Theor Appl Genet (1998) 96: 203–208

V. Korzun · S. Malyshev · N. Kartel
T. Westermann · W. E. Weber · A. Börner

A genetic linkage map of rye (*Secale cereale* L.)

Received: 5 August 1997 / Accepted: 2 September 1997

Abstract A genetic linkage map of rye composed of 91 loci (88 RFLP, two morphological and one isozyme markers) has been developed using two reciprocal crosses. The RFLP loci covering all seven chromosomes were detected by a selection of rye, wheat, barley and oat cDNA and genomic DNA probes. The level of polymorphism was dependent on the source of the clones, with a ranking of rye > wheat > barley > oat. Distorted segregations were detected in linkage groups of chromosomes 1R, 4R, 5R and 7R. When the recombination of the two reciprocal crosses was compared, no systematic increase or decrease in one or the other direction was observed suggesting that a combination of populations of reciprocal crosses is possible.

Key words Genetic mapping · Reciprocal crosses · RFLP · Rye · *Secale cereale* L.

Introduction

Because of its good adaptability to extreme climatic and soil conditions rye (*Secale cereale* L.) plays an important role in cereal production, mainly in the northern part of Europe. Rye is, however, also important as a genetical source for wheat improvement, and it is a constituent of triticale, a synthesized crop conceived with the aim to combine the nondemanding features of rye with the yield and quality characters of wheat.

As in many other cultivated crop species much effort is directed at the production of genetic maps of rye by employing molecular marker techniques. Restriction fragment length polymorphism (RFLP) or random amplified polymorphic DNA (RAPD) marker-based maps covering all seven rye chromosomes have been reported by Devos et al. (1993), Philipp et al. (1994) or Senft and Wricke (1996). Loarce et al. (1996) constructed a map containing six rye chromosomes (except 2R). RFLP markers have also been used for the tagging of genes affecting morphological traits and/or controlling plant development on single chromosomes; for example, the GA-insensitive dwarfing gene *ct2* and vernalisation response gene *Sp1* on chromosome 5R (Plaschke et al. 1993), the GA-insensitive dwarfing gene *ct1* on chromosome 7R (Plaschke et al. 1995), the GA-sensitive dominant dwarfing gene *Ddw1* and the hairy peduncle gene *Hp1* on chromosome 5R (Korzun et al. 1996a) or the mutant loci determining absence of ligules (*al*), waxless plant (*wa1*) and waxy endosperm (*Wx*) characters on chromosomes 2R, 7R and 4R, respectively (Korzun et al. 1997).

Not all of the published maps are comparable because different sets of markers were used. The maps presented here comprise a selection of 82 RFLP markers integrated in maps of rye (Devos et al. 1993; Philipp et al. 1994; Senft and Wricke 1996; Korzun et al. 1994, 1996b), wheat (Gale et al. 1995; Nelson et al. 1995), barley (Graner et al. 1991; Heun et al. 1991) or oat (O'Donoughue et al. 1995). The maps presented here originated from two reciprocal crosses and will help in merging mapping data of different marker sets and species in the future.

Communicated by G. Wenzel

V. Korzun · A. Börner (✉)
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany
Fax: 0049-39482-5155
E-mail: boerner@ipk-gatersleben.de

S. Malyshev · N. Kartel
Institute of Genetics and Cytology, 220074 Minsk, Belarus

T. Westermann · W. E. Weber
Institut für Pflanzenzüchtung und Pflanzenschutz, Martin-Luther-Universität Halle-Wittenberg, Berliner Strasse 2, D-06466 Gatersleben, Germany

Materials and methods

Plant materials, isozyme studies and DNA isolation

Two F_2 populations were created by reciprocally crossing the two rye inbred lines P87 and P105. Both lines were developed at the Institute of Genetics and Cytology, Minsk, Belarus, by Dr. T. S. Schilko. A selected inbred line (I_2) of the progeny of a cross between the varieties 'Polycrossnaja' × 'Bolgar dwarf' (*Ddw1*) was crossed with 'Litovskaja 3'. From the pedigree of that cross the two inbred lines P87 (I_6) and P105 (I_5) were selected. P105 carries the dwarfing and hairy peduncle genes *Ddw1* and *Hp1*, recently mapped on chromosome 5R by Korzun et al. (1996a). For further analysis 154 and 121 F_2 individuals of the P87 × P105 and P105 × P87 mapping populations, respectively, were available.

Isozyme (β-amylase) studies were performed as described for wheat by Liu (1991), using one half (endosperm end) of each individual F_2 dry grain.

DNA was extracted from fresh leaf material cut from F_2 seedlings of both mapping populations. The procedures for DNA extraction, restriction digesting, gel electrophoresis, Southern transfer, probe labeling and filter hybridization were performed as described by McCouch et al. (1988) and Devos et al. (1992). At flowering time the character hairy peduncle was scored. Final plant height was measured just before harvest.

DNA probes and statistical analysis

A selection of 139 cDNA and genomic DNA probes from various rye, wheat, barley and oat libraries developed at the Institute of Applied Genetics, University of Hannover, Germany (IAG clones), John Innes Centre, Norwich, UK (PSR, PSB clones), Institute for Resistance Genetics, Grünbach, Germany (MWG clones), Cornell University, Ithaca, USA (BCD, WG, CDO clones) or IPK Gatersleben, Germany (TRIA, SCB) were used. The probes were known to be distributed over all seven Triticeae chromosomes.

The genotypes were scored for the individual plants at each locus to construct linkage maps using the program MAPMAKER 2.0 supplied by E. S. Lander, Whitehead Institute of Biomedical Research, Cambridge, Massachusetts, USA. The maps were constructed for each population separately on the basis of 154 and 121 plants, respectively, and as joint maps including all 275 F_2 individuals of both crosses for all seven chromosomes. Recombination rates between reciprocal crosses were compared by *t*-test using standard errors for recombination rates estimated by the maximum likelihood (ML) method as described by Weber and Wricke (1994).

Results

Of the selected 139 RFLP probes 82 were polymorphic and detected 88 loci. The degrees of polymorphism are shown in Table 1. Although the number of clones used from different species varied, the percentage of polymorphic clones also varied, decreasing in the order rye (75%) > wheat (66%) > barley (44%) > oat (20%). Two loci were detected by the probes PSR109 (chromosomes 2R and 5R), PSR120 (chromosome 5R), MWG913 (chromosome 1R) and MWG2062 (chromosomes 1R and 5R), whereas WG110 detected 3 polymorphic loci on chromosomes 3R (2) and 7R (1). Comparing the two reciprocal combinations we found 4 markers to be polymorphic in only one cross. No polymorphism was detected for *Xpsr911* (chromosome 5R) and *Xwg110c* (chromosome 7R) in the P87 × P105 cross and for *Xpsr130* (chromosome 2R) and *Xpsr160* (chromosome 6R) in the P105 × P87.

Segregation data were analyzed for 88 RFLP markers, the isozyme marker (β-amylase) and the two morphological traits. Seventy-three loci (83%) and the β-amylase locus could be scored as co-dominant markers, whereas for 15 RFLP loci (17%) and the two morphological genes a dominant inheritance was observed. The number of markers per chromosome varies from a minimum of 5 (chromosome 3R) to a maximum of 24 (chromosome 5R). For chromosome 1R the maps generated from the two reciprocal crosses as well as the joint map including all F_2 individuals are given in Fig 1.

Distorted segregations significantly different from the expected 1:2:1 or 3:1 ratios (χ^2 test, $P > 0.05$) were observed for 11 RFLP markers and *Ddw1* in either one or the other of the two reciprocal crosses and for 1 RFLP marker and *β-Amy-R1* in both crosses (Table 2). For *Ddw1*, *β-Amy-R1* and 8 RFLP markers the distortion was also detected after the data of both combinations were summarized. Six of the loci formed two clusters close to the centromere on chromosome 4R.

For all chromosomes except 4R some recombination rates of reciprocals were significantly different at $P = 0.05$. The corresponding differences are boxed as are, those regions with a disturbed order of marker loci (Figs. 1, 2). The corresponding recombination rates and the *t*- and *P*-values of adjacent marker loci are given in Table 3. Small regions with a significantly disturbed order were found on chromosome 1R between *Xmwg913a* and *Xiag79*, on chromosome 3R between *Xwg110a* and *Xpsr1077* and on chromosome 5R at the short end.

Table 1 Total numbers and percentages of polymorphic clones

Species (Clones)	Number of selected clones	Number of polymorphic clones	Number of non-polymorphic clones	Percentage of polymorphic clones
Rye (IAG, SCB)	20	15	5	75
Wheat (PSR, WG, TRI)	71	47	24	66
Barley (MWG, BCD, PSB)	43	19	24	44
Oat (CDO)	5	1	4	20
Total	139	82	57	59

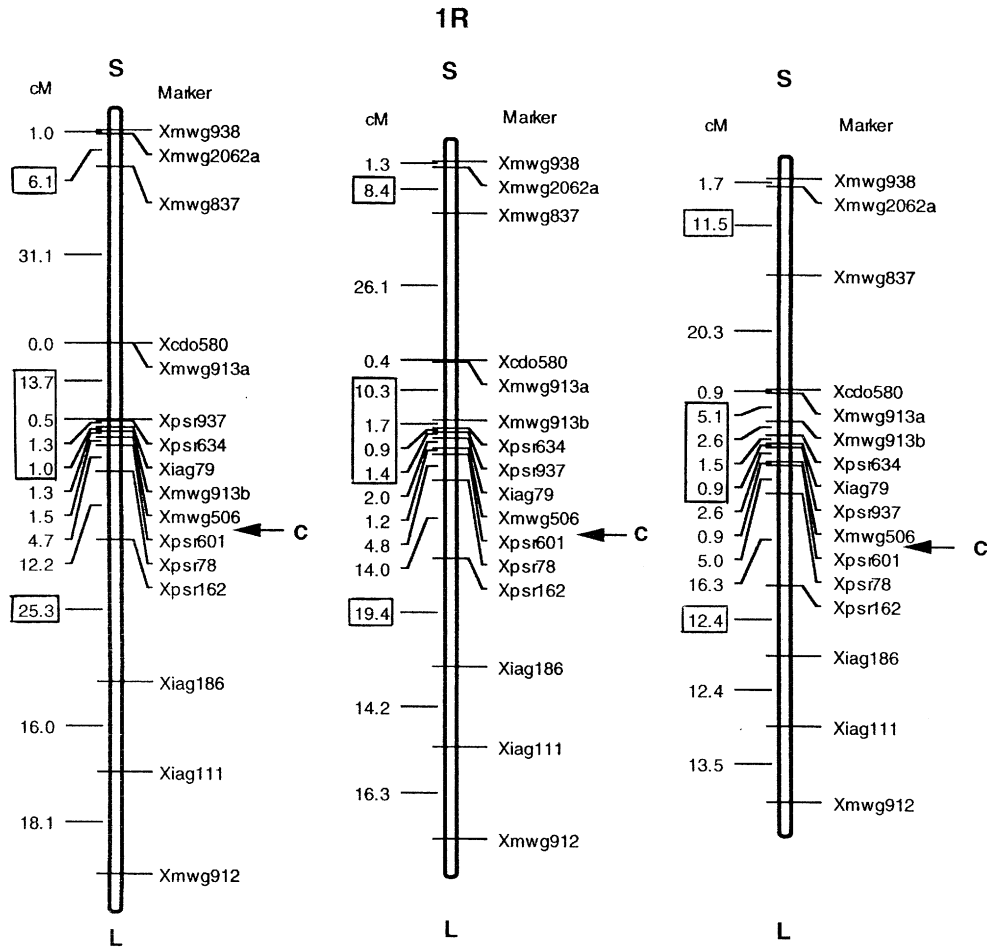

Fig. 1 RFLP linkage maps of chromosome 1R derived from the F_2 populations of the reciprocal crosses P87 × P105 (*left*) and P105 × P87 (*right*). The joint map is presented in the *middle*. Genetic distances are given in centiMorgans (cM). *c* Centromere, *S* short arm, *L* long arm

Table 2 Segregation values and χ^2 values for the loci with distorted segregation

Chromosome	Locus	Map								
		P105 × P87			P87 × P105			Joint map		
		Expected segregation	Observed segregation	χ^2 values[a]	Expected segregation	Observed segregation	χ^2 values	Expected segregation	Observed segregation	χ^2values
1R	*Xiag186*	3:1	114:40	0.08	3:1	69:35	**4.15***	3:1	183:75	2.28
4R	*Xiag120*	1:3	45:109	1.46	1:3	47:74	**12.37*****	1:3	92:183	**10.48****
4R	*Xpsr584*	1:3	42:112	0.42	1:3	43:76	**7.87****	1:3	85:188	**5.48***
4R	*Xpsr150*	1:3	39:113	0.04	1:3	44:76	**8.71****	1:3	83:189	**4.41***
4R	*Xiag115*	1:3	44:110	1.05	1:3	42:68	**10.19****	1:3	82:178	**5.93***
4R	*Xmwg530*	1:2:1	31:63:52	**7.02***	1:2:1	23:58:31	1.45	1:2:1	54:121:83	**6.69***
4R	*Xmwg539*	1:2:1	26:50:53	**13.54****	1:2:1	13:46:26	**6.83***	1:2:1	39:96:79	**14.88*****
5R	*Xpsr360*	1:2:1	20:80:35	**11.44****	1:2:1	18:57:27	3.84	1:2:1	38:137:62	**14.64*****
5R	*Xwg199*	1:2:1	39:59:49	**6.75***	1:2:1	27:44:29	1.02	1:2:1	66:103:78	**7.74***
5R	*Ddw1*	3:1	102:52	**6.31***	3:1	86:35	1.10	3:1	188:87	**6.46***
5R	*ß-Amy-R1*	1:2:1	37:49:53	**15.0*****	1:2:1	28:49:44	**7.18***	1:2:1	65:98:97	**21.01*****
7R	*Xpsr59*	1:2:1	44:74:36	0.98	1:2:1	25:73:22	**6.23***	1:2:1	69:147:58	2.58
7R	*Xpsr305*	1:2:1	31:74:35	0.74	1:2:1	18:65:21	**7.35***	1:2:1	49:139:56	5.47
7R	*Xiag119*	1:2:1	52:65:34	**6.02***	1:2:1	23:63:27	2.31	1:2:1	75:128:61	1.59

* $P \leqslant 0.05$, ** $P \leqslant 0.01$, *** $P \leqslant 0.001$

[a] Values in boldface are for loci showing distorted segregation

In general, maps for reciprocal crosses were in good agreement. The observed differences showed no systematic pattern. Recombination rates were not generally increased in one direction and reduced in the other. Therefore, the direction of the cross was not important, and both populations could be combined successfully to create joined maps for all seven chromosomes (Fig. 2).

Discussion

As expected the level of polymorphism in rye was relatively high, approximately 60% on average. Polymorphism was, however, influenced by the source of the clones. The same ranking for the amount of polymorphism described here was found by Loarce et al. (1996), with 66% for rye, 41% for wheat, 39% for barley and 32% for oat probes. When partial chromosomes were mapped using mainly wheat probes the level of polymorphism was 59% for chromosome 7R (Plaschke et al. 1995) or 72% for chromosome 5R (Korzun et al. 1996a), which is in accordance with the 66% obtained with wheat probes in the present study. In contrast to these data the degree of polymorphism described by Senft and Wricke (1996) was only 38% and 34% for the rye (IAG) and wheat (PSR) clones, respectively. The detection of 4 markers showing polymorphism only in one of the two reciprocal crosses is most probably due to the fact that the two parental inbred lines were still heterozygous for some loci.

Deviations from the expected Mendelian ratio were observed by several authors. Phillip et al. (1994) reported distorted segregations for chromosomes 1R and 6R, Wanous et al. (1995) for the chromosomes 1R, 6R and 7R, Plaschke et al. (1995) for chromosome 7R and Loarce et al. (1996) for chromosomes 5R and 6R. In the present study, however, in addition to chromosomes 1R, 5R and 7R chromosome 4R carries most of the loci with distorted segregation, resulting in two clusters of loci dividing chromosome 4R into three parts. It should be mentioned that a distorted segregation for

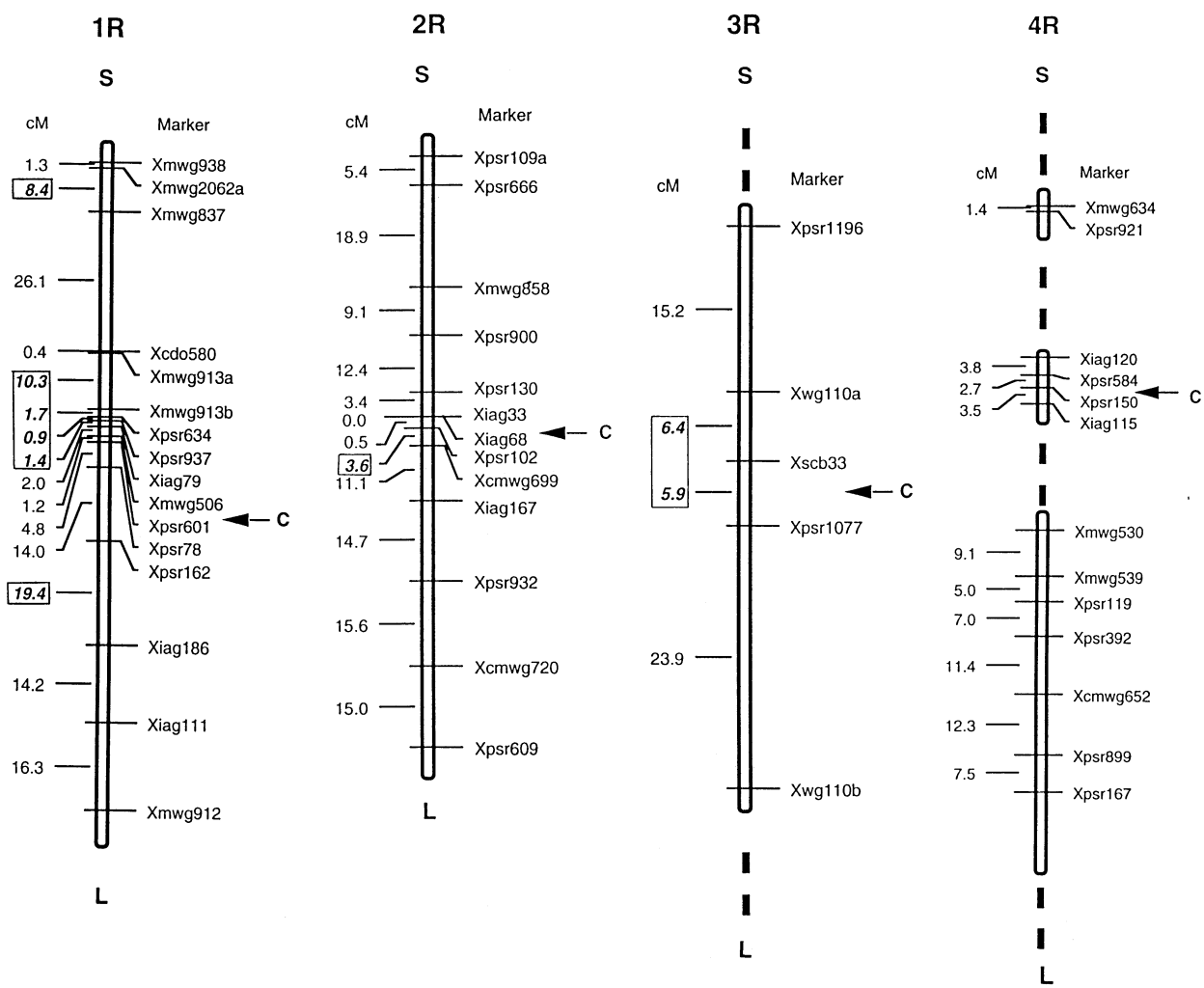

Fig. 2 See page 207 for legend

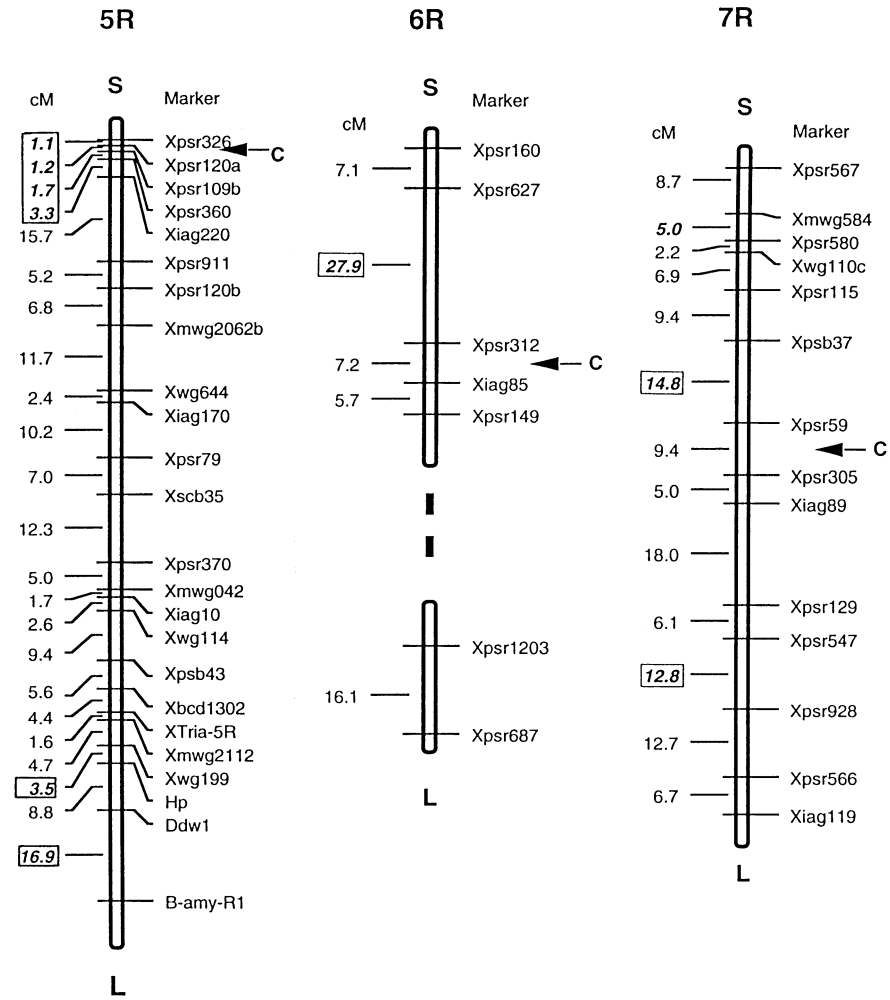

Fig. 2 Joint linkage maps of chromosomes 1R, 2R, 3R, 4R, 5R, 6R and 7R derived from two reciprocal crosses. Genetic distances are given in centiMorgans (cM). *c* Centromere, *S* short arm, *L* long arm

Table 3 Recombination rates of adjacent marker loci showing differences between reciprocal crosses

Chromosome	Marker 1	Marker 2	Recombination rate			
			P87 × P105	P105 × P87	*t*	*P*
1*R*	*Xmwg2062a*	*Xmwg837*	0.060	0.112	2.12	0.035
1R	*Xmwg913a*	*Xmwg913b*	0.135	0.053	2.99	0.002
1R	*Xpsr162*	*Xiag186*	0.242	0.114	2.50	0.012
2R	*Xpsr102*	*Xcmwg699*	0.015	0.054	2.54	0.011
3R	*Xwg110a*	*Xscb33*	0.024	0.108	2.81	0.005
3R	*Xscb33*	*Xpsr1077*	0.023	0.098	3.69	0.000
5R	*Xpsr326*	*Xpsr120a*	0.003	0.022	2.00	0.047
5R	*Xwg199*	*Ha1*	0.006	0.087	4.58	0.000
5R	*Ddw1*	*β-amy-R1*	0.213	0.129	2.16	0.031
6R	*Xpsr627*	*Xpsr312*	0.207	0.363	2.54	0.011
7R	*Xmwg584*	*Xpsr580*	0.074	0.022	2.70	0.007
7R	*Xpsb37*	*Xpsr59*	0.115	0.182	2.03	0.043
7R	*Xpsr547*	*Xpsr928*	0.186	0.054	4.24	0.000

a gene that restores male fertility and is located on the long arm of chromosome 4R was observed recently (Börner et al., unpublished data).

A comparison of our mapping data for the two morphological genes *Ddw1* and *Hp1* and the isozyme locus *β-Amy-R1* on chromosome 5RL (Fig. 2) to data already published by Korzun et al. (1996a) shows that although the distances between common markers are different the same order is present. The 3 loci in both maps are clearly located on the segment of chromosome 5RL which is homoeologous to other Triticeae 4L chromosomes.

Present knowledge of RFLP probes allowed cross hybridization within the small grain cereal genomes and resulted in detailed comparisons of maps of different species. It was shown that a substantial conservation in the order of DNA markers detected by the same probes is present for large segments of the cereal genomes (Moore et al. 1995; Van Deynze et al. 1995a, b). By using selected probes of already published maps of both 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 in the presented map potential users will be able to go back to those published maps to choose additional probes for saturating genome regions of their interest. A good example of this is Korzun et al. (1997) who mapped 3 mutant loci in rye. The authors used mapping data available for their genes of interest in wheat or barley and selected closely linked probes for mapping the homoeologous loci in rye. It is clear that the integration of mutant loci and molecular markers of several species will ultimately result in the construction of a grass genome consensus map, which will enable the consolidation of all the information available so far from genetical and molecular studies of separate species.

Acknowledgments We thank Drs. M. D. Gale, A. Graner, M. Sorrells and G. Wricke for providing the probes. V. Korzun and S. Malyshev thank INTAS Brussels (INTAS-93-355) for financial support.

References

Devos KM, Atkinson MD, Chinoy CN, Liu C, Gale MD (1992) RFLP-based genetic map of the homoelogous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp, China Agricultural Scientech Press, Beijing, pp 1333–1500

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Korzun V, Kartel N, Plaschke J, Börner A (1994) Construction and screening of a rye DNA library for RFLP mapping. Cereal Res Commun 22:151–157

Korzun V, Melz G, Börner A (1996a) RFLP mapping of the dwarfing (*Ddw1*) and hairy peduncle (*Hp*) genes on chromosome 5 of rye (*Secale cereale* L.). Theor Appl Genet 92:1073–1077

Korzun V, Balzer H-J, Balzer A, Bäumlein H, Börner A (1996b) Chromosomal location of three wheat sequences with homology to a pollen allergen encoding, DNA replication regulating and DNA (cytosine-5)-methyltransferase genes in wheat and rye. Genome 39:1213–1215

Korzun V, Malyshev S, Voylokov A, Börner A (1997) RFLP based mapping of three mutant loci in rye (*Secale cereale* L.) and their relation to homoeologous loci within the *Gramineae*. Theor Appl Genet 95:468–473

Liu CJ (1991) Biochemical marker genes in hexaploid wheat, *Triticum aestivum*. PhD thesis, University of Cambridge, Cambridge, UK

Loarce Y, Hueros G, Ferrer E (1996) A molecular linkage map of rye. Theor Appl Genet 93:1112–1118

McCouch SR, Kochet G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses, line up and form a circle. Curr Biol 5:737–739

Nelson JC, Sorrels ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721–731

O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Sorrells ME, Tanksley SD, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Autrique E, Van Deynze A (1995) A molecular linkage map of cultivated oat. Genome 38:368–380

Philipp U, Wehling P, Wricke G (1994) A linkage map of rye. Theor Appl Genet 88:243–248

Plaschke J, Börner A, Xie DX, Koebner RMD, Schlegel R, Gale MD (1993) RFLP-mapping of genes affecting plant height and growth habit in rye. Theor Appl Genet 85:1049–1054

Plaschke J, Korzun V, Koebner RMD, Börner A (1995) Mapping of the GA_3-insensitive dwarfing gene *ct1* on chromosome 7R in rye. Plant Breed 114:113–116

Senft P, Wricke G (1996) An extended genetic map of rye (*Secale cereale* L.). Plant Breed 115:508–510

Van Deynze AE, Nelson JC, Yglesias ES, Harrington SE, Braga DP, Mc Couch SR, Sorrells ME (1995a) Comparative mapping in grasses. Wheat relationships. Mol Gen Genet 248:744–754

Van Deynze AE, Nelson JC, O'Donoughue LS, Ahn SN, Siripoonwiwat W, Harrington SE, Yglesias ES, Braga DP, Mc Couch SR, Sorrells ME (1995b) Comparative mapping in grasses. Oat relationships. Mol Gen Genet 249:349–356

Wanous MK, Goicoechea PG, Gustafson JP (1995) RFLP maps of rye chromosomes 6R and 7R including terminal C-bands. Genome 38:999–1004

Weber WE, Wricke G (1994) Genetic markers in plant breeding. Adv Plant Breed 16:1–105