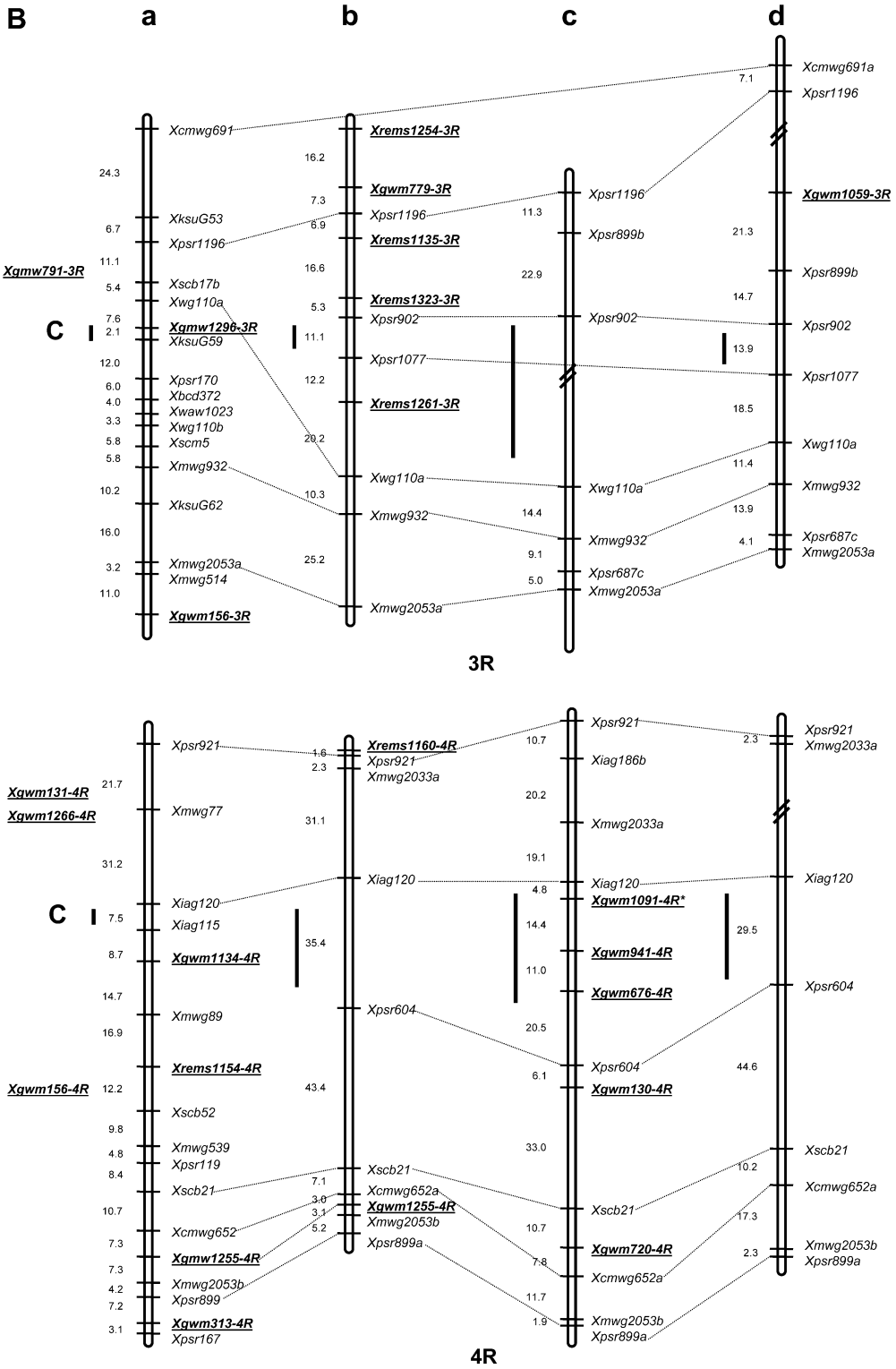


Fig. 1 Linkage map of rye based on the data from the crosses P87×P105 (*a*) (Korzun et al. 2001), N6×N2 (*b*), N7×N2 (*c*) and N7×N6 (*d*). The new microsatellite markers mapped in this study are *underlined*. Four loci mapped with a LOD score of less than 3.0 are indicated with an *asterisk*. *Xgwm* markers with homoeologous loci in wheat are marked with a *cross*. The centromeres (*C*) are indicated based on the data of Devos et al. (1993) and Korzun et al. (2001); the short arms of the chromosomes are at the *top* and the long arms at the *bottom*. *Xgwm* loci assigned to chromosome arms according to wheat-rye arm addition lines analysis are indicated on the *left*. Sequence information of the microsatellites can be obtained from the authors upon request

designed primer pairs (31.4%) amplified good-quality PCR products in the rye genome, ranging from quality scores of one to four according to the scale of Stephenson et al. (1998), with 36 (55.4%) amplifying 39 loci polymorphic at least in one of the four mapping populations. Table 1 shows the GeneBank numbers, repeat types and lengths, expected sizes (in basepairs) and numbers of detected alleles for markers mapped on chromosomes 5R and 7R as examples. Information for all other loci can be obtained from the authors upon request. Of the 65 REMS, 45 (69.2%) that amplified good PCR products in rye also amplified proper fragments in bread wheat (Chinese Spring).

Fig. 1 (continued)

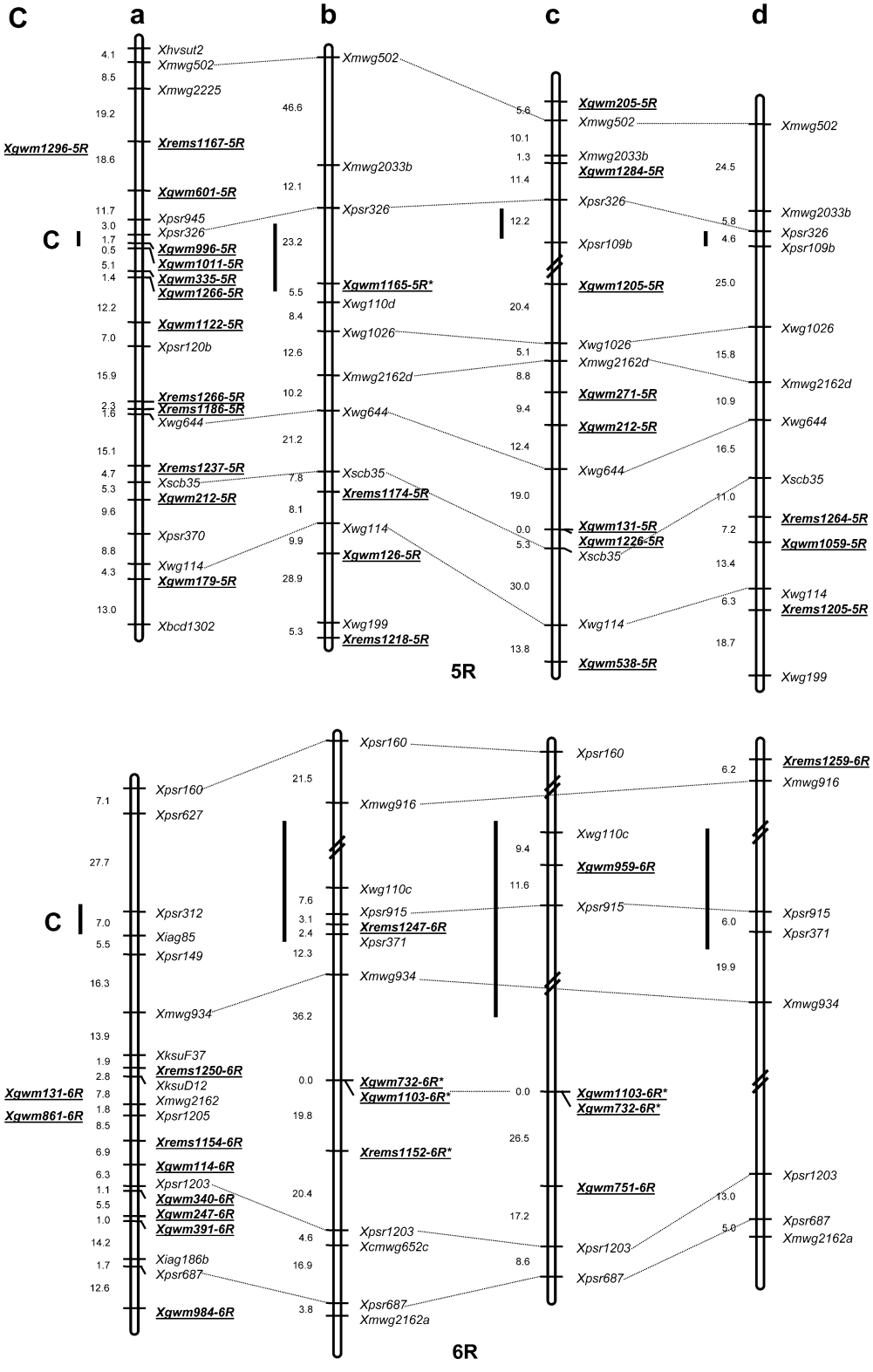


Of the 651 WMS tested, 81 primer pairs (12.4%) amplified good-quality PCR products in the rye genome, again ranging from 1 to 4 (Stephenson et al. 1998), with 56 amplifying 70 loci either polymorphic in one of the four mapping populations or located on a particular chromosome using the set of wheat-rye addition lines.

Microsatellite mapping/chromosome arm location

In total, 99 microsatellite-derived loci (39 *Xrems* and 60 *Xgwm*) were integrated into the RFLP frameworks of four mapping populations, seven of which had a LOD score of less than 3.0 (Fig. 1). The linkage maps we constructed

Fig. 1 (continued)



include the microsatellite loci covered lengths of 1,111 cM, 1,087 cM, 1,109 cM and 1,111 cM for populations P87×P105, N6×N2, N7×N2 and N7×N6, respectively. The total numbers of markers mapped for each chromosome were 8 (1R), 19 (2R), 8 (3R), 10 (4R), 26 (5R), 14 (6R) and 14 (7R).

In the five rye lines investigated here, the number of alleles detected by the mapped *Xrems* loci in each line ranged from two to five, with an average of 2.62 alleles per locus; the number of alleles detected by the mapped *Xgwm* loci in each line ranged from two to four alleles per locus with an average of 2.76 alleles per locus. Ten *Xgwm* loci were assigned to arms of chromosomes 1R (1), 3R (1), 4R (3), 5R (1), 6R (2) and 7R (2) using the set of wheat-rye (arm) addition lines (Fig. 1). On average, 15 new microsatellite-derived loci were mapped/located on each of the seven chromosomes of rye. Both the *Xrems* and *Xgwm* loci were evenly distributed on the chromosomes.

Discussion

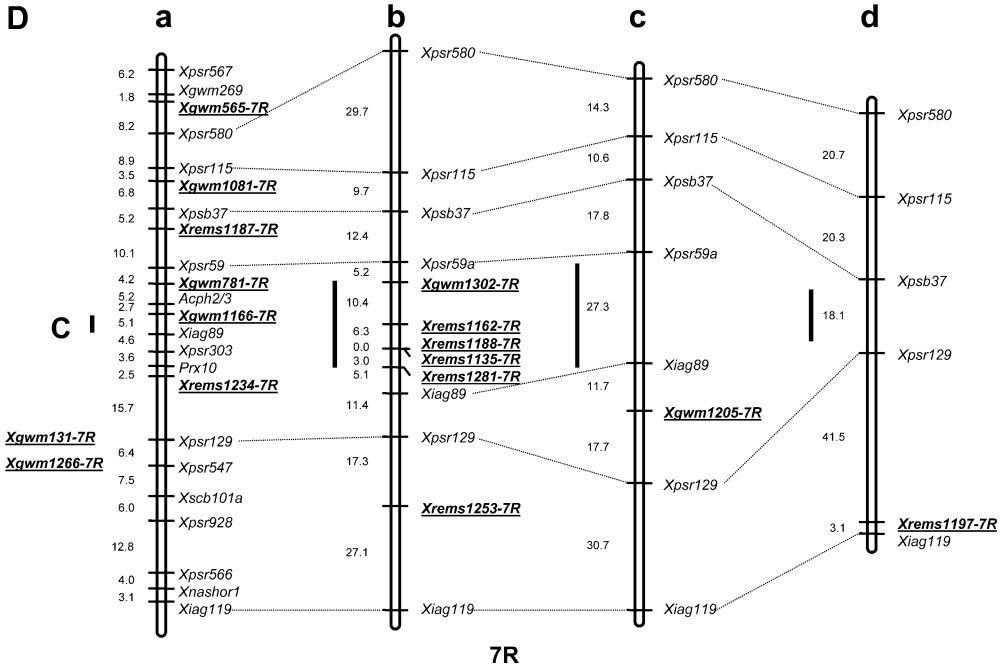
During the last 2 years ESTs have become widely used for developing new microsatellite markers in plants, including the cereals (Kota et al. 2001; Eujayl et al. 2002; Thiel et al. 2003). Varshney et al. (2002) estimated that the potential of cereal EST databases for the development of new microsatellite markers could be as high as 3% of the total ESTs. Hackauf and Wehling (2002b) assessed it to about 2% for rye ESTs. This was verified in the present study, and we were able to confirm that rye ESTs are a promising source of new microsatellite markers for rye.

Henry et al. (2000 reported that the exploitation of microsatellites across the cereal genera is more limited than that among other plant species. These authors also suggested that EST-derived microsatellites are better transferable between species than microsatellites derived from genomic libraries. This was supported by studies performed by Kantety et al. (2002), who identified a high number of cross-species matches among SSR-ESTs of cereals. The present study confirmed that microsatellites reveal respectable intergeneric transferability. Nearly 70% of the EST-derived SSRs amplifying good-quality PCR products in rye also amplified proper fragments in bread wheat and, therefore, can be assumed to be a potential source for mapping homoeologous loci in wheat. The transferability rate of rye EST-SSRs to wheat is comparable to that determined in earlier studies in cereals (Holton et al. 2002; Gao et al. 2003; Gupta et al. 2003).

For wheat more than 1,000 Gatersleben wheat microsatellite (GWM) markers have been developed at IPK Gatersleben or TraitGenetics GmbH, Gatersleben, of which about 650 have been screened for their usability for rye mapping, regardless of whether they represent microsatellites or sequence-tagged site (STS) markers. In a comparison of the locations of *Xgwm* loci amplified by the same primer pairs in wheat (Röder et al. 1998; M.S. Röder et al., unpublished data) and rye (present study) genomes, 44 of the 60 *Xgwm* loci mapped in orthologous positions considering multiple evolutionary translocations in the rye genome relative to those of hexaploid wheat and the other Triticeae species, as has been described in detail by Devos et al. (1993). The identification of cross-amplifying microsatellite (STS) loci is important for their further application in comparative studies among wheat and rye or even other cereals.

To date, genetic mapping in rye has been mainly performed using RFLP markers. Several genes determining reduced plant height, vernalization response, self-incompatibility and a range of morphological mutants have been mapped successfully (Plaschke et al. 1993, 1995b; Korzun et al. 1996, 1997; Senft and Wricke 1996;

Fig. 1 (continued)



Voylokov et al. 1998). Due to the fact that most RFLP markers allow cross-hybridisation the data obtained have been highly comparable to mapping data in wheat or barley. However, relative to other marker techniques, the disadvantages of RFLP markers include the higher amount of genomic DNA required, the low throughput and the need of isotopes.

In contrast, microsatellite markers are much more efficient and easier to apply. In addition to gene tagging they can be used to study genetic diversity, analysing high numbers of genotypes, as has been demonstrated for wheat by Huang et al. (2002) and Röder et al. (2002), who analysed about 1,000 genebank accessions and 500 European wheat varieties, respectively. The utilisation of rye microsatellites for studying the genetic integrity of genebank accessions of rye was demonstrated by Chebotar et al. (2003).

The availability of the 99 randomly distributed microsatellite-derived loci described in the present paper will enable rye geneticists to obtain more information on the genetic diversity present in existing rye collections but also to map genes of interest more quickly. The tagging of "rye-specific loci" determining adaptability to poor soil conditions or frost tolerance, which will be not detectable in other Triticeae species, will be of special interest in the future.

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