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RFLP-based mapping of three mutant loci in rye (*Secale cereale* L.) and their relation to homoeologous loci within the *Gramineae*

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Abstract Three mutant loci of rye determining absence of ligules (*al*), waxless plant (*wa1*) and waxy endosperm (*Wx*) characters were mapped in a single F_2 population, comprising 84 individual plants. The three loci could be clearly tagged in relation to 7 (*al* on chromosome 2R), 4 (*wa1* on chromosome 7R) or 6 (*Wx* on chromosome 4R) RFLP markers. The mapping data are compared with existing data for homoeologous regions containing equivalent mutants of wheat, barley, rice and maize. It is shown that the loci analysed are highly conserved across the cereal species, including rye.

Key words Comparative mapping · Absence of ligules · Waxless plant · Waxy endosperm · RFLP · *Secale cereale* L.

Introduction

At the present time molecular linkage maps are available for most of the *Gramineae* species. Due to the fact that restriction fragment length polymorphism (RFLP) probes allow cross-hybridisation within the small-grain cereal genomes a detailed comparison of the maps is possible. Examples of collinearity within the *Triticeae* (wheat, barley and rye) are given by Devos et al. (1993a, b) or Devos and Gale (1993) using common sets of RFLP probes. Furthermore, comparative mapping data for genomes of species belonging to different tribes within the *Poaceae* have become available recently showing substantial conservation in the order of DNA markers detected by the same probes for large segments of wheat, rice, maize or oat (Ahn and Tanksley 1993; Ahn et al. 1993; Kurata et al. 1994; Devos et al. 1994; Van Deynze et al. 1995 a, b, c).

In addition to the anonymous or known function DNA probes mainly used in comparative studies, a few examples are known where mutant genes have been incorporated. Börner et al. (1997) summarise present comparative RFLP mapping data for genes determining dwarfness (GA insensitive and GA sensitive), vernalisation response and photoperiodic response in wheat, rye and barley. Interestingly, collinearity was also determined by making comparisons between the map positions of major genes and quantitative trait loci (QTL) for flowering time in response to daylength and plant height by Laurie et al. (1995) and Bezant et al. (1996), respectively.

In this paper we describe the RFLP mapping of three rye mutant loci determining absence of ligules (*al*), waxless plant (*wa1*) and waxy endosperm (*Wx*) characters. Whereas for the *waxy* endosperm mutant locus of rye no data was available prior to our investigation, *al* (syn *el*) and *wa1* (syn *epr*) had been located using a translocation tester set (De Vries and Sybenga 1984) in combination with the linkage data of Smirnov and Sosnikhina (1981) on chromosome 2R and by trisomic analysis on chromosome 7R (Melz 1989), respectively. The rye mapping data will be compared with previously existing data for equivalent mutant loci of wheat, barley, rice and maize.

Materials and methods

Mapping population and DNA extraction

The three mutant loci were mapped in one F_2 population, which was produced by crossing the self-incompatible stock SI (ligules absent,

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waxless plant) of the Peterhof Rye Stock Genetical Collection, kindly supplied by Dr. V. G. Smirnov, St. Petersburg State University, Russia, with the self-fertile inbred line N6 of normal phenotype (ligules present, waxy plant). N6 was originally selected as a self-compatible plant from the Swedish variety 'Steel' by A. Muenzing and underwent selfing for 31 generations. It was shown to carry a single self-fertility (*Sf*) mutation located on chromosome 1R (Voylokov et al. 1994). After selfing one individual F_1 plant, 84 F_2 seeds were obtained. The F_2 plants were grown in a greenhouse to evaluate the target traits. Leaves from 5- to 6-week old seedlings were used for DNA extraction following McCouch et al. (1988). Whereas the traits absence/presence of ligules could be phenotypically determined from the early seedling stage till ripening (Fig. 1), the scoring of the character waxy/waxless plant was most easily done just before flowering. The mapping of the *waxy* endosperm locus was based on the detection of polymorphism for the functional cDNA probes pcwx27 cloned from barley and sequenced by Rhode et al. (1988) and pcSS22 isolated from hexaploid wheat by Clark et al. (1991).

RFLP analysis

The DNA of the inbred line N6 and the derived F_2 plants was digested with *Hin*dIII, *Dra*I, *Eco*RI and *Eco*RV and hybridised with selected cDNA and genomic DNA probes from various wheat (coded with PSR), barley (coded with BCD and MWG) and oat (coded with CDO) libraries according to the methods of Devos et al. (1992). The probe selection was based on the knowledge that the target genes are located on the *Triticeae* homoeologous groups 2 and 7. To enable comparisons to be made with equivalent mutants mapped in other species, we chose closely linked probes mapped in barley (Pratchett and Laurie 1994) and wheat (Van Deynze et al. 1995a). The genotypes were scored for individual plants at each locus to construct linkage maps with the MAPMAKER 2.0 computer program (Lander et al. 1987) using the Kosambi map unit function.

Results

Segregation of the mutant loci

The segregation ratios for the two characters presence/absence of ligules and waxy/waxless plants fitted the expected 3 : 1 ratio for monogenic inheritance. Whereas for the *al* mutation the observed ratio of 63 plants with ligules : 21 plants without ligules was identical with the expected proportion, the segregation ratio for *wa1* was less precise although it did not deviate significantly from the expected 3 : 1 ratio ($\chi^2 = 1.67$; $P > 0.20$). The functional probes pcwx27 and pcSS22 used for mapping the *waxy* endosperm locus detected co-dominant RFLPs which were not significantly different from the expected 1 : 2 : 1 ratio ($\chi^2 = 2.67$; $P > 0.25$).

Marker analysis and linkage mapping

Of the 24 probes tested 19 were polymorphic with at least one of the four enzymes and could therefore be mapped to chromosome 2R (7), 4R (8) and 7R (4). The segregation hybridisation patterns were scored, giving either 1 : 2 : 1 (15 loci) or 3 : 1 (*Xpsr150*, *Xps604*, *Xpsr609*, *Xpsr899*) ratios. No deviations from the expected ratios were found by the χ^2-test ($P > 0.10$).

The genetic map location of the *al* locus on the long arm of chromosome 2R is shown in Fig. 2 (left). It is closely linked to 3 distal markers (*Xpsr932*, 4.4 cM;

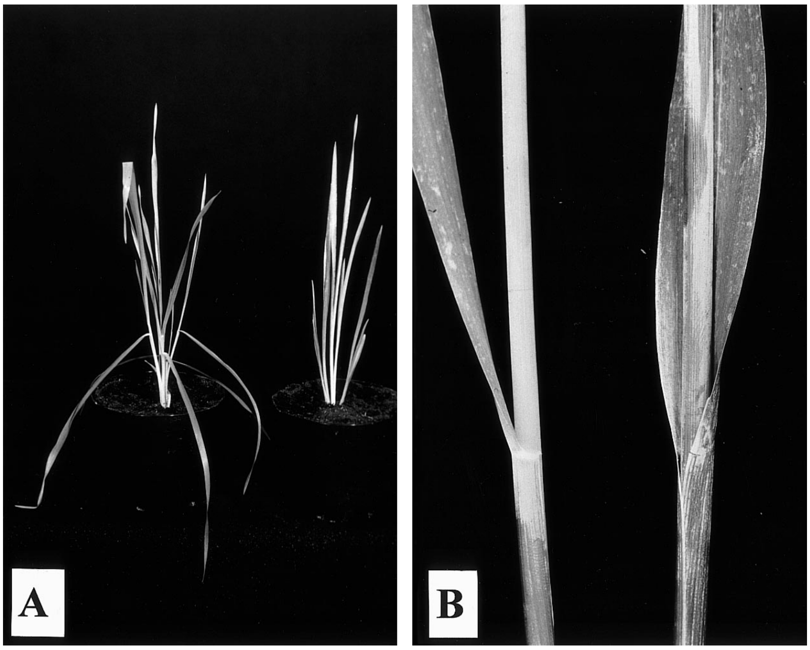


Fig. 1A, B Phenotypes of the lack of ligules mutation. **A** At the tillering stage (*left* wild type, *right* mutant), **B** leaves of adult plants (*left* wild type, *right* mutant)

Fig. 2 Partial RFLP map of rye chromosome 2R derived from the F_2 of the cross SI × N6 showing the position of the lack of ligules gene *al* and the alignment with homoeologous regions of barley, wheat, rice and maize. Genetic distances are given in centimorgans (cM).
c Centromere, *L* long arm

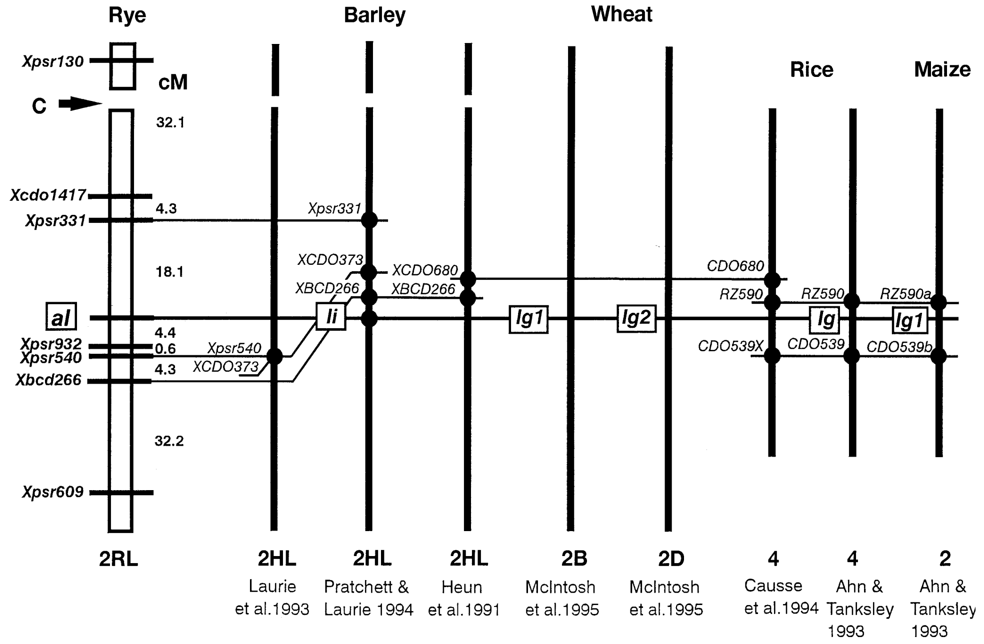
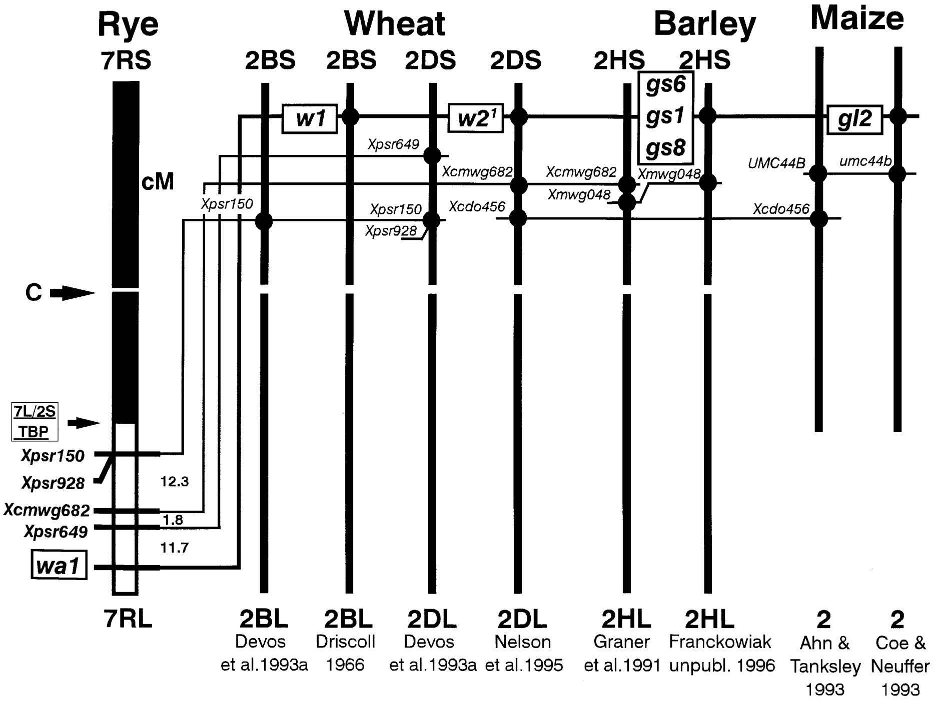


Fig. 3 Partial RFLP map of rye chromosome 7RL derived from the F_2 of the cross SI × N6 showing the position of the gene *wa1* that determines the waxless plant character and the alignment with homoeologous regions of wheat, barley and maize. Genetic distances are given in centimorgans (cM), *c* Centromere, *TBP* translocation break point, *S* and *L* indicate short and long arms, respectively



Xpsr540, 5.0 cM; *Xbcd266*, 9.3 cM), and by 18.1 cM to *Xpsr331*, which was placed proximal to *al*. In total, all seven polymorphic markers cover a total map distance of 96 cM.

The recessive *wa1* mutant determining the waxless plant character could be mapped in relation to four RFLP markers (Fig. 3, left). The three PSR markers are known to map 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 2 chromosomes (Rognli et al. 1992). No recombination was found between *Xpsr150* and *Xpsr928*. The marker *Xcmwg682* originally mapped on chromosome 2H by Graner et al. (1991) was found to be located between

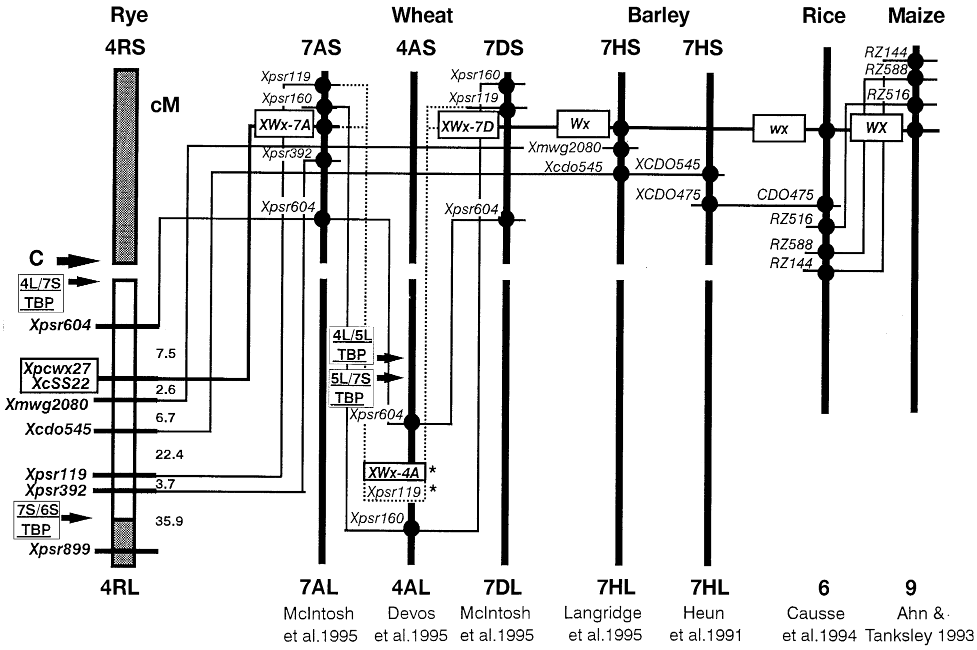


Fig. 4 Partial RFLP map of rye chromosome 4RL derived from the F_2 of the cross SI × N6 showing the position of the *waxy* endosperm locus and the alignment with homoeologous regions of wheat, barley, rice and maize. Genetic distances are given in centimorgans (cM). *c* Centromere, *TBP* translocation break point, *S* and *L* indicate short and long arms, respectively, * indicates two probes that were not mapped by Devos et al. (1995) but were assigned to 4AL by Chao et al. (1989)

Xpsr928 and *Xpsr649*. The target gene *wa1* maps in the most distal position, linked to *Xpsr649* by 11.7 cm.

The third mutant locus detected using the two probes pcwx27 and pcSS22 mapped on the long arm of chromosome 4R between *Xpsr604* and *Xmwg2080* and is tightly linked with the latter by 2.6 cM (Fig. 4, left). Five of the six markers mapped to this linkage group, including the two flanking loci, are known to be located on the wheat and barley homoeologous group 7S chromosomes. Thus, the *waxy* gene is located on a segment of chromosome 4RL which was translocated during the evolution of rye and which is homoeologous to other *Triticeae* 7S chromosomes. The most distally mapped marker *Xpsr899* is known to have homoeloci on other *Triticeae* group 6 chromosomes. Its location on chromosome 4RL is in agreement with mapping data of Devos et al. (1993b) showing that the distal end of 4RL has homoeology with the distal ends of the short arms of the wheat group 6 chromosomes.

Discussion

The evolutionary conservation of cereal chromosomes including the map positions of cross-hybridising probes and gene loci offers new approaches to genetic analysis. Gene location in one species can be carried out rapidly if information about its mapping position in related species is already known and transferable. Known translocation differences (Devos et al. 1993b; 1995) have to be considered.

Absence of ligules mutation

Mutants lacking ligule and auricle structures on all leaves have been known to occur in several cereals for many years. The map positions of the mutant loci have recently been determined to be on chromosomes 4 and 2, respectively, for rice and maize (Ahn and Tanksley 1993) and on chromosome 2HL for barley (Pratchett and Laurie 1994). The mapping data for rye presented here confirms the location of the *al* locus on chromosome 2R (De Vries and Sybenga 1984). As in barley the locus determining absence of ligules is located on the long arm of chromosome 2 (Fig. 2). Whereas in rye *al* maps proximal to *Xpsr540*, which is co-segregating with *XCDO373* (Laurie et al. 1993) and *Xbcd266*, the position of *li* is distal to *XCDO373* and *Xbcd266* in barley (Pratchett and Laurie 1994). At this stage it cannot be determined, if this difference is due to intrachromosomal rearrangements of this segment of the homoeologous group 2 chromosomes in barley and rye or whether it is due to the relatively small mapping populations used in both experiments. However, it is likely that *al* of rye and *li* of barley are the mutations of a common gene.

In wheat, two genes for the lack of ligules (*lg1* and *lg2*) are known on chromosomes 2B and 2D, respectively (McIntosh et al. 1995). Although no molecular mapping is available for either mutant it is suggested that rye, barley and wheat contain homoeoalleles determining this morphological trait. When two probes from chromosome 2H of the RFLP map of Heun et al. (1991) were used as a bridge, a connection to equivalent chromosome regions of rice (Causse et al. 1994; Ahn and Tanksley 1993) and maize (Ahn and Tanksley 1993) could be constructed, suggesting that liguleless mutants across the grasses are due to lesions of a conserved gene.

Waxless plant mutation

As expected, the locus determining the waxless plant character was mapped on the translocated part of the long arm of chromosome 7R, which is homoeologous to other *Triticeae* group 2S chromosomes. As described by Rognli et al. (1992) for the Ds2 × RxL10 mapping population and in the plant material studied here the orientation of the translocated segment relative to the centromere, which comprised only the distal portion of 2S, is consistent with the wheat homoeologous group 2S map described by Devos et al. (1993a).

From Fig. 3 it can be suggested that the *wa1* locus of rye is homoeoallelic to the glaucousness (waxiness) loci *w1* or *w2*^{1} of wheat. The locus *w2*^{1} has been mapped in relation to *Xcmwg682* at a distance of less than 20 cM by Nelson et al. (1995). *Xcmwg682* was also used in the SI × N6 cross. For *w1* no molecular mapping data is available, but Driscoll (1966) described an inhibitor of wax production (*w1*) on chromosome 2BL at a distance of at least 42 crossover units (independent) from the centromere.

In barley nine genes/alleles for glossy sheath/spike are reported to be located on five different chromosomes (Søgaard and von Wettstein-Knowles 1987). Three genes/alleles (*gs1*, *gs6*, *gs8*) are located on the short arm of chromosome 2H, linked to *Xmwg048* (Franckowiak unpublished). The RFLP marker *Xmwg048* was shown to be linked to *Xcmwg682* by Graner et al. (1991). A homoeoallelic relationship between rye *wa1* and barley *gs1*/*gs6*/*gs8* can therefore be suggested.

Interestingly, maize chromosome 2 also carries a gene, *gl2*, that alters cuticle wax and is linked to *umc44b* (Coe and Neuffer 1993). The utilisation of the mapping data of Ahn and Tanksley (1993) allows the maize *gl2* region to be aligned with the *w2*^{1} on chromosome 2DS of wheat (Nelson et al. 1995).

Waxy endosperm mutation

The term waxy endosperm was created by Collins (1909) to describe a new type of Indian corn from China that has an endosperm with an optical resemblance to wax. Today, the *waxy* locus has been cloned from a number of plants, including maize (Shure et al. 1983), rice (Wang et al. 1990), barley (Rhode et al. 1988) and wheat (Clark et al. 1991). Because no comparable mutants are known for rye, two functional probes isolated from wheat and barley, respectively, were used to detect the position of the *waxy* locus in rye. As can be seen in Fig. 4 the *waxy* locus could be detected on that part of chromosome 4R which show homoeology to the short arms of chromosomes 7AS and 7DS of wheat. The order and orientation relative to the centromere of the markers on the rye segment is consistent with the comparable wheat arms. The third *waxy* locus of wheat was located on the long arm of chromosome 4A utilising aneuploid genetic stocks (Chao et al. 1989); however this locus has not been mapped in relation to comparable markers. As at least three translocations involving chromosome 4A are known (Liu et al. 1992; Devos et al. 1995) homoeoallelism can again be suggested.

By using the barley probe MWG2080 for rye mapping we were able to compare our data to the data of Langridge et al. (1995): a close linkage between that particular probe with the *Wx* locus in barley was observed. Furthermore, an alignment to the *waxy* gene containing regions of the maize chromosome 6 and rice chromosome 9 is possible (Devos et al. 1994). It could be suggested that the gene controlling the *waxy* endosperm character is highly conserved across the cereal species, including rye.

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