Theor Appl Genet (2005) 110: 1011–1019
DOI 10.1007/s00122-004-1916-1

ORIGINAL PAPER

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Chromosomal rearrangements differentiating the ryegrass genome from the Triticeae, oat, and rice genomes using common heterologous RFLP probes

Received: 13 October 2004 / Accepted: 20 December 2004 / Published online: 2 March 2005

Abstract An restriction fragment length polymorphism (RFLP)-based genetic map of ryegrass (*Lolium*) was constructed for comparative mapping with other Poaceae species using heterologous anchor probes. The genetic map contained 120 RFLP markers from cDNA clones of barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), and rice (*Oryza sativa* L.), covering 664 cM on seven linkage groups (LGs). The genome comparisons of ryegrass relative to the Triticeae, oat, and rice extended the syntenic relationships among the species. Seven ryegrass linkage groups were represented by 10 syntenic segments of Triticeae chromosomes, 12 syntenic segments of oat chromosomes, or 16 syntenic segments of rice chromosomes, suggesting that the ryegrass genome has a high degree of genome conservation relative to the Triticeae, oat, and rice. Furthermore, we found ten large-scale chromosomal rearrangements that characterize the ryegrass genome. In detail, a chromosomal rearrangement was observed on ryegrass LG4 relative to the Triticeae, four rearrangements on ryegrass LGs2, 4, 5, and 6 relative to oat, and five rearrangements on ryegrass LGs1, 2, 4, 5, and 7 relative to rice. Of these, seven chromosomal rearrangements are reported for the first time in this study. The extended comparative relationships reported in this study facilitate the transfer of genetic knowledge from well-studied major cereal crops to ryegrass.

Communicated by J.S. Heslop-Harrison

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Introduction

Among the members of the genus *Lolium*, perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*L. multiflorum* L.) are the two primary species cultivated throughout the world. Perennial ryegrass is one of the major grass species used for turf and forage, and Italian ryegrass is cultivated for hay and silage and for rapidly establishing winter overseeding of warm-season turf. Ryegrasses are taxonomically related to many important cereal crops in the plant family Poaceae, which contains some 10,000 species, including rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), oat (*Avena sativa* L.), and sorghum (*Sorghum bicolor* L.). Ryegrasses are outcrossing, self-incompatible diploids ($2n = 2x = 14$) with relatively large genomes (approx. 2,000 Mb/haploid genome; http://www.rbgkew.org.uk/cval/homepage.html).

Comparative mapping analysis using a common set of heterologous restriction fragment length polymorphism (RFLP) probes in plants has revealed a remarkable conservation in gene content and order among related plant species over millions of years of evolution. For example, the genomes of tomato (*Lycopersicum esculentum* L.) and potato (*Solanum tuberosum* L.) show a high level of conserved synteny and colinearity (Bonierbale et al. 1988; Tanksley et al. 1992). The genome relationships among Poaceae species have been extensively studied, and conservation of gene content and order has been reported between maize and sorghum (Hulbert et al. 1990; Whitkus et al. 1992), rice (Ahn and Tanksley 1993), and wheat (Devos et al. 1994); wheat and rice (Kurata et al. 1994), rye (Devos et al. 1993), and barley (Namuth et al. 1994; Dubcovsky et al. 1996); wheat, oat, rice, and maize (Van Deynze et al. 1995b, c).

Recently, with the extensive public genomic and expressed sequence tag (EST) sequencing information of grass species, DNA sequence-based comparative mapping studies have been initiated. La Rota and

Sorrells (2004) aligned a large number of mapped wheat ESTs with the rice genome sequence for comparative DNA sequence analysis. They presented evidence of small-scale chromosomal rearrangements that had not been identified in the previous RFLP-based comparative maps. Furthermore, Francki et al. (2004) supported the rearrangements, showing detailed syntenic relationships between wheat homoeologous groups 3S, 7L and the rice genome.

However, the molecular marker-based genetic linkage mapping and the comparative mapping of *Lolium* species have not been studied in depth. The first molecular genetic linkage map of ryegrasses was developed from a population produced by crossing a perennial ryegrass × Italian ryegrass interspecific hybrid and an artificial homozygote produced through anther culture (Hayward et al. 1994, 1998). The map contained 106 loci, including restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), and a number of isozyme markers, and had a map length of 692 cM. A subsequent ryegrass genetic linkage map was developed based on the p150/112 reference mapping population developed from a cross between a multiple heterozygote from complex descent and an artificial homozygote of perennial ryegrass (Bert et al. 1999). The genetic linkage map by Bert et al. (1999) contained 463 amplified fragment length polymorphism (AFLP) markers and covered a distance of 930 cM. The genetic map of Jones et al. (2002) enhanced the reference map of Bert et al. (1999), covering 811 cM. The latter also established the comparative relationships between perennial ryegrass and other Poaceae species genomes such as wheat, oat, and rice. Recently, female (MFA-4) and male (MFB-2) genetic linkage maps of ryegrass, based on an interspecific population that was derived by crossing perennial ryegrass and Italian ryegrass, have been constructed using AFLP, SSR, RAPD, isozyme, and morphological markers as well as 16 heterologous RFLPs (Warnke et al. 2004). This interspecific mapping population structure is advantageous for constructing genetic maps using heterologous probes because of a higher level of polymorphisms detected by fewer restriction endonucleases.

The objectives of the present study were to enhance the genetic linkage map of the MFA × MFB population and to extend the existing comparative relationships of ryegrass with other Poaceae species via genetically anchored heterologous RFLP markers. This paper presents the detailed genome comparisons between ryegrass and the Triticeae, oat, and rice by providing enhanced syntenic relationships as well as new evidence of large-scale chromosomal rearrangements.

Materials and methods

Plant material

A three-generation interspecific population that was previously used to develop female (MFA-4) and male (MFB-2) genetic linkage maps using AFLP, SSR (simple sequence repeat), RAPD, RFLP, isozyme, and morphological markers (Warnke et al. 2004) was used in this study. In brief, two Italian ryegrass (*Lolium multiflorum* L.) clones from the variety *Floregon* were crossed with two perennial ryegrass (*L. perenne* L.) clones from var. *Manhattan*. Two interspecific F_1 plants (MFA-4 and MFB-2) were randomly chosen from the resultant F_1 populations. MFA-4 as the female parent was crossed with MFB-2 as the male parent to develop the genetic mapping population consisting of 156 progeny. This population was provided by R.E. Barker at USDA-ARS, Corvallis, Oregon, USA.

RFLP analysis

Genomic DNA was extracted from 500 mg of lyophilized leaf tissues harvested from greenhouse-grown plants using a modified cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). For Southern blotting, 10 µg of genomic DNA from each plant was digested with five different restriction enzymes, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, and *Hind*III (Promega, Madison, Wis.). The digested DNAs were run on 1% (w/v) agarose gels and transferred onto Hybond-N+ nylon membranes (Amersham, Piscataway, N.J.) by capillary transfer. Prehybridization and hybridization were performed in a rotary hybridization chamber (Techne, Princeton, N.J.) at 65°C. The probes were labeled with [^{32} P] by the random priming method. The membranes were washed in 0.5× SSC and 0.1% sodium dodecyl sulphate for 50 min at 65°C.

The heterologous probes used to construct the genetic linkage map of ryegrass consisted of a common set of 152 anchor probes (CDO: oat cDNA; BCD: barley

Table 1 Summary of heterologous RFLP probes screened and mapped in the MFA×MFB ryegrass population

Probe class	Probe source	Number of probes screened	Number of loci scored	Number of loci mapped[a]
BCD	Barley cDNA	65	32	27
CDO	Oat cDNA	112	89	84
RZ	Rice cDNA	34	10	9
Total		211	131	120

[a] Indicates the number of RFLP loci mapped in Fig. 1.

cDNA; RZ: rice cDNA) from Cornell University, Ithaca, New York (Van Deynze et al. 1998) (Table 1). Additional CDO and BCD clones were selected from the USDA probe depository (Albany, Calif.) according to their known map position in respective species. All of the probes were initially screened to detect polymorphism using the heterozygous mapping parents (MFA-4 and MFB-2). Polymorphic probes with a simple segregation pattern (mostly one locus and rarely two loci) and with a strong signal were selected and tested on a progeny set of up to 89 randomly selected individuals.

Genetic map construction and comparative mapping

Two genetic maps from each parent (MFA-4 and MFB-2) were previously constructed using 235 AFLP, 81 RAPD, 160 SSR, two isozyme, 16 RFLP, and two morphological markers (Warnke et al. 2004). In the current study, additional 104 RFLP markers were collected. Most of the RFLP markers were one of two segregation types [locus heterozygous in both parents (four alleles) and locus heterozygous in only one parent]. The RFLP markers were included to map along with previously published marker data to show the relative locations of them and integrate the two parent maps.

The linkage groups (LGs) from MFA-4 were determined with an logarithm of odds ratio (LOD) threshold of 6.0. The determination of linkage groups from MFB-2 was done with an LOD threshold of 7.0 with the exception of LGs5 and 6 where the LOD threshold of 6.0 was used. The LOD threshold scores were adjusted in order to retain the highest number of RFLP markers in each linkage group. Individual MFA-4 and MFB-2 maps were independently constructed and then integrated based on co-dominant RFLP markers using JOINMAP V. 3.0 (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl). Map distances were calculated using the Kosambi mapping function (Kosambi 1944).

For the comparative mapping study, a genetic linkage map in ryegrass was reconstructed using only RFLP markers because of their co-dominant nature and robustness. The linkage groups were determined with an LOD threshold of 5.0 with the exception of LGs2 and 6, for which the LOD threshold of 3.0 and 4.0 were used, respectively. Comparative mapping with the Triticeae was conducted using information from GrainGenes (http://www.wheat.pw.usda.gov/ggpages/maps.shtml) and comparison with the consensus maps of wheat homoeologous chromosomes 1, 2, 3, and 6 (Nelson et al. 1995a, b; Van Deynze et al. 1995a; Marino et al. 1996). A Triticeae consensus map, which was developed using a common set of RFLP markers mapped in wheat, barley, and rye, was used for genome comparison with ryegrass in this study. Comparative mapping with oat was conducted through comparison with the oat map of Van Denyze et al. (1995b) and data from the GrainGenes website. Comparative mapping with rice was conducted using data from Gramene (http://www.gramene.org/cmap) and comparison with the rice map of Ahn and Tanksley (1993). In addition, we used BLAST-X analysis at NCBI's web server (http://www.ncbi.nlm.nih.gov/BLAST) to determine the chromosome locations of the RFLP markers without known genetic map information in rice. A syntenic chromosomal segment between two species was defined if a segment containing more than three syntenic loci was not interrupted by more than two non-syntenic loci or a segment containing two syntenic loci was not interrupted by a non-syntenic locus. The number of syntenic loci in the syntenic chromosomal segments ranged from 2 to 19 for the Triticeae relationship, 2 to 11 for the oat relationship, and 2 to 11 for the rice relationship.

Results

Genetic map construction

A total of 211 heterologous probes from barley, oat, and rice were initially screened to detect RFLP between the two parents, MFA-4 and MFB-2 (Table 1). The probes showed significant variation with respect to the intensity and clarity of the hybridization signal as well as copy numbers (data not shown). One hundred and fifty-two probes showing clear hybridization signals and scorable polymorphisms were selected and tested with a progeny set of up to 89 individuals. Of these, a total of 131 loci were successfully scored (32 BCD, 89 CDO, and 10 RZ). Sixteen of these loci along with AFLP, RAPD, SSR, isozyme, and morphological markers were previously mapped by Warnke et al. (2004). In this study, an additional 104 RFLP loci were collected and mapped on the previous map of Warnke et al. (2004) (Table 1, Fig. 1). The number of RFLP loci per linkage group ranged from 11 on LG6 to 26 on LG4. Our integrated genetic linkage map covered a total map distance of 664 cM, with a range of 56 to 127 cM per linkage group.

The current ryegrass genetic map has four probes, CDO105, CDO395, CDO580, and CDO590, that generated duplicated loci. The two loci generated from CDO105 and CDO590 were mapped on the same linkage group: LG1 for CDO105 loci at 25 cM apart, and LG5 for CDO590 loci at 19 cM apart. In contrast, the two loci from CDO580 were mapped on LGs1 and 7, respectively. Although two loci were identified using the CDO395 probe, only one of them was mapped on ryegrass LG2 while the other locus currently remains unlinked.

Comparative genome relationships between ryegrass, the Triticeae, oat, and rice

A genetic linkage map reconstructed using only RFLP markers was used for comparative mapping. This map

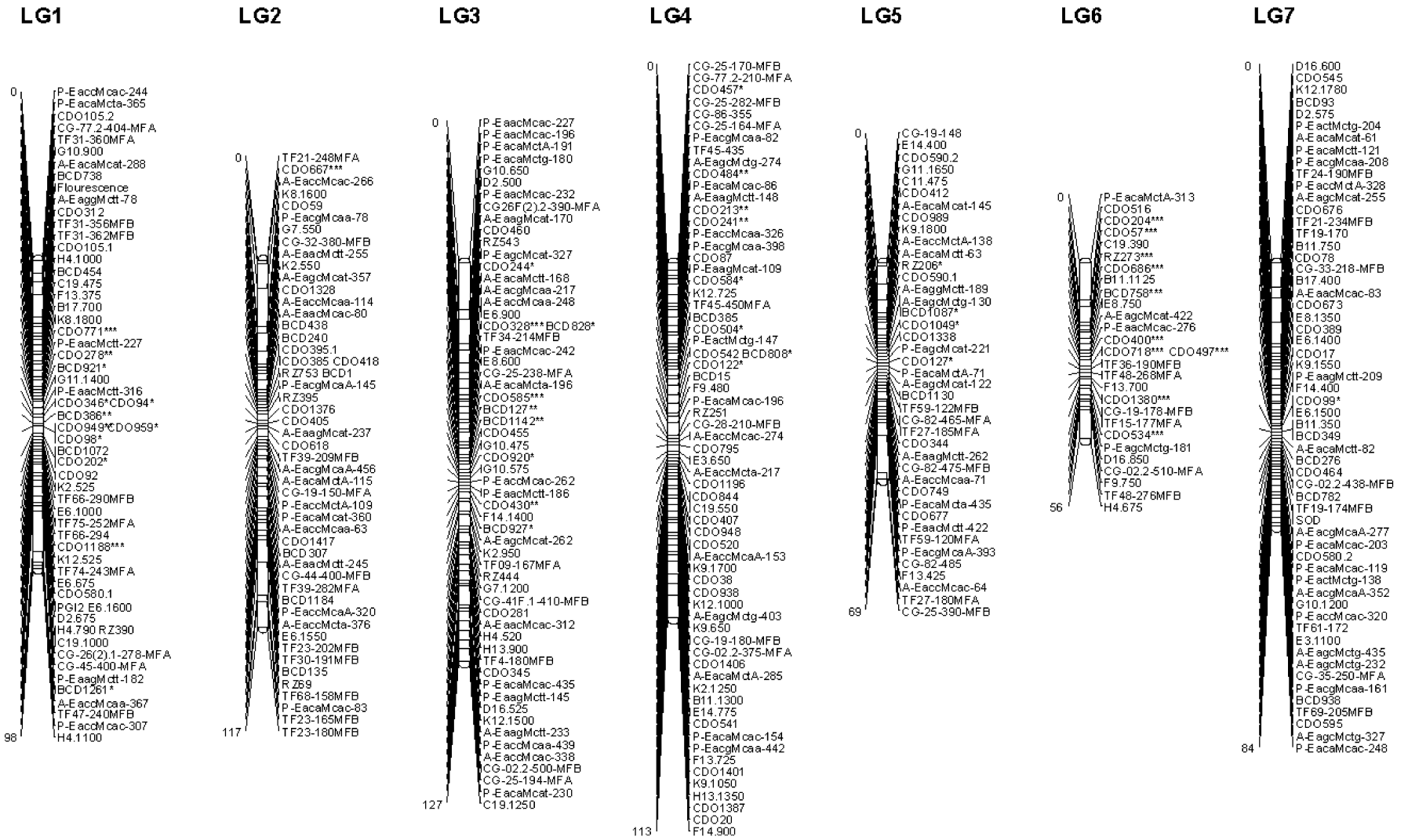

Fig. 1 An integrated genetic linkage map of the MFA×MFB ryegrass population derived from each parental map using RFLP markers. The *numbers* shown on the *left* of the linkage groups represent the map distances in centiMorgans based on the Kosambi mapping function. Locus names are listed to the *right* of the linkage groups. Duplicated RFLP loci are indicated by *x.1* (probe name + .1) and *x.2* (e.g., CDO1196.1 and CDO1196.2). The naming of the AFLP, RAPD, and SSR markers is described in Warnke et al. (2004). The RFLP markers showing distorted segregations are indicated by * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$)

covers a total map distance of 573 cM with a total of 123 loci, including 16 loci previously reported in Warnke et al. (2004). Three additional RFLP markers (CDO36 on LG3, BCD880 and BCD1860 on LG6), which were not mapped in Fig. 1, were included in the map. Of 123 loci, 112 were in common with the Triticeae consensus linkage map, 82 with the oat linkage map, and 108 with the rice linkage map (Table 2). Based on the sufficient number of common RFLP loci, we developed detailed genome relationships between ryegrass and the Triticeae, oat, and rice.

Triticeae

The 112 loci with known map locations in the Triticeae were evenly distributed on seven linkage groups of ryegrass. Ten chromosomal segments of the Triticeae represented all of the ryegrass linkage groups, indicating a high degree of syntenic relationship between the two species (Table 2, Fig. 2). With the exception of a few non-syntenic loci, three ryegrass linkage groups 1, 3, and 5 showed almost complete synteny with the corresponding homoeologous chromosomes of the Triticeae.

We detected a large-scale chromosomal rearrangement on ryegrass LG4 relative to Triticeae chromosomes 4 and 5 that was not reported in a previous study (Jones et al. 2002) and that can differentiate the ryegrass genome from the Triticeae genome (Fig. 2). In detail, ryegrass LG4 was represented by two Triticeae chromosomal segments. One segment from Triticeae chromosome 4 covered the region of ryegrass LG4, spanning from CDO457 to CDO504. The other region between CDO542 and CDO20 was represented by a segment of Triticeae chromosome 5. Furthermore, we found the possibility of large-scale chromosomal rearrangements in ryegrass LGs 2, 6 and 7. Although most loci on ryegrass LG2 were present on Triticeae chromosome 2, two non-syntenic loci, RZ395 and BCD1, were located on Triticeae chromosome 6. Similarly, ryegrass LG6 has two non-syntenic loci that are on Triticeae chromosome 5 separated by two non-syntenic loci, CDO400 and CDO718. In ryegrass LG7, two non-syntenic loci were present on Triticeae chromosome 3, and two distinct chromosomal segments of Triticeae chromosome 7 were separated by non-syntenic loci, BCD276, BCD782, and CDO673 on Triticeae chromosomes 6, 3, and 1, respectively.

Table 2 Genome comparisons between ryegrass and the Triticeae, oat, and rice based on common heterologous RFLP makers

Species	Number of common loci	Number of conserved syntenic segments	Number of chromosomal rearrangements
Triticeae	112	10	1
Oat	82	12	4
Rice	108	16	5

Oat

The ryegrass map contains 82 loci that have known map locations in diploid oat and well-covered ryegrass LGs. Comparative mapping between ryegrass and oat also revealed a high degree of synteny. Most regions of the ryegrass LGs were represented by 12 segments of seven oat chromosomes (Table 2, Fig. 3). In detail, ryegrass LGs1 and 7 showed almost complete syntenic relationships to oat chromosomes A and D, respectively. This is the first report of the syntenic relationship of ryegrass LG7 to oat chromosome D. Although ryegrass LG3 was represented by a segment of oat chromosome C, two loci, CDO455 and CDO345, indicated that the rearrangements of a segment of oat chromosome G might be involved in the relationship. In addition, we found that ryegrass LGs2, 4, 5, and 6 were explained by large-scale chromosomal rearrangements of oat chromosomes that differentiate the ryegrass genome from the oat genome (Fig. 3). Among them, the large-scale chromosomal rearrangements observed on ryegrass LGs2, 5 and 6 are originally reported in this study. In detail, ryegrass LG2 was represented by two segments from oat chromosomes B and C. Similarly, two segments that are derived from oat chromosomes E and F, respectively, represented ryegrass LG5. In ryegrass LG6, two distinct segments of oat chromosome G as well as a segment of oat chromosome E are involved to explain the relationship. Moreover, ryegrass LG6 has four non-syntenic loci, CDO534, CDO718, CDO686, and CDO57, indicating the possibility of more chromosomal rearrangements.

Rice

The 108 loci that are evenly distributed on ryegrass linkage groups were used for comparative mapping between ryegrass and rice. As shown in the Triticeae and oat relationships, a high degree of syntenic relationship was also observed between rice and ryegrass. Sixteen segments that are derived from all of the rice chromosomes, except for rice chromosome 12, explain the ryegrass linkage groups (Table 2, Fig. 4). At the current resolution, only CDO677 on ryegrass LG5 represents rice chromosome 12. In comparison with the rice genome, the most conserved syntenic relationships were observed on ryegrass LGs3 and 6 that were represented by rice chromosomes 1 and 2, respectively. In contrast to these two linkage groups, the other five ryegrass linkage groups (1, 2, 4, 5, and 7) showed large-scale chromosomal rearrangements. Three of these (1, 4, and 5) are reported for the first time in this study: (1) LG1 is represented by the insertion of a segment of rice chromosome 10 between two distinct segments of rice chromosome 5; (2) LG4 is represented by the insertion of a segment of rice chromosome 11 between two distinct segments of chromosome 3; (3) LG5 is represented by the insertion of a segment of rice chromosome 11 between two distinct segments of chromosome 9. Ryegrass

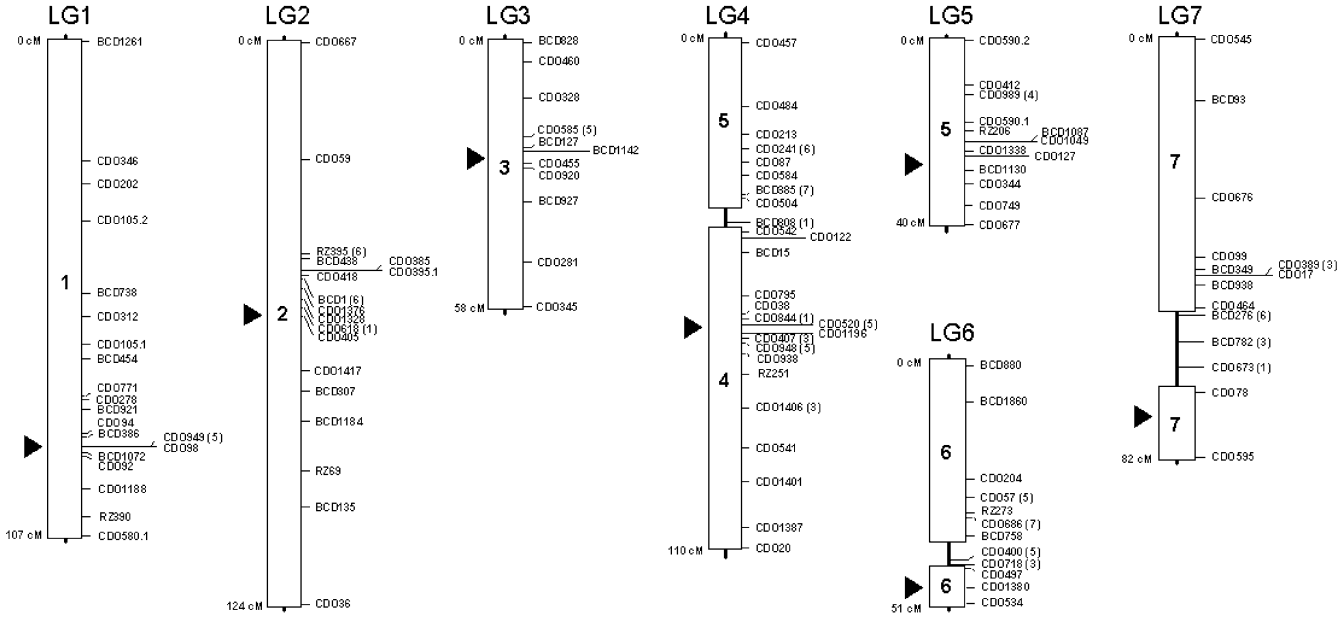

Fig. 2 Comparative relationship between ryegrass and the Triticeae genomes. Each *bar* represents each ryegrass linkage group of the RFLP-based genetic map. The *numbered boxes* indicate the conserved syntenic segments of Triticeae chromosomes. The position of non-syntenic markers is indicated *in parenthesis following* the marker names. The *arrowheads* indicate proposed locations of the centromere in ryegrass as deduced from comparisons with Triticeae chromosomes

LG4 shows the possibility of an additional insertion of rice chromosome 7 because it has two loci 5 cM apart—CDO38 and CDO407—that have been mapped on rice chromosome 7.

Discussion

Comparative mapping is a strategy to efficiently utilize the information given by well-studied model species. The studies of comparative mapping have focused on the economically important cereal crops. Although ryegrass is a major grass species for forage and turf and a member of the Poeae tribe of the subfamily Pooideae, relatively little comparative mapping of ryegrass has been done to date. Therefore, our study was conducted to extend the existing comparative mapping information of ryegrass using the MFA×MFB population derived from the cross between perennial ryegrass (*L. perenne* L.) and Italian ryegrass (*L. multiflorum* L.).

We first integrated two ryegrass linkage maps of the MFA×MFB population that were previously developed (Warnke et al. 2004) by adding a number of RFLP markers (Fig. 1). The total map length (664 cM) of the integrated map is similar to the 692 cM map reported by Hayward et al. (1998) but is smaller than the 930 cM and 811 cM maps reported by Bert et al. (1999) and Jones et al. (2002), respectively. In the present study, markers were grouped and ordered using the JOINMAP program, but Bert et al. (1999) and Jones et al. (2002) used JOINMAP to determine linkage groups and then ordered the markers using the MAPMAKER program (Lander et al. 1987). All of the studies used the Kosambi mapping function to calculate map distance. Van Ooijen et al. (1994) and Castiglioni et al. (1998) indicated that the MAPMAKER program tends to generate genetic maps with longer lengths than the JOINMAP program. Therefore, the discrepancy in map lengths is most likely due to the use of different mapping programs in each study.

In the current ryegrass genetic map, a small number of probes (CDO105, CDO395, CDO580, and CDO590) produced duplicated loci, which were mapped on the same or different linkage groups. The production of duplicate loci from seven probes was also reported by Jones et al. (2002). The low frequency of duplicated loci that we observed may be mainly due to a bias in selecting probes that only produced a simple banding pattern from the parental screening step and partly due to the selection criteria originally used in the development of the 152 anchor probes (Van Deynze et al. 1998). In fact, a higher number of probes that might generate more than two loci were observed (unpublished data). Interestingly, two of four duplicated loci in the present map, CDO395 and CDO580, were reported to be a single locus by Jones et al. (2002). In contrast, BCD1072, CDO38, CDO328, and CDO542 were reported to be duplicated loci in the map of Jones et al. (2002), but as a single locus in the present map. One possible explanation for the discrepancy is that all of the loci would be multiple loci and either the study of Jones et al. (2002) or our current study detected only one locus due to the use of different restriction enzymes.

Ryegrass has high taxonomic affinity to oat and the Triticeae because it is also a member of the subfamily

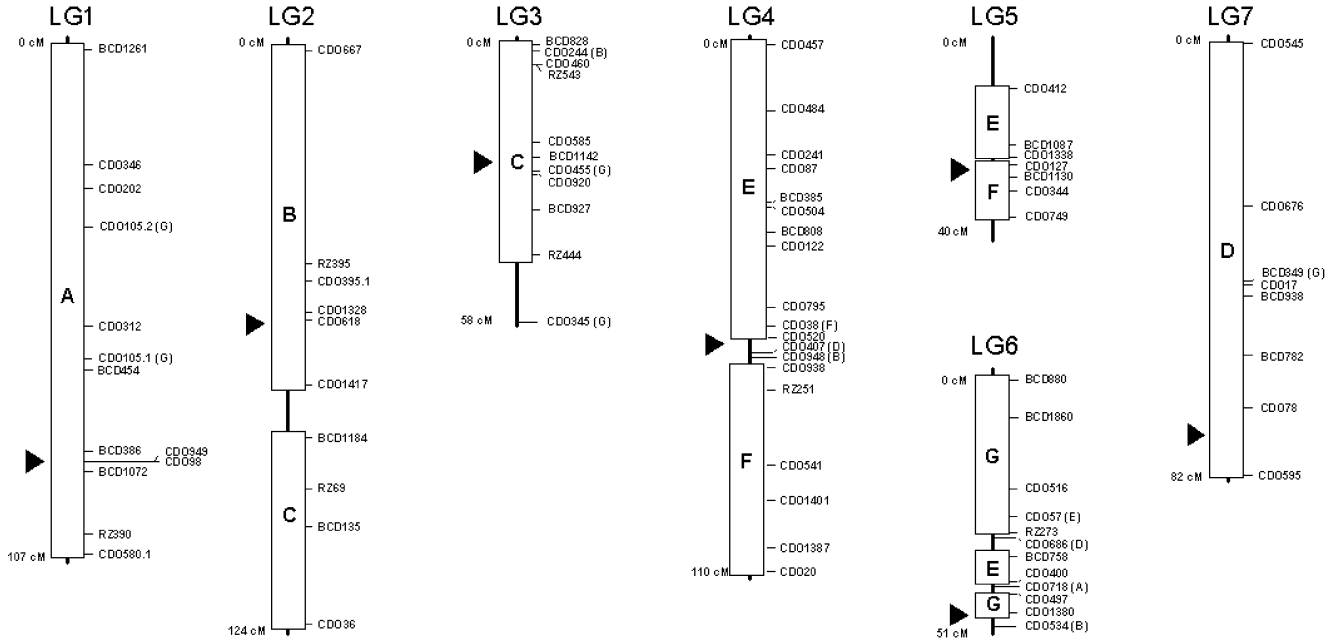

Fig. 3 Comparative relationship between ryegrass and oat genomes. Each *bar* represents each ryegrass linkage group of the RFLP-based genetic map. The *boxes with capital letters* indicate the conserved syntenic segments of oat chromosomes. The position of non-syntenic markers is indicated *in parenthesis following* the marker names. The *arrowheads* indicate proposed locations of the centromere in ryegrass as deduced from comparisons with Triticeae chromosomes

Pooideae within the Poaceae family (Soreng and Davis 1998). Ryegrass is also closely related to rice even though rice belongs to a different subfamily, Oryzoideae. In addition, previous comparative mapping studies of the Poaceae species have revealed a remarkable synteny among species. Therefore, we would expect a high level of synteny between ryegrass, the Triticeae, oat, and rice as well as large-scale chromosomal rearrangements that differentiate the ryegrass genome from other species' genomes. In fact, we found that the ryegrass genome has significant syntenic relationships to all of the other grass species used in this study. Furthermore, unique chromosomal rearrangements as well as common evolutionary chromosomal rearrangements between ryegrass and the other species were observed. At the current resolution of comparative mapping, we found a total of ten large-scale chromosomal rearrangements on seven ryegrass linkage groups relative to the Triticeae, oat, and rice. However, several non-syntenic loci suggest the existence of the additional chromosomal rearrangements on ryegrass linkage groups, especially on LGs2, 6, and 7 in the Triticeae relationship, LGs3 and 6 in the oat relationship, and LG4 in the rice relationship. The study of Jones et al. (2002) supports the additional chromosomal rearrangements on LGs3 and 6 in the oat relationship. They found a chromosomal rearrangement explained by two segments from oat chromosomes C and G for LG3 as well as another explained by two segments from oat chromosomes D and G.

Gale and Devos (1997) reported three evolutionary chromosomal rearrangements differentiating between rice and the Triticeae: rice chromosome 10 (R10) is inserted into R5 to form the Triticeae chromosome 1, R7 into R4 to form the Triticeae chromosome 2, and R8 into R6 to form the Triticeae chromosome 7. Of these, the corresponding chromosomes of all studied Pooideae species, including oat (Van Deynze et al. 1995b) and meadow fescue (*Festuca pratensis*) (Alm et al. 2003), show the R5-R10-R5 and R6-R8-R6 chromosomal rearrangements. Jones et al. (2002) detected strong evidence for the R6-R8-R6 rearrangement, but the R5-R10-R5 arrangement was claimed by one syntenic locus mapped on R10. In this study, we observed that ryegrass LGs1 and 7 are strongly represented by these chromosomal rearrangements in rice (Fig. 4). Therefore, these chromosomal rearrangements appear to characterize the subfamily Pooideae. Furthermore, it suggests that genome rearrangements derived from a common ancestor would be maintained among the species over the long evolutionary process. The other chromosomal rearrangement explained by R4-R7-R4 is not common in all Pooideae species. Oat and meadow fescue, which are species of the supertribe Poodae within subfamily Pooideae, show R7-R4 rearrangements in their genomes (Van Deynze et al. 1995b; Alm et al. 2003). In ryegrass, which also belongs to the Poodae, both the study of Jones et al. (2002) and our study found evidence of R7-R4 rearrangements on LG2, suggesting that these chromosomal rearrangements appear to differentiate the supertribe Poodae from Triticodae.

Ryegrass LG6 has the most disturbed and inconsistent syntenic relationship with oat chromosomes. Jones et al. (2002) found that ryegrass LG6 has a

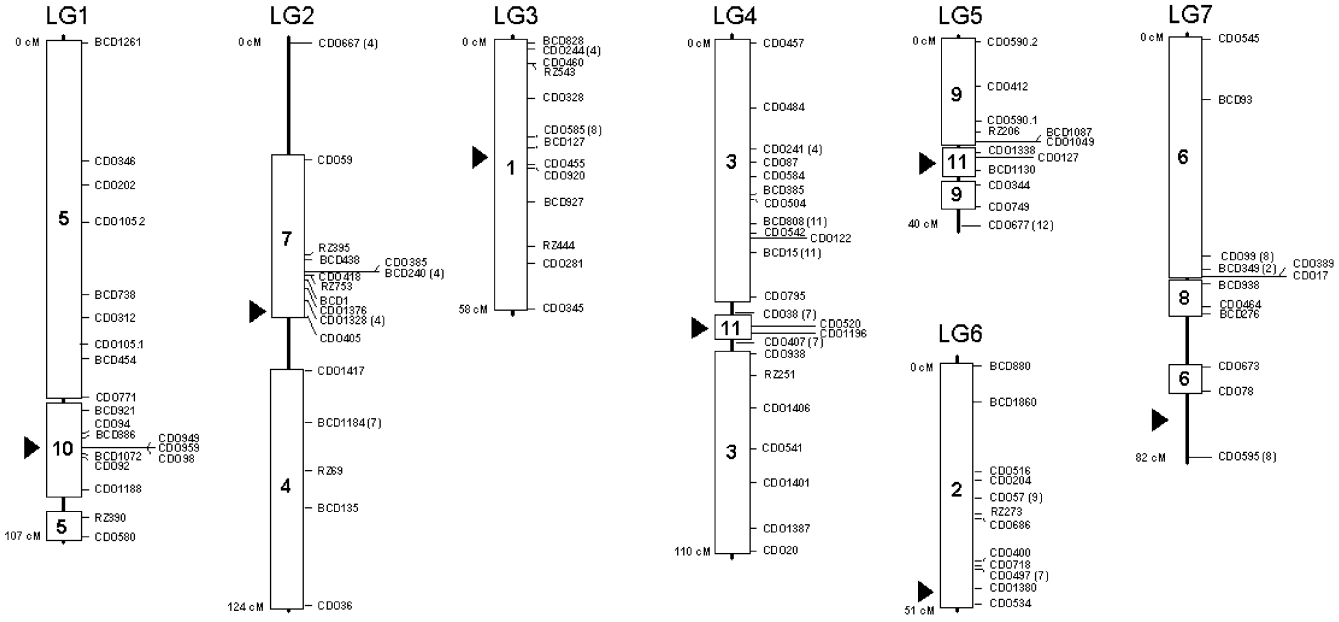

Fig. 4 Comparative relationship between ryegrass and rice genomes. Each *bar* represents each ryegrass linkage group of the RFLP-based genetic map. The *numbered boxes* indicate the conserved syntenic segments of rice chromosomes. The position of non-syntenic markers is indicated *in parenthesis following* the marker names. The *arrowheads* indicate proposed locations of the centromere in ryegrass as deduced from comparisons with Triticeae chromosomes

syntenic relationship with oat chromosomes D and G rather than the expected relationship with oat chromosomes A, B, and G. Similarly, our study indicated that ryegrass LG6 shows synteny with oat chromosomes E and G, although there are non-syntenic markers with oat chromosomes A, B, and D. These discrepancies may be due to an insufficient number of markers mapped on LG6 in both studies. The resolution of the linkage groups needs to be improved by adding more informative common markers in order to better understand how this linkage group is rearranged relative to oat chromosomes.

In the previous genetic linkage maps of the MFA×MFB population (Warnke et al. 2004), clusters of markers (mostly AFLP) with distorted segregation were present on LG6 in both of the parents' maps, especially on LG6 of the male map which showed 90% of the markers having distorted segregation. In the present study, over 90% of additionally mapped RFLP markers on LG6 also showed distorted segregation. However, clusters of markers with distorted segregation ratios on LG6 were not observed by Jones et al. (2002) and Hayward et al. (1998). Furthermore, the cause of the distorted segregation could not be explained by the known Z and S self-incompatibility loci that were mapped to LGs1 and 2, respectively (Thorogood et al. 2002). Therefore, significant recombination suppression observed on LG6 is most likely due to a biological effect in our particular cross.

We were able to extend the existing comparative information of the ryegrass genome relative to the Triticeae, oat, and rice genomes by providing new information on the conserved syntenic relationships as well as strong evidence of large-scale chromosomal rearrangements. Although the identification of small-scale chromosomal rearrangements at the DNA sequence level remains necessary to establish a high resolution of comparative map between ryegrass and other grass species, the present comparative genome relationships established in this study will provide a basis for transferring genetic information from well-characterized grass species to ryegrass for the identification of markers and candidate genes of agronomic importance at the molecular marker level.

Acknowledgements We thank Dr. S. McCouch of Cornell University, Ithaca, N.Y. and the USDA probes depository, Albany, Calif. for kindly providing the heterologous RFLP probes. We also thank Dr. Shawn Kaeppler, Dr. Rebecca Abler, and Dr. Zahi Atallah for their valuable comments and critical review and Lori Evans for handling plant materials. The Wisconsin Sod Producers Association and the Wisconsin Turfgrass Association provided partial funding.

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