Theor Appl Genet (1998) 97:255–260

C. Chen · D. A. Sleper · G. S. Johal

Comparative RFLP mapping of meadow and tall fescue

Received: 23 May 1997 / Accepted: 15 January 1998

Abstract Molecular markers based on restriction fragment length polymorphism (RFLP) were used to construct a genetic linkage map in diploid meadow fescue, *Festuca pratensis* Huds. ($2n = 2x = 14$, genomic designation PP), and to compare its genomic relationship with a related species, hexaploid tall fescue (*Festuca arundinacea* Schreb.; $2n = 6x = 42$, $PPG_1G_1G_2G_2$). Using a collection of 66 tall-fescue (heterologous) markers, an RFLP linkage map was constructed in *F. pratensis*. This map, which has a total length of 280.1 cM, includes seven linkage groups. A comparison of 33 markers that were mapped in both *F. pratensis* and *F. arundinacea* detected highly conserved linkage groups between these two species. Our data are consistent with the proposal that one of the genomes of *F. arundinacea* was derived from *F. pratensis*. However, since significant changes in marker sequences, map distances, and homoeologous linkage groups were also detected between the two species, it appears that the P genome diverged substantially during evolution from the diploid to the hexaploid *Festuca*.

Key words RFLPs · Genome mapping · *Festuca pratensis* · *F. arundinacea*

Introduction

The detection of RFLPs in plants has permitted genetic mapping to be accomplished at the molecular level. As a result, detailed linkage maps have been constructed in many plant species. While rapid and significant progress has been made in most crop species (Bernatzky and Tanksley 1986; Bonierbale et al 1988; McCough et al. 1988; Chao et al. 1989; Song et al. 1991), molecular maps have been constructed in only a few forage species such as *Medicago sativa* L. (Brummer et al. 1993) and *Festuca arundinacea* Schreb. (Xu et al. 1995). An informative RFLP linkage map is useful in analyzing the structural organization of genomes (Berhan et al. 1993), locating and cloning agriculturally important genes such as those conferring disease resistance (Martin et al. 1993), generating physical maps of specific chromosomes through in situ hybridization using DNA markers (Werner et al. 1992; Wanous and Gustafson 1995), and improving the agronomic attributes of the crop plant by marker-assisted selection. In addition, comparative RFLP mapping of related species has the potential of providing significant insights into the evolution of plant genomes (Ahn and Tanksley 1993; Huang and Kochert 1994).

The objectives of the present research were two-fold: first, to develop an RFLP linkage map in meadow fescue, a diploid ($2n = 2x = 14$) cool-season forage grass species, grown mainly in the temperate zone of Europe (Sleper and Buckner 1995); and secondly, to assess the evolutionary relationship of meadow fescue with the hexaploid ($2n = 6x = 42$) tall fescue (*F. arundinacea* var. *genuine* Schreb.). Tall fescue, which is cultivated worldwide, serves as an important source of forage in the USA, Canada, Australia, North Africa and some parts of Europe (Borill et al. 1976; Sleper 1985).

Both meadow fescue and tall fescue belong to the genus *Festuca*, and a number of studies have suggested a close genomic relationship between them. Based largely on morphological characteristics and the presence of chromosome pairing (meiotic bivalents) in *F. arundinacea* × *F. pratensis* F_1 hybrids (Chandrasekharan and Thomas 1971; Sleper 1985), the diploid genome (PP) of *F. pratensis* has been proposed as one of the progenitors of the hexaploid genome

Communicated by G. E. Hart

C. Chen · D. A. Sleper (✉) · G. S Johal
Department of Agronomy, University of Missouri, Columbia, MO 65211, USA

($PPG_1G_1G_2G_2$) of *F. arundinacea*. This hypothesis is further strengthened by recent studies involving RFLPs and genomic in situ hybridization between the two species (Xu and Sleper 1994; Humphreys et al. 1995).

Despite the widespread use of meadow fescue in agriculture, this species lacks useful genetic markers, and, as a result, no genetic map is available for meadow fescue. This deficiency prompted us to develop an RFLP linkage map for this forage crop, which we have constructed with the aid of tall-fescue RFLP markers already available in our laboratory (Xu et al. 1995). In addition, these molecular markers have allowed us to compare and contrast the genomic organization of meadow fescue with that of tall fescue.

Materials and methods

F_2 mapping population

A total of 56 F_2 individuals that served as the mapping population were derived by self-pollinating a single F_1 hybrid plant from a cross between two genotypes of diploid *F. pratensis*, 307.1–1 and 309.1–2. The crossing of these two parents was accomplished through manual emasculation and pollination. The resulting 18 F_1 seeds were harvested when still young and germinated on Murashige and Skoog (MS) medium (pH 5.7) to form a large clonal population from each F_1, followed by transplanting the F_1 plants in the field from September to December to accomplish vernalization. Plants were then moved to the greenhouse and four out of 18 F_1 plants were randomly chosen to generate the F_2s. Before anthesis, each F_1 plant was placed in isolation in a different greenhouse room and forced to self-pollinate by employing emasculation and multiple pollination. The efficiency of seed set ranged from 1.2 to 16.5% in the four F_1 plants. One of the plants yielded 216 F_2 seeds, 98 of which were planted and randomly selected to generate the final F_2 mapping population of 56 plants.

DNA clones and RFLP detection

The heterologous RFLP markers used in this study were originally isolated to construct an RFLP map in tall fescue; their isolation from a *Pst*I-genomic DNA library has been described previously (Xu et al. 1991). A total of 267 clones were randomly chosen from the library but all P-genome-specific probes were selected to screen for polymorphism. These RFLP clones were designated with a prefix (TF) and a laboratory number. The procedures for genomic DNA isolation, restriction-enzyme digestion, electrophoresis, Southern blotting, probe preparation and labeling, hybridization, and washing have been described elsewhere (Chen et al. 1995). To maximize the detection of polymorphism between the parental stocks of *F. pratensis*, six different restriction enzymes, *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Sst*I and *Xho*I, were used individually in this study.

Genetic map construction

Multipoint maximum-likelihood linkage analysis was performed using Mapmaker version 2.0 of Dupont (Lander et al. 1987) on a Power Macintosh 7100/66AV computer. The suspected linkage group for co-dominant markers was established by two point/group functions at a minimum LOD = 3.0 and maximum theta = 0.40. An initial marker order for each linkage group was determined using the three point/first order command. The LOD table command was then used to select a core of six to seven markers that were not closely linked but possessed high LOD scores. A multipoint maximum-likelihood core map was generated using the compare command. Markers, which were associated with the linkage group established previously by the Group command, were then added to the core map using the Try command. After each Try, the map was checked with the Ripple command to confirm the best order with the new marker. It was found that the Ripple command showed a value of -3.0, or less, for all adjacent triplets. After all 55 co-dominant markers were mapped, 15 dominant (presence/absence) markers were analyzed. Finally, the map was run through the Drop Marker command; however, localized distortions in marker distance were not found. The Show Raw data command was run to check for double crossovers. Autoradiographs were re-examined for markers with double crossovers. Recombinant frequency was converted to centiMorgans (cM) using the Haldane mapping function (Haldane and Waddington 1931).

Results

Polymorphism

Overall, a high level of polymorphism between the two parental genotypes of *F. pratensis* was detected using heterologous tall-fescue clones. Of 267 probes employed, an average of 77.5% (207) hybridized to *F. pratensis* DNA, of which 49.4% (132) detected polymorphism with one or more enzymes. Only slight differences were observed in the ability of each of the six restriction enzymes to produce polymorphisms, which varied from 26.8 (*Sst*I) to 34.5% (*Eco*RI). Through a combination of *Eco*RI and *Hin*dIII, two relatively inexpensive enzymes, tall-fescue RFLP probes detected 41.2% of the polymorphisms present in the two parents of *F. pratensis* used in this study. For reasons that remain unexplained, DNA digested by *Bam*HI usually exhibited stronger hybridization signals during Southern analysis than did the DNA digested with *Sst*I or *Xho*I.

Segregation in the F_2

Of the 70 RFLP loci mapped, 26 (37.1%) showed segregation distortion, evident as a deviation from the expected progeny ratios of 1:2:1 for co-dominant loci or 3:1 for dominant loci when tested by chi-square analysis ($P < 0.05$, Table 1). Heterozygotes were favored at approximately 70% (18 of 26) of the loci with skewed segregation. Only 19% (4 of 26) were highly skewed to favor male homozygotes. No significant distortion was found toward the female genotype. What caused these RFLP loci to segregate unevenly in the progeny remains to be determined, possibly it was due to self-incompatibility or inbreeding depression.

Table 1 *F. pratensis* RFLP loci with skewed segregation ratios (B : H : A)

Linkage group	Locus	Ratio (B : H : A)	Linkage group	Locus	Ratio (B : H : A)	Linkage group	Locus	Ratio (B : H : A)
Toward H (heterozygote)								
I	tf07	10 : 38 : 7	I	tf32A	10 : 38 : 7	I	tf65	11 : 38 : 7
I	tf168	10 : 38 : 7	I	tf189	11 : 38 : 7	II	tf50	4 : 33 : 19
II	tf151	3 : 36 : 17	IV	tf01	1 : 54 : 1	IV	tf96	3 : 52 : 1
IV	tf140	2 : 53 : 1	IV	tf153	5 : 44 : 7	IV	tf215	1 : 54 : 1
IV	tf223L	1 : 54 : 1	IV	tf249	1 : 54 : 1	IV	tf504B	2 : 53 : 1
IV	tf520	5 : 49 : 2	VI	tf219	2 : 35 : 19			
Toward A (male parent)								
VI	tf32B	–:–:28	VI	tf44	3 : 25 : 28	VI	tf130	–:–:36
VI	tf198	1 : 17 : 38	VI	tf542	–:–:38			
Toward A and H								
II	tf45	3 : 33 : 20	II	tf124	3 : 32 : 21	II	tf543	3 : 33 : 20
VI	tf74A	5 : 30 : 21						

Meadow-fescue map

Out of the 70 RFLP markers mapped using an F_2 population of 56 plants, four did not exhibit linkage with any marker, and therefore were not used in the construction of the map (Fig. 1). The other 66 markers allowed the meadow-fescue genome to be divided into seven linkage groups with a total length of 280.1 cM (Table 2, Fig. 1). On average, each linkage group had 9.4 loci with a separation of 4.2 cM between adjacent loci. The longest was linkage group VI with ten loci spanning 70.8 cM. Only two loci were placed on linkage group VII.

Only seven RFLP markers represented duplicate loci and, except for tf358, which mapped to two separate locations in linkage group VI, all duplicate RFLP markers mapped to different linkage groups. Duplicate loci mapped to five out of the current seven linkage groups of *F. pratensis*.

A total of eight markers that displayed dominant inheritance were mapped in this study. Except for the dominant marker TFl83, which caused a significant alteration of marker distance, and therefore was discarded, all other dominant markers were mapped (Fig. 1).

Map comparison of *F. pratensis* with *F. arundinacea*

A comparison of RFLP markers that could be localized in both the *F. pratensis* and *F. arundinacea* linkage maps showed the presence of highly conserved linkage groups between these species. Of these 33 common markers mapped in both species, 70% (23) were located in corresponding linkage groups in *F. pratensis* and *F. arundinacea*. Eight of the nine common markers that mapped to linkage group I in *F. pratensis* were present in the corresponding linkage group 1 a of *F. arundinacea*. Although conservation of linkage to this extent was not observed in other linkage groups, large blocks were obviously retained in linkage groups II, III, and IV of *F. pratensis* and their counterparts in *F. arundinacea*. However, even in regions that are conserved among the two species, one difference was apparent – the extent of linkage between markers. On average, distances between adjacent markers were greater in the *F. arundinacea* map (6.6 cM) than in the *F. pratensis* map (3.4 cM). Of the six probable homoeologous linkage groups, marker distance was increased in three, decreased in one, and remained relatively similar in the remaining two linkage groups in *F. arundinacea* (Table 2).

The linear arrangement or order of loci within conserved linkage blocks of *F. pratensis* and *F. arundinacea*, while remaining unchanged for some markers, was substantially rearranged for others. For example, common markers in four pairs of linkage groups, I/1 a, II/2 c, III/3 b and IV/4 c are nearly co-linear. However, markers of linkage group VI of *F. pratensis* are completely rearranged in *F. arundinacea*. Three common markers, TF74B, TF235 and TFl99B, of linkage group VI of *F. pratensis* are located in three different linkage groups of the *F. arundinacea* map (Fig. 2).

By using a set of 22 polymorphic markers mapped in conserved linkage groups from both species, we also assessed evolutionary changes in the copy (band) number of markers among these species (Table 2). A change in copy number between the two species was observed with 77% (17/22) of the probes, with 13 (72%) detecting a higher copy number in *F. arundinacea* than in *F. pratensis*. On average, the copy number of these markers increased from 2.4 in *F. pratensis* to 4.1 in *F. arundinacea*.

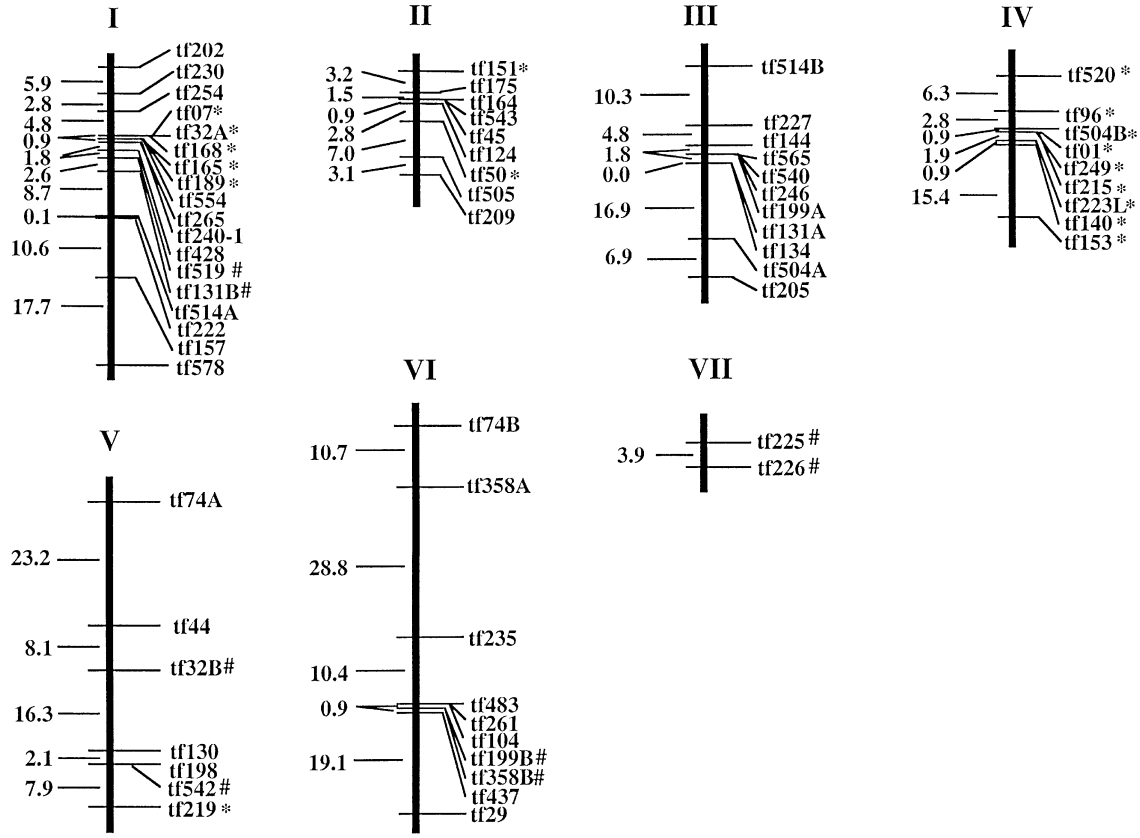

Fig. 1 RFLP-based genetic map of *F. pratensis*. Linkage groups are represented as thick *vertical lines*, with their number being indicated at the top. Names of markers are on the right. Distances are given in centiMorgans (cM) using the Haldane mapping function and are shown to the left of the *thick vertical lines*. Duplicated loci detected with same probe are labeled with *uppercase letters*, A or B. Symbols * and # denote markers with distorted segregation ratios and dominant markers, respectively

Table 2 Comparison of common loci and suspected homoeologous linkage groups detected in *F. pratensis* (*F.p.*) and *F. arundinacea* (*F.a.*)

Linkage group		Mapped common loci	No. of bands		Ave. cM of adjacent loci	
F.p.	*F.a.*		*F.p.*	*F.a.*	*F.p.*	*F.a.*
I	1a	8	14	28	3.8	10.6
II	2b	4	8	16	3.5	4.3
III	3b	3	6	9	2.2	9.0
IV	4c	3	5	15	0.0	2.4
V	19	2	12	13	11.6	0.8
VI	2a	2	7	9	2.0	2.6
Weighted mean		3.7	2.4	4.1	3.4	6.6

Discussion

Comparison of meadow-fescue and tall-fescue genomes

One objective of this study was to determine the degree of genomic conservation between meadow fescue and tall fescue. A number of studies have reported the close genomic relationship of *F. pratensis* with tall fescue. The initial evidence largely emerged from the morphology of F_1 plants between these species and chromosome pairing behavior in their hybrids (Malik and Thomas 1967). Recent results of RFLP comparisons and genomic in situ hybridization between the two species have supported the hypothesis that *F. pratensis* may be the donor for the P genome of tall fescue (Xu and Sleper 1994; Humphreys et al. 1995).

Genome-specific probes were identified on the basis of their Southern hybridization with DNA from two progenitor species, diploid *F. pratensis* and tetraploid *F. glaucescens*, of tall fescue (Xu et al. 1991). P-genome-specific probes only hybridized to DNA of *F. pratensis* and was mapped on group IV of meadow fescue and on 5a of tall fescue. However, none of 14 G_1 or G_2 genome-specific probes was mapped on the P genome of meadow fescue. Comparative RFLP mapping between the *F. pratensis* and *F. arundinacea* genomes has shown the presence of highly conserved linkage groups and marker sequences, thereby providing further evidence that the ancestor of the P genome in the hexaploid *F. arundinacea* may be the diploid meadow fescue. Because a number of rearrangements have also been detected in the location of a number of RFLP markers, and marker distance has also generally

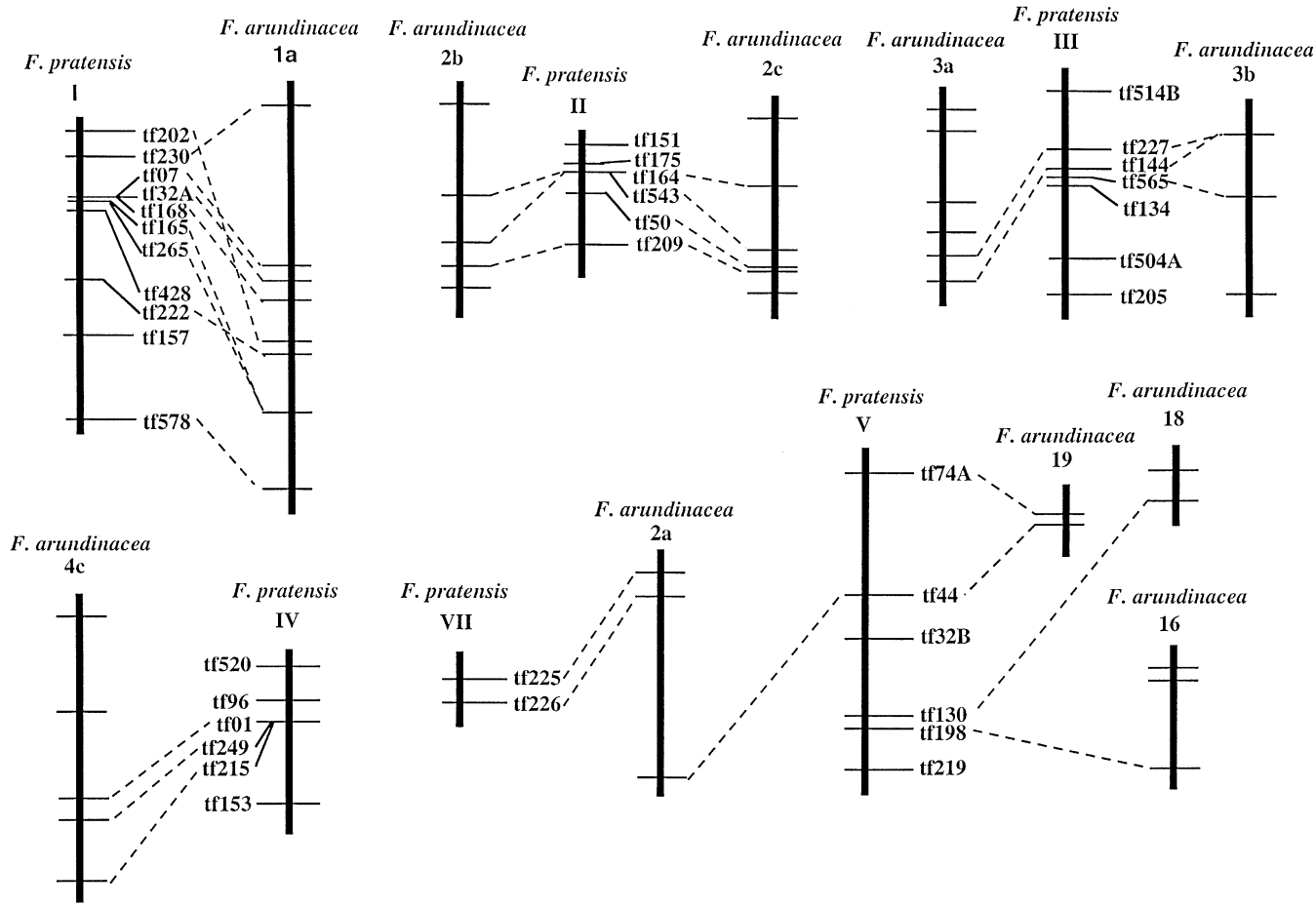

Fig. 2 Comparison of RFLP-based genetic maps between meadow fescue and tall fescue based on the meadow-fescue linkage groups. The co-linearity of markers between these two species is shown by *dashed lines* across suspected homoeologous linkage groups

increased in tall fescue relative to meadow fescue, the results suggest that the P genome has evolved in the hexaploid tall fescue since its acquisition from the diploid meadow fescue. It is apparent from a comparison of the two maps that some of the changes that may have contributed to the evolution of the P genome may be brought about by gross chromosomal rearrangements (Devos et al. 1993).

Cytogenetic analysis based on predictive mathematical formulae to quantify relationships between genomes of different species also predicted that the perennial ryegrass genome was more closely related to one of the tall-fescue genomes than to the other two (Kleijer 1984). Our comparative mapping study is also compatible with this prediction; not only was there a conserved P genome between *F. pratensis* and *F. arundinacea*, but most of the linkage groups from the P genome were closely related to those from the other two genomes, G_1 and G_2, of *F. arundinacea* (Fig. 2). Compared to the tall-fescue map, linkage group I of *F. pratensis* has a close relationship to 1a, 1b, and 1c of tall fescue (Xu et al. 1995), suggesting the relatedness of P to G_1 and G_2. Close relationships among P, G_1 and G_2 explain why more than seven bivalents sometimes formed in $4 \times F.\ pratensis \times F.\ arundinacea$ hybrids.

The three genomes, P, G_1 and G_2, of tall fescue have complicated chromosomal relationships. G_1 can sometimes pair with G_2. Even though P is distantly related to either G_1 or G_2, pairing between P and G_1 or G_2 also occurs (Jauhar 1993; Sleper 1995). This is validated by the genome mapping of tall fescue. Homoeologous chromosomes were found to share high levels of linkage similarity (Xu et al. 1995). Comparative mapping between tall fescue and meadow fescue further confirmed this relationship. For instance, linkage group I of meadow fescue not only had a high level of sequence conservation with linkage 1a of tall fescue, but also shared some similarity with homoeologous groups 1b and 1c (data not shown).

Implications for *Festuca* breeding and genome evolution

The high degree of linkage conservation observed between the genomes of meadow fescue and tall fescue indicates that it should be possible to begin uniting the

genetics and breeding of the *Festuca* species, and it is likely that most DNA markers can probably be used interchangeably between them with the purpose of saturating RFLP maps. These RFLP markers should be useful for tagging genes for important traits, such as high palatability and digestibility, contributed by the *F. pratensis* genome.

References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Berhan AM, Hulbert SH, Butler LG, Bennetzen JL (1993) Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. Theor Appl Genet 86:598–604

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bonierbale M, Plaisted R, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Borill M (1976) Temperate grasses. In: Simmonds NW (ed) Evolution of crop plants. Longman, London New York, pp 137–142

Brummer EC, Bouton JH, Kochert G (1993) Development of an RFLP map in diploid alfalfa. Theor Appl Genet 86:329–332

Chandrasekharan P, Thomas H (1971) Studies in *Festuca*. V. Cytogenetic relationships between species of bovinae and scariosae. Z Pflanzenzuchtg 65:345–354

Chao S, Sharp PJ, Worland EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495–504

Chen C, Sleper DA, West CP (1995) RFLP and cytogenetic analyses of hybrids between *Festuca mairei* and *Lolium perenne*. Crop Sci 35:720–725

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

Haldane JBS, Waddington CH (1931) Inbreeding and linkage. Genetics 16:357–374

Huang H, Kochert G (1994) Comparative RFLP mapping of an allotetraploid wild rice species (*Oryza latifolia*) and cultivated rice (O. *sativa*). Plant Mol Biol 25:633–648

Humphreys MW, Thomas HM, Morgan WG, Meredith MR, Harper JA, Thomas H, Zwierzykowski Z, Ghesquiere M (1995) Discriminating the ancestral progenitors of hexaploid *Festuca arundinacea* using genomic in situ hybridization. Heredity 75:171–174

Jauhar PP (1993) Cytogenetics of the *Festuca-Lolium* complex. Springer-Verlag, New York

Kleijer G (1984) Cytogenetic studies of crosses between *Lolium multiflorum* Lam. and *Festuca arundinacea* Schreb. 1. The parents and the F_1 hybrids. Z Pflanzenzuchtg 93:1–22

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

Malik CP, Thomas PT (1967) Cytogenetical relationships and genome structure of some *Festuca* species. Caryologia 20:1–39

Martin GB, Brommonschenkei SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu, T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

McCough SR, Kochert G, Yu ZH, Wand ZY, Kush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Sleper DA (1985) Breeding tall fescue. J Plant Breed Rev 3:313–342

Sleper DA, Buckner RC (1995) The fescues. In: Barnes RF, Miller DA, Nelson CJ (eds) Forages. Iowa State University Press, Ames, pp 345–356

Song KM, Suzuki JY, Slocum MK, Williams PH, Osborn TC (1991) A linkage map of *Brassica rapa* (syn. Campestris) based on restriction fragment length polymorphism loci. Theor Appl Genet 82:296–304

Wanous MK, Gustafson JP (1995) A genetic map of rye chromosome 1R integrating RFLP and cytogenetic loci. Theor Appl Genet 91:720–726

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311

Xu WW, Sleper DA (1994) Phylogeny of tall fescue and related species using RFLPs. Theor Appl Genet 88:685–690

Xu WW, Sleper DA, Hoisington DA (1991) A survey of restriction fragment length polymorphisms in tall fescue and its relatives. Genome 34:686–692

Xu WW, Sleper DA, Chao S (1995) Genome mapping of polyploid tall fescue (*Festuca arundinacea* Schreb.) with RFLP markers. Theor Appl Genet 91:947–955