Theor Appl Genet (2001) 102:709–717 © Springer-Verlag 2001

V. Korzun · S. Malyshev · A.V. Voylokov · A. Börner

A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci

Received: 11 May 2000 / Accepted: 12 July 2000

Abstract Among the cereals, rye (*Secale cereale* L.) can be grown under extreme climatic and poor soil conditions and, is a major crop in North Europe. In the present paper, we report the development of a genetic linkage map of rye using a pooled F_2 mapping population created from a reciprocal cross of two self-fertile inbred lines. The 183 mapped markers consist 139 RFLPs, 19 isozyme and protein markers, 13 microsatellites, 10 known function sequences and two morphological genes. The markers are randomly distributed on the seven chromosomes with a maximum of 38 on chromosome 5R and a minimum of 19 on chromosome 3R. In addition, 23 gene loci and 25 quantitative trait loci were aligned to chromosome regions. For some of the mapped or aligned genes comparable loci are present in other cereals. The homoeologous relationships of these loci are discussed. The potential of the new map for further genetic studies is outlined.

Keywords Genetic mapping · Isozymes · Microsatellites · RFLP · Rye ·*Secale cereale* L.

Introduction

On a global scale rye (*Secale cereale* L.) is a minor crop, its production being about 5% that of wheat or rice. However, in northern European countries with extreme climatic and poor soil conditions, rye may occupy up to 30% of the acreage (Madej 1996). The main advantages of rye over other winter cereals are its excellent tolerance to low temperatures and the ability to realize relatively high grain yields under environmental conditions in which other crops perform poorly. Rye is also known to have the lowest requirements for chemical treatments like fertilizers or pesticides, which makes it an ecologically and economically sound crop for specific regions.

During the last few years molecular maps based mainly on restriction fragment length polymorphism (RFLP) markers have been published by several authors (Devos et al. 1993; Philipp et al. 1994; Korzun et al. 1994, 1996a, b; Senft and Wricke 1996; Loarce et al. 1996). DNA markers also have been used to tag several genes determining morphological and/or agronomically important traits. Korzun et al. (1998) published a map comprising 88 RFLP, one isozyme and two morphological markers integrating probes from other maps of rye (Devos et al. 1993; Philipp et al. 1994; Senft and Wricke 1996; Korzun et al. 1994; Korzun et al. 1996a, b), 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). A modified version of that map was used by Börner et al. (2000) for quantitative trait locus (QTL) mapping.

In the present paper, the original map is extended by additional 51 RFLPs, 18 isozyme and protein markers and 13 microsatellite markers.

Communicated by B.S. Gill

V. Korzun · A. Börner (✉)
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK),
D-06466 Gatersleben, Germany
Fax: +49-39482-5155
e-mail: boerner@ipk-gatersleben.de

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

A.V. Voylokov
St. Petersburg State University, 199034 St. Petersburg, Russia

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

Materials and methods

Plant materials and DNA isolation

A pooled F_2 population consisting of 275 individuals was created by combining two populations originated from a reciprocal cross of the two rye inbred lines P87 and P105. The pedigrees of both lines, developed at the Institute of Genetics and Cytology, Minsk, Belarus and the details for DNA extraction are given by Korzun et al. (1998).

DNA probes

A range of 240 RFLP clones consisting of both cDNA and genomic DNA probes known to be distributed over all seven *Triticeae* chromosomes were selected and used to screen for polymorphisms. The sources of these probes were discribed previously by Korzun et al. (1998). The functional probes used were kindly supplied by B. Keller, Institute for Plant Biology, University of Zurich, Switzerland (Lrk10), W.A. Wilson, Cornell University, Ithaca, USA (WAW1023), R. Kunze, Institute for Genetics, University of Munich, Germany (MSH2, MSH3, MSH6), W. Weschke, IPK Gatersleben, Germany (HvSUT1, HvSUT2), H. Bäumlein, IPK Gatersleben, Germany (TriaIII, NASHOR1) and U. Baumann, Waite Agricultural Research Institute, Glen Osmond, Australia (Bm2).

Isozyme and protein studies

Analyses were carried out with extracts prepared from grains or young leaves. The endosperm ends of individual F_2 dry grains were used for studying β-amylase (*β-amy-R1*). From the endosperm of ten bulked F_3 grains we extracted esterase (*Est-R5*), peroxidase (*Eper*), phosphatase (*Ph-2*), water soluble protein (*Wsp-R1*) and iodine binding factor (*Ibf-R1*). For these markers only 95 F_3 families of the cross P87×P105 were analyzed. The enzyme and protein extractions, resolutions and staining were performed as described for wheat by Liu (1991). Ten isozyme systems were studied by using either frozen leaves of F_2 plants or fresh leaves of F_3 plants collected at the tillering stage. For analyzing glucosephosphate isomerase (*Gpi-R1*), leucine aminopeptidase (*Lap1*), superoxide dismutase (*Sod-R1*), diaphorase (*Dia2*), endopeptidase (*Ep-R1*), acid phosphatase (*Acp2/3*) and glutamate oxaloacetate transaminase (*Aat2*) electrophoresis with polyacrylamide gels were applied (Davis 1964). For resolution of peroxidase (*Prx7*, *Prx10*), malate dehydrogenase (*Mdh*) and esterase (*Est2*, *Est4*, *Est10*), isoelectric focusing (IEF) in a pH 3.5–10 gradient of ampholites was used. The details of enzyme extraction, resolution and staining are presented in Priyatkina et al. (1994). The same authors describe the used nomenclature of esterase and leucine aminopeptidase loci used here. The symbols containing the capital letter R followed by a number are given to loci belonging to well defined homoeologous sets, as recommended by McIntosh et al. (1998). The loci *Ph-2*, *Acp2/3* and *Prx7* were first described by Jaaska (1983) or Wehling (1985).

Microsatellite investigation

A collection of 13 primer pairs representing rye microsatellites (SCM) and 30 primer pairs representing wheat microsatellites (WMS) were chosen for the analysis. The isolation and primer sequences of the rye and wheat microsatellites were previously described by Saal and Wricke (1999) and Röder et al. (1998), respectively. Polymerase chain reactions (PCR) and fragment detection were performed as described by Röder et al. (1998). Fragment analysis was carried out using an automated laser fluorescence (ALF) sequencer (Pharmacia), and fragment sizes were calculated using the computer program FRAGMENT MANAGER V. 1.2 (Pharmacia) by comparison with internal standards. For mapping of the microsatellites a subpopulation consisting of 78 plants of the P87×P105 cross was used.

Statistical analysis

The individual plants were genotyped for each locus to build linkage maps for all seven chromosomes. Because the single maps for the two reciprocal crosses were in good agreement (Korzun et al. 1998) joint maps were assembled including all 275 F_2 individuals of both crosses. Only one cross (P87×P105) was used for genotyping the isozymes/proteins extracted from the endosperms of F_3 grains and the microsatellites. The maps were constructed by using the MAPMAKER 2.0 computer program (Lander et al. 1987). Centimorgan distances were calculated applying the Kosambi map-unit function (Kosambi 1944).

Results

Marker performance

RFLP markers

Out of the 240 selected RFLP probes originating from rye (32), wheat (103), barley (100) and oat (5), 131 (55%) were found to be polymorphic and detected 139 loci. As previously reported (Korzun et al. 1998), the level of polymorphism was influenced by the source of the clones decreasing in the order rye (70%)>wheat (55%)>barley (45%)>oat (20%). In addition to 123-single copy probes 8 low-copy probes were used, detecting 2 loci (IAG186, MWG913, MWG2053, MWG2062, PSR109, PSR120, WG110, SCB101). Codominant and dominant alleles were scored for 112 (81%) and 27 (19%) RFLP loci, respectively. The number of RFLP markers over all seven chromosomes ranged from a minimum of 13 (on 6R) to a maximum of 26 (on 5R).

Biochemical markers

The 19 biochemical markers polymorphic in the present population were found to be located on six of the seven rye chromosomes. The loci *Est4*, *Ep-R1*, *Prx10*, *Mdh*, *Dia2*, *Acp2/3*, *Lap1*, *Eper* and *Ibf-R1* were scored as dominant (3:1) markers; the others were scored as codominant (1:2:1) markers.

Microsatellite markers

Out of the 13 rye microsatellites (SCM) 8 (61%) were polymorphic in the mapping population and detected 9 loci. Two loci were detected by SCM138 in the centromeric regions of 5R and 7R. Only 8 out of 30 wheat microsatellites (WMS) produced a specific amplification product and only 4 (13%) of these could be mapped, 1 each on rye chromosomes 3R, 5R, 6R and 7R.

Gene loci

Major genes

In addition to the DNA and biochemical markers, gene loci were either directly mapped, scoring the pooled F_2 population of the reciprocal cross of P87 and P105 (12 loci), or aligned to chromosome regions based on mapping data published recently (23 loci). This alignment became possible because common markers could be used as anchors. From the 12 mapped gene loci, the positions of the 2 loci controlling reduced plant height (*Ddw1*) and

Table 1 Information on genes aligned to chromosome regions in the map presented in Fig. 1

Gene symbol	Trait	Chromosome	First report
al	Absence of ligules	2RL	Korzun et al. (1997a)
cb	Culm brown	6RL	Malyshev et al. (2000)
Chl	Chlorophyll deficiency	5RL	Senft and Wricke (1996)
CreR	Resistance against cereal cyst nematode	6RL	Taylor et al. (1998)
ct1	Compactum (Reduced plant height)	7R	Plaschke et al. (1995)
ct2	Compactum (Reduced plant height)	5RL	Plaschke et al. (1993)
Hs	Hairy leaf sheath	5RL	Senft and Wricke (1996)
Lr-a	Reaction to leaf rust	6RL	Ruge et al. (1999)
Lr-c	Reaction to leaf rust	1R	Ruge et al. (1999)
Lr-g	Reaction to leaf rust	1R	Ruge et al. (1999)
mp	Multi pistils	7RL	Malyshev et al. (2000)
np	Nana postratum (Reduced plant height)	4RL	Malyshev et al. (2000)
Pm	Reaction to powdery mildew	1RS	Wricke et al. (1996)
Rfg1	Male fertility restoration	4RL	Börner et al. (1998)
S	Self fertility	1RS	Senft and Wricke (1996), Voylokov et al. (1998)
S5	Self fertility	5R	Voylokov et al. (1998)
Sec2	Secalins	2RS	Malyshev et al. (1998)
Sec5	Secalins	2RS	Malyshev et al. (1998)
Sp1	Spring growth habit (Vernalization response)	5RL	Plaschke et al. (1993)
w	Waxless plant with waxy nodes	4RL	Malyshev et al. (2000)
wa1	Waxless plant	7RL	Korzun et al. (1997a), Malyshev et al. (2000)
Wx	Waxy endosperm	4RL	Korzun et al. (1997a)
Z	Self fertility	2RL	Senft and Wricke (1996), Voylokov et al. (1998)

hairy peduncle (*Hp1*) and of the loci of the sequences with homology to a pollen allergen (*XtriaIII*) and to mismatch repair genes (*Xmsh2*, *Xmsh3*, *Xmsh6*) have been described earlier (Korzun et al. 1996a, b, 1998, 1999b). The remaining 6 loci were mapped by us here for the first time by the detection of polymorphisms for a receptor-like kinase gene (*Xlrk10*), a wheat VIVIPAROUS-1 ortholog (*Xwaw1023* syn. *Xlars10(taVp1)*), a nicotianamine synthase gene (*Xnashor1*), a self-incompatibility-related gene (*Xbm2*) or two sucrose transporter genes (*Xhvsut1*, *Xhvsut2*) (Feuillet et al. 1997; Bailey et al. 1999; Herbik et al. 1999; Li et al. 1994; Weschke et al. 2000).

The loci aligned to chromosome regions are listed in Table 1 and comprise genes controlling the traits self-fertility (*S*, *Z*, *S5*), reduced plant height (*ct1*, *ct2*, *np*), reaction to powdery mildew (*Pm*), reaction to leaf rust (*Lr-a*, *Lr-c*, *Lr-g*), reaction to cereal cyst nematode (*CreR)*, Secalins (*Sec2*, *Sec5*), absence of ligules (*al*), waxy endosperm (*Wx*), male fertility restoration (*Rfg1*), chlorophyll deficiency (*Chl*), hairy leaf sheath (*Hs*), vernalization response (*Sp1*), waxless plant (*wa1, w*), brown stem (*cb*) and multiple pistils (*mp*). All these genes have been mapped using molecular makers during the last 7 years by Plaschke et al. (1993, 1995), Senft and Wricke (1996), Korzun et al. (1997a), Börner et al. (1998b), Malyshev et al. (1998, 2000), Voylokov et al. (1998), Wricke et al. (1996), Taylor et al. (1998) or Ruge et al. (1999).

Quantitative trait loci

The nomenclature used for the loci in the present paper was adapted to that recommended for wheat by McIntosh et al. (1998). The basic symbol *Q* is followed by a trait designator, a laboratory designator, a hyphen and the symbol for the chromosome in which the QTL is located. The trait designators used are *Ht* (plant height), *Pdl* (peduncle length), *Eal* (ear length), *Ean* (ear number), *Fln* (floret number), *Grn* (grain number), *Tgw* (thousand grain weight), *Flt* (flowering time), *Eyd* (ear yield) and *Syd* (straw yield). The laboratory designators are *ipk* (Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany), *stp* (St. Petersburg State University, St. Petersburg, Russia) and *psr* (John Innes Centre, Norwich, UK). Whereas 21 QTLs were detected by analyzing F_3 plants of the presented mapping population under four different environments, the loci *QEal.stp-5R.1*, *QFln.stp-5R.1*, *QHt.stp-5R.1* and *QPdl.stp-5R.1* were identified in another population also segregating for the major dwarfing gene *Ddw1*. Common markers were used as anchors to extrapolate the map positions. Detailed descriptions of the QTLs (LOD scores, interval lengths) are given in Börner et al. (1999, 2000).

The genetic map

The linkage map generated from 275 individuals is shown in Fig. 1. In total, the map spans 1,063.4 cM and consists of 183 loci. These loci formed seven linkage groups representing the seven rye chromosomes. In size, the linkage groups varied from 118.9 cM (chromosome 3R) to 205.6 cM (chromosome 5R). The distance between precisely mapped markers (omitting cosegregants) ranges from 0.2 to 39.5 cM. Three gaps greater than 25 cM were found on chromosomes 4R, 5R and 6R.

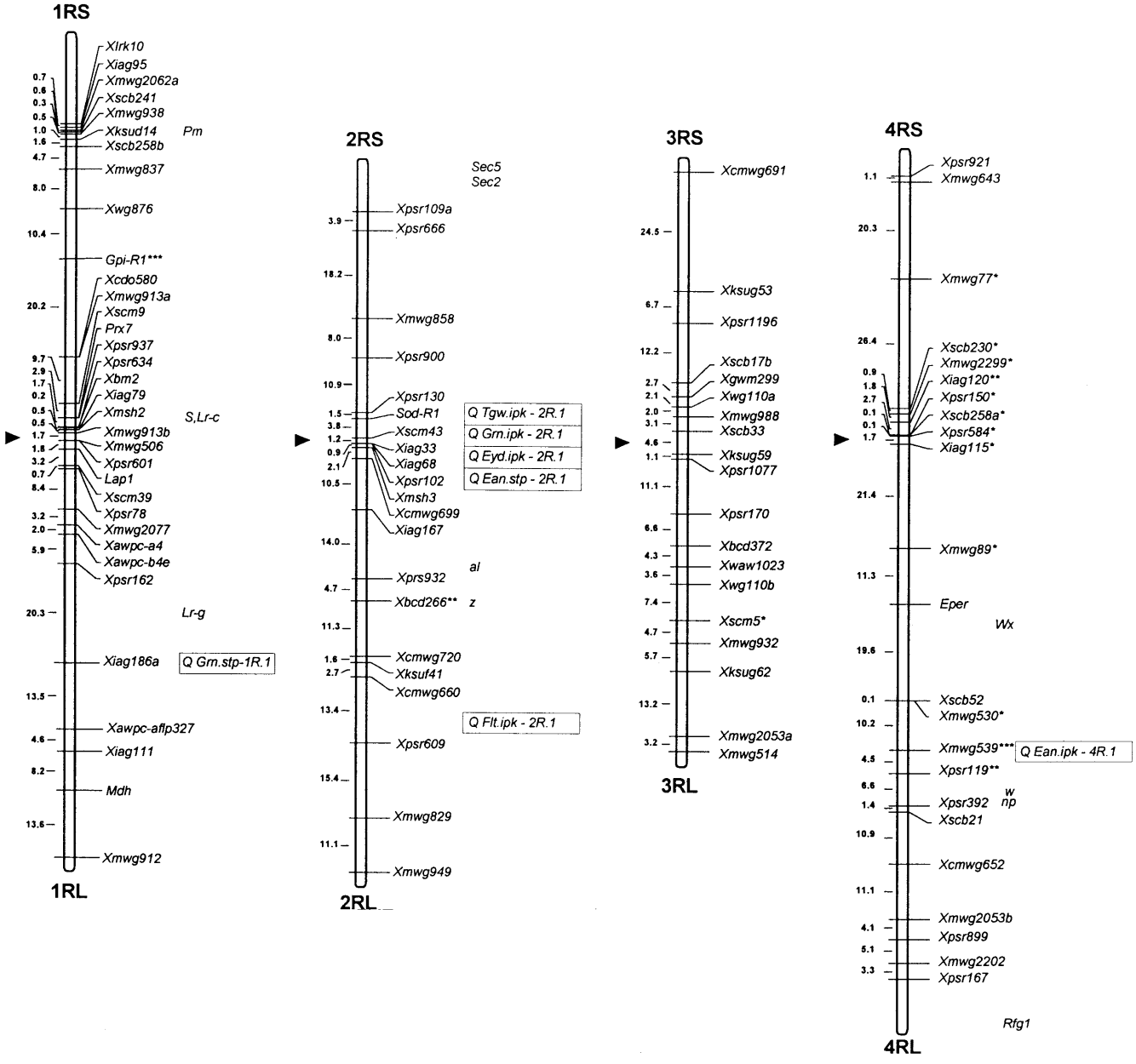

Fig. 1 Genetic linkage maps of chromosomes 1R, 2R, 3R, 4R, 5R, 6R and 7R of rye The aligned major genes and quantitative trait loci (QTLs) are placed in the most probable position on the *right hand side* of the framework. The QTLs are *boxed*. Genetic distances are given in centimorgans (cM). The *black triangles* indicate the estimated centromere position. The *asterisks* indicate loci with distorted segregation (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). *S* Short arm, *L* long arm

On average, the genetic map consists of about 26 markers per chromosome with a maximum of 38 on chromosome 5R and a minimum of 19 on chromosome 3R. Twenty-two loci (12%) deviated significantly from the expected 1:2:1 or 3:1 ratios (χ^2 test, $P<0.05$), as shown by the asterisks in Fig. 1. More than 50% of loci showing a distorted segregation were found to map in two domains on chromosome 4R in the centromere region, including the proximal part of the short arm, and about 40 cM distal from the centromere on 4RL. A further cluster was identified in the distal region on chromosome 5RL.

In addition to the 183 precisely mapped markers, another 23 gene loci could be aligned to certain chromosome regions (Table 1). These loci were placed in the most probable positions on chromosomes 1R (4 loci), 2R (4 loci), 4R (4 loci), 5R (5 loci), 6R (3 Loci) and 7R (3 loci). Of the 25 QTLs detected in total, 12 were found to map on chromosome 5RL in the region of *Ddw1* and to determine plant height, peduncle length, flowering time and yield components. An additional cluster consisting of 4 QTLs controlling flowering time and yield components was discovered in the centromere region of chromosome 2R.

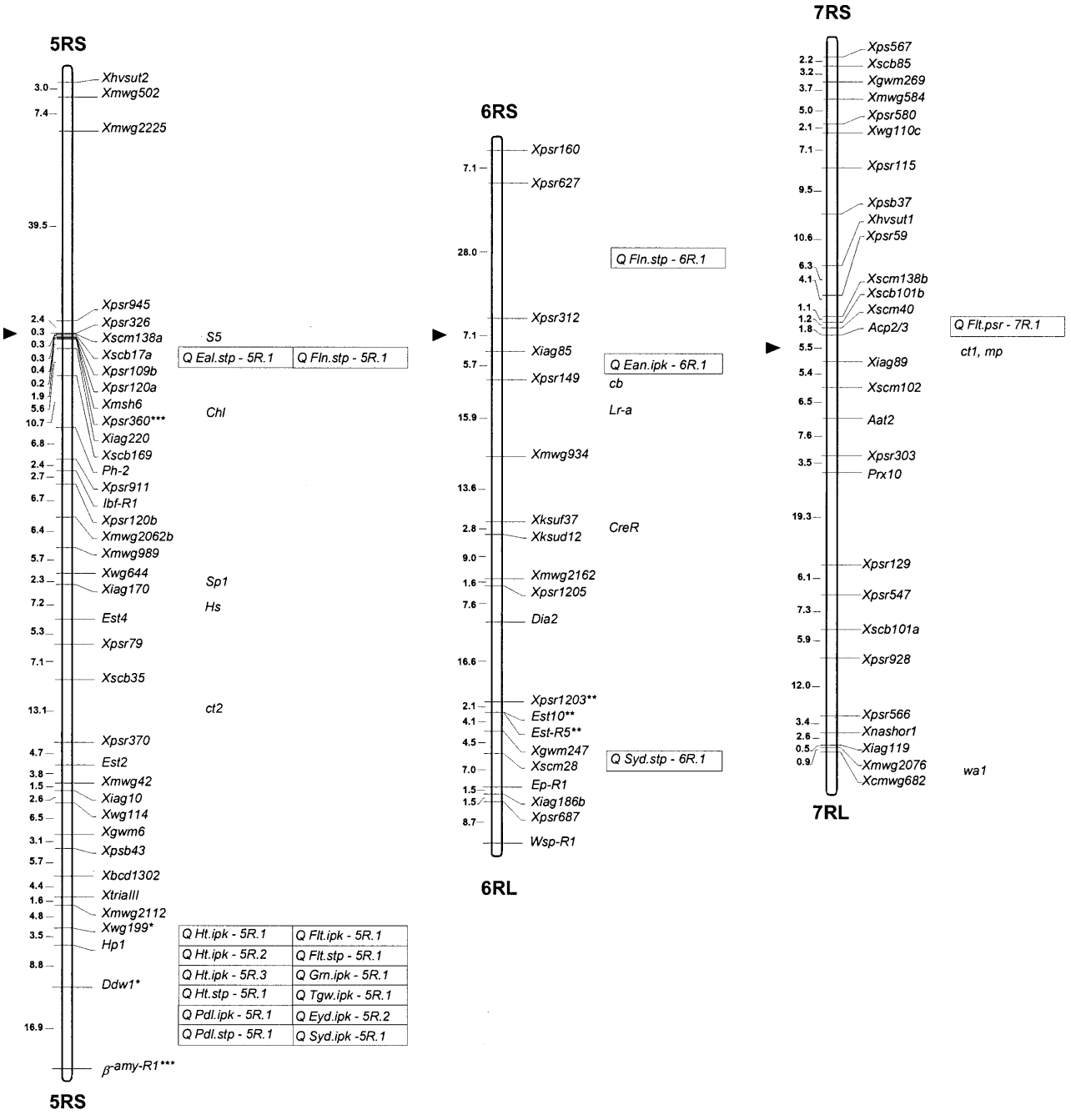

Fig. 1 continued

Discussion

The linkage map of rye presented here consits of 139 RFLP markers, 19 isozyme/protein loci and 13 microsatellites. Furthermore, 10 gene homologue sequences and two morphological genes were mapped. Chromosome 4R (centromere region) was found to carry most of the loci with distorted segregation. Because an unequal number of homozygous classes of alleles was observed, the markers seem to be linked with a selection locus. Taking into consideration that chromosome 4R is translocated with respect to wheat and barley and is homoeologous to groups 4S, 7S and 6S (Devos et al. 1993) it should be mentioned here that Faris et al. (1998) detected regions with skewed segregation ratios on chromosomes 4DS and 7DS of *Aegilops tauschii*.

Nineteen biochemical markers were integrated into this molecular map of rye. In most cases the map positions are in agreement with previously described linkage data published by Wehling (1985), Benito et al. (1990, 1996), Liu et al. (1992), Devos et al. (1993) or Priyatkina et al. (1994, 1995). The distal map position of the locus *Mdh* (1R) does not correspond to any of 2 *Mdh1* and *Mdh2a* loci described in literature (Benito et al. 1990). These 2 loci are closely linked to each other in the centromere

region of chromosome 1R. The distal map location of *Mdh* locus described here on 1RL corresponds with the map position of the *Xadh* locus, revealed using the untranslated region of *Adh1A* as a probe (Devos et al. 1993). One may suggest that one of the numerous bands of MDH activity on the IEF zymograms is really the ADH isozyme, developed in the presence of traces of ethyl alcohol. Thus, *Mdh* is only a tentative designation.

The locus designated as *Prx10* was discovered for the first time. The mapping population segregated for an active peroxidase band in the acidic part of IEF zymogram in a ratio 3 (present):1 (absent). For the *Eper* locus mapped here it was not possible to relate it to any of the linked peroxidase loci (*Eper1, Eper2, Eper3*) also mapped on chromosome 4R (Vaquero et al. 1990) because we studied the segregation of only one of the whole set of the peroxidase bands developed using a different way of separation.Three known function clones, *XPer1, XPer2* and *XPer3*, were mapped on chromosomes 4R, 2R and 7R, respectively, by Devos et al. (1993). It has been suggested that *XPer1* (2RS) corresponds with the isozyme marker *Per-2* (Devos et al. 1993). The peroxidase loci *Eper* (4RL) and *Prx10* (7RL) may correspond with *XPer1* and *XPer3*, respectively of Devos et al. (1993), as suggested from their map position relative to common RLFP markers.

Locus *Ph-2* described by Jaaska (1983) was localized and mapped on chromosome 5R for the first time, as was locus *Wsp-R1* on 6RL. Taking into consideration the existing translocations between rye and the other *Triticeae* members *Wsp-R1* was found to map at a position homoeologous to the group 7L chromosomes of wheat or barley, distal to the endopepdidase locus (Liu 1991).

Cosegregation was observed for leaf esterase *Est10* and grain esterase *Est-R5*, which may be due to tight linkage of 2 complex loci differentially expressed in leaf and grain. The close linkage of such loci was also observed in wheat (Liu 1991). *Est10* may correspond to *Est8* or *Est9*, which are closely linked to each other and mapped in a highly comparable position on chromosome 6RL (Senft and Wricke 1996).

Five of the eight rye microsatellites (SCM39, SCM43, SCM138, SCM28, SCM40) mapped at positions highly comparable to those previously shown for rye by Saal and Wricke (1999), whereas SCM5 was mapped in a more distal region. However, segregation for this marker was distorted in the present population. For SCM138, a second locus was detected on chromosome 7R. Microsatellites SCM9 and SCM102 were mapped for the first time. The four polymorphic wheat microsatellites were found to be located in homoeologous positions compared to those in wheat (Röder et al. 1998), taking into consideration the translocations existing between wheat and rye (Devos et al. 1993).

One of the two morphological genes mapped here was the gibberellic acid (GA)-sensitive major dwarfing gene *Ddw1*. It was shown to map in exactly the same position as described earlier by Korzun et al. (1996a) using a different mapping population. The pedigree data of the cross used here give clear indication that the dwarf character originated from the mutant Bolgar dwarf (Korzun et al. 1998). After searching for further information we discovered that a synonym for Bolgar dwarf is K10028, a catalog number of the St. Petersburg genebank collection. Although K10028 has been described to carry the dominant dwarfing gene *Ddw2*, located on chromosome 7R (Melz 1989), our results suggest that this dwarf carries an allele at the *Ddw1* locus on chromosome 5R. Based on earlier investigations of Kobyljanski (personal communication) K10028 and EM1, the *Ddw1* carrier, were thought to be very closely related. The main difference between these two is the higher frost tolerance of K10028. To clarify this situation an allelic cross between Bolgar dwarf and EM1 will be necessary. Comparative mapping studies in wheat revealed the presence of a GA sensitive dwarfing gene (*Rht12*) in a homoeologous position on chromosome 5 A (Korzun et al. 1997b).

The second morphological gene determining the trait hairy peduncle (*Hp1*) was found to be closely linked to *Ddw1* (Korzun et al. 1996a). *Hp1* determines both hairy peduncles and hairy leaf sheaths. It should be mentioned here that Korzun et al. (1999a) recently mapped a gene for hairy pubescence in barley (*Hordeum bulbosum*) designated Hs_b. A comparison of the mapping positions of both loci indicated that Hs_b and *Hp1* are homoeoallelic.

The 10 gene loci mapped by using their sequences were all isolated from species different from rye. Some of them have been mapped previously in other cereals and, therefore, the map positions can be compared to each other. On chromosome 1R one of the three sequences (Lrk10) has been mapped in wheat, barley and rice (Gallego et al. 1998). Under high-stringency conditions for hybridization *XLrk10* was mapped on the distal parts of chromosomes 1AS and 1HS in wheat and barley, respectively, as in the present study on chromosome 1RS. However, a gene partially homologous to Lrk10 was also found on group 3 S chromosomes of wheat and barley if low-stringency conditions were used. Under those conditions a homoeologous locus may be expected on rye chromosome 3RS as well. Because *Xlrk10* in wheat is very closely linked to *Lr10*, a leaf rust resistance gene on chromosome 1A, this marker may become useful for the detection of a homoeologous resistance gene in rye.

Bm2 was isolated from pollen of *Phalaris coerulescens* Desf., a diploid perennial grass (Li et al. 1994), with the aim of finding a sequence determining the self-incompatibility of this species. Initially, the authors strongly suggested that Bm2 represents either the self-incompatibility gene *S* of *Phalaris* itself or a closely linked gene. However, subsequent detailed investigations of Baumann et al. (2000) show that Bm2 is closely linked to the *S*-locus, however, it is definitely not the *S*-gene. Interestingly, in rye, Bm2 maps in the centromere region of chromosome 1RS and is linked to one of the loci for self-incompatibility (*S*). It is possible that the region around the loci determining self-incompatibility is highly conserved within the grasses. *Xbm2* was mapped in a comparable position on the 1R chromosome by Senft and Wricke (1996).

From maize it is known that the transcription factor VIVIPAROUS-1, encoded by the *Vp1* gene, plays a critical role in the induction and maintenance of dormancy. Mutants lacking VP1 activity are viviparous, i.e. the immature embryos germinate precociously on the cob. A wheat *Vp1* ortholog (*Xlars10(taVp1)*) was used by Bailey et al. (1999) for mapping in wheat and rice. In wheat, *Xlars10(taVp1)* was found to map on the long arms of chromosomes 3 A, 3B and 3D, more than 25 cM proximal to the *R* loci, which also affect dormancy in wheat. As expected, in the present rye map *Xwaw1023* [syn. *Xlars10(taVp1)*] was found on the long arm of chromosome 3R in a homoeologous position.

The *nas* genes, which encode nicotianamine synthase and nicotianamine synthase-like proteins, play an important role in graminaceous plants grown under iron deficiency-stress conditions. Five *nas* genes were isolated by Higuchi et al. (1999). At the same time Herbik et al. (1999) isolated a nicotianamine synthase gene from barley (NASHOR1) that was subsequelly used in our studies. This gene was mapped on the distal part of chromosome 7R, which is translocated and homoeologous to the chromosome 2S in comparison to other *Triticeae* species. Therefore, in the barley genome the locus of the nicotianamine synthase-like gene is located on chromosome 2H (Korzun et al. unpublished data).

Two cDNAs encoding the sucrose transporters in developing seeds were cloned using a barley caryopsis library (Weschke et al. 2000). Again rye was used for mapping both sequences, designated HvSUT1 and HvSUT2 (*Hordeum vulgare* putative sucrose transporter DNA). *Xhvsut1* was mapped in the centromere region of chromosome 7RS, whereas *Xhvsut2* was linked distal to the 2 RFLP markers *Xmwg502* and *Xmwg2225* on the short arm of chromosome 5R. Although the gap between the two centromere-related markers *Xpsr945* or *Xpsr326* and *Xmwg2225* is about 40 cM, from homoeologous relationships between rye and barley, it can be strongly concluded that *Xhvsut1* is located on 5RS. *Xmwg502* has been shown to map in the distal region of chromosome 5HS (Graner et al. 1991).

Mismatch repair (MSH) and pollen allergen genes in rye map in correspondence to their homoelogous positions in wheat (Korzun et al. 1996, 1999b).

All genes which have been tagged in the past or will be tagged in the near future and having linked common RFLP markers can easily be aligned. The 23 already aligned gene loci shown in Fig. 1 cover a broad range of agronomically important genes. For 5 genes, homoeologous loci in other *Triticeae* or even *Poaceae* species are known. For the vernalization response gene *Sp1* on chromosome 5R homoeoallelic loci have been described on barley chromosome 5H and on the wheat chromosomes 5A, 5B and 5D. Our present knowledge of the homoeologous relationships of genes determining flowering time and reduced plant height within the *Triticeae* is summarized by Börner (1998a). Korzun et al. (1997a) compared the mapping data of the mutant loci *al*, *wa1* and *Wx* with already existing data for homoeologous regions containing equivalent mutants of wheat, barley, rice and maize. It was clearly shown that these loci are highly conserved across the cereal species. Four additional mutants, *cb*, *np*, *mp* and *w*, may have homoeoallelic loci in barley and/or wheat (Malyshev et al. 2000). A similar situation was postulated by Wricke et al. (1996) for the powdery mildew resistance gene *Pm* located at the end of the short arm of chromosome 1R.

The QTLs shown in Fig. 1 are based on the analysis of F_3 plants originating from two mapping populations. For further studies of complex characters like quality or yield, however, field plot experiments trials over several years are often necessary, as larger amounts of seeds are needed. Therefore, we commenced a program to develop recombinant inbred lines from single plants of each F_3 family of the present mapping population; these are now in the F_6 generation. After bulking up the stocks they can be used for further QTL analysis for any trait of interest.

The map presented here provides the possibility of integrating additional molecular markers, including isolated sequences of known function. Due to the fact that in rye the level of polymorphism is high, this mapping population can be used to map sequences not polymorphic in other cereals such as wheat or barley. Although rye has undergone a number of interchromosomal translocations compared to the other *Triticeae* members, collinearity is retained within the translocated chromosome segments and, therefore, the map positions of the probes of interest in other cereals may be postulated. There is also a possibility to align further genes mapped in different mapping populations using common markers as anchors. Moreover, due to the fact that the origin of the DNA probes used here is not only rye but also wheat, barley and oat, their utilization as anchor markers for comparative mapping analysis is possible. Mapping data available for genes of interest in wheat or barley could be used for mapping the homoeologous loci in rye and *vice versa*. Lastly, soon to be developed recombinant inbred lines may be used for performing further QTL analyses. By adding more probes, the present average of 26 markers per chromosome will increase and, therefore, the efficiency of such analyses will improve.

Acknowledgments We thank Drs. M.D. Gale, A. Graner, M. Sorrells, G. Wricke, B.S. Gill, B. Keller, W.A. Wilson, R. Kunze, W. Weschke, H. Bäumlein and U. Baumann for providing the probes. I. Egorova, St. Petersburg State University and R. Voss, IPK Gatersleben are acknowledged for performing isozyme studies and excellent technical assistance, respectively. We are grateful to the Ministry of Agriculture (Project No. 102) for financial support.

References

Bailey PC, McKibbin RS, Lenton JR, Holdsworth MJ, Flintham JE Gale MD (1999) Genetic map locations for orthologous *Vp1* genes in wheat and rice. Theor Appl Genet 98:281–284

Baumann U, Juttner J, Bian X, Langridge P (2000) Self-incompatibility in the grasses. Ann Bot 85 (Suppl A):203-209

Benito C, Frade JM, Orellana J, Carrillo JM (1990) Linkage and cytogenetic maps of genes controlling endosperm storage proteins and isozymes in rye (*Secale cereale* L.) Theor Appl Genet 79:347–352

Benito C, Romero MP, Henriques-Gil N, Lorente F, Figueiras AM (1996) Sex influence on recombination frequency in *Secale cereale* L. Theor Appl Genet 93:926–931

Börner A (1998) Comparative molecular mapping of agronomically important genes. In:Slinkard AE (ed) Proc 9th Int Wheat Genet Symp, vol. 1. University Extension Press, Saskatoon, Sask., Canada, pp 20–23

Börner A, Polley A, Korzun V, Melz G (1998) Genetics and molecular mapping of a male fertility restoration locus (*Rfg1*) in rye (*Secale cereale* L.). Theor Appl Genet 97:99–102

Börner A, Korzun V, Voylokov AV, Weber WE (1999) Detection of quantitative trait loci on chromosome 5R of rye (*Secale cereale* L.). Theor Appl Genet 98:1087–1090

Börner A, Korzun V, Voylokov AV, Worland AJ, Weber WE (2000) Genetic mapping of quantitative trait loci in rye (*Secale cereale* L.). Euphytica (in press)

Davis BJ (1964) Disc electrophoresis II. Method and application to human serum proteins. Ann NY Acad Sci 121:404–427

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

Faris JD, Laddomada B, Gill BS (1998) Molecular mapping of segregation distortion loci in *Aegilops tauchii*. Genetics 149: 319–327

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

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

Gallego F, Feuillet C, Messmer M, Penger A, Graner A, Yano M, Sasaki T, Keller B (1998) Comparative mapping of the two wheat leaf rust resistance loci *Lr1* and *Lr10* in rice and barley. Genome 41:328–336

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

Herbik A, Koch G, Mock H-P, Dushkov D, Czihal A, Thielmann J, Stephan UW, Bäumlein H (1999) Isolation, charakterization and cDNA cloning of nicotianamine synthase from barley. Eur J Biochem 256:231–239

Higuchi K, Suzuki K, Nakanishi H, Yamaguchi H, Nishizawa N-K, Mori S (1999) Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores. Plant Physiol 119:471–479

Jaaska V (1983) *Secale* and Triticale. In: Tanksley D, Orton TJ (eds) Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam, pp 79–101

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 (1997a) 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

Korzun V, Röder M, Worland AJ, Börner A (1997b) Intrachromosomal mapping of genes for dwarfing (*Rht12*) and vernalization response (*Vrn1*) in wheat by using RFLP and microsatellite markers. Plant Breed 116:227–232

Korzun V, Malyshev S, Kartel N, Westermann T, Weber WE, Börner A (1998) A genetic linkage map of rye (*Secale cereale* L.). Theor Appl Genet 96:203–208

Korzun V, Malyshev S, Pickering RA Börner A (1999a) RFLP mapping of a gene for hairy leaf sheath using a recombinant line from *Hordeum vulgare* L.×*Hordeum bulbosum* L. cross. Genome 42:960–963

Korzun V, Börner A, Siebert R, Malyshev S, Hilpert M, Kunze R, Puchta H (1999b) Chromosomal location and genetic mapping of the mismatch repair gene homologs *MSH2, MSH3 and MSH6* in rye and wheat. Genome 42:1255–1257

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg I (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li X, Nield J, Hayman D, Langridge P (1994) Cloning a putative self-incompatibility gene from the pollen of the grass *Phalaris coerulescens*. Plant Cell 6:1923–1932

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

Liu CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Nonhomoeologous translocations between group 4, 5 and 7 chromosomes within wheat and rye. Theor Appl Genet 83: 305–312

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

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In:Slinkard AE (ed), Proc 9th Int Wheat Genet Symp, vol. 5, pp 1–236. University Extension Press, Saskatoon, Sask., Canada, pp 1-236

Madej LJ (1996) Worldwide trends in rye growing and breeding. Vortr Pflanzenzuecht 35:1–6

Malyshev S, Khmyl TO, Zabenkova KI, Voylokov AV, Korzun VN, Kartel NA (1998) RFLP-based mapping of the *Sec-2* and *Sec-5* loci encoding 75 K *y*-secalins of rye. Plant Breed 117: 329–333

Malyshev S, Korzun V, Voylokov AV, Smirnov V, Börner A (2000) Linkage mapping of mutant loci in rye (*Secale cereale* L.). Theor Appl Genet (in press)

Melz G (1989) Beiträge zur Genetik des Roggens (*Secale cereale* L.). PhD thesis, Berlin

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

Priyatkina SN, Linz A, Fam TF, Voylokov AV (1994) Isozyme markers in the genetic studies of rye *Secale cereale* L. In:Markert CL, Scandalios JG, Lim HA, Serov OL (eds) Isozymes: organization and roles in evolution, genetics and physiology. (Proc 7th Int Congr Isozymes). Word Scientific Publ, Novosibirsk, pp 191–201

Priyatkina SN, Voylokov AV, Linz A, Fam TF (1995) Genetic mapping in rye (*S. cereale* L.). In: Börner A, Worland AJ (eds) Proc 9th EWAC Conf 1994. Gatersleben-Wernigerode, pp 134–139

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Ruge B, Roux SR, Linz A, Wehling P (1999) Erschliessung und molekulare Charakterisierung von Resistenzen gegen Braunrost. Vortr Pflanzenzuecht 46:169–176

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42:964–972

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

Taylor C, Shepherd KW, Langridge P (1998) A molecular genetic map of the long arm of chromosomes 6R of rye incorporating the cereal cyst nematode resistance gene, *CreR*. Theor Appl Genet 97:1000–1012

Vaquero F, Rebordinos L, Vences FJ, Perez de la Vega M (1990) Genetic mapping of isozyme loci in *Secale cereale* L. Theor Appl Genet 80:88–94

Voylokov AV, Korzun V, Börner A (1998) Mapping of three selffertility mutations in rye (*Secale cereale* L.) by using RFLP, isozyme and morphological markers. Theor Appl Genet 97:147–153

Wehling P (1985) Electrophoretic analysis of 10 enzyme systems in rye:linkage relationships and chromosomal location of isozyme loci. In: Proc. EUCARPIA Meet Cereal Sect Rye. Svalov, Sweden, pp 101–124

Weschke W, Panitz R, Sauer N, Wang Q, Neubohn B, Weber H, Wobus U (2000) Sucrose transport into barley seeds:molecular characterization of two transporters and implications for seed development and starch accumulation. Plant J 21:455–467

Wricke G, Dill P, Senft P (1996) Linkage between a major gene for powdery mildew resistance and an RFLP marker on chromosome 1R of rye. Plant Breed 115:71–73