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ORIGINAL PAPER

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Linkage map construction in allotetraploid creeping bentgrass (*Agrostis stolonifera* L.)

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Abstract Creeping bentgrass (*Agrostis stolonifera* L.) is one of the most adapted bentgrass species for use on golf course fairways and putting greens because of its high tolerance to low mowing height. It is a highly outcrossing allotetraploid species ($2n=4x=28$, A_2 and A_3 subgenomes). The first linkage map in this species is reported herein, and it was constructed based on a population derived from a cross between two heterozygous clones using 169 RAPD, 180 AFLP, and 39 heterologous cereal and 36 homologous bentgrass cDNA RFLP markers. The linkage map consists of 424 mapped loci covering 1,110 cM in 14 linkage groups, of which seven pairs of homoeologous chromosomes were identified based on duplicated loci. The numbering of all seven linkage groups in the bentgrass map was assigned according to common markers mapped on syntenous chromosomes of ryegrass and wheat. The number of markers linked in coupling and repulsion phase was in a 1:1 ratio, indicating disomic inheritance. This supports a strict allotetraploid inheritance in creeping bentgrass, as suggested by previous work based on chromosomal pairing and isozymes. This linkage map will assist in the tagging and eventually in marker-assisted breeding of economically important quantitative traits like disease resistance to dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) and brown patch (*Rhizoctonia solani* Kuhn).

Keywords *Agrostis stolonifera* L. · Creeping bentgrass · Allotetraploid · Linkage map · Homoeologous chromosomes · Molecular markers

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Introduction

Agrostis, or bentgrass, is a large genus of over 200 species in the Poaceae family (Hitchcock 1951). Only five species are used as turfgrass in the United States: colonial (*Agrostis capillaris* L.), velvet (*Agrostis canina* L.), dryland (*Agrostis castellana* Boiss. and Reut.), redtop (*Agrostis gigantea* Roth) and creeping (*Agrostis stolonifera* L.). These species are perennial, outcrossing cool-season grasses used for lawns, athletic fields, and golf courses. Currently, the stoloniferous, allotetraploid creeping bentgrass ($2n=4x=28$, A_2 and A_3 subgenomes) is the most adapted species for use on golf course fairways and greens because of its fine texture and high tolerance to low mowing height (Warnke 2003). This demand has created $50 million in sales in the turf seed industry, making creeping bentgrass an economically important turfgrass species in the United States (Cook 1996).

Molecular marker-based genetic linkage maps have been developed in a number of economically important cereal crops such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), oat (*Avena* spp.), barley (*Hordeum* spp.), and maize (*Zea mays* L.) (Rayapati et al. 1995), and also in pasture and turfgrass species like perennial ryegrass [(*Lolium perenne* L.) Hayward et al. 1994; Jones et al. 2002; Warnke et al. 2004; Sim et al. 2005], Italian ryegrass [(*Lolium multiflorum* Lam.) Inoue et al. 2004], and meadow fescue [(*Festuca pratensis* Huds.) Alm et al. 2003]. Plant breeders and geneticists are increasingly using molecular markers to study genome structure and to manipulate genes. Furthermore, many Poaceae species possess significant chromosomal regions of conserved synteny between their genetic maps constructed using a common set of heterologous RFLP probes (Gale and Devos 1998). Since gene order of perennial ryegrass and Triticeae cereals are highly conserved, their individual maps have been merged (Jones et al. 2002; Sim et al. 2005). Such map integration and comparative map analysis can be used for the improvement of less well-studied species like bentgrass.

Linkage maps have been constructed in allotetraploid species such as cotton (*Gossypium* sp.), tef [*Eragrostis tef* (Zucc) Trotter], white clover (*Trifolium repens*), and *Brassica napus* L. (Osborne et al. 1997; Brubaker et al. 1999; Zhang et al. 2001; Barrett et al. 2004). However, these species are self-compatible, and therefore, mapping populations have a maximum of two alleles per locus, whereas creeping bentgrass is a self-incompatible allotetraploid consisting of two diploid genomes with a maximum of four alleles per locus (Warnke et al. 1998). Moreover, in some species like *Brassica* sp., double haploid lines have been developed that reduce the number of alleles to two per locus. Maps have also been constructed from known diploid progenitors, which reduce the complexity of interpreting codominant banding patterns in segregating progeny. This linkage map in creeping bentgrass will be the first one constructed for an outcrossing, allotetraploid monocot species with no available diploid progenitors.

In this paper, our objectives were to construct a genetic linkage map from a cross between two highly heterozygous creeping bentgrass clones using RAPD, AFLP, and heterologous cereal and homologous bentgrass cDNA RFLP markers, and to designate each of the seven pairs of homoeologous chromosomes in bentgrass respective to syntenous chromosomes of wheat and ryegrass. We also discuss whether creeping bentgrass follows a disomic segregation pattern.

Materials and methods

Plant material and full-sib mapping population

Two highly heterozygous parental creeping bentgrass clones, 372 and 549, were selected from 700 clones collected from golf course fairways and putting greens throughout Wisconsin that are at least 75 years old (Casler et al. 2003). Both clones are from fairways, and clone 372 was collected from Lake Forest Golf Course, Eagle River, Wis., and clone 549 was obtained from Ojibwa Golf Course, Chippewa Falls, Wis. (Wang et al. 2005). These two creeping bentgrass clones exhibit dramatic differences in leaf color, shoot density, root depth, and disease response to snow mold (*Typhula* spp.) (Wang et al. 2005) and dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) (Chakraborty et al. 2003). Clone 372 is lighter green in leaf color with higher shoot density, shorter internodes, and narrower leaves than the dark-green clone 549.

During the spring of 2002, a full-sib mapping population named 549 × 372 consisting of 697 progeny was developed in a greenhouse, Madison, Wis., from a single controlled cross between the creeping bentgrass clones 372 and 549. Seeds were collected in late June from the maternal parental clone 549. Seeds were sown in a 4-in. round pot, and after germination and growth in the greenhouse, individual seedlings were transferred to 3-in. square pots and assigned a number. Of 697 progeny available, 94 progenies along with the parental clones were randomly selected and used in mapping in an initial RAPD analysis, and subsequently, 90 progeny of the original 94 were used in AFLP and RFLP analysis for map construction.

RAPD analysis

DNA extraction for RAPD analysis was performed according to Scheef et al. (2003). Initially, 523 primers (Operon Technologies, Alameda, Calif. USA) were screened against the two parents and 65 primers with the highest number of polymorphic bands were chosen to be tested on the 94 progenies. RAPD reactions were performed in 96-well plates with a total volume of 10 μl in an MJ PTC-100 thermal cycler (MJ Research, Watertown, Mass., USA) with the same thermal cycling conditions as described by Scheef et al. (2003) of 91°C for denaturation, 42°C for annealing, and 72°C for elongation. PCR products were run in a 1.5% (w/v) agarose gel and stained in ethidium bromide (1.5 μg/ml) for 30 min and subsequently destained in distilled water for 20–30 min.

Each segregating RAPD marker was named using its approximate size in base pairs, combined with the Operon primer name. Three banding pattern classes were identified: (1) bands present in both parents and segregating 3:1 in progeny (indicated by marker names beginning with D); (2) band present only in the female parent (549) and segregating as 1:1 in the progeny (name of the marker beginning with 5); and (3) band present only in the male parent (372) and segregating as 1:1 in the progeny (name of the marker beginning with 3).

AFLP analysis

DNA digestions, adaptor ligations, and pre-selective and selective amplifications were performed according to instructions provided with an AFLP analysis kit purchased from Invitrogen (Gathersburg, Md., USA), and standard AFLP procedures (Vos et al. 1995). The selective amplifications with three selective bases per primer were carried out using 6-carboxy fluorescein fluorescent primers labeled on the 5′ nucleotide. The amplified fragments were detected with an ABI3730xl instrument (PE Applied Biosystems, Foster City, Calif., USA). Each sample lane included the GeneScan 500-LIZ internal lane standard (Larson et al. 2001). The capillary electrophoresis procedures were performed by the Bovine Functional Genomics Lab (Beltsville, Md., USA). Fluorescent fragments between 50 nucleotides (nt) and 500 nt were identified by GeneScan 3.1 software (PE Applied Biosystems). GeneScan trace files were then analyzed for the presence or absence of AFLP products, in 1-nt intervals using the computer program Genographer (Benham et al. 1999).

RFLP analysis

Young leaf tissue from greenhouse-grown plants was harvested, lyophilized, and genomic DNA was extracted from 500 mg of ground tissue using a modified cetyltrimethylammonium bromide method (Saghai-Maroof et al. 1984). Approximately 10 μg of genomic DNA for each sample was digested with restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII), run on a 1% agarose gel with 1X TAE buffer, and blots were made with Hybond-N$^+$ (Amersham) according to the technique of Southern (1975). Random primer labeling method was used and the membranes were prehybridized, hybridized, and washed according to the protocol described in Warnke et al. (2004).

A common set of 152 anchor heterologous probes that contain CDO: oat cDNA, BCD: barley cDNA, and RZ: rice cDNA from Cornell University, Ithaca, N.Y. (Van Deynze et al. 1998), and additional heterologous CDO and BCD probes (the USDA probe depository, Albany, Calif., USA) were obtained. The probes were initially selected based on their known map location in wheat and ryegrass and not because of any biological reason. To detect polymorphism, 221 of the selected probes were screened against the parental clones. Twenty-four of those probes with strong signal intensity, simple and scorable segregation pattern (i.e., typically two and rarely single, three, or four loci), were selected and tested on a progeny set that consisted of 90 randomly selected individuals. The common RFLP markers that are previously mapped in ryegrass (Sim et al. 2005) and wheat and rice (Van Deynze et al. 1998) were used to designate the specific linkage groups in creeping bentgrass.

In addition, two cDNA libraries were constructed each from two creeping bentgrass parental clones, and the resulting cDNA clones were used as RFLP probes. In brief, total RNA was isolated separately from vigorously growing leaf tissue of the two clones using RNA Isolator (Genosys, The Woodlands, Tex., USA), and then purified using the RNeasy Plant MiniKit (Qiagen, Valencia, Calif., USA). This was done with the goal of making one separate library from each plant clone. Next, mRNA isolation was done using the Micro-Poly(A)Pure kit (Ambion, Austin, Tex., USA). First- and second-strand cDNA synthesis, adapter ligations, size fractionation, ligation of cDNA into the Uni-ZAP XR vector, packaging into lambda phage, amplification of the library, and mass excision were all performed as directed in the ZAP-cDNA Synthesis and ZAP-cDNA Gigapack III Gold Cloning kit (Stratagene, La Jolla, Calif., USA). After plating colonies, PCR using M13 forward and reverse primers was used to determine insert size. Cycle sequencing of recombinant clones was done using BigDye, version 3.1, reagents and T7 primer, to generate single-pass sequences from the 3′ end of the cDNA clones. Reactions were then purified using CleanSeq reagents (Agencourt, Beverly, Mass., USA), and then run on an ABI3700 sequencer at the University of Wisconsin Biotechnology Center.

Parental clones were screened with 140 bentgrass cDNA probes (termed "Ast" markers for *Agrostis* spp.), and a subset of 20 polymorphic probes were chosen for progeny evaluation based on the criteria detailed above for heterologous probes. For 36 Ast markers generated from the 20 creeping bentgrass cDNA clones, sequence similarity with rice was analyzed using a *japonica* rice cDNA clone collection to determine syntenous chromosome locations in rice (Kikuchi et al. 2003).

Linkage map construction

The marker data, both dominant and codominant types, were scored according to the coding systems described in JoinMap, version 3.0 (van Ooijen and Voorrips 2001; Warnke et al. 2004). The RFLP markers were of several segregation types, and the most common types were ab × cd (locus heterozygous in both parents, four alleles), and ef × eg (locus heterozygous in both parents, three alleles). Other segregation types, mainly observed for RAPD and AFLP markers but also for some RFLP markers, were scored as follows: locus heterozygous in female parent (lm × ll), in male parent (nn × np), and in both parents with two alleles (hk × hk). Data from all the markers collected from both parents were used to produce one genetic linkage map representing both parents using JoinMap (van Ooijen and Voorrips 2001). A LOD value (logarithm of odds ratio) ranging from 4.0 to 15.0 was selected for the initial formation of linkage groups. To estimate recombination and order of markers for each linkage group, the default parameters resident in JoinMap were used. These include a pairwise recombination estimation using $r < 0.40$, a LOD score > 1.0, a χ^2 threshold for removal of loci with respect to jumps in goodness of fit 5.0, and the use of the Kosambi mapping function (Kosambi 1944). In JoinMap, the deviation of all the mapped markers from the expected segregation ratios (1:1, 1:1:1:1, 3:1, or 1:2:1) was determined using χ^2 contingency tables.

We considered a linkage group to be homoeologous to another linkage group thereby representing the two chromosomes each originating from one of the two genomes, based on the presence of at least two pairs of duplicate loci. Each pair of linkage groups was designated with the group numbers based on synteny followed by an arbitrary ".1" or ".2" (Fig. 1) to show that they represent the two different creeping bentgrass diploid genomes.

Examination of chromosomal pairing behavior

One method used to determine meiotic behavior, or more specifically, to distinguish between disomy and tetrasomy, is the comparison of the number of linked loci identified in coupling and repulsion phase (Sorrells 1992; Wu et al. 1992). Polysomy is characterized by low

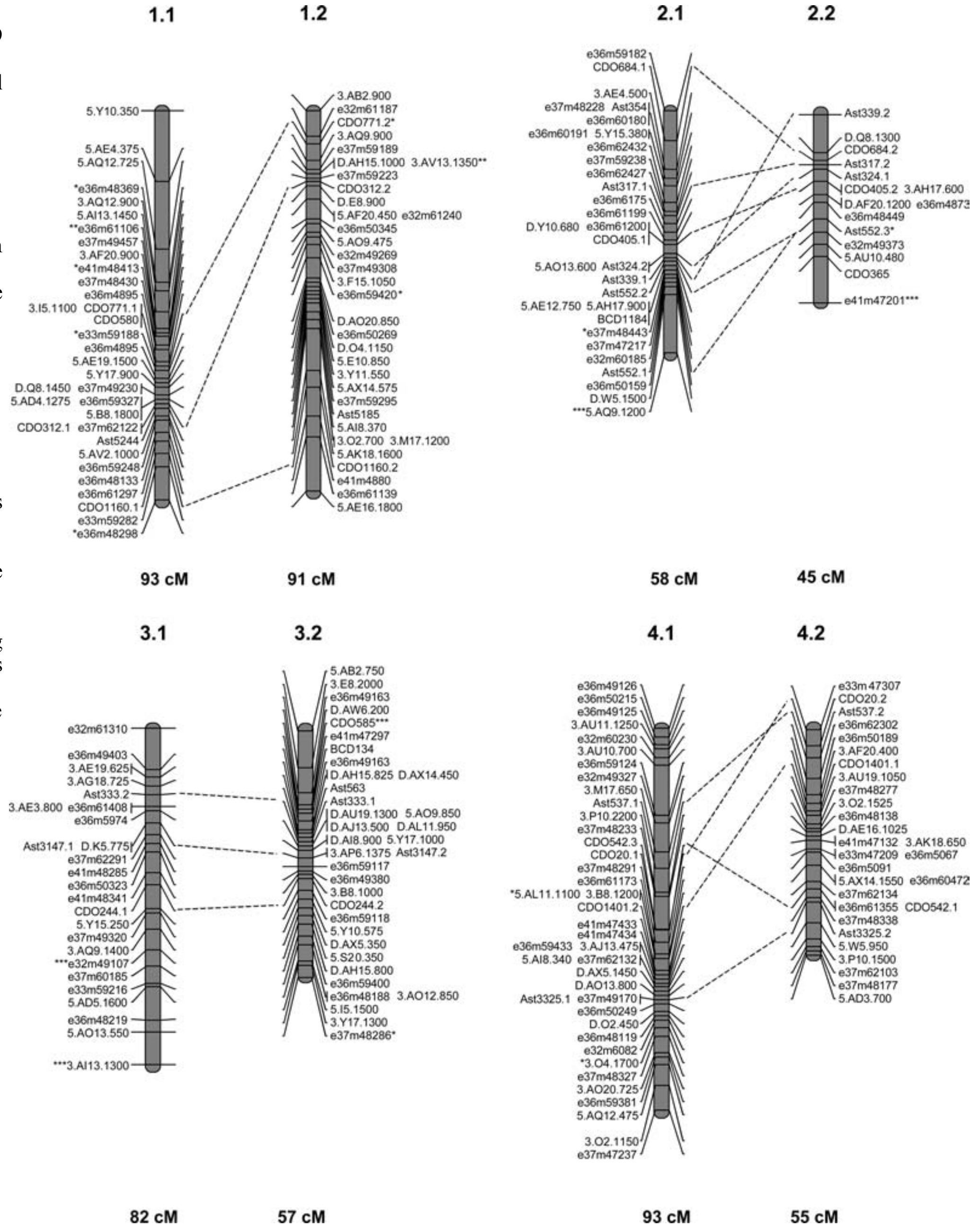

Fig. 1 Genetic linkage map in creeping bentgrass based on 549 × 372 population using AFLP, RAPD, and heterologous cereal and homologous bentgrass cDNA RFLP markers. The linkage groups are numbered according to Sim et al. (2005) and Van Deynze et al. (1998) based on common RFLP markers. Genetic length of each homologue is indicated below each group. The *asterisks* beside loci represent segregation distortion (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). Each segregating RAPD marker was named using its approximate size in base pairs, combined with the Operon primer name. Creeping bentgrass cDNA clones used as RFLP probes are indicated as "Ast" plus the clone name and the heterologous RFLP loci are indicated as the probe name followed by ".1" or ".2" to show duplicate loci representing two different creeping bentgrass diploid genomes (e.g., CDO1160.1 and .2). The above notation was not used if only one marker was mapped. The AFLP loci are indicated in the format of exxmzzzz (e.g., e37m48338)

frequencies or absence of repulsion-phase linked markers, whereas in diploids or strict allopolyploids, the number of markers linked in coupling and repulsion phase is expected to be in a 1:1 ratio.

Therefore, the ratio of coupling to repulsion phase linkages was used to assess the level of homoeologous chromosome pairing in tetraploid creeping bentgrass (Qu and Hancock 2001). The AFLP marker data set was split into male and female data sets to represent markers coming from each of the parents in the mapping population (coupling-phase data set). The marker data was duplicated and then inverted 0 to 1 and 1 to 0 to create a repulsion-phase data set. The combined coupling- and repulsion-phase data sets for each parent were analyzed for linkages using the computer program MAPMAKER 3.0 EXP. The default linkage was set to 37.5 cM, which is the maximum detectable recombination fraction for our population size of 90 (Wu et al. 1992).

Additionally, with disomic segregation, codominant markers will segregate with only two alleles per locus in each parent, and each progeny will receive only one of the two alleles (Lespinasse et al. 2000). Thus, examination of the banding configuration of the RFLP markers was undertaken as another method of examining the chromosomal pairing behavior.

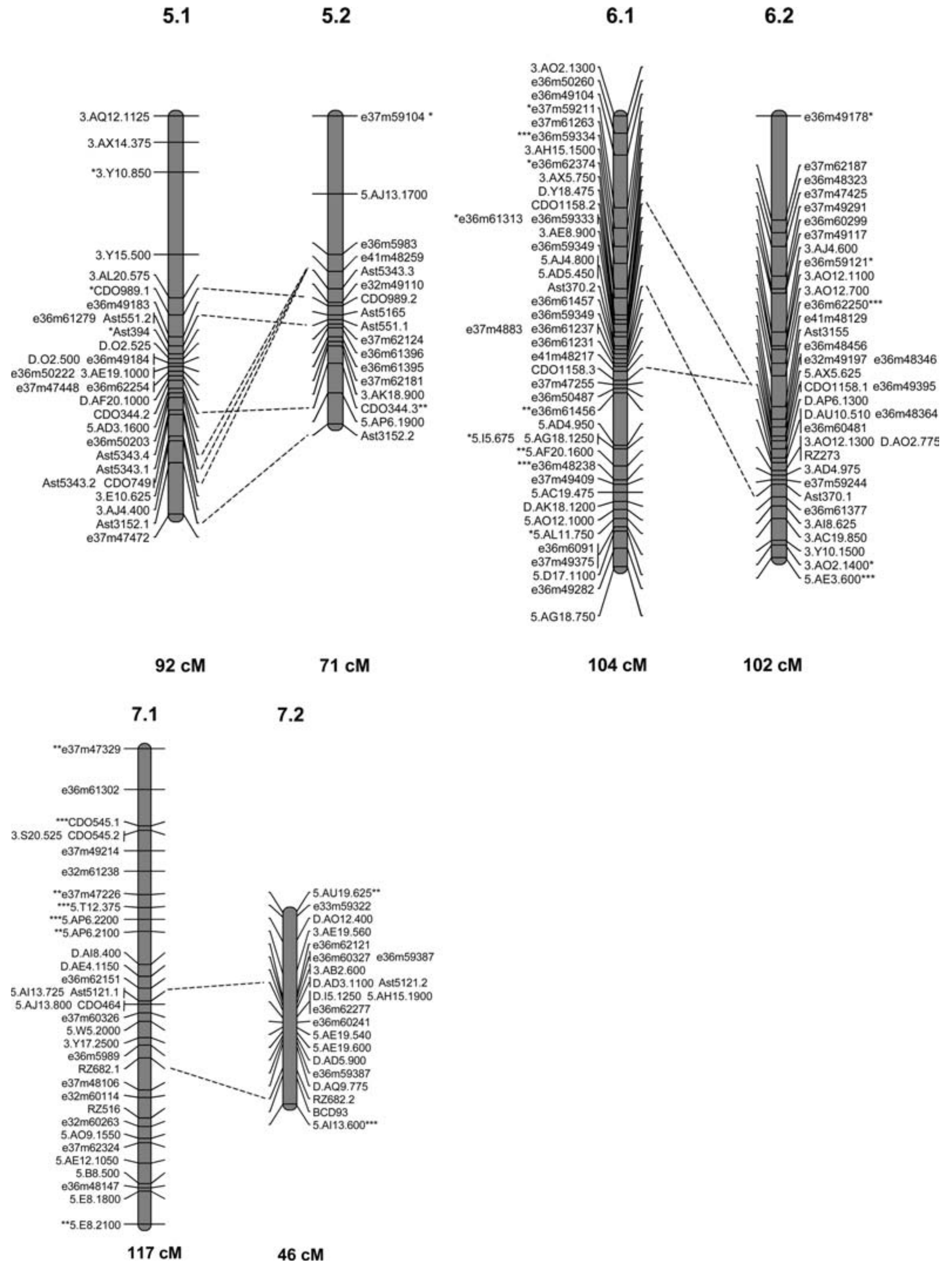

Fig. 1 (Contd.)

Results

RAPD markers

One hundred and sixty-nine RAPD polymorphic fragments were mapped using 94 progeny. Of these, 38 markers segregated 3:1, 69 segregated 1:1, with band present in the female parent (549), and 62 segregated 1:1, with band present in the male parent (372). Whereas the average number of polymorphic bands per primer was three, marker distribution over the linkage groups varied from 4 to 20 per linkage group.

AFLP markers

A total of 219 AFLP markers were scored in 90 progeny, and 180 of these markers were evenly placed on the linkage map. Twenty-two different primer combinations

Table 1 AFLP markers scored and the ratio of coupling- to repulsion-phase linkages. *maxR*Maximum recombination fractions

Parent	Markers scored	Repulsion linkages *maxR* = 37.5 cM	Coupling linkages *maxR* = 37.5 cM	*P*-value 1:1
Female 549	149	299	302	0.90
Male 372	117	123	127	0.80

were used to identify, on an average, ten markers per primer combination. The primer combination ACC and CTG produced the largest number of scored fragments (20), and the ACG and CTG combination yielded the lowest (1). These markers were among all 14 linkage groups with linkage group (LG)6.1 having the most (23) and LG2.2 possessing 4 AFLP markers (Fig. 1).

RFLP markers

Seventy-five RFLP markers were identified using the 24 heterologous cereal probes (18 CDO, 3 BCD, and 3 RZ) as well as the 20 homologous bentgrass probes from the two creeping bentgrass cDNA libraries. Those mapped markers were 32 CDO, 3 BCD, 4 RZ, and 36 Ast markers. Fifty-two percent of the mapped probes detected two loci with a maximum of four alleles in each parent, whereas some probes (45%) generated one polymorphic locus and one monomorphic locus in each parent. Two probes (Ast552 and CDO1158) produced three mapped loci. One probe, Ast5343 generated four mapped loci. Probes that produced complex banding patterns during the parental screening were not used in progeny. However, for most probes it was possible to resolve segregation pattern of the bands into various numbers of scorable loci (1–4). The percentage of RFLP marker loci showing various segregation ratios and types were 45% as 1:1 (lm × ll or nn × np), 44% as 1:1:1:1 (ab × cd or ef × eg), and only 10% of the markers with 1:2:1 (hk × hk).

Examination of chromosomal pairing behavior

The two-point linkage coupling- and repulsion-phase linkage analysis resulted in 299 repulsion-phase linkages and 302 coupling-phase linkages for the female parent (549). The male parent (372) had 123 repulsion-phase linkages and 127 coupling-phase linkages (Table 2). The results are very close to a 1:1 ratio of coupling- to repulsion-phase linkages, which would be expected with disomic inheritance in a tetraploid species (Qu and Hancock 2001). If tetrasomic inheritance through random bivalent pairing or multivalent formation were occurring, far fewer repulsion-phase linkages would be expected than were observed.

Additionally, based on allelic configuration of the RFLP markers, it was determined that each parent never possessed more than two alleles per locus, and only one

Table 2 cDNA clones from creeping bentgrass cDNA libraries mapped on the 549 × 372 population of creeping bentgrass. The *e*-value of the matches to database (blastx) and the map position of the bentgrass ESTs relative to rice (Kikuchi et al. 2003) are indicated

cDNA clone	Putative function	*e*-Value	Putative location on chromosomes	
			Rice	Creeping bentgrass
Ast5185	Inhibition of virus replication (IVR)-like protein [*Nicotiana tabacum*)	5e−56	5	1
Ast5244	Protein kinase MK6 (*Mesembryanthemum crystallinum*)	1e−60	5	1
Ast317	*S*-Adenosylmethionine decarboxylase precursor (*Triticum aestivum*)	1e−3	4	2
Ast324	Plastid-lipid associated protein PAP-related/fibrillin-related (*A. thaliana*)	1e−37	4	2
Ast339	Similar to GDSL-motif lipase/hydrolase (*Oryza sativa*)	4e−19	7	2
Ast354	Mitochondrial F0 ATP synthase D chain (*A. thaliana*)	7e−50	8	2
Ast552	Putative alpha subunit of F-actin capping protein (*O. sativa*)	1e−117	7	2
Ast3147	Expressed protein (*A. thaliana*)	5e−14	1	3
Ast333	Putative MtN3 (*O. sativa*)	3e−3	1	3
Ast563	Fructose-bisphosphate aldolase (*O. sativa*)	2e−70	11	3
Ast3325	Putative leucine-rich repeat transmembrane protein kinase (*O. sativa.*)	2e−69	3	4
Ast537	Knotted 6 (*Hordeum vulgare*)/Putative homeobox gene (*O. sativa*)	3e−60	3	4
Ast394	One-helix protein (*Deschampsia antarctica*)	1e−49	12, 5	5
Ast551	emsy N terminus domain-containing protein (*A. thaliana*)	2e−42	9, 8	5
Ast5165	PS I rxn centre subunit N, chloroplast precursor psaN barley	3e−70	12	5
Ast5343	Putative wound inductive gene (*O. sativa*)	1e−101	8	5
Ast3152	No hit	–	–	5
Ast3155	Putative isoleucyl-tRNA synthetase (*O. sativa*)	8e−76	2	6
Ast370	Expressed protein (*A. thaliana*)	2e−43	2, 4	6
Ast5121	NADH-dependent glyceraldehyde-3-phosphate dehydrogenase (*O. sativa*)	1e−106	8	7

of these two alleles was ever present in any progeny (data not shown). This is typical of the pattern seen in disomic segregation (Lespinasse et al. 2000). Both of these results strongly support disomic inheritance in tetraploid creeping bentgrass.

Segregation distortion

In total, 9% of the RAPD, 12% of the AFLP and 10% of the RFLP markers examined yielded distorted segregation ratios ($P < 0.05$). All LGs possessed markers having distorted segregation ranging from 5% in LG4.1 to 20.5% in LG7.1. However, considering all the marker types, 10.8% of the markers mapped in our map showing distorted segregation ratios is relatively low compared to other studies (Jones et al. 2002; Warnke et al. 2004).

Genetic linkage map and comparative relationships with rice

A. stolonifera is allotetraploid with $2n=4x=28$ (two subgenomes, A_2 and A_3) based on studies on chromosome pairing, isozyme analysis, along with flow cytometry studies (Jones 1956a, b, c; Warnke et al. 1998; Bonos et al. 2002). As expected, 14 LGs were detected that covered a total of 1,110 cM using 169 RAPD, 180 AFLP, and 75 RFLP markers (Fig. 1). LG length varied from 45 cM to 117 cM, and marker distribution ranged from 15 to 45 markers per LG.

Based on the presence of duplicate RFLP loci between two LGs, we have identified seven pairs of homoeologous chromosomes. Such pairing is characteristic of species with allopolyploid genomes. The number of pairs of duplicate loci in each LG varied from two pairs (LG6 and LG7), three pairs (LG1 and LG3), five pairs (LG4 and LG5), and six pairs (LG2). Of the 44 probes tested, 23 (CDO244, CDO542, CDO20, CDO1401, CDO1160, CDO771, CDO344, CDO312, CDO989, CDO405, CDO684, RZ682, Ast317, Ast324, Ast3152, Ast551, Ast333, Ast537, Ast3325, Ast339, Ast370, Ast3147, and Ast5121) generated two loci between pairs of LGs (Fig. 1). In addition, probe Ast5343 generated a total of four marker loci, of which three mapped on LG5.1 and one mapped on LG5.2. Although both Ast552.1 and Ast552.2 mapped on LG2.1 (5 cM apart), Ast552.3 mapped on LG2.2 (Fig. 1). Similarly, CDO1158.2 and CDO1158.3 mapped on LG6.1 (14 cM apart), and CDO1158.1 mapped on LG6.2. In another case, CDO545 generated two marker loci mapped on the same LG7.1 (1 cM apart).

As indicated by the duplicate loci present in different LGs and also the common markers mapped on LGs in ryegrass (Sim et al. 2005) and the Triticeae (Van Deynze et al. 1995c, 1998) that belong to the Pooideae subfamily, we assigned the numbering of all seven LGs in the bentgrass map. Seventeen heterologous probes that were mapped in bentgrass could be assigned to corresponding LGs (at least two probes per LG) in ryegrass map. Only one probe common in ryegrass map was mapped on bentgrass LG6. In the Triticeae, map locations of five Ast clones (Ast5244, Ast5185, Ast339, Ast354, and Ast552) were deduced from rice–wheat synteny information (Sorrells et al. 2003; http://wheat.pw.usda.gov/pubs/2003/Sorrells/). The 27 loci (22 anchor and five Ast) with known map locations in the Triticeae were mapped on seven LGs of bentgrass, in which at least two common loci were contained. The number of syntenic markers mapped on bentgrass LGs 1, 3, 4, 5, 6, and 7 and on respective homoeologous chromosomes of the Triticeae was 4, 2, 3, 4, 2, and 5, respectively. Bentgrass LG2 contained five (Ast552.1, Ast552.2, BCD1184, CDO405.1, and CDO684.1) and two (Ast339.1 and Ast354) syntenic markers on homoeologous chromosomes 2, and 4, respectively of the Triticeae.

In our results, 75 RFLP loci (39 anchor and 36 Ast) that are evenly distributed on bentgrass LGs were used for assessing comparative syntenous relationships with rice. Map locations of all 19 Ast clones (except Ast3152) derived from the creeping bentgrass cDNA clones were successfully deduced from sequence similarity of bentgrass ESTs with rice (Kikuchi et al. 2003; Table 1). Based on 43 common loci (23 anchor and 20 Ast), we detected some syntenic relationships between rice and bentgrass. The most conserved syntenic segments with a minimum of four common markers were observed on bentgrass LGs 1, 2, 3, 4, 5, 6, and 7 that were represented by rice chromosomes 5, 7, 1, 3, 8, 2, and 6, respectively. In addition, the bentgrass LGs 2, 5 and 7 contained chromosomal segments with two syntenic markers, which represent rice chromosomes 4 (CDO684.1, Ast317, and Ast324.2), 9 (CDO 989.1, Ast551.2, and CDO344.2), and 8 (Ast5121.1 and CDO464), respectively.

Discussion

Bentgrass species that are less studied genetically will gain most from well-studied cereal species such as rice, wheat and oat via the comparative mapping approach, which relies on co-dominant markers such as heterologous RFLP probes (Rayapati et al. 1995; Van Deynze et al. 1995a, b, c, 1998). Therefore, construction of a linkage map in bentgrass using a common set of RFLP cereal probes, and homologous RFLP bentgrass probes with known sequence is extremely important.

In this study, we have observed that 59% of the mapped probes hybridized to duplicate loci mapped on two paired LGs. This suggests that these are hybridizing to the two different diploid genomes of the ancestors of creeping bentgrass. This pattern of disomic inheritance with multiple loci per probe provides further evidence of the allotetraploid nature of the creeping bentgrass genome, thus supporting previous findings using

chromosomal pairings by Jones (1956b), and isozymes by Warnke et al. (1998). The number of markers linked in coupling- and repulsion-phase is in a 1:1 ratio, which also suggests disomic inheritance.

Further studies are necessary to identify exactly which LG originated from either the A_2 or A_3 subgenomes. Moreover, studies by Jones (1956b) have shown that colonial (*A. capillaris* A_1 and A_2 subgenomes) and creeping bentgrass are both allotetraploid and share one ancestral subgenome (A_2) in common. Therefore, the construction of linkage maps with same set of RFLP markers in colonial bentgrass combined with comparative mapping analysis might be valuable in finding which chromosomes were derived from the common A_2 subgenome. Interestingly, four (9%) of the mapped RFLP probes detected duplicated loci with various recombination distances within each of the four LGs (LGs 2.1, 5.1, 6.1, and 7.1), suggesting that there might be intragenomic segmental duplication that played a role in recent evolution of creeping bentgrass, as in most flowering plant genomes suggested by Masterson (1994).

Our results indicated some syntenic relationships between bentgrass and rice and the Triticeae based on common locations of anchor and bentgrass EST probes between the bentgrass map presented here and published rice and wheat maps. However, the number of relevant markers was very low for definite conclusion. Addition of further heterologous RFLP and bentgrass EST markers will eventually reveal the syntenic regions and the chromosomal rearrangements that differentiate the bentgrass genome from the other members of Poaceae. Another benefit of such work is that putative orthologous loci or QTL with major effects on important traits such as disease resistance can be readily compared across a broad range of grass genera including turfgrass. Recently, researchers have shown synteny between heading date QTL in perennial ryegrass and a major heading date locus in rice (Armstead et al. 2004). Similar work has revealed consistent locations of QTL and candidate genes between turf or forage grass species and cereals, for example, genes involved in lignin biosynthesis (Cogan et al. 2005) and crown rust resistance (Dumsday et al. 2003). Therefore, this work will also provide the foundation for comparative mapping research in creeping bentgrass and other turfgrass species.

Future use of this map will be detection of QTL for resistance to fungal diseases such as dollar spot, Pythium blight, brown patch, and snow molds, and it will also serve to utilize genetic information from well-studied model species like rice for locating genes for traits of interest in bentgrass. Markers tightly linked to the QTL for various fungal diseases will be used for marker-assisted selection in bentgrass breeding programs.

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