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Linkage mapping of mutant loci in rye (*Secale cereale* L.)

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Abstract Eight mutant loci determining the traits waxy plant (*w* and *wa1*), brown culm (*cb*), multiple pistils (*mp*), weak plant with reduced plant height (*np*), monoculm growth habit (*mc*), compactum growth habit (*ct3*) and anthocyaninless (*an*) were mapped on rye chromosomes 4R (*w, np*), 6R (*cb, mc*) and 7R (*mp, wa1, ct3, an*). For five mutants (*w, wa1, cb, mp, np*) molecular and biochemical markers were applied, whereas for *mc*, *ct3* and *an* a classical linkage analysis was performed. Furthermore, it could be demonstrated that homoeologous relationships exist between most of the mapped rye loci and comparable mutants in wheat and barley. It was confirmed not only that genes controlling fundamental aspects of plant biology are highly conserved across the Triticeae species but so also were many mutant loci.

Keywords Genetic mapping · Homoeology · Mutant loci · RFLP · *Secale cereale* · Triticeae

Introduction

Since molecular maps became available for the main cereal species including rye (*Secale cereale* L.), many efforts have been made on the precise mapping of gene loci (Senft and Wricke 1996; Korzun et al. 2000). Besides the tagging of major genes following Mendelian segregation, high-density maps allowed the integration of quantitative inherited traits designated as QTLs (quantitative trait loci) (Börner et al. 1999, 2000).

Comparing the map positions of gene loci mapped both in rye and in other cereals a high degree of collinearity was obtained for certain loci. Korzun et al. (1997) compared the mapping data of genes determining the absence of ligules (*al*), waxless plants (*wa1*) and waxy endosperm (*Wx*) characters, with already existing data for homoeologous regions containing equivalent mutants of wheat, barley, rice and maize. It was clearly shown that the loci are highly conserved across the cereal species. To-date it is not yet clear, whether the described mutant loci may be of any significance in cereal breeding. However, features like erect leaves, extended peduncle or waxy plants may become important traits for a character described as ‘disease escape’ as an alternative to plant disease resistance.

In the present paper we describe the molecular mapping of five loci determining brown culm (*cb*), multiple pistils (*mp*), weak plants with reduced plant height (*np*) and waxy plants (*w* and *wa1* syn. *epr*). Whereas *wa1* has already been studied by Korzun et al. (1997) the remaining loci were mapped for the first time. A classical genetic linkage analysis was performed for two (brown culm = *cb,* and monoculm = *mc*) and three (anthocyaninless = *an* syn. viridis = *vi*, multiple pistils = *mp,* and compactum 3 = *ct3*) mutant loci on chromosomes 6R and 7R, respectively. The origin and detailed description of these mutants is given by Smirnov and Sosnikhina (1984). From previous investigations it was known that the target genes may be located on chromosomes 4R, 6R and/or 7R (De Vries and Sybenga 1984; Smirnov and Voylokov, unpublished data). Homoeologous relationships between the rye loci and comparable mutants of the Triticeae members wheat and barley are discussed.

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Materials and methods

Plant materials and target-trait analysis

The molecular mapping studies were performed by analyzing two segregating F_2 populations. Population 1 was created by crossing two inbred lines differing in alleles at the loci *np* (nana postratum), determining a weak plant with reduced plant height, and *w* (waxless plant), having no wax layer on all parts of the plant except the nodes. One single F_1 plant was used to produce 149 F_2 seeds.

Population 2, consisting of 128 F_2 plants, was obtained from one F_1 plant in the cross of two inbred lines segregating for the genes *wa1* (waxless plant), *cb* (culm brown), and *mp* (multiple pistils). The genotypes carrying the gene *wa1* produce no visible wax layer on any part of the plant, which is in contrast to the *w* mutant of population 1. The locus *cb* induces a light-brown/orange coloration of the stems (nodes and internodes) and spikes (rachis, glumes and awns), and the third segregating locus of population 2, designated *mp*, determines a crucial change of the florets. Instead of one pistil and three anthers, four pistils are constructed (Fig. 1). Therefore, the mutant can only be maintained via heterozygous plants.

In order to analyze the linkage between *cb* (culm brown) and *mc* (monoculm) on chromosome 6R, and *an* (anthocyaninless), *mp* (multiple pistils) and *ct3* (compactum 3) on chromosome 7R, the segregating F_2 populations listed in Table 1 were studied.

The F_2 plants were grown in the greenhouse and the traits were phenotyped at different stages. Whereas the trait anthocyaninless was scored in young seedlings (coleoptile color) scoring for the waxy plant and brown culm characters was most effective just before flowering. The *mp* mutants were identified after anthesis. Plants showing no anthers were very easily detectable. The traits monoculm growth habit, compactum growth habit and weak plants with reduced plant height were recorded just before harvest. At harvest time the final plant height was measured in population 1.

Isozyme and GA response studies

Isozyme analysis was carried out with extracts prepared from fresh leaves of F_2 plants collected at the tillering stage. The enzyme extraction, resolution and staining were performed as described by Priyatkina et al. (1994). For the *np* mutant, determining a plant habit with reduced height, the GA_3 seedlings test was performed as described by Börner (1991). The average length of 30 seedlings treated with GA_3 was compared with those of 30 control plants, grown under the same conditions but omitting GA_3.

Molecular-marker analysis

Fresh leaf material cut from 5–6 week-old F_2 seedlings of populations 1 and 2 was used for extracting DNA by the procedure of

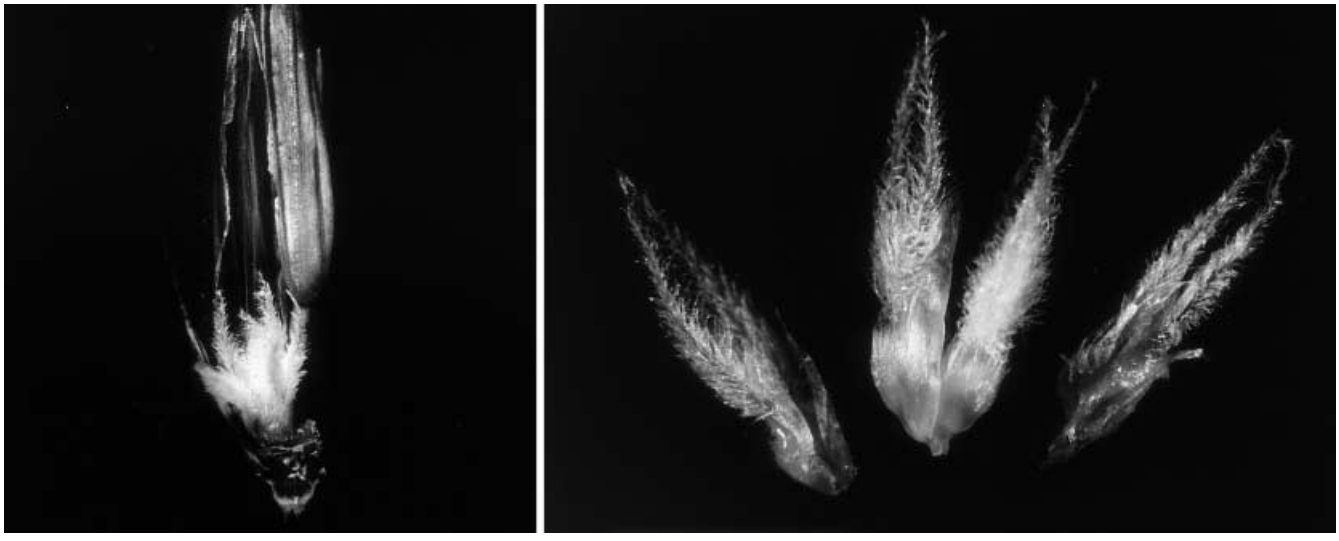

Fig. 1 Complete floret (left) and dissected floret (right) from a spike of the multi-pistil mutant. Compared to the wild-type four pistils are present instead of one pistil and three anthers

Table 1 Number of F_2 plants, segregation values and χ^2-tests for mapping the populations analyzed

Linkage group	Mapping population			
	Number of F_2 plants	Segregating gene loci	Observed segregation ratio	χ^2 value (3 : 1)
cb – mc	216	*cb*	168 : 48	0.88, $P > 0.30$
		mc	175 : 41	4.17, $P > 0.02$
an – mp	899	*an*	658 : 241	0.85, $P > 0.30$
		mp	670 : 229	0.11, $P > 0.70$
mp – ct3	198	*mp*	152 : 46	0.33, $P > 0.55$
		ct3	159 : 39	2.97, $P > 0.05$
an – ct3	429	*an*	312 : 117	1.18, $P > 0.25$
		ct3	345 : 84	6.72, $P > 0.009$

McCouch et al. (1988). Applying the methods of Devos et al. (1992), restriction digesting with enzymes *Hin*dIII, *Dra*I, *Eco*RI and *Eco*RV, gel electrophoresis, Southern transfer, probe labeling and filter hybridization were performed. Selected cDNA and genomic DNA probes from various wheat (coded with PSR, KSU), barley (coded with MWG) and rye (coded with IAG and SCB) libraries were used. The probe selection was based on the knowledge that the target genes may be located on chromosomes 4R, 6R and 7R. Individual F_2 plant genotypes were determined for each locus and linkage maps were constructed with the MAPMAKER 2.0 computer program (Lander et al. 1987) using the Kosambi map-unit function.

Results

Segregation of the mutant loci, final plant height measurements and GA response

In population 1, segregating for the traits waxless plants (*w*) and weak plants with reduced plant height (*np*), monogenic inheritance was obtained for both characters. Of the 149 plants 39 produced no visible wax at the internodes and leaves, whereas 110 did. The ratio tested by χ^2 fitted the expected 1 : 3 for a monogenic recessive inheritance of *w* ($\chi^2 = 0.110$, $P > 0.70$). A very similar ratio of 40 : 109 was obtained scoring the population for *np* ($\chi^2 = 0.224$, $P > 0.60$). With the exception of two plants, all waxless genotypes also showed the *np* character, indicating a close linkage of both loci. Analyzing the final plant-height measurements of population 1, however, no clear cut 1 : 3 segregation was obtained (Fig. 2). Several plants with a moderate plant height were found, not

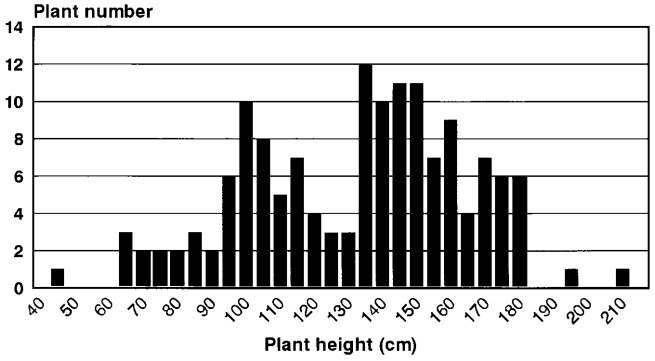

Fig. 2 F_2 segregation pattern for final plant height in population 1

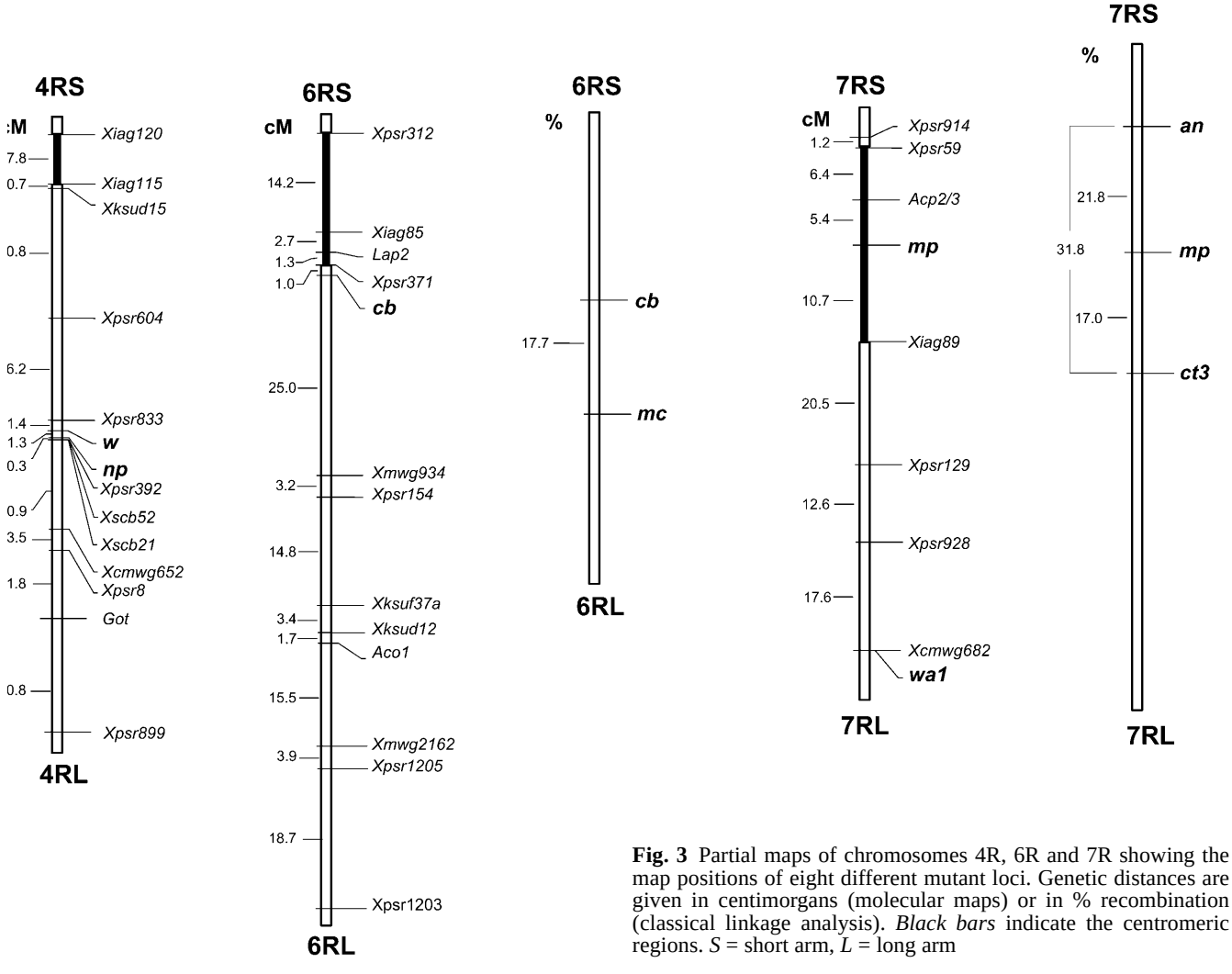

Fig. 3 Partial maps of chromosomes 4R, 6R and 7R showing the map positions of eight different mutant loci. Genetic distances are given in centimorgans (molecular maps) or in % recombination (classical linkage analysis). *Black bars* indicate the centromeric regions. *S* = short arm, *L* = long arm

showing the typical phenotype (weaker growth habit) of *np*. Therefore, for the linkage studies the data obtained from visual phenotypic scoring were used. The GA_3 seedlings test gave clear indication that *np* is a very GA_3-sensitive mutant with a 208% response in seedling elongation after treatment.

In population 2 the waxless plant trait, determined here by the gene *wa1*, was again recorded. Twenty-seven of the 128 plants in total were waxless. For the expected segregation ratio of 1 : 3 a χ^2 = 1,042 was calculated (P >0.30). For the character brown culm, 35 plants were scored showing the brown/orange color, whereas 93 plants were wild-type indicating a recessive inheritance of the trait (χ^2 = 0.375, P >0.50). Only 126 of the 128 plants developed spikes and, therefore, were scorable for the trait multiple-pistils. Again a 1 : 3 Mendelian segregation ratio of 26 mutant phenotypes to 100 wild-type phenotypes was observed (χ^2 = 1.280, P >0.25).

The segregation ratios and χ^2-values for the F_2 populations analyzed in order to find linkage between *cb* and *mc*, *an* and *mp*, *an* and *ct3*, or *mp* and *ct3* are listed in Table 1.

Marker analysis and gene mapping

In total, 30 of the 49 RFLP probes that hybridized to filters were polymorphic in one of both mapping populations (61%). Three markers could not be linked and, therefore, the maps presented in Fig. 3 consist of 11 (chromosome 4R), ten (chromosome 6R) and six (chromosome 7R) RFLP markers. In addition four isozyme markers could be integrated on chromosomes 4R (*Got*), 6R (*Lap2*, *Aco1*) and 7R (*Acp2/3*). For two closely linked RFLP markers in the centromere region of chromosome 4R (*Xiag115* and *Xksud15*) distorted segregations were detected. For all other loci no segregations significantly different to the expected 1:2:1 or 3:1 ratios (χ^2 test P >0.05) were observed.

Five of the mutant loci were mapped in relation to RFLP and/or isozyme markers (Fig. 3). On the long arm of chromosome 4R the two target genes *w* and *np* were found to be closely linked (4.4 cM) and to map about 40 cM distal to the centromere. The closest markers are *Xpsr833* for *w* and *Xpsr392* for *np*, with a distance of 1.5 cM and 1.3 cM, respectively. The gene *cb*, determining brown/orange stems is located close (distal) to the centromere on chromosome 6RL. It is flanked by the two RFLP markers *Xpsr371* and *Xmwg934* located 6.2 cM proximal and 28.4 cM distal to the gene locus. Two mutant loci were mapped on chromosome 7R. Whereas *wa1* was co-segregating with *Xcmwg682* at the distal end of 7RL, *mp* was mapped in the centromere region of chromosome 7R between the isozyme marker *Acp2/3* and the RFLP marker *Xiag89*, with distances of 9.5 cM and 13.5 cM, respectively.

Beside the molecular maps Fig. 3 also shows the recombination frequencies calculated between the mutant loci *cb*, *mc*, *an*, *mp* and *ct3*. Whereas a frequency of 17.7% was detected between *cb* and *mc* located on chromosome 6R, the loci *an*, *mp* and *ct3* were found to build one linkage group on chromosome 7R.

Discussion

In the present paper several mutant loci were studied. For *np* and *w* mapped on chromosome 4R it can be concluded that they are located on that part of the long arm of 4R which was translocated with, and shows homoeology to, the 7S chromosomes of other Triticeae species (Devos et al. 1993). Interestingly, in wheat a GA-sensitive dwarfing gene designated *Rht9* was located on the 7BS arm of the translocated chromosome 5BS/7BS in the Italian variety 'Mara' (McIntosh et al. 1998). The height reduction was associated with a large yield reduction of about 20% (Worland et al. 1984). Because *np* was also shown to be GA-sensitive and to determine weak plants with a lower grain yield, a homoeologous relationship to *Rht9* may be suggested. In barley two recessive dwarfing genes, designated *brh1* (*brachytic 1*) and *wnd* (*winding dwarf*), are known to be located about 60 and 30 cM , respectively, distal to the centromere on chromosome 7HS (Franckowiak 1997; Lundqvist et al. 1997). All here-mentioned homoeologous group-7S dwarfing genes of the Triticeae seem not to be usable for breeding high yielding lodging-resistant varieties.

The mapping and homoeologous relationships of one of the two genes determining a lack of the wax layer on the plant (*wa1*) was already described by Korzun et al. (1997). The data described by these authors are comparable with those shown here. The gene is located on the distal part of chromosome 7RL which is translocated with respect to wheat and barley and is homoeologous to a distal region of the group-2S chromosomes (Devos et al. 1993). Homoeologous loci may be present on chromosomes 2BS (*w1*) and 2DS ($w2^l$) of wheat or 2HS (*gs1*, *gs6*, *gs8*) of barley (Korzun et al. 1997). For *w*, which was linked to the dwarfing gene *np* on chromosome 4RL, no homoeologous loci are described on wheat group-7S chromosomes. However, in barley the gene *gsh3* determining an absence of a surface wax-coating on the spike, leaf sheath and stem, is located on chromosome 7HS 6.5–12.9 cM proximal to the *brh1* locus (Franckowiak 1997; Lundqvist et al. 1997).

The mapping position of the recessive *cb* mutant distal to *Xpsr371* gives some indication that it is located on the long arm of chromosome 6R close to the centromere. Again, in barley a homoeologous locus determining an orange pigmentation of the plant and designated *rob* is described as being located on chromosome 6H close to the centromere, though on the short arm. The *rob* mutant is linked at about 2.2 cM to the uniculm locus *cul2* located on the opposite chromosome arm 6HL (Franckowiak 1997; Lundqvist et al. 1997). A linkage between *cb* and the monoculm mutant *mc* of rye was also found in the present paper. Therefore, it is very likely that the *mc* locus may be located distal to *cb* on 6RL. In wheat no

comparable gene determining the orange/brown color of the plants has been identified, although an old German variety named 'Rimpaus Brauner Bastard' ('Rimpaus brown bastard') is known to have such a phenotype. For comparative mapping studies, crosses were initiated at IPK Gatersleben using that variety.

As for the other mutants described here, synteny may be also postulated for the flower mutant *mp*, mapped in the centromere region of chromosome 7R. From the data presented it cannot be decided whether *mp* is located on the translocated Triticeae group 4L part (7RS) or on the 7L part of the 7R chromosome. However, taking into consideration that a comparable recessive mutant designated *multi-ovary 5* (*mov5*) determining the same phenotype is known to be closely linked to the hull-less seed gene *nud* on chromosome 7H in barley (Tazhin 1980), it may be concluded that *mp* is located on 7L. Further indication for the location of *mp* on homoeologous group seven comes from studies of wheat in the early Fifties. Sears (1954), in his outstanding publication 'The aneuploids of common wheat', describes the nullisomics of chromosome XI (7A) to have spikes which show considerable pistillody and which are relatively infertile.

Linked to *mp*, and most probably located on 7RS, the locus *an* determining the trait anthocyaninless was discovered. Loci, which may be homoeologous, were already described on barley chromosome 7HS near the centromere, designated *ant1* (Franckowiak 1997; Lundqvist et al. 1997), and on wheat chromosomes 7DS, 7A and 7B, designated *Rc3*, *Rc1* and *Rc2*, respectively (Mc Intosh et al. 1998). Also linked to *mp* the mutant *ct3* was found. Whether this mutant is allelic to *ct1* mapped by Plaschke et al. (1995) in the centromere region of chromosome 7R can only be speculated. Allelic test crosses are being initiated to clarify this.

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References

Börner A (1991) Genetical studies of gibberellic acid insensitivity in rye (*Secale cereale* L.). Plant Breed 106: 53–57

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 116:203–209

De Vries JN, Sybenga J (1984) Chromosomal location of 17 monogenetically inherited morphological markers in rye (*Secale cereale* L.) using the translocation tester set. Z. Pflanzenzüchtg 92: 117–139

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

Franckowiak JD (1997) Revised linkage maps for morphological markers in barley, *Hordeum vulgare*. Barley Genet Newslett 26: 9–21

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

Korzun V, Malyshev S, Voylokov A, Börner A (2001) A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, microsatellite and gene loci. Theor Appl Genet (in press)

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

Lundqvist U, Franckowiak JD, Konishi T (1997) New and revised descriptions of barley genes. Barley Genet Newslett 26: 22–516

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

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. University Extension Press, University of Saskatchewan, pp 1–236

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 Congress on Isozymes, Word Scientific Publishing Co Pte Ltd, pp 191–201

Sears ER (1954) The aneuploids of common wheat. University of Missouri Agricultural Experiment Station Research Bulletin 572

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

Smirnov VG, Sosnikhina SP (1984) Genetics of rye (in Russian). Leningrad University Press, Leningrad

Tazhin OT (1980) The linkage of the genes *mo5* and *n* in barley. Barley Genet Newslett 10: 69–72

Worland AJ, Law CN, Parker BB (1984) Alternative semi-dwarfing genes. Annual Report, Plant Breeding Institute Cambridge, 1983, pp 59–61