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Extending a RFLP-based genetic map of rye using random amplified polymorphic DNA (RAPD) and isozyme markers

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Abstract RFLP-based genetic map of rye, developed previously using a cross of lines DS2×RXL10 (F_2 generation), was extended with 69 RAPD and 12 isozyme markers. The actual map contains 282 markers dispersed on all seven chromosomes and spans a distance of 1,140 cM. The efficiency of mapping RAPD markers was close to ten loci per 100-screened arbitrary primers. A strong selection of polymorphic, intensive and reproducible fragments was necessary to reveal individual marker loci that could be assigned to rye chromosomes. Newly mapped markers cover a substantial part of the rye genome and constitute a valuable tool suitable for map saturation, marker-aided selection and phenetic studies. A specific nomenclature for the RAPD loci mapped on individual rye chromosomes, which could be helpful in managing of accumulating data, is proposed.

Keywords Genetic map · *Secale cereale* L. · RAPD · Isozymes · RFLP

Introduction

Since 1990, random amplified polymorphic DNA (RAPD) markers have been successfully applied for identification of DNA polymorphism in various plant species (Williams et al. 1990). They are often used for screening of a wide range of genetic stocks in order to find linkage with traits of agronomic significance (Kelly 1995).

Suitability of RAPD markers for the construction of genetic maps, fingerprinting and phenetic studies has been proved by many authors (Newbury and Ford-Lloyd 1993; Lodhi et al. 1995; Yang and Quiros 1995; Nilsson et al. 1997; Divaret et al. 1999). This PCR-based molecular marker method is straightforward, does not require prior knowledge on DNA sequence and can be carried out using as little as 1 ng/µl of template DNA isolated from plant tissue according to simplified procedures (Caetano-Anolles 1994; Thomson and Henry 1995). The efficiency of polymorphism-detection by RAPD markers is decreased by the necessity of selecting primers that produce strong and reproducible amplicons (Senft and Wricke 1996). It is also necessary to optimise and then to unify analysis conditions in respect to the DNA concentration, temperature and time profile of PCR, and the source and concentration of *Taq* polymerase and $MgCl_2$ (Devos and Gale 1992; Staub et al. 1996).

So-far reports regarding the application of RAPD markers in studies on polymorphism in rye (*Secale cereale* L.) are contradictory. Koebner and Martin (1994) found it difficult to determine the chromosomal assignment of individual DNA fragments, which suggested a multilocus origin of the RAPD bands. Other authors presented examples of the high efficiency of RAPD markers in revealing polymorphism and in the fingerprinting of genetic stocks (Iqbal and Rayburn 1994; Gallego et al. 1998).

As compared to other marker systems, RAPDs have not been widely used in producing rye linkage maps (Schlegel et al. 1998). Genetic maps of the rye genome have been preferentially constructed using RFLP markers (Devos et al. 1993; Senft and Wricke 1996; Korzun et al. 1998). Two independent attempts to include RAPD markers into the framework of a RFLP-based maps suggest low efficiency (Loarce et al. 1996; Senft and Wricke 1996). However, for the purpose of extending the existing maps, RAPD markers still offer a choice (Beaumont et al. 1996; Nilsson et al. 1997), especially if they can be shown to be a potential markers for traits like cytoplasmic male sterility (Börner et al. 1998), aluminium tolerance (Gallego et al. 1998) and sprouting resistance (Masojć et al. 1998).

The objective of the present study was to saturate a RFLP map of rye, developed by Devos et al. (1993), with RAPD and isozyme markers.

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Table 1 Sequences of arbitrary primers for rye genome mapping (APR) and chromosomal location of the RAPD marker loci

Marker symbol (primer code)	Primer sequence 5´–3´	Fragment length bp	Chromosome location	Distance from centromere cM
APR1.1	GACTACGGGG	1,600	1RL	7.7
APR1.2 (OPF05)	CCGAATTCCC	1,400	1RS	10.2
APR1.3	ACGCCCAGAC	520	1RS	6.8
APR1.4	ATGGATCCGC	550	1RL	39.5
APR1.5	GCACGTAGAT	950	1RS	52.9
APR1.6	ACTCACTACA	850	1RS	8.9
APR1.7	ACTCACTACA	1,030	1RL	1.0
APR2.1	TGTCCAGCTT	1,200	2RL	68.0
APR2.2 (CS1519)	TCGCCCCATT	900	2RS	14.1
APR2.3	AGTTCGTCTG	1,000	2RS	12.5
APR2.4 (OPB08)	GTCCACACGG	400	2RL	33.7
APR2.5 (OPC09)	CTCACCGTCC	750	2RS	72.6
APR2.6 (OPE14)	TGCGGCTGAG	650	2R	0.0
APR2.7	GCAACTACGT	1,200	2RS	18.2
APR2.8	CTCGAGGTAA	1,030	2RS	21.7
APR2.9 (OPF02)	GAGGATCCCT	570	2RL	2.6
APR2.10	TCCGACAAGA	450	2RS	59.0
APR2.11	CACCATCCAA	840	2RS	14.1
APR2.12 (OPC09)	CTCACCGTCC	1,100	2RL	9.6
APR3.1	CCCTACCGAC	550	3RS	12.2
APR3.2	AACGCGTTCT	2,000	3RS	6.8
APR3.3	CGTACGGATA	2,000	3RL	22.2
APR3.4 (OPF12)	ACGGTACCAG	850	3RL	73.6
APR3.5 (OPH01)	GGTCGGAGAA	530	3RS	61.6
APR3.6	ATGGATCCGC	870	3RS	26.3
APR3.7	CAAACGTCGC	700	3RS	26.0
APR4.1	CGTCGTGGAA	900	4RL	30.4
APR4.2	CCTTGCAACT	850	4RL	30.0
APR4.3	TCAGCCCCTG	800	4RS	7.3
APR4.4	GCACGTAGAT	500	4RL	9.3
APR4.5 (OPE20)	AACGGTGACC	750	4RL	14.5
APR4.6 (OPE20)	AACGGTGACC	1,600	4RL	76.2
APR4.7 (OPF05)	CCGAATTCCC	1,100	4RS	12.7
APR4.8	GTGATCGCAG	530	4RL	59.7
APR4.9 (OPF20)	GGTCTAGAGG	910	4RS	20.0
APR4.10 (OPF20)	GGTCTAGAGG	800	4RL	25.3
APR4.11 (CS1514)	ATGACGTTGA	830	4RS	10.3
APR4.12 (OPB04)	GGACTGGAGT	900	4RL	111.3
APR5.1	CCACTGTTAG	350	5RS	11.7
APR5.2	AGAGATCTCC	430	5RL	123.4
APR5.3 (OPE09)	CTTCACCCGA	350	5RS	56.1
APR5.4 (OPE09)	CTTCACCCGA	550	5RS	39.7
APR5.5	AACGCGTTCT	460	5RL	44.8
APR5.6	GACTACGGGG	500	5RS	3.8
APR5.7 (OPH16)	TCTCAGCTGG	650	5RS	11.7
APR5.8	GGGCCACGCT	840	5RL	24.7
APR5.9	AGAATCGGGG	520	5RS	3.8
APR6.1 (OPC07)	GTCCCGACGA	750	6RL	23.6
APR6.2	TCCGCGGTCT	750	6RS	1.0
APR6.3	AGGGTGTACG	350	6RL	128.6
APR6.4 (OPA10)	GTGATCGCAG	1,200	6RL	44.8
APR6.5 (OPG03)	GAGCCCTCCA	680	6RS	4.0
APR6.6 (OPE07)	AGATGCAGCC	580	6RL	136.6
APR6.7	GGTCTAGAGG	510	6RL	60.8
APR6.8	ACGATGAGCT	590	6RS	76.0
APR6.9	GTGATCGCTG	460	6RL	142.0
APR7.1	TGTCCAGCTT	400	7RS	46.4
APR7.2 (OPC07)	GTCCCGACGA	350	7RS	3.6
APR7.3	TGCCGCTAAG	900	7RL	79.7
APR7.4	TCGCGCTGTC	700	7RL	7.0
APR7.5	GTGTACGGAT	800	7RL	36.5
APR7.6 (OPA10)	GTGATCGCAG	330	7RL	3.5
APR7.7 (OPG11)	TGCCCGTCGT	750	7RL	28.8
APR7.8 (OPG14)	GGATGAGACC	660	7RL	8.9
APR7.9 (CS1510)	TCCCGAACCG	830	7RL	24.8
APR7.10	GGGCCACGCT	1,400	7RL	79.7
APR7.11	ACGCCCAGGG	1,350	7RS	70.2
APR7.12	ACGCCCAGGG	500	7RL	3.3
APR7.13 (OPP01)	CCTCTGCCCA	1,100	7RL	3.3

Table 2 Loci encoding isozymes and proteins mapped on rye chromosomes

Locus	Electrophoretic technique	Allele identification pI (IEF), Rf (PAGE)	Chromosomal location	Distance from centromere cM
Sod2	IEF	4.0/null	2RS	11.0
	PAGE	0.55/0.64		
Est6	IEF	9.8/10.0	2R	0.0
Est2	IEF	8.0/null	3RL	2.6
Isa1	IEF	7.0/6.7	2RL	3.8
Ia1	IEF	5.0/6.0	3RS	9.0
Aat4	PAGE	0.15/0.21	3RS	4.6
Aat2	PAGE	0.64/null	7RL	1.2
Per4	IEF	8.4/8.0	3RL	4.0
Per5	IEF	9.7/null	7RS	1.6
Wsp1	IEF	4.6/4.8	2RL	7.2
Ssp1	PAGE	0.79/null	4RS	63.5
Ssp3	PAGE	0.48/null	5RL	51.9

Materials and methods

Plant material

Genetic mapping was carried out using a population of 99 F_5 deriviatives of F_2 plants from the original mapping population (Devos et al. 1993). They were all a progeny of the cross between inbred rye lines DS2×RXL10. Each F_2 plant was represented by 8–10 lines, that were propagated in isolation.

RAPD markers

DNA was extracted from 2-mm^2 leaf discs using the single-step method described by Thomson and Henry (1995). The PCR mixture (25 µl) contained 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 100 µM of each dNTP, 2,5 µg of BSA, 1.5 U of *Taq* polymerase (MBI Fermentas), 35 ng of 10-mer primer (Ransom Hill Bioscience, Inc., Genset S.A.) and 2 ng of DNA (1 µl of crude template).

Following an initial incubation at 94°C for 1 min, 45 PCR cycles were performed. The profile of the first 10 cycles was 94°C for 5 s, 37°C for 30 s, and 72°C for 30 s. The remaining 35 cycles were carried out at 94°C for 5 s, 37°C for 30 s and 72°C for 60 s. All PCRs were performed in a PTC-200 DNA Engine (MJ Research, Inc.).

Amplification products were separated by electrophoresis in TBE buffer (0.45 M TRIS-borate, 0.01 M EDTA pH 8.0) in 1.5% agarose gels containing 0.5 mg/l of ethidium bromide. Electrophoresis was carried out at 100 V for 2.5 h in a Sub Cell Model 96 System (Bio-Rad) at 5°C. Amplification products were visualised and documented by UV transillumination and a Polaroid DS-34 camera.

Protein markers

Four enzyme systems, i.e. superoxide dismutase (SOD), esterase (EST), aspartate aminotransferase (AAT or GOT) and peroxidase (PER), two alpha-amylase inhibitors (ISA1 and IA1) together with water-soluble (WSP) and salt-soluble (SSP) proteins were separated by means of isoelectric focusing (IEF) and/or polyacrylamide gel-electrophoresis (PAGE), as described by Milczarski et al. (1996).

Segregation analysis

The segregation data were analysed by the software package MAPMAKER v.3.0b, supplied by E.S. Lander (Lander et al. 1987). The Kosambi function was used to convert recombination values to genetic distances (cM). *Default linkage criteria* were set at LOD 3.0. and a 50-cM distance, and the *error detection* function was always on. The RFLP segregation and mapping data, containing published loci (Devos et al. 1993) and 47 additional markers, were kindly provided by Dr. K. Devos (John Innes Centre, Norwich, UK).

Results

Among 700 10-mer primers, each having a different arbitrary sequence, 56 produced fragments showing single-locus segregation in the DS2×RXL10 F_2 intercross. Usually, one polymorphic locus per primer was found; however, segregation of two or three fragments was also observed. Segregation for all RAPD loci was consistent with a 3:1 ratio, expected for dominant markers. Consequently, the positions of 69 RAPD loci dispersed over all seven chromosomes were determined (Table 1, Fig. 1).

In an attempt to build up the RAPD marker nomenclature system for each rye chromosome, a marker symbol was given the letters *APR* (arbitrary primers for rye), followed by the number of the chromosome to which it belongs and an identification number reflecting the order of its integration into the map. From seven (1R, 3R) to 13 (7R) new marker loci were assigned to each of the seven rye chromosomes.

The distribution of RAPD markers along the chromosome maps was generally random, with no effects of clustering in the centromeric regions, which was typical for RFLP loci in the Devos et al. (1993) map. Groups consisting of 3–5 linked RAPD markers were mapped on the proximal parts of the 1RS-5RS and 7RL chromosome arms. Except for chromosome 1R and 4RS, all chromosome arms had RAPD loci on their distal ends.

Both polyacrylamide gel-electrophoresis (PAGE) and isoelectric focusing (IEF) proved to be useful in revealing polymorphic loci that encode isozymes or proteins (Table 2). They allowed the localisation of an additional 12 isozyme and protein markers on five rye chromosomes (except 1R and 6R). Seven markers (*Sod2, Est6, Est2, Isa1, Ia1, Aat4* and *Wsp1*) segregated in accordance with the 1:2:1 ratio characteristic for codominant alleles. The remaining five markers exhibited recessive *null* alleles and a 3:1 phenotypic ratio. In cases when the electrophoresis was carried out on both IEF and PAGE gels, segregation ratios within the same loci were scored

and the one containing most information (usually co-dominant) was finally applied for mapping. Distorted segregation was found for the *Aat2* (7RL) and *Est2* (3RL) loci.

Altogether, 81 (69 RAPD and 12 isozyme and protein) markers, that were linked to the previously (Devos et al. 1993) located RFLP loci, extended the map of the rye genome up to 1,140 cM. This was mainly caused by an addition of loci to the distal parts of chromosome arms (24%) but also by the integration of new loci within the existing framework of RFLP markers (9%). Consequently, individual chromosome length approached 140 cM for 1R-3R, 150 cM for 7R, 180 cM for 4R and 5R, and 218 cM for 6R.

The presented map contains 282 marker loci with a mean number of 40 markers per chromosome, from 31 on 6R to 50 on 1R. The average density of the extended map is close to 4.0 cM, varying from 2.8 (1R) to 7.0 (6R) in individual chromosomes. The map distances between the closest loci often do not exceed 5 cM, and generally are below 15 cM. However, there are still few fragments of low density on chromosomes 1RS (25.7 cM), 3RL (25.9 cM), 4RS (29.9 cM) and 6RS (34.6 cM).

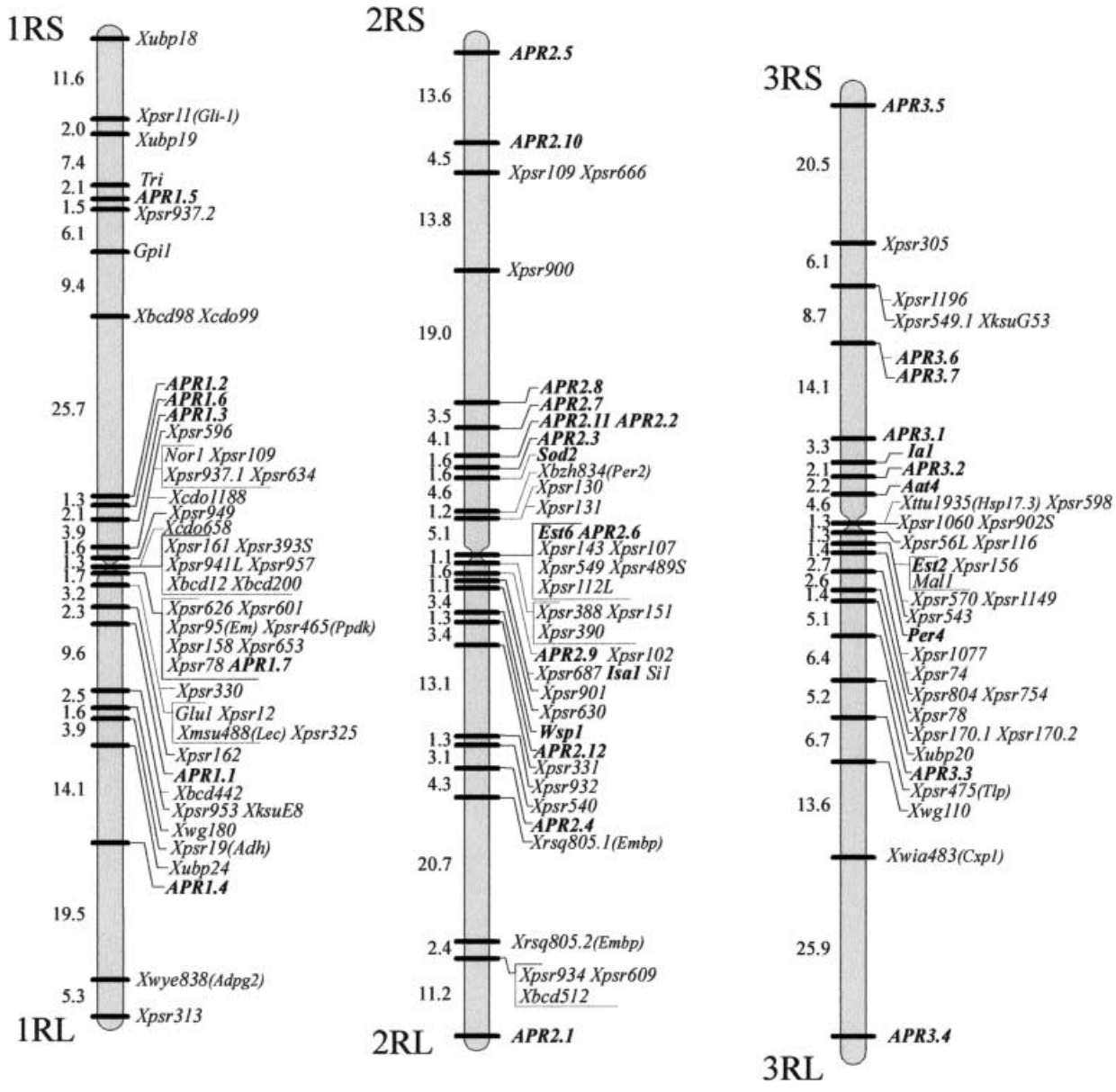

Fig. 1 Genetic map of the rye genome, containing RFLP, RAPD and isozyme markers. Newly mapped RAPD (*APR*) and isozyme markers are in bold. Map distances given on the left are in cM. Probes for RFLP loci other than *Xpsr* (JIC) were supplied by: P. Spagnoletti (*Xubp*), M.E. Sorrels (*Xcdo*, *Xbcd*, *Xwg*), B.S.Gill (*Xksu*), H. Nguyen (*Xttu*, *Xwia*), Pioneer Hi-Bred International (*Xnpi*, *Xphp*), E.H. Coe (*Xumc*), B. Burr (*Xbnl*), B.G.Lane (*Xbgl*), A. Kleinhofs (*Xak466*), J. Forster (*Xabl*), P. Jourdier (*Xmtd862*), C.C. Ainsworth (*Xwye1958*), R. Dudler (*Xbzh834*) and R.S. Quatrano (*Xrsq806*)

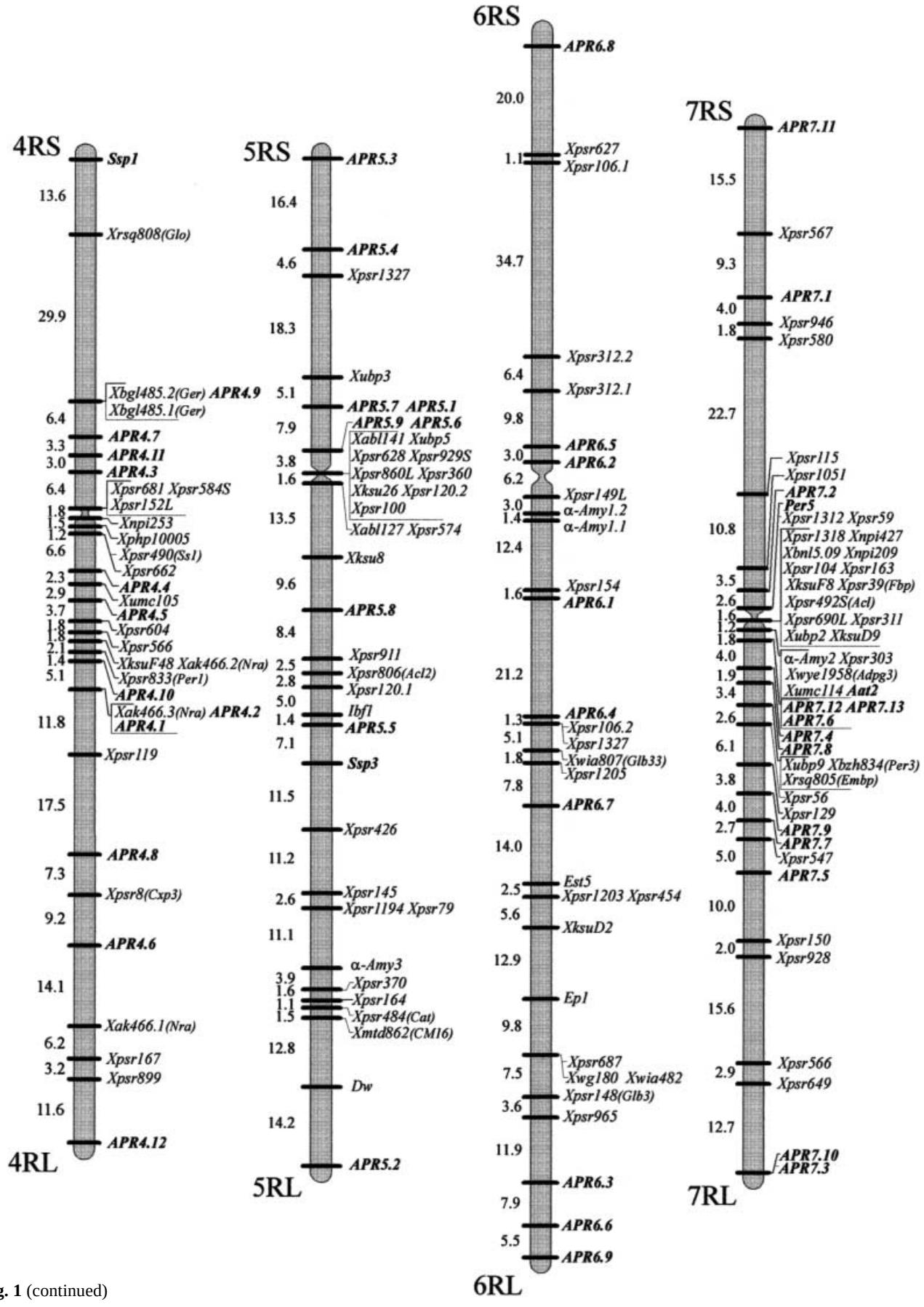

Fig. 1 (continued)

Discussion

RAPDs are among the most-widely used markers of economically important traits in cultivated plants (Mohan et al. 1997). They are also applied in plant genome mapping programmes (Yang and Quiros 1995; Beaumont et al. 1996; Nilsson et al. 1997). Although the number of RAPD loci that can be mapped is potentially unlimited, the efficiency of searching for strong and reproducible polymorphic fragments may appear rather low. Senft and Wricke (1996) screened 280 random primers detecting only 28 reliable and polymorphic loci. In a similar investigation, Loarce et al. (1996) found that 180 10-mers produced as little as 5.5% polymorphic fragments and, consequently, only 17 RAPD markers could be included in their map. Low efficiency of finding segregating, strong RAPD fragments was also experienced in this study where analysis of 700 primers yielded 69 polymorphic loci in spite of the fact that about 140 of these 10-mers revealed differences between the parental lines.

There were several reasons that the majority of fragments differentiating parental genotypes proved to be useless for mapping purposes. In most cases the poor reproducibility of fragments did not allow for the identification of genotypes in the segregating population. Also, some fragments were segregating other than in a 3:1 ratio, thus showing non-Mendelian inheritance. Finally, there were fragments showing expected segregation ratios but no linkage to any of the mapped loci was found.

The accumulating evidence on the necessity of selecting suitable primers out of a large number of 10-mers, separately for each studied species, shows the need of developing an information system on the sequences assigned to individual chromosomes. In an attempt to do so we started to built lists of RAPD markers linked to each rye chromosome. Until now the sets of RAPD markers identified using the DS2×RXL10 F_2 intercross contain from seven (1R, 3R) to 13 (7R) individual loci of known distance from the centromeres. There are also a number of Operon primers given by Loarce et al. (1996), Gallego et al. (1998) and Börner et al. (1998), that are known to be useful in rye map construction.

Interestingly only one common marker, *APR7.6*, was detected in two independent studies (Gallego et al. 1998 and this paper). This observation suggests that many of DNA fragments, segregating in one specific cross, might show a low level of polymorphism in wider plant material. Therefore, the presented list of RAPD markers for each rye chromosome should be enriched through the accumulation of new segregation data from other mapping populations. It will eventually contain enough markers to be useful in the identification of rye chromosomes or their fragments, and in marker-assisted selection carried out in any newly studied genetic stocks.

In spite of well-known drawbacks, RAPD markers seem to be a valuable tool in the construction of genetic maps. The main advantage of RAPDs, as was shown in this study and elsewhere (Senft and Wricke 1996), is apparently the random distribution of marker loci over the entire length of rye genome. This property is advantageous when a marker system for map saturation is sought. Usefulness of RAPD markers for extending RFLP maps was also demonstrated in maize (*Zea mays* L.) and sugar beet (*Beta vulgaris* L.) by Beaumont et al. (1996) and Nilsson et al. (1997), respectively.

Isozymes and proteins are usually of limited value for map construction due to their restricted number and low polymorphism level. This group of molecular markers should be considered as a comparatively inexpensive means of map saturation in specific regions. The present paper presents the location of several isozyme loci, which have already been mapped by other authors. The examples are *Est 6* from chromosome 2R, located near the centromere, and *Isa1*, an endogenous alpha-amylase and subtilisin inhibitor locus (Masojc and Gale 1990), found in the same position on chromosome 2RL as the homologous subtilisin inhibitor locus (*Si1*) previously mapped by Devos et al. (1993). Similar locations as presented here were also found for *Aat4* (*Got4*) on chromosome 3R and *Aat2* (*Got2*) on chromosome 7R (Senft and Wricke 1996). The map positions of *Sod2* (2RS), *Est2* and *Ia1* (3RS) loci are consistent with data presented by Schlegel et al. (1998). Homoeoloci for the exogenous alpha-amylase inhibitor (*Ia1*) were also located on the short arms of 3B and 3D in wheat (Sanches-Monge et al. 1986). Identified map positions of protein loci *Ssp1*, *Ssp3*, *Wsp1* and two peroxidase loci (*Per4*, *Per5*) were not found elsewhere.

So-far reported studies in rye, maize and sugar beet, show that it is possible to saturate RFLP-based maps with RAPD markers, although the efficiency of this method may be relatively low. On the other hand, a potentially unlimited number of loci per genome, which could be found in any region of interest and, if necessary, converted to more specific markers, suggest RAPDs as useful, alternative tools for developing genetic maps in plants.

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