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Comparative Genome Analysis between Agrostis stolonifera and Members of the Pooideae Subfamily Including Brachypodium distachyon

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COMPARATIVE GENOME ANALYSIS BETWEEN *AGROSTIS STOLONIFERA* AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING *BRACHYPODIUM DISTACHYON*

A Thesis Presented

by

LORETO ARANEDA

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Plant and Soil Sciences
COMPARATIVE GENOME ANALYSIS BETWEEN AGROSTIS STOLONIFERA
AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING
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DEDICATION

A mi familia.

Thanks to God for everything I have experienced.

My parents Manuel and Sara……all their love and efforts have made me the person I am now.

My brothers and sister, Manolo, Juan Pablo and Paola……my playmates during my childhood and that make me proud.

My nieces and nephews, Belen, Florencia, Simon and Matias……they have filled my heart of love.

Carlos and Marjorie……for being the happiness of my sister and brother.

And my loved husband Roberto the light in my life.
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ABSTRACT

COMPARATIVE GENOME ANALYSIS BETWEEN *AGROSTIS STOLONIFERA* AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING *BRACHYPODIUM DISTACHYON*

SEPTEMBER 2011

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Understanding of grass genome structure and evolution has been significantly advanced through comparative genomics. The genomes of most cool-season forage and turf grasses, belonging to the Pooideae subfamily of the grasses, remain understudied. Creeping bentgrass (*Agrostis stolonifera*, allotetraploid $2n=4x=28$) is one of the most important cool-season turfgrasses widely used on golf courses due to its low mowing tolerance and aggressive growth habit. Genome analysis of this recently domesticated polyploidy, creeping bentgrass, can add to the understanding of the evolution and domestication of grasses. In this study, an RFLP genetic map of creeping bentgrass using 229 RFLP markers derived from cereal (91) and creeping bentgrass (68) EST-RFLP probes was constructed for a comparative genome analysis. The genetic map of creeping bentgrass (Aveneae tribe) was compared with those of perennial ryegrass (Poeae tribe), oat (Aveneae tribe) and wheat (Triticeae tribe) of the Pooideae subfamily, and rice (Oryzeae tribe) of the Ehrhartoideae subfamily. Large-scale chromosomal rearrangements between the map of creeping bentgrass and the respective maps of the Triticeae, oat, and rice were observed. However, despite their membership in different
tribes of Pooideae, no evidence of chromosomal rearrangements between the maps of creeping bentgrass and perennial ryegrass was detected, suggesting that these recently domesticated species might be closely related than expected. Further comparative genome analysis of creeping bentgrass was performed with whole genome sequences of *Brachypodium distachyon* (Brachypodieae tribe, Pooideae subfamily) using sequences of the above-mentioned RFLP mapped markers and 8,470 publicly available *A. stolonifera* EST (AgEST) sequences. A total of 24 syntenic blocks were identified between the *Agrostis* linkage groups and the *B. distachyon* chromosomes. Orthologous loci of six hundred seventy-eight AgESTs were identified in the *B. distachyon* genome, and these loci can be utilized in further comparative mapping of Pooideae species. Insights from comparative genomics with *B. distachyon* will be useful for genetic improvement of *Agrostis* spp. and provide a better understanding of the evolution of the Pooideae species.
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CHAPTER 1
COMPARATIVE GENOME ANALYSIS BETWEEN *AGROSTIS STOLONIFERA*
AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING
*BRACHYPODIUM DISTACHYON*

**Introduction**

Eukaryotic genomes differ by gene content on chromosomes as well as the order of these genes, which are known as synteny and collinearity, respectively. Angiosperm genomes vary in size and arrangement, even within closely related species. Despite the frequent whole-genome duplication in angiosperms, the number of chromosomes is less variable than genome size. Analyses of changes in genomic structure, such as specific gene rearrangements, insertions or deletions, provide an informative way to clarify relationships among lineages. In the grass family Poaceae, significant variation in DNA content and chromosome number suggest that the evolution of these genomes is fast and dynamic (Tang et al. 2008).

Comparative genomics studies in temperate cereals have been carried out using rice as a model system due to its compact and well-annotated genome sequence. However, the active changes in rice genome sequences, the lack of some biological features, characteristics of a model organism (easy to grow, short plant and life cycle) and the phylogenetic distance from the Pooideae subfamily (that include wheat, barley and temperate forage and turf grasses) lead one to question the utility of this species as a model system. Moreover, rice does not display all the traits that are applicable to study in
temperate grasses, such as fungal resistance, freezing and heat tolerance, wear and injury tolerance and others (Draper et al. 2001; Pacurar, 2009).

A new species, *Brachypodium distachyon* is proposed as a better model system for temperate grasses than rice. *Brachypodium distachyon* is an annual wild grass member of the Brachypodieae tribe and the Pooideae subfamily. Besides its small and complete sequenced genome, *B. distachyon* has additional desirable biological features and a closer phylogenetic relationship to temperate cereals, forage and turf grasses (Draper et al. 2001; Pacurar, 2009; Kumar et al. 2009; Vogel et al. 2010; Garvin et al. 2010).

Comparative genome analysis using a common set of RFLP probes in plants has demonstrated shared gene content and collinearity of genes among related Poaceae species, such as maize, wheat, barley, oat and rice. When whole-genome sequences or physical maps are not available, marker-based mapping is the best approach for a better understanding of the whole-genome (Devos et al. 1993; Van Deynze et al. 1995a, 1995b; Gaut, 2002).

Species of the genus *Agrostis* (known as bentgrasses) are perennial, self-incompatible, and C3 cool-season grasses and are native to the temperate and subarctic climates of Western Europe. The four species commonly accepted as turfgrass types are: 1) creeping bentgrass *A. stolonifera* L. (*2n*=4*¥*=28, A$_2$ and A$_3$ genomes), 2) velvet bentgrass *A. canina* L. (*2n*=2*¥*=14, A$_1$ genome), 3) colonial bentgrass *A. capillaris* L. (*2n*=4*¥*=28, A$_1$ and A$_2$ genomes), and 4) redtop bentgrass *A. gigantea* L. (*2n*=6*¥*=42, A$_1$, A$_2$, and A$_3$ genomes). Creeping bentgrass is the most widely used for golf course because of its fine texture, excellent tolerance to low mowing, strong stoloniferous growth habit
and cold tolerance. The genus *Agrostis* is placed in the subfamily Pooideae of the grass family Poaceae that include rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), oat (*Avena sativa* L.), ryegrass (*Lolium* spp.), and sorghum (*Sorghum bicolor* L.). *Agrostis* is assigned to the tribe Aveneae, which also includes oat (Renvoize and Clayton, 1992; Watson, 1990). Further, the tribes Aveneae and Poeae are closely related (Kellogg, 1998) and in some phylogenies have been combined, but this relationship remains unresolved (Grass Phylogeny Working Group, 2001).

A few studies on molecular marker-based genetic mapping and trait mapping have been conducted in *Agrostis*. The first DNA marker based genetic linkage map of creeping bentgrass (Chakraborty et al. 2005) was developed from a full-sib reference mapping population by crossing two naturalized clones (549 and 372) collected from old golf courses in Wisconsin (Wang et al. 2005). The map covered a distance of 1,110 cM, and contained 14 linkage groups, which were mapped with 424 loci, including expressed sequence tag-restriction fragment length polymorphism (EST-RFLP), randomly amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP). Seven pairs of the homoeologous chromosomes were identified based on duplicated RFLP loci and numbered according to syntenous chromosomes of perennial ryegrass and wheat (Chakraborty et al. 2005). In addition, first genetic linkage map of colonial bentgrass using the backcross population of colonial bentgrass x creeping bentgrass (recurrent parent) mapped with AFLP and gene-based markers was published (Rotter et al. 2009). Recently, QTL mapping of field resistance to dollar spot caused by *Sclerotinia homoeocarpa* was investigated in an enhanced map of the 549 x 372
population using additional EST-RFLP loci (Chakraborty et al. 2006). Comparative genome relationships between *Agrostis* and Poacea spp. need to be established so that genome resources from well-studied species can be utilized to understand genome of underrepresented species for improvement.

One objective of this study was to investigate comparative genome relationships between the EST-RFLP based genetic map of the creeping bentgrass 549 x 372 population and previously published maps of the Triticeae, perennial ryegrass, oat, and rice. For the first time, the creeping bentgrass map is integrated with those members of Poaceae family. A second objective of this study was to explore the utility of *B. distachyon* as a model system for members of Pooideae subfamily. Comparative genome analysis of *A. stolonifera* was performed with whole genome sequences of *B. distachyon* using publicly available sequences of 8,470 *A. stolonifera* EST and sequences of the above-mentioned EST-RFLP mapped markers.

**Materials and Methods**

**Plant material**

A two-way pseudo-testcross population, which was developed from the cross between two highly heterozygous creeping bentgrass parental clones 549 and 372, was previously used to develop a genetic map (Chakraborty et al. 2005) using AFLP, RAPD, heterologous cereals and creeping bentgrass EST-RFLP markers. The map density was further enhanced for QTL analysis of dollar spot resistance by mapping additional EST-
RFLP markers (Chakraborty et al. 2006). For comparative genome analysis, an EST-RFLP genetic map was constructed in the current study.

In brief, the controlled crosses were performed in a greenhouse by bagging panicles of both parents with a pollination bag after a field vernalization process. Two parental clones, 549 and 372 were chosen due to differences in morphological traits such as leaf color, shoot density, and root depth, and a significant difference in disease responses to snow mold (caused by *Typhula* spp.) (Wang et al. 2005) and dollar spot (caused by *Sclerotinia homoeocarpa*) (Chakraborty et al. 2006). Six hundred ninety-seven progeny were obtained from seeds harvested from the female clone 549. Of them, a randomly selected progeny of 90 individuals was used for the linkage map construction and for the comparative genome analysis.

**RFLP analysis**

Young leaves from greenhouse grown plants were harvested, immediately lyophilized and finely ground using a homogenizer. Genomic DNA was extracted from 500 mg of ground tissue using a modified CTAB method (Saghai-Marooof et al. 1984). Ten μg of genomic DNA from each plant was digested with five restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, and *Hind*III) (Promega, Madison, WI). Digested DNAs were loaded onto 1% (w/v) agarose gels and the restriction fragments were separated based on size by electrophoresis for 28 hours at 30V. The DNA from the gels was transferred to Hybond-N+ nylon membranes (Amersham, Piscataway, NJ) by capillary transfer overnight and then treated with optimal crosslink in a UV microwave.
Prehybridization and hybridization were performed in a rotary hybridization chamber (Techne, Inc.) at 65°C. Probes were labeled with $^{32}$P by a random priming method (Feinberg and Vogelstein 1983). The membranes were washed in 0.5X SSC and 0.1% SDS for 1 hour at 65°C. Membranes were exposed to X-ray film with intensifying screening for five days at -80°C. The membranes were re-probed 10 to 15 times by first treating them with 0.2M NaOH for 20 min and then with 0.5M Tris/HCL pH 7.5, 0.1X SSC, 0.1% SDS for 20 min at room temperature.

A common set of 152 heterologous anchor probes which contain oat cDNA (CDO), barley cDNA (BCD), and rice cDNA (RZ) from Cornell University, Ithaca, N.Y. (Van Deynze et al. 1998) and additional CDO and BCD probes (the USDA probe depository, Albany, CA) were obtained. Probes with known map locations in the other grass species such as perennial ryegrass, wheat, and oat were initially selected for a further screening step. In addition, 140 creeping bentgrass EST-RFLP probes named “Ast” obtained from two cDNA libraries for each parental clone of the 549 x 372 population (Chakraborty et al. 2005) were first screened against two mapping parents, 549 and 372. Polymorphic probes with a simple and scoreable segregating pattern, showing mostly single or two loci (rarely three or four loci), were selected and tested on the progeny.

**Linkage map construction**

An EST-RFLP based genetic map was constructed using JoinMap 3.0TM software (Van Ooijen and Voorrips 2001), which can handle out-crossing species using
any marker types with various modes of segregation. For example, analyzed RFLP markers were categorized into one of five segregation types: 1) a heterozygous locus in both parents with four alleles, 2) a heterozygous locus in both parents with one common allele, 3) a heterozygous locus in both parents with two common alleles, 4) a heterozygous locus in the maternal parent, and 5) a heterozygous locus in the paternal parent.

LOD thresholds ranged from 4.0 to 10.0 were used for grouping of markers. The numbering of each pair of homoeologous linkage groups (LGs) was followed according to Chakraborty et al. (2006) and map distances were calculated using the Kosambi mapping function (Kosambi, 1944).

Comparative genome analysis between creeping bentgrass and Triticeae, oat, perennial ryegrass, rice and B. distachyon

The bentgrass 549 x 372 EST-RFLP genetic map was compared with the consensus genetic map of three Triticeae species developed among T. aestivum, T. tauschii, and Hordeum spp. available from the GrainGenes website (http://www.wheat.pw.usda.gov/ggpages/maps.shtml) (Van Deynze et al. 1995c). Comparative map analysis with perennial ryegrass was conducted by comparison with the previously published ryegrass maps (Jones et al. 2002; Sim et al. 2005) and the updated ryegrass map using 13 bentgrass Ast probes (Sim et al. 2007). Comparative map analysis with oat was performed through comparison with the oat map of Van Deynze et al. (1995b). Comparative map analysis with rice was conducted using maps obtained
from Gramene website (http://www.gramene.org/cmap) and with the rice map of Ahn and Tanksley (1993). For the purpose of the comparative genome analysis in this study, a syntenic block between the two species was defined when a segment containing two syntenic loci was not disrupted by a non-syntenic locus or a segment with more than two syntenic loci was not disrupted by two consecutive non-syntenic loci.

Those bentgrass EST-RFLP markers mapped were further analyzed for their putative function by blasting their sequences against protein sequences of known genes at E-value $\leq 1 \times 10^{-5}$ as a cut-off using BLASTX on the NCBI’s web server. Map locations of Ast clones were deduced from syntenous chromosome locations in rice using sequence similarity with a Japonica rice cDNA clone collection (Kikuchi et al. 2003) and wheat synteny information (Sorrells et al. 2003; http://wheat.pw.usda.gov/pubs/2003/Sorrells). Marker locations and order, detected with the common probes in each respective maps of the Triticeae, ryegrass, oat, and rice, were represented according to the published information cited above.

Because the EST-RFLP markers found in the creeping bentgrass map do not have genetically mapped information in B. distachyon, the comparative analysis with this species was performed using BLASTN at B. distachyon web site (www.brachybase.org and www.phytozone.net) to determine the chromosomal location, selecting the alignments with E-values $\leq 1 \times 10^{-10}$. Moreover, sequences of 8,470 A. stolonifera EST (AgEST) publicly available (Rotter et al. 2007) were blasted against the B. distachyon genome to find orthologous loci between these two species. Before the BLAST analysis all the redundant AgEST sequences were removed using the CD-HIT-EST program with a sequence identity cut off $\geq 90\%$ (Li and Godzik, 2006). The identification of orthologs
was based on two more stringent parameters CIP (Cumulative Identity Percentage) and CALP (Cumulative Alignment Length Percentage), and values of CIP ≥60% and CALP ≥70% were used as thresholds (Salse et al. 2008; Kumar et al. 2009).

Results

Linkage map construction

Two hundred-twenty one heterologous RFLP probes (62 BCD, 121 CDO and 38 RZ) were chosen from a common set of 152 anchor probes (Van Deynze et al. 1998) and from additional CDO and BCD probes (the USDA probe depository, Albany, CA) because of their known positions on perennial ryegrass and Triticeae maps (Jones et al. 2002; Sim et al. 2005; Van Deynze 1995a and c). These probes were screened against bentgrass mapping parents and those with strong hybridization signals were selected to test in the progeny (Chakraborty et al. 2005 and 2006). Fifty-five and sixty-two percentages of the barley BCD and oat CDO probes tested, respectively detected simple and scoreable band patterns (data not shown). However, most of the rice RZ probes showed weaker signals than those BCD and CDO probes. Some of the probes with the strong signals were not used due to their complex band patterns with more than two segregating loci per parent, which generated many allelic combinations that made them difficult to score. For example, probe CDO344 generated the most complex band patterns, showing 18 different segregation patterns. In total, 115 probes with simple and clear hybridization signals were tested to determine their segregation among progeny.
Among the tested probes 91 of them were scored and generated 126 markers, which were successfully mapped on 14 LGs (Fig. 1). The other twenty-two probes tested could not be scored because of their faint or smeared bands.

In addition, a total of 140 creeping bentgrass EST-RFLP probes (Ast) were tested for first screening between two mapping parents, 549 and 372. Seventy-two of them exhibited a simple and scoreable segregating pattern, showing mostly single or two loci (rarely three or four loci), were selected and tested on the progeny individuals. Sixty-eight probes generated 103 markers, which were successfully mapped on 14 LGs (Fig. 1).

Five types of RFLP segregation patterns among progenies were detected (ab x cd, ef x eg, hk x hk, lm x ll and nn x np). One locus, heterozygous in one of the parents (lm x ll or nn x np) was the most dominant among the segregation types. The lm x ll type (66%), heterozygous only in the 372 parent, was much more frequent than the nn x np type. Ninety three percent of the mapped markers segregated 1:1 or 1:1:1:1, with a few that segregated 1:2:1. Deviations from the expected segregation ratios (1:1, 1:1:1:1 or 1:2:1) were calculated for all of the mapped markers based on Chi-square contingency tables. Twenty-six percent of the mapped markers deviated significantly from the expected ratios. Particularly on LG5.2, one half of the mapped markers were significantly distorted at $P<0.05$. 
Figure 1. EST-RFLP genetic linkage map of creeping bentgrass. The total genetic length (cM) of each linkage group is indicated below it. The creeping bentgrass, oat, barley and rice cDNAs used as RFLP markers are indicated as Ast, CDO, BCD and RZ followed by the clone number (clone numbers plus ‘.1’, ‘.2’, ‘.3’ or ‘.4’ which show duplicated loci that where linked by a black dotted line). The segment on LGs 6.1 and 6.2, spanning three RFLP markers (CDO1380, CDO1158 and CDO534) and highlighted in orange indicated an inversion and translocation between the two homoeologous LGs.
The final map utilized in the current study included 229 markers generated from 159 RFLP probes including a 42% of those probes that were duplicated (Table 1). The total number of markers was separated into 14 LGs at LOD thresholds ranging from 4.0 to 10.0 (Fig. 1). The numbers for each of the linkage groups (LG1 to LG7) were assigned according to the common probes mapped in genetic maps of the Triticeae (Van Deynze et al. 1995c) and perennial ryegrass (Jones et al. 2002; Sim et al. 2005 and 2007). The numbering of each pair of the homoeologous LGs followed Chakraborty et al. (2005 and 2006). A total of 865 cM was covered in the current genetic map and the genome coverage varied from 30 cM to 91 cM for each LG. The number of mapped marker distribution was from 11 to 22 per LG and the average interval between markers was 3.8 cM in size (Fig. 1).

**Comparative genome relationships between creeping bentgrass and Triticeae, oat, perennial ryegrass, rice and B. distachyon**

Comparative genome relationships between the map of creeping bentgrass and the respective map of the Triticeae, rice, perennial ryegrass and oat using a common set of heterologous EST-RFLP cereal probes and EST-RFLP creeping bentgrass probes were shown in Figure 2. Overall, comparative mapping showed highly conserved syntenic relationships between creeping bentgrass and the Triticeae, ryegrass, oat and rice.

Besides, the sequences of the Ast probes in the current study were BLAST searched, and 39 of them showed similarity to known genes. Moreover 47 and 32 Ast
probes showed a putative location on rice and wheat chromosomes, respectively (Table 2).

**Triticeae**

The 53 EST-RFLP loci with known map locations in the Triticeae were evenly distributed on seven pairs of creeping bentgrass LGs (Fig. 2). These markers covered 98% of the bentgrass map (Table 3). Bentgrass LGs 1, 2, 6, and 7 except for a few non-syntenic loci, showed almost complete synteny with corresponding homoeologous chromosomes of the Triticeae. We detected a chromosomal rearrangement on bentgrass LG3.2 relative to Triticeae chromosomes 1 and 3. Bentgrass LG3.2 is represented by the insertion of a segment (covered by Ast5166.2 and Ast5170) of the Triticeae chromosome 1 between two distinct segments of Triticeae chromosome 3. Bentgrass LGs 4.1 and 4.2 contained large-scale chromosomal rearrangements relative to the Triticeae chromosomes 4 and 5. A distal end segment of the Triticeae chromosome 5 flanked by CDO484.1 and CDO504.2 loci on LG4.1 was inserted into the Triticeae chromosome 4. For LG4.2 the same segment flanked by the duplicated loci, CDO484.2 and CDO504.1 was observed. However, due to disruption by a non-syntenic marker (BCD808), a block of synteny could not be established. In addition, bentgrass LG5.1 was represented by segments of Triticeae chromosomes 4 and 5. A segment of Triticeae chromosome 4 flanked by Ast357 and CDO989.1 loci was inserted into Triticeae chromosome 5. In total, we detected three large-scale chromosomal rearrangements in LGs 3, 4, and 5, which can differentiate the bentgrass genome from the Triticeae genome.
Figure 2. Comparative genome relationships between creeping bentgrass genetic linkage map and the genetic maps of rice (R), Triticeae (W), oat (O) and ryegrass (Rg), respectively, represented for each of the black columns. The markers indicated on the right correspond to those mapped in the creeping bentgrass linkage map (Fig. 1). The number or letter inside the boxes shows the segments of chromosomes or linkage groups from each of the genomes (R, W, O, Rg) that are syntenic to the bentgrass linkage groups. The arrowheads indicate the deduced location of the centromere in bentgrass from the comparisons with Triticeae chromosomes. The total genetic length (cM) of each linkage group is indicated on bottom left.
Figure 2. Continued.
Oat

Relatively small coverage of the bentgrass map (76%) was detected by 42 common loci with oat, which were caused by lack of common probes and resulted in a weak syntenic block on bentgrass LG2 (Table 3). Our comparative map indicated that bentgrass LGs 4 and 5 consisted of rearrangements (reciprocal translocation) of oat chromosomes E and F (Fig. 2). Bentgrass LG4.2 was composed of two segments of oat chromosomes E and F. A segment from the most distal area in oat chromosome F between CDO1395 and CDO20.2 loci was inserted between CDO38 and BCD450 loci in oat chromosome E. Bentgrass LGs 5.1 and 5.2 also included two segments of chromosomes E and F. Therefore bentgrass LGs 4 and 5 were differentiated from oat chromosomes E and F. In addition, we detected a large-scale chromosomal rearrangement on bentgrass LG3.1 relative to oat chromosomes B and C. The LG3.1 is represented by the attachment of a segment (flanked by CDO244.1 and BCD98.2 loci) of the oat chromosome B to the distal end of oat chromosome C.

Perennial ryegrass

The 104 common loci were assigned to their locations on the ryegrass map, which covered 92% of the bentgrass map (Table 3). Despite the most number of the common loci shared between the two species, except for some non-syntenic loci, all bentgrass LGs have complete syntenic relationships with those of ryegrass (Fig. 2). Even for bentgrass LGs 4 and 5 which are different from ones of Triticeae and oat, no evidence of large-scale chromosomal rearrangement at the present map resolution was observed between bentgrass and ryegrass.
**Rice**

The 83 common loci mapped on rice and bentgrass genetic maps covered 82% of the bentgrass map (Table 3). The genome coverage was higher because map locations of the Ast mapped loci were deduced from sequence similarity of bentgrass ESTs with rice. Twenty-nine segments that are derived from all of the rice chromosomes, except for rice chromosome 11, explain all the bentgrass linkage groups (Fig. 2). However, BCD808 on LG4.2 represents rice chromosome 11 and the three duplicated loci generated from Ast563 probe, which were mapped on LGs 5.1, 5.2, and 3.2 represent rice chromosome 11. With the exception of a few non-syntenic loci, both homoeologous pairs of LGs 3 and 6 showed the most conserved syntenic relationships with the homologous chromosomes 1 and 2 of rice, respectively. On the other hands, the other five bentgrass LGs (1, 2, 4, 5, and 7) showed large-scale chromosomal rearrangements relative to rice. Bentgrass LG1.2 was comprised of two syntenic segments from rice chromosomes 5 and 10. A segment containing BCD921 and CDO94 from rice chromosome 10 was inserted into two segments of rice chromosome 5. Only CDO98 on LG1.1 represents rice chromosome 10. Bentgrass LGs 2.1 and 2.2 are represented by segments of rice chromosomes 4 and 7. Bentgrass LG4.2 is represented by the insertion of rice chromosome 7 between two distinct segments of rice chromosome 3. Bentgrass LGs 5.1 and 5.2 are represented by two segments of rice chromosomes 9 and 12. Lastly, LGs 7.1 and 7.2 are represented by the insertion of rice chromosome 8 between two distinct segments of rice chromosome 6.
B. distachyon

Chromosomal location of the EST-RFLP loci mapped in the bentgrass genetic map was deduced by similarity between sequences of the EST-RFLP markers and the B. distachyon chromosome sequences (Table 4). One hundred fourteen out of the 160 EST-RFLP markers with significant alignments (E≤1x10^-10) to B. distachyon genome were used for analysis of comparative relationship between these two species. Twenty-four segments derived from all the five B. distachyon chromosomes were distributed in the 14 bentgrass LGs (Fig. 3). However at the current resolution, only two markers CDO684 and Ast5162 duplicated on LGs 2.1 and 2.2 established a block of synteny with chromosome 5 of B. distachyon. Markers CDO1328 on LG2.1, Ast567 on LG2.2, CDO244.1 on LG3.1 and CDO244.2 on LG3.2 also represent chromosome 5 of B. distachyon. The most conserved syntenic relationships were found between LG1.1 and chromosome 2, LGs 3.1-3.2 and chromosome 2, LGs 4.1-4.2 and chromosome 1, LG5.2 and chromosome 4, and LGs 6.1-6.2 and chromosome 3 of B. distachyon. Out of them, the largest inferred region showing 14 syntenic loci was detected between bentgrass LG4.2 and B. distachyon chromosome 1. However, on LGs 3.1 and 3.2 still remain large regions with unknown syntenic relationship to sections of B. distachyon chromosomes.

The rest of the bentgrass LGs showed large-scale chromosomal rearrangements (LGs 1.2, 2.1, 2.2, 5.1, 7.1 and 7.2). Bentgrass LG1.2 presents synteny with two segments of B. distachyon chromosomes 2 and 3; linkage group 2.1 has two blocks of synteny with chromosomes 1 and 5.
Figure 3. Comparative genome relationship between creeping bentgrass and Brachypodium distachyon. Each of the bars represents the bentgrass linkage groups (total length in cM, below the bar) as showed in Fig.1. The boxes correspond to the conserved synteny to a segment of Brachypodium chromosome (number inside). The position of nonsyntenic markers within a block of synteny is showed in parenthesis next to the marker name. Markers in bold, within a block of synteny, were additionally located in a different chromosome (indicated in parenthesis next to the marker). The collinearity is represented by the color lines that link the markers with the highly similar sequences located in B. distachyon chromosomes (Bd).
Figure 3. Continued.
Figure 3. Continued.
Linkage group 5.1 is represented by the insertion of a segment of chromosome 3 between two segments of chromosome 4, a similar rearrangement in LGs 7.1 and 7.2 but in these cases the insertion of chromosome 3 is between two segments of *B. distachyon* chromosome 1.

Linkage group 2.2 did not show chromosomal rearrangement but only showed four loci in a block syntenic to *B. distachyon* chromosome 1 (Fig. 3). However the rest of the loci (6) were found either in chromosomes 1, 4 or 5 and this could represent a possibility of additional insertions of one or all these *B. distachyon* chromosomes.

The orthology analysis between 8,470 *A. stolonifera* ESTs (AgEST) and *B. distachyon* genome showed 678 non-redundant AgEST orthologs to *B. distachyon* [196 AgEST in Ch1 (29%), 136 AgEST in Ch2 (20%), 151 AgEST in Ch3 (22%), 104 AgEST...
in Ch4 (15%) and 91 AgEST in Ch5 (14%)] in Figure 4. Ninety five percent of these orthologous loci corresponded to unique loci and the rest were found duplicated among and within *B. distachyon* chromosomes (Table 5).

**Figure 4.** Creeping bentgrass ESTs orthologous to *Brachypodium distachyon* and their location in the physical map of *B. distachyon* (in base pairs). Bd1, Bd2, Bd3, Bd4 and Bd5 represent the *B. distachyon* chromosomes (1 to 5) and horizontal lines for the position of the 678 orthologs. The segments of different colors indicate the creeping bentgrass linkage groups that have synteny with a specific region of *B. distachyon* chromosomes. The arrowheads indicate the centromeric region.
Discussion

Creeping bentgrass EST-RFLP map

The total RFLP-based map size is 865 cM, which is shorter than a previously published creeping bentgrass genetic map (1,110 cM in Chakraborty et al. 2005 and 2006). This difference may be due to the number and type of markers mapped (229). Other marker systems such as AFLP may make the linkage map length longer due to double crossing-overs. For example, AFLP maps in perennial ryegrass have the longest length (Bert et al. 1999). Genotyping errors should also be considered.

In this study, sixty-eight Ast probes generated 103 RFLP markers, which were mapped on 14 LGs (Fig. 1). The sequences of those Ast probes showed similarity to known genes and a putative location on rice and wheat chromosomes (Table 3). These results reflect that Ast probes have the high potential for use in genomic analysis not only for creeping bentgrass but also other for bentgrass spp.

Two subgenomes of creeping bentgrass

Agrostis species are suggested to have some of the most complex genome structures among other genera within the grass family (Bonos et al. 2002). Early cytological studies performed by Jones (1956a, b, and c), studies of disomic inheritance of polymorphic isozymes, the published RFLP based linkage map with duplicated markers likely originated from the two subgenomes (A₂ and A₃) and the ratio of coupling
vs. repulsion linkages between markers, suggest that creeping bentgrass is a strict allotetraploid comprised of two $A_2A_2$ and $A_3A_3$ subgenomes (Warnke et al. 1998; Chakraborty et al. 2005 and 2006). At the current map resolution, a high percentage (42%) of the mapped RFLP markers were duplicated and evenly distributed on the seven pairs of homoeologous LGs (Fig. 1) which further confirmed an allotetraploid origin suggested by Jones (1956a, b, and c), Warnke et al. (1998), and Chakraborty et al. (2005). Duplication of markers within and among LGs in Figure 1 were detected in following probes: Ast5244, Ast39, Ast5343, Ast552, Ast563, BCD98 and CDO99. Evidence of inversion and translocation between distal end segments of two homoeologous LGs 6.1 and 6.2 indicated that two subgenomes of creeping bentgrass could have gone through major chromosomal rearrangements. However, more research is required to confirm whether duplications of markers are within homologous chromosomes before homoeologous chromosomes are differentiated. Unfortunately, diploid bentgrass species with either $A_2$ or $A_3$ genome are presently unknown. Colonial and velvet bentgrasses have been proposed to have genome compositions $A_1A_1A_2A_2$ and $A_1A_1$ based on cytological evidence, respectively, where the $A_1A_1$ genome is common. Recent DNA sequences based phylogenic analysis suggested that genome designation for velvet bentgrass should be $A_2$ genome (Rotter et al. 2010). Comparison of these two species’ maps mapped with the same set of RFLP probes uniquely present in each genome should reveal the chromosomes of the $A_2$ genome common in creeping and colonial bentgrasses.
Synteny between creeping bentgrass and Triticeae, ryegrass, oat, rice and B. distachyon, and genome level affinity

A high level of conserved synteny between creeping bentgrass and other grass species such as Triticeae, ryegrass, oat and rice were observed in the current study. Comparative mapping studies within Poaceae species also revealed evidence of unique chromosomal rearrangements. Kellogg (1998) studied genome structure within the grass family using comparative mapping and suggested that all species belonging to the Pooideae subfamily have two unique chromosomal rearrangements (5-10-5 and 6-8-6) relative to rice. As a member of the Pooideae subfamily, creeping bentgrass in the current study clearly shows the 5-10-5 and the 6-8-6 rice rearrangements on creeping bentgrass LGs 1 and 7, respectively as observed in ryegrass (Sim et al. 2005).

The bentgrass LGs fell in the 12 groups of orthology between rice and wheat defined by Salse et al. (2008), however LGs 4 and 5 show differences due to lack of synteny with chromosome 11 of rice. When we compare creeping bentgrass with rice at this level of map resolution, only four markers (CDO365, CDO520, BCD808 and Ast563) represent this rice chromosome in six different bentgrass LGs (2.2, 3.2, 4.1, 4.2, 5.1, and 5.2). The poor synteny with this rice chromosome may be attributable to the high fraction of repetitive sequences described in rice chromosomes 11 and 12 (29.5% and 31.6%, respectively) producing a disruption of the synteny (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005).

Based on comparisons of morphological characters, bentgrass and oat belong to the same Aveneae tribe, while ryegrass belongs to the Poeae tribe (Renvoize and Clayton,
Results of our comparative genome analyses show strong syntenic relationship between bentgrass and ryegrass, with only four non-syntenic markers. On the other hand, events of large-scale chromosomal rearrangements relative to oat were detected on bentgrass LGs 3, 4 and 5. Results suggest that bentgrass is more closely related to ryegrass than to oat in terms of genome structure, despite a closer taxonomic affinity between bentgrass and oat. These results combined with previous results in the recently domesticated species ryegrass provide strong evidence to dispute the currently accepted taxonomic relationship among species within Pooideae subfamily, which Jones et al. (2002) and Sim et al. (2005) first brought into question. Additionally, Rotter et al. (2007) carried out the first and only phylogenetic analysis that included two members of Agrostis genus (A. stolonifera “creeping bentgrass” and A. capillaris “colonial bentgrass”) based on sequences of conserved ortholog sets (COS). This study clustered creeping and colonial bentgrasses more closely to tall fescue (tribe Poeae) than to oat (tribe Aveneae). Therefore, these recently domesticated species, bentgrass and ryegrass, might be closely related and “wild” grass species of the Poaceae family.

Based on the common sets of markers, when creeping bentgrass is compared to rice, the number of chromosomal rearrangements is eight, on the LG1.2, 2.1-2.2, 4.2, 5.1-5.2 and 7.1-7.2. The same types of rearrangements were previously reported in ryegrass when it is compared to rice (Sim et al., 2005). However when creeping bentgrass is compared to B. distachyon, this number is reduced to six because LGs 4.1 and 4.2 do not show any evidence of chromosomal rearrangements. Nevertheless we observed many changes in the order of markers within blocks of synteny across the different LGs (Fig.3), a situation that could indicate that gene movements within chromosome have taken place.
with a relatively high frequency (de la Rota, 2004). Despite all the *B. distachyon* chromosomes are represented across the 14 LGs of creeping bentgrass, chromosome 5 of *B. distachyon* is significantly underrepresented (only two syntenic blocks defined by two markers, Fig. 3). According to Vogel et al. (2010) the high LTR retrotransposon density found in this *B. distachyon* chromosome could increase syntenic disruptions.

We believe that one way to establish a more accurate syntenic relationship with *B. distachyon* chromosomes is to develop molecular markers from the creeping bentgrass EST orthologous to the *B. distachyon* genome identified in this study, which could serve as a roadmap in order to enhance the *Agrostis* linkage map. As a preliminary attempt we have designed intron-spanning primers (data not shown) based on *B. distachyon* genome sequences and creeping bentgrass EST orthologs (Feltus et al. 2006). These primers were screened in nine species from the Aveneae, Poeae and Brachypodieae tribes and around 70% of them amplified strong polymorphic bands in all the species. Future linkage studies could be performed using intron-spanning markers, considering the close proximity of introns to exons and the conserved position of introns.

A previous study detected the most robust QTL for dollar spot resistance on LG7.1 of the creeping bentgrass linkage map (Chakraborty et al. 2006). The same authors mention that QTL for SHB-TRS (sheath blight resistance caused by *Rhizoctonia solani*) could be detected close to the dollar spot resistance QTL, since both fungi may have similar pathogenicity. Based on our comparative mapping, there is a highly similar genome structure between creeping and ryegrass. Previous studies have located QTL for crown rust and powdery mildew resistance in linkage group 7 of ryegrass genetic map (Sim et al. 2007 and Schejbel et al. 2008). With this limited information we could suggest
that LG7.1 of creeping bentgrass may have a more important role in disease resistance than the rest of the linkage groups. When we compare creeping bentgrass with rice for syntenic QTL information, we found that the QTL for SHB-TRS resistance is located on chromosome 11 of rice (Channamallikarjuna et al. 2010) and several genes of agronomic importance (blast, bacterial, virus, insect resistance and others) have been mapped in rice chromosomes 11 and 12, moreover the resistance and defense response genes [leucine-rich repeat (LRR), protein kinase and NB-ARC domains] are enriched on these two chromosomes relative to the whole genome of rice (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005). However due to lack of synteny with this rice chromosome, it is not possible to establish a link between the genetic information of rice and the QTL information of creeping bentgrass at an equivalent level of resolution.

In addition to this, when we examined the putative function of the complete data set of creeping bentgrass EST used in this study we found 13 of them showed similarity to genes associated with defense or disease resistance. The bentgrass ESTs with hits from the *B. distachyon* proteome and homologous genes were localized mainly to *B. distachyon* chromosomes 1, 2 and 3 (Table 6). As shown in Figure 3, chromosomes 1 and 3 of *B. distachyon* show synteny with bentgrass LG7.1 where the major QTL for field resistance to dollar spot was found. Additionally, Chakraborty et al. (2006) detected QTL with smaller effect in LGs 7.2, 2.1, 3.2, 4.1, 4.2 and 6.2 that present synteny with *B. distachyon* chromosomes 1, 2 and 3. A recent study indicated a higher level of synteny and conservation of resistance gene positions between *B. distachyon* and barley, as compared with rice and barley (Drader and Kleinhofs, 2010). Although rice has been extensively used as a model system for grasses, genome information of *B. distachyon*
could be easily transferable for comparative studies, at least in Pooideae spp. due to evolutionary closeness. Future studies should explore the relationship between QTL of traits of interest in the bentgrass linkage map and genes located in syntenic areas of *B. distachyon*.

In conclusion, the current genetic map of allotetraploid creeping bentgrass was, for the first time, used for comparative genome analysis with cereals based on a common set of cereal and creeping bentgrass EST-RFLP probes. This comparative map establishes a first look in elucidating genome organization among bentgrass species as well as understanding the genome relationships of bentgrass with Triticeae, ryegrass, oat, and rice. Evidence of large-scale chromosomal rearrangements, which differentiate the bentgrass genome from genomes of Triticeae, oat, and rice, were observed based on syntenic markers, and need further validation. However, no evidence of large-scale chromosomal rearrangement was detected between two recently domesticated species, bentgrass and ryegrass suggesting that these two species might be more closely related than expected.

Our future goal is to utilize genetic information from well-studied model species like rice and now *B. distachyon* through comparative mapping, to locate ortholog genes of traits of interest in bentgrass and eventually to develop improved creeping bentgrass cultivars using classical and molecular breeding approaches. Currently, this map has served to detect a QTL for field resistance to dollar spot (Chakraborty et al. 2006) and will be utilized for mapping QTLs and functional markers for important traits of interest in the golf industry such as drought, cold, heat, salt and disease tolerance, as well as leaf color, aggressiveness, and shoot density.
APPENDIX

TABLES

Table 1. Duplicated RFLP loci derived from a common set of heterologous cereal and creeping bentgrass EST-RFLP probes that were mapped in the 549 x 372 mapping population of creeping bentgrass.

| Source of probe | No. of probes generating multiple loci |
|-----------------|---------------------------------------|
|                 | 1 locus | 2 loci | 3 loci | 4 loci | Total |
| Ast             | 37      | 28     | 2      | 1      | 68    |
| BCD             | 20      | 9      | 0      | 0      | 29    |
| CDO             | 32      | 24     | 0      | 0      | 56    |
| RZ              | 4       | 2      | 0      | 0      | 6     |
| Total           | 93      | 63     | 2      | 1      | 159   |

*Ast, BCD, CDO, and RZ probes derived from creeping bentgrass, barley, oat, and rice cDNAs, respectively.*
Table 2. Creeping bentgrass cDNA clones mapped on 14 linkage groups of the creeping bentgrass reference population (549 x 372) (Chakraborty et al. 2005 and 2006). The e-value of the cDNA sequence that matches to database (BLASTX) and the map position of the bentgrass ESTs relative to rice (Kikuchi et al. 2003) and wheat (Sorrels et al. 2003; http://wheat.pw.usda.gov/pubs/2003/Sorrells/) are indicated.

| Bentgrass cDNA clone | Putative function | e-value | Putative location on chromosomes | Bentgrass LG |
|----------------------|-------------------|---------|----------------------------------|--------------|
| Ast5151              | putative U2 snRNP auxiliary factor [Oryza sativa] | 7e-08   | Wheat 4, Rice 5 |
| Ast54                | No significant similarity found. | -       | Wheat 1, Rice 5 |
| Ast355               | phosphoglycerate kinase, putative [Arabidopsis thaliana] | 6e-09   | Wheat 1, Rice 5 |
| Ast3151              | unknown protein [A. thaliana] | 1e-40   | Wheat 1, Rice 5 |
| Ast5208              | glycine cleavage system H protein, mitochondrial, putative [A. thaliana] | 2e-63   | Wheat 1, Rice 10 |
| Ast39                | chitinase (EC 3.2.1.14) precursor [Beta vulgaris] | 2e-06   | Wheat 2, Rice 4 |
| Ast348               | No strong matches | -       | - |
| Ast3102              | Mg-chelatase subunit XANThA-F [Hordeum vulgare] | 2e-53   | Wheat 2, Rice 3 |
| Ast540               | homeobox-leucine zipper protein homolog [Glycine max] | 2e-14   | Wheat 4 |
| Ast5145              | ribosomal protein L12.1 precursor, chloroplast [Secale cereale] | 2e-53   | Wheat -, Rice 7 |
| Ast580               | No hit | -       | Wheat 2, Rice 4 |
| Ast567               | thylakoid lumen 15.0 kDa protein [A. thaliana] | 2e-39   | Wheat -, Rice 7 |
| Ast5162              | chlorophyll A-B binding protein, chloroplast (LHCB6) [A. thaliana] | 1e-47   | Wheat 2, Rice 4 |
| Ast33                | No hit | -       | Wheat -, Rice 1 |
| Ast3277              | putative histidine kinase [O. sativa] | 1e-17   | Wheat 3, Rice 1 |
| Ast555               | oxygen-evolving enhancer protein 3-1, chloroplast precursor (16 kDa subunit photosystem II) [O. sativa] | 1e-45   | Wheat -, Rice 1 |
| Ast564               | No hit | -       | Wheat -, Rice 1 |
| Ast571               | oat victorin binding protein [Avena sativa] | 5e-64   | Wheat 3 |
| Ast5166              | metallothionein-like protein type 3 [H. vulgare] | 6e-14   | Wheat 1, Rice 3 |
| Accession | Description                                                                 | Score | E-value | Query Coverage (%) | Database Coverage (%) |
|-----------|------------------------------------------------------------------------------|-------|---------|---------------------|-----------------------|
| Ast5170   | Aux/IAA protein [Solanum tuberosum]                                           |       | 1e-34   | 1                   | 1                     |
| Ast388    | No hit                                                                       |       | -       | 4                   | 3                     |
| Ast349    | unknown protein [O. sativa]                                                  |       | 2e-11   | 3                   | 3                     |
| Ast572    | putative methylenetetrahydrofolate reductase [O. sativa]                    |       | e-117   | 3                   | 4                     |
| Ast546    | putative serine/threonine-specific protein kinase [O. sativa]                |       | 3e-62   | 4                   | 4                     |
| Ast5103   | putative serine/threonine kinase [O. sativa]                                 |       | 1e-43   | 4                   | 4                     |
| Ast5103   | No hit                                                                       |       | -       | -                   | -                     |
| Ast5103   | nonspecific lipid transfer protein Cw-18 precursor - barley [H. vulgare]     |       | 6e-27   | 2                   | 12                    |
| Ast53     | Serine hydroxymethyltransferase, mitoch. Precursor [S. tuberosum]           |       | 0.0     | 4                   | 3                     |
| Ast357    | PS I 10.8 kDa reaction center subunit IV, chloroplast precursor (PSI-E) [H. vulgare] |       | 6e-21   | 4                   | 7                     |
| Ast323    | No hit                                                                       |       | -       | -                   | 9                     |
| Ast3110   | No hit                                                                       |       | -       | 3                   | 4                     |
| Ast369    | fructose-bisphosphate aldolase (EC 4.1.2.13) [O. sativa]                     |       | 4e-47   | 5                   | 11                    |
| Ast3163   | expressed protein [A. thaliana]                                              |       | 3e-59   | 1                   | 2                     |
| Ast3115   | putative brown planthopper susceptibility protein Hd002A [O. sativa]         |       | 2e-34   | -                   | 2                     |
| Ast3199   | chloroplast translational elongation factor Tu [O. sativa]                   |       | e-115   | 6                   | 2                     |
| Ast528    | putative WRKY9-10 protein [H. vulgare]                                       |       | 4e-05   | 6                   | 2                     |
| Ast529    | phosphoribulokinase; ribulose-5-phosphate kinase [T. aestivum]               |       | 2e-57   | 7                   | 2                     |
| Ast32     | putative auxin-regulated protein [O. sativa]                                 |       | 2e-54   | 1                   | 8                     |
| Ast369    | ALDH2b mRNA for mitochondrial aldehyde dehydrogenase [Secale cereale]       |       | 0.0     | -                   | -                     |
| Ast3213   | chlorophyll a/b binding protein precursor [H. vulgare]                       |       | 2e-97   | -                   | 6                     |
| Ast56     | putative UOS1 [O. sativa] (Pred. nucleoside-diphosphate-sugar epimerases: weaker) |       | 5e-96   | 7                   | 6                     |
| Ast533    | knotted 6 [H. vulgare] / Putative homeobox gene [O. sativa]                 |       | 3e-60   | 7                   | 6                     |
| Ast554    | vacuolar proton-inorganic pyrophosphatase [H. brevisubulatum]                |       | 3e-58   | 7                   | 6                     |
| ID    | Description                                                                 | E-value | Bit-score | Length [A] | Length [O] |
|-------|-----------------------------------------------------------------------------|---------|-----------|------------|------------|
| Ast5381 | Ras-related GTP-binding family protein [A. thaliana]                        | 4e-50   | -         | 6          | 7          |
| Ast5213 | putative cytochrome B5 [O. sativa]; susceptibility homeodomain transcription factor [O. sativa] | 1e-43   | 3         | 6          | 7          |
| Ast568  | putative MATE efflux family protein [O. sativa]                            | 3e-82   | 3         | 3          | 7          |
| Ast574  | No hit                                                                      |         | -         | 6          | 7          |
| Ast5150 | nucleosidase-related [A. thaliana]                                         | 5e-22   | -         | 1          | 7          |
| Ast5115 | putative protein kinase [O. sativa]                                         | 1e-23   | -         | 6          | 7          |
Table 3. Genome comparisons of the 549 x 372 map of *Agrostis stolonifera* with previously published maps of *Triticum, Lolium, Avena*, and *Oryza sativa* based on a common set of heterologous cereal and creeping bentgrass EST-RFLP probes.

| Species          | Number of common loci | Number of conserved syntenic block | Number of chromosomal rearrangements | Genome coverage (%)<sup>a</sup> |
|-----------------|-----------------------|------------------------------------|-------------------------------------|----------------------------------|
| *Triticum*<sup>b</sup> | 53                    | 24                                 | 3                                   | 98                               |
| *Lolium*<sup>c</sup>   | 104                   | 14                                 | 0                                   | 92                               |
| *Avena*<sup>d</sup>    | 42                    | 19                                 | 4                                   | 76                               |
| *Oryza sativa*        | 83                    | 29                                 | 8                                   | 82                               |

<sup>a</sup>Genome coverage in bentgrass (%) = (a/b)x100, where a = total map length (cM) represented in bentgrass with probes mapped in each species (Triticeae, ryegrass, oat, or rice), b = total map length (cM) of bentgrass.

<sup>b</sup>Triticeae tribe consensus maps for *T. aestivum, T. tauschii*, and *Hordeum* spp.

<sup>c</sup>*Lolium* map was derived from diploid MFA x MFB interspecific cross between *L. multiflorum* Lam. and *L. perenne* L.

<sup>d</sup>*Avena* map was derived from diploid interspecific cross between *A. atlantica* and *A. hirtula*
Table 4. *B. distachyon* chromosome location of sequences of EST-RFLP markers mapped on genetic map of creeping bentgrass.

| Bentgrass LG<sup>a</sup> | Marker (EST ID)<sup>b</sup> | Bd chromosome<sup>c</sup> | Bd chromosome location<sup>d</sup> |
|--------------------------|-----------------------------|--------------------------|-----------------------------------|
|                          |                             | 2                        | Start | End                  |
| 1.2                      | CDO202                      | 2                        | 13312741 | 13313054          |
| 1.1-1.2                  | BCD1261                     | 2                        | 13918752 | 13919099          |
| 1.1-1.2                  | CDO1160                     | 2                        | 13959438 | 13959911           |
| 1.1                      | Ast5244 (DY543632)          | 2                        | 17734568 | 17737309           |
| 1.2                      | BCD738                      | 2                        | 18485474 | 18485955           |
| 1.2                      | Ast355 (DY543397)           | 2                        | 18485698 | 18485954           |
| 1.2                      | CDO89                       | 2                        | 19921391 | 19922005           |
| 1.2                      | Ast3151<sup>e</sup>         | 2                        | 22117132 | 22119285           |
| 1.1-1.2                  | CDO771                      | 2                        | 22596648 | 22597674           |
| 1.1                      | BCD454                      | 2                        | 23921191 | 23921611           |
| 1.1-1.2                  | BCD1072                     | 2                        | 30224103 | 30224321           |
| 1.1                      | BCD200                      | 2                        | 30230242 | 30230609           |
| 1.2                      | Ast5185 (DY543548)          | 2                        | 36219723 | 36221116           |
| 1.1                      | CDO580                      | 2                        | 38466405 | 38467009           |
| 1.2                      | RZ444                       | 2                        | 53296932 | 53297140           |
| 1.2                      | BCD921                      | 3                        | 20772298 | 20772621           |
| 1.1                      | CDO98                       | 3                        | 30319151 | 30321086           |
| 1.2                      | BCD386                      | 3                        | 32086894 | 32087540           |
| 1.2                      | Ast5208 (DY543591)          | 3                        | 32651725 | 32653364           |
| 1.2                      | CDO94                       | 3                        | 32721646 | 32722742           |
| 2.1                      | BCD1184                     | 1                        | 13478584 | 13478963           |
| 2.1-2.2                  | Ast552 (DY543414)           | 1                        | 14908782 | 14909109           |
| 2.1-2.2                  | Ast5145 (DY543455)          | 1                        | 15743376 | 15743751           |
| 2.1-2.2                  | Ast3102<sup>e</sup>         | 1                        | 19259600 | 19259999           |
| 2.1-2.2                  | CDO405                      | 1                        | 20953161 | 20953582           |
| 2.1                      | BCD1709                     | 1                        | 23483880 | 23484122           |
| 2.1                      | Ast354 (DY543396)           | 1                        | 24105397 | 24107492           |
| 2.1                      | BCD438                      | 1                        | 53957117 | 53957391           |
| 2.1                      | BCD1184                     | 1                        | 64356335 | 64357924           |
| 2.2                      | CDO365                      | 4                        | 17638266 | 17638709           |
| 2.2                      | Ast567 (DY543447)           | 5                        | 4337487 | 4339261            |
| 2.1-2.2                  | Ast5162 (DY543463)          | 5                        | 15617190 | 15617775          |
| 2.1                      | CDO1328                     | 5                        | 15844066 | 15847157          |
| 2.1-2.2                  | CDO684                      | 5                        | 21100968 | 21101188           |
| 3.2                      | Ast563 (DY543444)           | 2                        | 841745  | 842266             |
| 3.1-3.2                  | Ast5166 (DY543467)          | 2                        | 4664467 | 4665522            |
| 3.2                      | Ast5170 (DY543421)          | 2                        | 6119943 | 6123643            |
| 3.1                      | CDO328                      | 2                        | 6867698 | 6868202            |
| 3.2                      | BCD127                      | 2                        | 8725766 | 8726125            |
| 3.1-3.2                  | RZ995                       | 2                        | 9220956 | 9221418            |
| 3.1  | BCD1495 | 2  | 9496671 | 9497017 |
|-----|---------|----|---------|---------|
| 3.1  | BCD1495 | 2  | 10491102 | 10491445 |
| 3.1  | BCD1495 | 2  | 10495830 | 10496174 |
| 3.1-3.2  | BCD134 | 2  | 41622340 | 41623472 |
| 3.1-3.2  | BCD828 | 2  | 47227872 | 47228154 |
| 3.2  | CDO1174 | 2  | 47437527 | 47437914 |
| 3.1  | Ast571 (DY543450) | 2  | 48360330 | 48361363 |
| 3.1-3.2  | Ast3277 (DY543641) | 2  | 56909915 | 56911116 |
| 3.1-3.2  | Ast3277 (DY543641) | 2  | 58867188 | 58867992 |
| 3.1-3.2  | Ast3277 (DY543641) | 2  | 58868700 | 58869561 |
| 3.1  | BCD98 | 3  | 44128540 | 44129179 |
| 3.2  | CDO99 | 3  | 44130090 | 44130609 |
| 3.2  | CDO585 | 4  | 7093558  | 7093796  |
| 3.2  | Ast563 (DY543444) | 4  | 29522820 | 29523351 |
| 3.1-3.2  | CDO244 | 5  | 24490389 | 24490639 |
| 4.1-4.2  | CDO484 | 1  | 1366964  | 1367495  |
| 4.1  | Ast572 (DY543451) | 1  | 2811156  | 2813423  |
| 4.1  | Ast349 (DY543394) | 1  | 3103523  | 3104213  |
| 4.2  | BCD808 | 1  | 4998784  | 4999139  |
| 4.1-4.2  | CDO504 | 1  | 5962259  | 5962873  |
| 4.2  | BCD450 | 1  | 5988342  | 5988796  |
| 4.2  | BCD808 | 1  | 7318997  | 7319438  |
| 4.1-4.2  | CDO542 | 1  | 8350647  | 8351378  |
| 4.1  | CDO122 | 1  | 8567153  | 8567563  |
| 4.1-4.2  | CDO795 | 1  | 11769377 | 11770588 |
| 4.1-4.2  | BCD385 | 1  | 12074984 | 12075099 |
| 4.2  | BCD808 | 1  | 19135955 | 19136422 |
| 4.2  | CDO938 | 1  | 62486394 | 62487017 |
| 4.2  | CDO38 | 1  | 62730415 | 62730828 |
| 4.2  | Ast5103 (DY543488) | 1  | 63213682 | 63215164 |
| 4.2  | CDO1395 | 1  | 68017621 | 68018050 |
| 4.1-4.2  | Ast3325 (DY543730) | 1  | 68060410 | 68061124 |
| 4.1-4.2  | CDO1401 | 1  | 71738939 | 71739260 |
| 4.1-4.2  | CDO20 | 1  | 72945681 | 72946010 |
| 4.1-4.2  | CDO20 | 1  | 72951260 | 72951645 |
| 4.1  | CDO241 | 4  | 11555301 | 11555602 |
| 4.1  | CDO520 | 4  | 14398184 | 14398326 |
| 4.2  | Ast559 (DY543419) | 4  | 31093780 | 31094131 |
| 5.1  | Ast357 (DY543398) | 1  | 24421682 | 24422146 |
| 5.2  | CDO1049 | 3  | 19476497 | 19477936 |
| 5.1-5.2  | Ast5343 (DY543747) | 3  | 21176323 | 21178461 |
| 5.1-5.2  | Ast551 (DY543413) | 3  | 21620907 | 21621429 |
| 5.1  | CDO749 | 4  | 1558290 | 1559232 |
| 5.1-5.2  | CDO677 | 4  | 2023698 | 2024300 |
| 5.1  | CDO344 | 4  | 3112832 | 3113225 |
| 5.1  | Ast394 | 4  | 6307762 | 6308465 |
| 5.2  | CDO1049 | 4  | 7497676 | 7500061 |
| 5.1  | CDO344 | 4  | 8453360 | 8453651 |
| 5.1  | CDO344 | 4  | 8457255 | 8457560 |
|   |   |   |   |   |
|---|---|---|---|---|
|5.1| CDO344| 4| 8460278| 8460619|
|5.1| CDO344| 4| 8464909| 8465214|
|5.1| CDO344| 4| 8471117| 8471457|
|5.1| CDO344| 4| 8474194| 8474511|
|5.1| CDO344| 4| 8480368| 8480693|
|5.1| CDO344| 4| 8483793| 8484112|
|5.1| CDO344| 4| 8486603| 8486922|
|5.1| CDO344| 4| 8493131| 8493419|
|5.1| CDO344| 4| 8497047| 8497331|
|5.1| CDO344| 4| 8502867| 8503174|
|5.1| CDO344| 4| 8544334| 8544651|
|5.1| CDO344| 4| 8547916| 8548212|
|5.1-5.2| Ast563 (DY543444)| 4| 29522820| 29523351|
|5.2| BCD1087| 4| 33816927| 33817270|
|5.1-5.2| CDO989| 4| 36917718| 36917857|
|5.1-5.2| CDO412| 4| 38688244| 38688602|
|5.2| BCD1088| 4| 46698455| 46698943|
|6.1-6.2| CDO1158| 1| 27070854| 27071168|
|6.1| CDO686| 1| 42666761| 42667080|
|6.1-6.2| CDO1158| 3| 3998204| 3998548|
|6.1-6.2| CDO534| 3| 5115466| 5116010|
|6.1| Ast3163 (DY543439)| 3| 48111250| 48114561|
|6.1-6.2| CDO497| 3| 49305661| 49306768|
|6.1| Ast3199 (DY543537)| 3| 49359330| 49359740|
|6.1-6.2| CDO1380| 3| 50900443| 50900807|
|6.1| Ast370 (DY543428)| 3| 53533432| 53533752|
|6.1-6.2| BCD1860| 3| 54188671| 54188821|
|6.2| RZ273| 3| 54190265| 54190547|
|6.2| CDO507| 3| 55685645| 55686559|
|6.1-6.2| Ast3115e| 3| 56863663| 56686855|
|6.1-6.2| Ast3155e| 3| 57450800| 57451023|
|6.1-6.2| CDO516| 3| 58868011| 58868332|
|6.1| Ast5165 (DY543466)| 4| 45075939| 45076791|
|7.1-7.2| Ast568 (DY543448)| 1| 12573959| 12576486|
|7.1| RZ508| 1| 25302337| 25302576|
|7.1| Ast554 (DY543415)| 1| 25880365| 25881182|
|7.1-7.2| RZ682| 1| 26511824| 26511978|
|7.1| CDO673| 1| 33021887| 33022102|
|7.1-7.2| Ast5381 (DY543790)| 1| 34370529| 34371141|
|7.1| Ast5213 (DY543594)| 1| 35552937| 35553492|
|7.1| Ast3213 (DY543567)| 1| 39989003| 39990068|
|7.1-7.2| CDO17| 1| 40844963| 40844982|
|7.1-7.2| Ast369 (DY543427)| 1| 41580006| 41581370|
|7.2| Ast5115 (DY543497)| 1| 46751576| 46751864|
|7.1| CDO545| 1| 49180863| 49181176|

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| 7.1  | RZ516 | 1 | 49336575 | 49336816 |
| 7.2  | BCD93 | 1 | 50094042 | 50094446 |
| 7.1  | **RZ508** | 1 | 72954229 | 72954468 |
| 7.2  | Ast5150 (DY543458) | 2 | 6103348 | 6103548 |
| 7.1  | **Ast5213** (DY543594) | 2 | 24920787 | 24921343 |
| 7.1  | **RZ508** | 3 | 928721 | 928957 |
| 7.1  | BCD349 | 3 | 11439298 | 11439995 |
| 7.1  | BCD276 | 3 | 38400248 | 38401418 |
| 7.1-7.2 | Ast5121 (DY543501) | 3 | 39274997 | 39275975 |
| 7.1  | CDO464 | 3 | 41048884 | 41046423 |
| 7.1  | CDO595 | 3 | 42643624 | 42643830 |
| 7.2  | BCD98 | 3 | 44128540 | 44129179 |
| 7.2  | CDO99 | 3 | 44130090 | 44130609 |

*a* Creeping bentgrass linkage group (LG) where the marker was mapped.

*b* The markers in bold correspond to those that matched in more than one *B. distachyon* chromosome.

*c* *B. distachyon* chromosome where the marker was found after the BLASTN search.

*d* Location of the alignment between the marker and the *B. distachyon* chromosome (start and end correspond to base pair number).

*e* Sequence not available at NCBI
Table 5. Creeping bentgrass EST orthologs to *B. distachyon* chromosomes.

| Agrostis EST ID | Bd chromosome location<sup>a</sup> | Bd chromosome<sup>b</sup> | Duplications<sup>c</sup> | Among (Bdch)<sup>d</sup> |
|-----------------|-------------------------------|-----------------|-----------------|-----------------|
| DV860292        | 58122365 - 58122254            | 1               |                 |                 |
| DV860299        | 44145666 - 44145859            | 1               |                 |                 |
| DV860408        | 48045416 - 48045508            | 1               |                 |                 |
| DV860460        | 5767669 - 5767618              | 1               |                 |                 |
| DV860498        | 20235456 - 20235377            | 1               |                 |                 |
| DV860500        | 64837233 - 64838211            | 1               |                 | Bd3             |
| DV860573        | 4696595 - 4696055              | 1               | 3               | Bd4             |
| DV860604        | 69418662 - 69418945            | 1               |                 |                 |
| DV860646        | 18654987 - 18654820            | 1               |                 |                 |
| DV860829        | 45677406 - 45677354            | 1               |                 |                 |
| DV860890        | 60661123 - 60661243            | 1               |                 |                 |
| DV860997        | 50443381 - 50443446            | 1               |                 |                 |
| DV861094        | 61951160 - 61951233            | 1               |                 |                 |
| DV861115        | 58331771 - 58331708            | 1               |                 |                 |
| DV861154        | 5190381 - 5190651              | 1               |                 |                 |
| DV861286        | 9087868 - 9087689              | 1               |                 |                 |
| DV861287        | 7148045 - 7148191              | 1               |                 |                 |
| DV861317        | 8149957 - 8150325              | 1               |                 |                 |
| DV861320        | 50081458 - 50081603            | 1               |                 |                 |
| DV861326        | 45677260 - 45677197            | 1               |                 |                 |
| DV861401        | 16199480 - 16198922            | 1               | 2               |                 |
| DV861438        | 5190249 - 5190675              | 1               |                 |                 |
| DV861460        | 13812451 - 13812769            | 1               |                 |                 |
| DV861483        | 74281932 - 74281970            | 1               |                 |                 |
| DV861541        | 4094647 - 4094675              | 1               |                 |                 |
| DV861568        | 13812524 - 13812769            | 1               |                 |                 |
| DV861577        | 70726749 - 70726604            | 1               |                 |                 |
| DV861613        | 72499792 - 72499653            | 1               |                 |                 |
| DV861633        | 63754352 - 63754049            | 1               |                 |                 |
| DV861655        | 7976981 - 7976902              | 1               |                 |                 |
| DV861676        | 62732883 - 62732736            | 1               |                 |                 |
| DV861700        | 48423826 - 48424626            | 1               |                 |                 |
| DV861736        | 21906435 - 21906124            | 1               |                 |                 |
| DV861802        | 33222172 - 33221876            | 1               |                 |                 |
| DV861828        | 9773669 - 9773516              | 1               |                 |                 |
| DV861866        | 63094796 - 63094718            | 1               |                 |                 |
| DV861915        | 63607740 - 63607830            | 1               |                 |                 |
| DV862048        | 9019073 - 9019421              | 1               |                 | Bd2, 3, 4       |
| DV862055        | 66614079 - 66614109            | 1               |                 |                 |
| DV862135        | 4109009 - 4109080              | 1               |                 |                 |
| DV862142        | 61924029 - 61923976            | 1               | 2               |                 |
| DV862161        | 20665137 - 20664943            | 1               |                 |                 |
| DV862168        | 1827223 - 1827426              | 1               |                 |                 |
| DV862191        | 21169471 - 21169737            | 1               |                 |                 |
| DV862253        | 69507164 - 69506853            | 1               |                 |                 |
| Code     | Value1     | Value2     | Value3 | Value4 | Value5 | Value6 | Value7 | Value8 | Value9 | Value10 | Value11 | Value12 |
|----------|------------|------------|--------|--------|--------|--------|--------|--------|--------|---------|----------|---------|
| DV862293 | 14550469   | 14550194   |        |        |        |        |        |        |        |          |          |         |
| DV862417 | 40736060   | 40735806   |        |        |        |        |        |        |        |          |          |         |
| DV862436 | 62984506   | 62984239   |        |        |        |        |        |        |        |          |          |         |
| DV862455 | 8567980    | 8567530    |        |        |        |        |        |        |        |          |          |         |
| DV862491 | 15300725   | 15300920   |        |        |        |        |        |        |        |          |          |         |
| DV862584 | 6390655    | 6390506    |        |        |        |        |        |        |        |          |          |         |
| DV862694 | 17151824   | 17151560   |        |        |        |        |        |        |        |          |          |         |
| DV862801 | 50540315   | 50540420   |        |        |        |        |        |        |        |          |          |         |
| DV862836 | 7703418    | 7703152    |        |        |        |        |        |        |        |          |          |         |
| DV862946 | 14212470   | 14212518   |        |        |        |        |        |        |        |          |          |         |
| DV862967 | 28326959   | 28327251   |        |        |        |        |        |        |        |          |          |         |
| DV863053 | 12482891   | 12483277   |        |        |        |        |        |        |        |          |          |         |
| DV863161 | 4167967    | 4167607    |        |        |        |        |        |        |        |          |          |         |
| DV863251 | 3337557    | 3337358    |        |        |        |        |        |        |        |          |          |         |
| DV863289 | 41623175   | 41623058   |        |        |        |        |        |        |        |          |          |         |
| DV863339 | 54705284   | 54705750   |        |        |        |        |        |        |        |          |          |         |
| DV863383 | 50093974   | 50094303   |        |        |        |        |        |        |        |          |          |         |
| DV863417 | 68743290   | 68743522   |        |        |        |        |        |        |        |          |          |         |
| DV863428 | 62356412   | 62356589   |        |        |        |        |        |        |        |          |          |         |
| DV863533 | 8312570    | 8312298    |        |        |        |        |        |        |        |          |          |         |
| DV863568 | 57063707   | 57063877   |        |        |        |        |        |        |        |          |          |         |
| DV863585 | 27644182   | 27644136   |        |        |        |        |        |        |        |          |          |         |
| DV863610 | 68584591   | 68584504   |        |        |        |        |        |        |        |          |          |         |
| DV863711 | 41875484   | 41875567   |        |        |        |        |        |        |        |          |          |         |
| DV863776 | 68237345   | 68237305   |        |        |        |        |        |        |        |          |          |         |
| DV863894 | 66404574   | 66404632   |        |        |        |        |        |        |        |          |          |         |
| DV863905 | 7319394    | 7318933    |        |        |        |        |        |        |        |          |          |         |
| DV863972 | 63265235   | 63265356   |        |        |        |        |        |        |        |          |          |         |
| DV863977 | 70427108   | 70426975   |        |        |        |        |        |        |        |          |          |         |
| DV864001 | 31324229   | 31324410   |        |        |        |        |        |        |        |          |          |         |
| DV864040 | 1827016    | 1827060    |        |        |        |        |        |        |        |          |          |         |
| DV864053 | 20665860   | 20665690   |        |        |        |        |        |        |        |          |          |         |
| DV864078 | 61924016   | 61923976   |        |        |        |        |        |        |        |          |          |         |
| DV864090 | 65468808   | 65468574   |        |        |        |        |        |        |        |          |          |         |
| DV864141 | 66405253   | 66404970   |        |        |        |        |        |        |        |          |          |         |
| DV864224 | 67130227   | 67130126   |        |        |        |        |        |        |        |          |          |         |
| DV864253 | 70031121   | 70030905   |        |        |        |        |        |        |        |          |          |         |
| DV864353 | 15392991   | 15393057   |        |        |        |        |        |        |        |          |          |         |
| DV864466 | 62192267   | 62192146   |        |        |        |        |        |        |        |          |          |         |
| DV864484 | 65941385   | 65941133   |        |        |        |        |        |        |        |          |          |         |
| DV864629 | 58231019   | 58230905   |        |        |        |        |        |        |        |          |          |         |
| DV864659 | 61653588   | 61653529   |        |        |        |        |        |        |        |          |          |         |
| DV864674 | 24421769   | 24421956   |        |        |        |        |        |        |        |          |          |         |
| DV864721 | 50442967   | 50443035   |        |        |        |        |        |        |        |          |          |         |
| DV864795 | 67267510   | 67267295   |        |        |        |        |        |        |        |          |          |         |
| DV864836 | 68520537   | 68520642   |        |        |        |        |        |        |        |          |          |         |
| DV864856 | 32343727   | 32343608   |        |        |        |        |        |        |        |          |          |         |
| Code     | Value1   | Value2   | Value3   | Value4 |
|----------|----------|----------|----------|--------|
| DV864873 | 64721655 | 64721282 | 1        |        |
| DV864877 | 53393692 | 53393817 | 1        |        |
| DV864895 | 33459406 | 33459594 | 1        |        |
| DV864903 | 34368864 | 34368949 | 1        |        |
| DV864919 | 20234724 | 20234779 | 1        |        |
| DV864929 | 5005786  | 5005835  | 1        |        |
| DV864935 | 64592884 | 64593196 | 1        |        |
| DV865001 | 32280141 | 32280519 | 1        |        |
| DV865003 | 68683540 | 68683948 | 1        |        |
| DV865043 | 28915490 | 28915411 | 1        |        |
| DV865075 | 1867227  | 1867175  | 1        |        |
| DV865098 | 6677073  | 6677035  | 1        |        |
| DV865127 | 46551540 | 46551366 | 1        |        |
| DV865133 | 7647707  | 7647819  | 1        |        |
| DV865195 | 8341195  | 8341289  | 1        |        |
| DV865392 | 10068223 | 10068158 | 1        |        |
| DV865471 | 62627778 | 62627626 | 1        |        |
| DV865532 | 62984068 | 62983804 | 1        |        |
| DV865533 | 15415853 | 15415740 | 1        |        |
| DV865669 | 54824816 | 54824718 | 1        |        |
| DV865674 | 54728480 | 54727963 | 1        |        |
| DV865696 | 64744478 | 64744433 | 1        |        |
| DV865701 | 23554411 | 23553534 | 1        |        |
| DV865708 | 54728507 | 54727963 | 1        |        |
| DV865724 | 20997621 | 20996942 | 1        |        |
| DV865739 | 6796569  | 6797013  | 1        |        |
| DV865785 | 66593521 | 66593487 | 1        |        |
| DV865804 | 54728502 | 54727963 | 1        |        |
| DV865826 | 54728480 | 54727963 | 1        |        |
| DV865841 | 72877071 | 72876983 | 1        |        |
| DV865868 | 69647995 | 69647889 | 1        |        |
| DV865884 | 8115004  | 8115227  | 1        |        |
| DV865886 | 43151697 | 43151965 | 1        |        |
| DV865950 | 41875866 | 41875939 | 1        |        |
| DV865964 | 3828360  | 3828166  | 1        |        |
| DV865986 | 19136181 | 19135848 | 1        |        |
| DV866003 | 72138556 | 72138667 | 1        |        |
| DV866026 | 17183424 | 17183235 | 1        |        |
| DV866076 | 25391399 | 25391539 | 1        |        |
| DV866214 | 29785607 | 29785382 | 1        |        |
| DV866247 | 20109516 | 20109787 | 1        |        |
| DV866274 | 44341630 | 44341496 | 1        |        |
| DV866364 | 3217278  | 3217478  | 1        |        |
| DV866367 | 5802618  | 5802512  | 1        |        |
| DV866392 | 74078223 | 74078355 | 1        |        |
| DV866394 | 6819372  | 6819658  | 1        |        |
| DV866507 | 14019536 | 14019453 | 1        |        |
| DV866687 | 30385706 | 30385357 | 1        |        |
| DV866702 | 35760109 | 35759575 | 1        |        | 3        |
| Code     | First 12 Chars | Second 12 Chars | 1st 8 Chars | 2nd 8 Chars | Category |
|----------|---------------|----------------|-------------|-------------|----------|
| DV866776 | 31324341      | 31324410       | 1           |             |          |
| DV866824 | 65468866      | 65468574       | 1           |             |          |
| DV866921 | 8340282       | 8340361        | 1           |             |          |
| DV866922 | 44166696      | 44166921       | 1           |             |          |
| DV866929 | 2149343       | 2149313        | 1           |             |          |
| DV867049 | 6966390       | 6966466        | 1           |             |          |
| DV867162 | 10462049      | 10462189       | 1           |             |          |
| DV867180 | 68584555      | 68584504       | 1           |             |          |
| DV867204 | 69506655      | 69506731       | 1           |             |          |
| DV867236 | 16199534      | 16198927       | 1           | 2           |          |
| DV867322 | 55153808      | 55153276       | 1           |             |          |
| DV867327 | 62269705      | 62269766       | 1           |             |          |
| DV867350 | 66515083      | 66515016       | 1           |             |          |
| DV867402 | 18722397      | 18722044       | 1           |             |          |
| DV867410 | 44107086      | 44106533       | 1           |             |          |
| DV867431 | 3846565       | 3846703        | 1           |             |          |
| DV867599 | 22258140      | 22257826       | 1           |             |          |
| DV867624 | 3033162       | 3032946        | 1           |             |          |
| DV867642 | 50442963      | 50443044       | 1           |             |          |
| DV867821 | 22255772      | 22255637       | 1           |             |          |
| DV867864 | 65333302      | 65332849       | 1           | 2           | Bd2, 4  |
| DV867906 | 64232845      | 64232686       | 1           |             |          |
| DV867939 | 69319357      | 69319205       | 1           |             | Bd2     |
| DV867983 | 8332686       | 8332424        | 1           |             |          |
| DV868011 | 65871029      | 65870796       | 1           |             |          |
| DV868178 | 35676838      | 35677115       | 1           |             |          |
| DV868185 | 64232480      | 64232170       | 1           |             |          |
| DV868389 | 61315864      | 61315929       | 1           |             |          |
| DV868473 | 30732130      | 30732203       | 1           |             |          |
| DV868474 | 5005531       | 5005619        | 1           |             |          |
| DV868568 | 21392853      | 21393410       | 1           |             |          |
| DV868569 | 47050436      | 47050913       | 1           |             | Bd2     |
| DV868571 | 8567287       | 8567477        | 1           |             |          |
| DV868589 | 9106557       | 9106458        | 1           |             |          |
| DV868657 | 16184528      | 16184225       | 1           |             |          |
| DV868673 | 46551481      | 46551366       | 1           |             |          |
| DV868696 | 35767393      | 35767306       | 1           |             | Bd3     |
| DV860440 | 58828213      | 58828652       | 2           |             |          |
| DV860462 | 8130081       | 8130213        | 2           |             |          |
| DV860544 | 1942380       | 1942420        | 2           |             |          |
| DV860576 | 34715619      | 34715470       | 2           |             |          |
| DV860677 | 44943932      | 44943639       | 2           |             |          |
| DV860798 | 54950742      | 54950566       | 2           |             |          |
| DV860863 | 54885244      | 54884948       | 2           |             |          |
| DV861137 | 22735692      | 22736079       | 2           |             |          |
| DV861142 | 49899123      | 49899365       | 2           |             |          |
| DV861250 | 20686994      | 20687069       | 2           |             |          |
| DV861296 | 45586147      | 45586321       | 2           |             |          |
| DV861316 | 9053108       | 9053172        | 2           |             |          |
| DV861558 | 21810268 | 21810563 | 2 |
| DV861598 | 48682792 | 48682886 | 2 |
| DV861788 | 14654541 | 14654289 | 2 |
| DV861816 | 52560350 | 52560458 | 2 |
| DV861913 | 44385570 | 44385704 | 2 |
| DV862008 | 50520435 | 50520160 | 2 |
| DV862047 | 57071793 | 57071582 | 2 |
| DV862048 | 14366890 | 14366544 | 2 |
| DV862081 | 42711075 | 42710606 | 2 |
| DV862120 | 45267064 | 45267100 | 2 |
| DV862122 | 53025570 | 53025669 | 2 |
| DV862342 | 39007020 | 39007129 | 2 |
| DV862368 | 23277511 | 23277543 | 2 |
| DV862417 | 27605443 | 27605162 | 2 |
| DV862428 | 48361001 | 48361277 | 2 |
| DV862475 | 25704451 | 25704594 | 2 |
| DV862658 | 34973349 | 34973455 | 2 |
| DV862727 | 42772559 | 42772621 | 2 |
| DV862859 | 27937279 | 27937380 | 2 |
| DV862967 | 34823412 | 34823373 | 2 |
| DV862973 | 42699139 | 42698868 | 2 |
| DV862975 | 31967633 | 31967706 | 2 |
| DV863075 | 10775828 | 10776170 | 2 |
| DV863175 | 79191 | 79132 | 2 |
| DV863230 | 54913122 | 54913017 | 2 |
| DV863397 | 4131594 | 4131919 | 2 |
| DV863415 | 48702669 | 48702487 | 2 |
| DV863483 | 9426644 | 9426437 | 2 |
| DV863513 | 51187722 | 51187665 | 2 |
| DV863731 | 13885525 | 13885704 | 2 |
| DV863768 | 23277511 | 23277543 | 2 |
| DV863834 | 52751022 | 52751229 | 2 |
| DV863912 | 41541235 | 41541312 | 2 |
| DV864036 | 14654768 | 14654655 | 2 |
| DV864079 | 4874407 | 4873819 | 2 |
| DV864101 | 56324416 | 56324554 | 2 |
| DV864179 | 21525833 | 21526263 | 2 |
| DV864372 | 25387498 | 25387430 | 2 |
| DV864419 | 26128710 | 26128637 | 2 |
| DV864452 | 34559406 | 34559520 | 2 |
| DV864519 | 79103 | 78833 | 2 |
| DV864523 | 36805299 | 36805225 | 2 |
| DV864592 | 5535433 | 5535613 | 2 |
| DV864635 | 54698355 | 54698422 | 2 |
| DV864651 | 53521548 | 53521800 | 2 |
| DV864662 | 8647615 | 8647514 | 2 |
| DV864717 | 56324456 | 56324554 | 2 |

Bd1, 3, 4
Bd1, 3, 4, 5
Bd1
Bd4, 5
Bd3, 4, 5
| DV864728 | 52517704 | 52517931 | 2 |     |
| DV864805 | 22829085 | 22828968 | 2 |     |
| DV864807 | 50017778 | 50017805 | 2 |     |
| DV864815 | 52389674 | 52389726 | 2 |     |
| DV864816 | 35174238 | 35174298 | 2 |     |
| DV864916 | 17396249 | 17396293 | 2 |     |
| DV864951 | 46310901 | 46311067 | 2 |     |
| DV864983 | 37775080 | 37775200 | 2 |     |
| DV865067 | 15854979 | 15854897 | 2 |     |
| DV865205 | 52015014 | 52015374 | 2 |     |
| DV865327 | 23489561 | 23489395 | 2 |     |
| DV865346 | 7157879  | 7157779  | 2 |     |
| DV865350 | 20681982 | 20681751 | 2 |     |
| DV865377 | 14730897 | 14730938 | 2 |     |
| DV865384 | 46083505 | 46083720 | 2 |     |
| DV865915 | 55881959 | 55881898 | 2 |     |
| DV865926 | 48854284 | 48854619 | 2 |     |
| DV866059 | 47349872 | 47349902 | 2 |     |
| DV866122 | 56980899 | 56980647 | 2 |     |
| DV866138 | 13931197 | 13931114 | 2 |     |
| DV866190 | 34514488 | 34514569 | 2 |     |
| DV866201 | 58846752 | 58846528 | 2 |     |
| DV866217 | 34715190 | 34715333 | 2 |     |
| DV866231 | 56169003 | 56169106 | 2 |     |
| DV866278 | 820190  | 819649   | 2 |     |
| DV866368 | 48360036 | 48359974 | 2 |     |
| DV866408 | 35945804 | 35945581 | 2 |     |
| DV866416 | 47445763 | 47445494 | 2 |     |
| DV866460 | 6723285  | 6723355  | 2 |     |
| DV866463 | 25704893 | 25704620 | 2 |     |
| DV866611 | 51077167 | 51077282 | 2 |     |
| DV866712 | 48360423 | 48360330 | 2 |     |
| DV866724 | 53137472 | 53137067 | 2 |     |
| DV866729 | 40630011 | 40629580 | 2 |     |
| DV866808 | 20797882 | 20797998 | 2 |     |
| DV866845 | 18329053 | 18329267 | 2 |     |
| DV866876 | 35296479 | 35296115 | 2 |     |
| DV867055 | 10495399 | 10495784 | 2 |     |
| DV867425 | 45787873 | 45787907 | 2 |     |
| DV867712 | 11493479 | 11494377 | 2 |     |
| DV867831 | 25508196 | 25508283 | 2 |     |
| DV867864 | 20792906 | 20792554 | 2 |     |
| DV867908 | 52480946 | 52480863 | 2 |     |
| DV867939 | 7218453  | 7218301  | 2 |     |
| Code         | Value 1          | Value 2          | Operation | Result |
|--------------|------------------|------------------|-----------|--------|
| DV868191     | 21033688         | 21033740         | 2         |        |
| DV868386     | 34339496         | 34339419         | 2         |        |
| DV868415     | 49834388         | 49834740         | 2         |        |
| DV868462     | 42039673         | 42039736         | 2         |        |
| DV868512     | 46502209         | 46502009         | 2         |        |
| DV868569     | 15977039         | 15976552         | 2         | BD1    |
| DV868618     | 52190423         | 52190047         | 2         |        |
| DV868699     | 18081321         | 18081370         | 2         |        |
| DV868717     | 58846361         | 58846404         | 2         |        |
| DV860413     | 57592315         | 57592604         | 3         |        |
| DV860467     | 41827196         | 41826633         | 3         |        |
| DV860484     | 14660906         | 14660670         | 3         |        |
| DV860500     | 26475457         | 26476433         | 3         | BD1    |
| DV860566     | 43602264         | 43602433         | 3         |        |
| DV860613     | 9538980          | 9539361          | 3         |        |
| DV860681     | 55140095         | 55140373         | 3         |        |
| DV860756     | 24423176         | 24423053         | 3         |        |
| DV860895     | 55140137         | 55140373         | 3         |        |
| DV861045     | 55820244         | 55819898         | 3         |        |
| DV861113     | 36891442         | 36891133         | 3         |        |
| DV861170     | 5230478          | 5230712          | 3         |        |
| DV861212     | 28301395         | 28301269         | 3         |        |
| DV861458     | 49035386         | 49035420         | 3         |        |
| DV861464     | 35237938         | 35238426         | 3         |        |
| DV861493     | 45748962         | 45748911         | 3         |        |
| DV861509     | 20773241         | 20772793         | 3         |        |
| DV861526     | 4234010          | 4234423          | 3         |        |
| DV861537     | 53307319         | 53307496         | 3         |        |
| DV861784     | 5230712          | 5230469          | 3         |        |
| DV861786     | 6822935          | 6822678          | 3         |        |
| DV861842     | 33515698         | 33515503         | 3         |        |
| DV861859     | 18005976         | 18005900         | 3         |        |
| DV861876     | 54190217         | 54190548         | 3         |        |
| DV861883     | 3589294          | 3589356          | 3         |        |
| DV862048     | 44114069         | 44114417         | 3         | BD1, 2, 4 |
| DV862087     | 51607353         | 51607297         | 3         |        |
| DV862175     | 52059922         | 52060115         | 3         |        |
| DV862209     | 27093561         | 27093458         | 3         | BD4, 5 |
| DV862232     | 47189109         | 47189388         | 3         |        |
| DV862248     | 46634216         | 46634359         | 3         |        |
| DV862254     | 11310459         | 11309975         | 3         |        |
| DV862334     | 6584285          | 6584137          | 3         |        |
| DV862417     | 31678620         | 31678309         | 3         | BD1, 2, 4, 5 |
| DV862562     | 53982648         | 53982619         | 3         |        |
| DV862591     | 34216918         | 34217129         | 3         |        |
| DV862624     | 2683106          | 2682823          | 3         |        |
| DV862689     | 41216693         | 41216912         | 3         |        |
| DV862703     | 54139041         | 54138920         | 3         |        |
| DV862767     | 2116200          | 2116120          | 3         |        |
DV862948  29195056  29195153  3
DV863002  53349136  53349260  3
DV863101  54190304  54190548  3
DV863192  54190241  54190546  3
DV863431  36345148  36345300  3
DV863446  13270142  13269770  3
DV863555  55140258  55140373  3
DV863594  37368021  37368100  3
DV863664  53348929  53349260  3
DV863673  49306759  49306504  3
DV863873  17661430  17661745  3
DV864207  14785676  14785599  3
DV864229  58563247  58562887  3
DV864230  11902479  11902181  3
DV864327  2601565  2601748  3
DV864343  33702  336766  3
DV864382  49951702  49952106  3
DV864458  58898957  58899115  3
DV864525  46632856  46633079  3
DV864553  14438066  14437928  3
DV864557  47725037  47725109  3
DV864561  41861655  41861108  3
DV864592  9405379  9405482  3
DV864600  52766934  52766564  3
DV864630  24456747  24456584  3
DV864680  1832061  1831933  3
DV864793  56413098  56413248  3
DV864818  50076027  50075975  3
DV864850  52767894  52767791  3
DV864994  44093484  44093418  3
DV865022  41334364  41334141  3
DV865074  15179324  15179068  3
DV865130  55140018  55140373  3
DV865247  6737570  6737609  3
DV865446  3799323  3799259  3
DV865545  52611933  52612227  3
DV865674  28296881  28296365  3
DV865689  38984160  38984084  3
DV865701  59882148  59881463  3
DV865707  7587848  7588313  3
DV865708  28296908  28296365  3
DV865723  43626765  43626818  3
DV865804  28296903  28296365  3
DV865826  28296881  28296365  3
DV865884  1260196  1260104  3
DV866009  49035313  49035420  3
DV866029  48566785  4856680  3
DV866042  16249343  16249408  3
DV866055  46867731  46867839  3
DV866058  53308274  53308036  3
DV866079  41216070  41216163  3
DV866085  54190311  54190546  3
DV866097  48293931  48293961  3
DV866118  42077347  42077437  3
DV866134  53986653  53986595  3
DV866137  53212701  53212662  3
DV866171  21962376  21962519  3
DV866329  2116116  2116161  3
DV866488  39621881  39621971  3
DV866557  55686086  55686020  3
DV866702  26473816  26473283  3  2  Bd1, 5
DV866764  36079096  36079123  3
DV866879  55473386  55473254  3
DV866906  50602552  50602678  3
DV866945  5324854  5325172  3
DV866947  19004259  19004342  3
DV867088  27093144  27092988  3  2  Bd4, 5
DV867365  32196364  32196984  3
DV867452  50073043  50072981  3
DV867607  4790646  4791079  3
DV867630  39009952  39010016  3
DV867631  57693525  57693466  3
DV867712  28307335  28306471  3  Bd2
DV867720  54698904  54698832  3
DV867767  32000422  32000393  3
DV867874  53307496  53307259  3
DV867975  42609633  42609386  3
DV868003  34849437  34849296  3
DV868026  12516792  12516856  3
DV868235  48294174  48294120  3
DV868245  27093371  27092986  3  Bd4, 5
DV868314  995185  995357  3
DV868382  34231259  34231467  3
DV868445  53307984  53307889  3
DV868526  38400966  38400870  3
DV868603  44238525  44238425  3  Bd4
DV868608  35809225  35808972  3
DV868655  36450614  36451259  3
DV868696  33554020  33554205  3  Bd1
DV869056  5997972  5998061  4
DV869073  12827931  12827393  4  Bd1
DV869017  23335842  23335470  4
DV869016  6943307  6943097  4
DV869125  2860524  2860959  4
DV869147  41342428  41342398  4
DV869147  9381570  9381465  4
| Key   | Value       | Description     |
|-------|-------------|-----------------|
| DV861636 | 4905440 | 4905332 | 4 |
| DV861734 | 7477414 | 7477380 | 4 |
| DV861753 | 32664644 | 32664666 | 4 |
| DV862048 | 42403184 | 42403529 | 4 | Bd1, 2, 3 |
| DV862209 | 7941422 | 7941479 | 4 | Bd3, 5 |
| DV862417 | 41377627 | 41377317 | 4 | Bd1, 2, 3, 5 |
| DV862668 | 3061596 | 3061518 | 4 |
| DV862928 | 35863629 | 35863411 | 4 |
| DV862938 | 40523915 | 40526007 | 4 |
| DV863232 | 2311169 | 2311316 | 4 | Bd5 |
| DV863635 | 13767568 | 13767051 | 4 | 3 |
| DV864085 | 7547767 | 7547646 | 4 | 2 |
| DV864184 | 8423213 | 8423511 | 4 |
| DV864355 | 37802805 | 37802859 | 4 |
| DV864369 | 33709656 | 33709864 | 4 |
| DV864424 | 37613848 | 37613993 | 4 |
| DV864475 | 26439698 | 26440071 | 4 |
| DV864528 | 200144 | 199930 | 4 |
| DV864592 | 8131489 | 8131669 | 4 | 8 | Bd2, 3, 5 |
| DV864830 | 33887378 | 33887554 | 4 |
| DV864889 | 33709656 | 33709864 | 4 |
| DV864966 | 32725916 | 32725872 | 4 |
| DV865096 | 45035187 | 45035264 | 4 |
| DV865392 | 757131 | 757066 | 4 | Bd1 |
| DV865666 | 5569885 | 5570111 | 4 |
| DV865884 | 3951132 | 3951040 | 4 | 5 | Bd1, 2, 3 |
| DV865890 | 8422997 | 8423093 | 4 |
| DV865966 | 31049668 | 31049571 | 4 |
| DV866068 | 45035166 | 45035264 | 4 |
| DV866159 | 24618071 | 24617821 | 4 |
| DV866573 | 8423334 | 8423511 | 4 |
| DV866645 | 41721218 | 41721185 | 4 | 2 |
| DV866653 | 27015231 | 27015379 | 4 | 2 |
| DV866717 | 1071167 | 1071376 | 4 |
| DV866731 | 36215370 | 36215424 | 4 |
| DV866847 | 16568599 | 16568411 | 4 |
| DV866866 | 32295119 | 32294863 | 4 |
| DV866921 | 17458499 | 17458445 | 4 | Bd1 |
| DV866965 | 43773471 | 43773595 | 4 |
| DV867026 | 30355538 | 30355387 | 4 |
| DV867088 | 7942029 | 7941877 | 4 | 2 | Bd3, 5 |
| DV867116 | 37910205 | 37910075 | 4 |
| DV867418 | 3357310 | 3357338 | 4 |
| DV867542 | 36215197 | 36215424 | 4 |
| DV867756 | 35744568 | 35744514 | 4 |
| DV867864 | 3420284 | 3420648 | 4 | Bd1, 2 |
| DV867978 | 41146228 | 41146272 | 4 |
| DV868017 | 29882056 | 29881815 | 4 | 2 |
| Code    | First_number | Second_number | Count |
|---------|--------------|---------------|-------|
| DV868027 | 30354683     | 30355233      | 4     |
| DV868078 | 41808838     | 41808767      | 4     |
| DV868245 | 7546855      | 7547238       | 4     |
| DV868253 | 7497078      | 7496803       | 4     |
| DV868381 | 8423364      | 8423464       | 4     |
| DV868411 | 8423333      | 8423512       | 4     |
| DV868417 | 23699443     | 23699364      | 4     |
| DV868603 | 43417370     | 43417462      | 4     |
| DV868616 | 39121245     | 39121177      | 4     |
| DV868639 | 17457309     | 17457234      | 4     |
| DV860321 | 556999       | 556867        | 5     |
| DV862105 | 26152340     | 26152471      | 5     |
| DV862209 | 5280265      | 5280384       | 5     |
| DV862417 | 2371119      | 2371408       | 5     |
| DV862454 | 9879775      | 9879416       | 5     |
| DV862599 | 13475166     | 13475477      | 5     |
| DV862716 | 22418989     | 22419077      | 5     |
| DV862906 | 9617332      | 9617129       | 5     |
| DV862933 | 22419478     | 22419344      | 5     |
| DV862973 | 323610       | 323650        | 5     |
| DV863161 | 18173955     | 18173595      | 5     |
| DV863274 | 1636986      | 1637171       | 5     |
| DV863372 | 20009427     | 20009345      | 5     |
| DV863522 | 15228513     | 15228449      | 5     |
| DV863707 | 25383635     | 25383680      | 5     |
| DV863732 | 26268894     | 26268812      | 5     |
| DV863777 | 17060599     | 17060864      | 5     |
| DV863851 | 19043205     | 19042880      | 5     |
| DV863980 | 9812091      | 9811993       | 5     |
| DV863987 | 19757998     | 19758208      | 5     |
| DV864085 | 5280260      | 5280384       | 5     |
| DV864145 | 9810291      | 9810267       | 5     |
| DV864301 | 6768918      | 6768952       | 5     |
| DV864385 | 3127656      | 3127514       | 5     |
| DV864567 | 9618288      | 9618248       | 5     |
| DV864592 | 2865773      | 2865950       | 5     |
| DV864607 | 17666190     | 17666155      | 5     |
| DV864930 | 17418268     | 17418535      | 5     |
| DV865067 | 23463062     | 23462980      | 5     |
| DV865147 | 21116726     | 21116851      | 5     |
| DV865243 | 19226028     | 19225990      | 5     |
| DV865579 | 22418954     | 22419077      | 5     |
| DV865701 | 5536         | 4659          | 5     |
| DV865833 | 19197894     | 19197970      | 5     |
| DV865909 | 16097438     | 16097918      | 5     |
| DV866106 | 9704864      | 9704790       | 5     |
| DV866389 | 20009378     | 20009345      | 5     |
| DV866651 | 14833840     | 14833799      | 5     |
| DV866689 | 12974472     | 12974588      | 5     |
| ID          | Start 1  | Start 2  | End  | Chromosome |
|------------|---------|---------|------|------------|
| DV866702   | 25646469| 25647003| 5    | Bd1, 3     |
| DV867088   | 5281337 | 5281181 | 5    | Bd3, 4     |
| DV867307   | 22015019| 22015084| 5    |            |
| DV867384   | 4345446 | 4345302 | 5    |            |
| DV867516   | 16032678| 16032634| 5    |            |
| DV867620   | 15704364| 15704614| 5    |            |
| DV867663   | 10410936| 10411180| 5    |            |
| DV868245   | 5281340 | 5280955 | 5    | Bd3, 4     |
| DV868429   | 22094662| 22094785| 5    |            |
| DV868456   | 25681656| 25681962| 5    |            |

\( ^a \) Location of the alignment between the marker and the *B. distachyon* chromosome (start and end correspond to base pair number).

\( ^b \) *B. distachyon* chromosome where the creeping bentgrass EST was found as ortholog.

\( ^c \) Number of multiple matches of Creeping bentgrass EST orthologs within one *B. distachyon* chromosome.

\( ^d \) Additional *B. distachyon* chromosome where the creeping bentgrass EST was found as ortholog.
Table 6. Disease resistance and defense response genes identified in creeping bentgrass ESTs and their homologous peptides in *B. distachyon*.

| Agrostis EST ID | Brachypodium locus ID | Brachypodium chromosome | Protein family          |
|-----------------|-----------------------|-------------------------|-------------------------|
| DV863491        | Bradi1g72430          | 1                       | Leucine rich repeat     |
| DV864848        | Bradi1g05950          | 1                       | Protein kinase domain   |
| DV861655        | Bradi1g10970          | 1                       | Protein kinase domain   |
| DV867398        | Bradi1g58400          | 1                       | Protein kinase domain   |
| DV861137        | Bradi2g24930          | 2                       | Protein kinase domain   |
| DV866278        | Bradi2g01320          | 2                       | Protein kinase domain   |
| DV867860        | Bradi2g06060          | 2                       | Protein kinase domain   |
| DV865535        | Bradi2g20200          | 2                       | Protein kinase domain   |
| DV863115        | Bradi3g47600          | 3                       | Protein kinase domain   |
| DV860937        | Bradi4g26410          | 4                       | Protein kinase domain   |
| DV862787        | Bradi4g33110          | 4                       | Protein kinase domain   |
| DV865579        | Bradi5g19430          | 5                       | Protein kinase domain   |
| DV863880        | Bradi5g07360          | 5                       | Protein kinase domain   |
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