SSR Marker Development, Linkage Mapping, and QTL Analysis for Establishment Rate in Common Bermudagrass

Yuanwen Guo, Yanqi Wu,* Jeff A. Anderson, Justin Q. Moss, Lan Zhu, and Jinmin Fu

Abstract
Common bermudagrass has been widely used as a major warm-season turf, forage, and soil stabilization grass in the southern United States. However, codominant marker development, linkage, and quantitative trait loci (QTL) mapping resources are limited in the important taxon. Accordingly, the objectives of this study were to develop simple sequence repeat (SSR) markers, construct a genetic map, and identify genomic regions associated with establishment rate. Five genomic SSR libraries were constructed, sequenced, and used in the development of 1003 validated SSR primer pairs (PPs). A linkage map was constructed using a first-generation selfed population derived from a genotype A12359 (2n = 4x = 36). A total of 249 polymorphic SSR PPs were mapped to 18 linkage groups (LGs). The total length of the map is 1094.7 cM, with an average marker interval of 4.3 cM. Ninety-eight out of 252 mapped loci (39%) were found to be distorted from the Mendelian 1:2:1 segregation ratio. Among the other 154 nondistorted loci, 88 coupling vs. 66 repulsion linkage phases were observed to confirm the allopolyploid origin of the parent. Ground coverage (GCR) phenotypic data in the establishment stage were collected in two replicated field trials. Quantitative trait loci mapping identified five genomic regions significantly related to the trait. The findings of this study provide valuable genetic tools and resources for genomic research, genetic improvement, and breeding new cultivars in the species.

Cynodon is taxonomically classified into nine species, and common bermudagrass (C. dactylon var. dactylon) is the most important because of its wide distribution, enormous variability, and extensive use for turf, livestock forage, and soil stabilization in the world (Taliaferro, 1995). Ploidy levels of the taxon range from diploid (2n = 2x = 18 somatic chromosomes) to hexaploid (2n = 6x = 54; Harlan et al., 1970; Forbes and Burton, 1963), while tetraploid (2n = 4x = 36) is the prevalent cytological form (Wu and Anderson, 2011). Common bermudagrass is a cross-pollinated species enforced with self-incompatibility (Burton, 1947; Taliaferro and Lamle, 1997; Tan et al., 2014). The sexual reproduction behavior dictates that bermudagrass genome is highly heterozygous at many loci. Therefore, codominant DNA markers, such as SSR markers, are required to accurately identify genotypes at individual loci, subsequently useful for developing genetic maps, identifying clonal cultivars, analyzing genetic diversity, and using in other genomic research in common bermudagrass.

The SSR markers or microsatellites are tandemly repeated sequences with 1 to 6 nucleotides long as one repeat unit with variable repeating numbers. Simple


Abbreviations: ADO, allelic dropout; BLAST, Basic Local Alignment Search Tool; EST, expressed sequence tag; GCR, ground coverage; GIS, Genetic Identification Services; LB, Lysogeny broth; LG, linkage group; LOD, log-likelihood of the odds; NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction; PKS, Perkins, OK; PP, primer pair; QTL, quantitative trait locus; RF, recombination frequency; SDRF, single-dose restriction fragment; SSR, simple sequence repeat; STW, Stillwater, OK.
sequence repeats have been extensively used in the identification of inheritance pattern, linkage analysis, and QTL mapping in multiple crops like sorghum [Sorghum bicolor (L.) Moench], wheat (Triticum aestivum L.), and pear (Pyrus spp.) as they are codominant, multiallelic, reliable, and based on polymerase chain reaction (PCR; Diwan et al., 2000; Wu and Huang 2006; Peleg et al., 2008; Wu et al., 2014). Using 9414 Cynodon dactylon unigenes derived from expressed sequence tags (ESTs), Kim et al. (2008) reported approximately 1.5% ESTs contained SSRs, which were used to design 95 EST-SSR PPs. Jewell et al. (2010) developed 16 EST-SSR markers by characterizing the EST-SSR primer sequences of Kim et al. (2008). Harris-Shultz et al. (2010) developed 53 EST-SSR PPs using the same EST sequences of Kim et al. (2008). Tan et al. (2010) reported 65 SSRs transferred from Sorghum bicolor to C. dactylon and C. transvaalensis, and the development of 230 EST SSR PPs using 20,237 Cynodon ESTs at National Center for Biotechnology Information (NCBI). Kamps et al. (2011) developed 25 genomic SSR markers from a genomic library enriched for the CA/GT repeat motif. Simple sequence repeats are a desirable marker system of choice to construct genetic maps in common bermudagrass, again due to the expected heterozygosity in the genome.

Linkage map is among the most important genetic tools used in contemporary genomic and genetic investigations in crops. Linkage maps have been widely used to establish marker-trait associations and to indirectly select for economically important traits. High density genetic maps are valuable for some reference whole genome sequence projects (Koning-Boucoiran et al., 2012; Liu et al., 2012). Linkage maps developed from different populations or resources are necessary to understand genome information and inheritance (Semagn et al., 2010). However, limited efforts have been made to construct genetic maps in common bermudagrass. Using an F1 population between a tetraploid common bermudagrass (2n = 4x = 36) and a diploid African bermudagrass (2n = 2x = 18; C. transvaalensis Burtt-Davy), Bethel et al. (2006) reported two framework linkage maps of single-dose restriction fragment (SDRF) markers, one for each parent. The tetraploid map covers 1837.3 cM with 155 SDRF markers, while the African diploid map covers 973.4 cM with 77 markers. The map of common bermudagrass was estimated to cover about 60% of its whole genome (Bethel et al. 2006). Harris-Shultz et al. (2010) mapped additional 35 alleles of SSR markers onto the tetraploid map of Bethel et al. (2006). But the newer map spanned just 1055 cM (Harris-Shultz et al., 2010). In addition to many gaps to be filled in the existing maps, most mapped markers were detected using restriction fragment length polymorphism probes based on a DNA hybridization technique that is rarely used now. We have developed a first-generation selfed (SI) population from a tetraploid common bermudagrass, which exhibited disomic inheritance (Guo et al., 2015).

Vegetative propagation is a common and effective method for commercial production and scientific research in forage and turf bermudagrass (Busey and Myers, 1979; Hanna and Anderson, 2008), therefore establishment rate is one important trait to be evaluated in field trials from breeder’s selection nurseries to national tests (i.e., National Turfgrass Evaluation Program; Morris, 2001). Ground coverage is widely used to represent turfgrass establishment rate during the grow-in stage. Brosnan and Deputi (2008) indicated through vegetative propagation bermudagrass can rapidly establish and spread after planting. Richardson et al. (2001) utilized digital photographs to generate turf GCR through dividing the total number of green pixels by the total number of pixels in the image taken with a camera mounted on a light-box. But the genetic determinants for establishment rate are basically unknown. Quantitative trait loci mapping has been widely used in identifying genome regions associated with phenotype variation in major crops in the last two decades. Quantitative trait loci mapping for turfgrass has mainly been focused on disease and stress tolerance, and reproductive development related traits in ryegrass (Lolium perenne L.), zoysiagrass (Zoysia japonica Steud.), and St. Augustinegrass [Stenotaphrum secundatum (Walter) Kuntze; Forster et al., 2004; Ding et al., 2010; Jessup et al., 2011; Mulkey et al., 2014]. To date, no QTL mapping for establishment rate in bermudagrass has been reported. Accordingly, the objectives of this study were to develop SSR markers, construct a genetic map, and identify genomic regions associated with establishment rate in common bermudagrass.

Materials and Methods

Plant Materials and Field Trials

Common bermudagrass ‘Zebra’ (2n = 4x = 36), a variegated F1 plant found in a C. dactylon population (Johnston and Taliaferro, 1975), was selected to isolate genomic DNA for constructing SSR enriched libraries. Zebra DNA was also used in the initial screening of designed SSR PPs. A first-generation selfed progeny population from a common bermudagrass genotype A12359 (2n = 4x = 36; Wu and Huang, 2006) was used for linkage map construction. This population was selected for this linkage mapping study because it exhibited a lower amount of distortion compared with a selfed progeny population of Zebra in an SSR marker segregation analysis as recently described by Guo et al. (2015). Within the selfed population of A12359, 130 selfed progenies were randomly selected and used for genetic map construction and QTL analysis.

In May 2014, two field trials of the mapping population were established in a randomized complete block design with three replications at Cow Creek Bottom, Stillwater, OK (STW) and Cimarron Valley Research Station, Perkins, OK (PKS). Soil types for STW and PKS were Easpor loam or Ashport silty clay loam, and Teller fine sandy loam respectively. The spacing between two
neighboring plants in a row and between adjacent rows was 1.5 m. Base fertilizer 17–5–5 (N–P–K) was applied at a rate of 448 kg ha⁻¹ before transplanting. Immediately after transplanting, plants of the mapping population into the trials in STW on 14 May and PKS on 21 May, Ronstar 50WSP [3-(2,4-dichloro-5-(1-methylethoxy)phenyl)-5-(1,1-dimethylethy)-1,3,4-oxadizol-2(3H)-one] was applied at both sites at 2.24 kg, a.i. ha⁻¹.

Alleys (60 cm) were cleaned up by repeated applications of Roundup [glyphosate: isopropylamine salt of N-(phosphonomethyl) glycine; Monsanto, St. Louis, MO] at 2.24 kg a.i. ha⁻¹, as necessary.

The fasta file containing all trimmed sequences was analyzed with the SSR Locator program of da Maia et al. (2008). This program identified SSR sequences and designed PPs. The parameters for the SSR primer design were default with changes as follows: 5-di-, tri-, tetranucleotide; 4-penta-, hexanucleotide; 3-hepta-, octa-, nona-, and decanucleotide. The defaults for the primer design were chosen as follows: ampiclon size 140 to 350 bp; GC clamp 0; primer size range 18 to 22 bases; optimum primer size 20; melting temp (Tₘ) 55 to 61°C with an optimum of 59°C; G/C content 45 to 50%; start and end point automatic; and end stability at 250. Individual forward primers designed by SSR Locator were modified by adding a M13 sequence (5′-CAGGAC-GTTGTAAGACGAC-3′; Integrated DNA Technologies) to the 5′ end of forward primer sequence. The trimmed fasta files of all sequences found to contain SSRs were further analyzed with the CAP3 program of Huang and Madan (1999) to form contigs and singletons to eliminate all redundant clones.

To prevent any redundancy with already published bermudagrass SSR primers, a comparison was performed between the forward and reverse genomic DNA primers of Kamps et al. (2011) and the trimmed sequences from the Zebra genomic libraries. Each set of sequences were compared by a specialized NCBI BLAST (Basic Local Alignment Search Tool) program bl2seq for aligning two (or more) sequences together. This specialized BLAST program was optimized for highly similar sequences (megabLAST) per NCBI default parameters with the exception that the word size algorithmic parameter was changed from 28 to 16 due to the size of the primers (20–21 bp).

**Linkage Map Construction**

A total of 810 SSR PPs developed from the small-insert genomic DNA libraries described above were screened for polymorphism using a small panel of DNA samples consisting of two A12359 replicates and its six selfed progenies. Respective genomic DNA of A12359 and its progeny plants was extracted following the CTAB method of Doyle with minor modifications (Doyle, 1990), then DNA concentration was quantified using a NanoDrop ND-1000 spectrophotometer (Nanodrop Products), and each DNA sample was diluted to 10 ng/μL, which was prepared for PCR. Then PCR was performed to amplify SSR markers in a Biosystems 2720 Thermal Cycler (Applied Biosystems Inc., CA) with the reaction conditions as described by Guo et al. (2015). The PCR products were separated on a 4300 LI-COR DNA Analyzer (LI-COR Biosciences, Lincoln, NE) for collecting genotypic data of SSR markers in the progeny
population. Polymorphic markers were tested to amplify one DNA plate encompassing two replicates of the parent and single samples of 62 progenies, and those producing stable and heritable bands were used to genotype the other DNA plate encompassing two parent replicates and additional 62 selfed progeny samples.

Allelic data for each polymorphic SSR PP of the 130 progeny were recorded into an Excel worksheet. The segregation type <hk × hk> was used for scoring alleles amplified with each polymorphic SSR PP because only two alleles existed at most loci in the parent and segregated in the progeny population. For a heterozygous locus of the parent with two alleles, h and k, if only one upper band was amplified in a progeny sample, the band pattern was scored as "hh"; if only one lower band in a progeny it was scored as "kk"; and, if both bands amplified, it was scored as "hk"; missing genotype (no band showing up for a sample) was encoded as "--". For these SSR PPs, each producing stable but segregating four bands, termed multiallele markers, two sets of loci were recorded and analyzed separately on the basis of their distinguishing allele sizes and segregation patterns (Liu et al., 2012).

Chi-square test was performed to examine the Mendelian segregation ratio (i.e., 1:2:1) using Joinmap 4.0 (Van Ooijen, 2006a). Markers distorted from the expected segregation ratio were marked as *, **, and *** in the linkage map described below for P < 0.01, P < 0.001, and P < 0.0001, respectively, to denote different significance levels (Liu et al., 2012). Linkage map was constructed using the same software in which “cross pollinator” was used as type code for the selfed population (Van Ooijen, 2006a). According to Van Ooijen (2006a), it was recommended to start with a higher log-likelihood of the odds (LOD) value indicating a relatively stringent level to construct a map, although it may lead to a map with more LGs than the actual number, and then LOD value with reduced stringency was decreased to regroup the map properly. Therefore, initially, a minimum of LOD value of 10.0 was used to group loci, and other linkage parameters was set as follows: Show weak linkages with a recombination frequency (RF) larger than 0.45, or a LOD smaller than 0.05; Show strong linkages with an RF smaller than 0.01, or a LOD larger than 10; Show linkages as suspected if RF estimates are larger than 0.5; Number of maximum linkages to show per locus = 2; Determine linkage phases using pairs with a LOD > 1; Show heterogeneity tests with P-values < 0.1. Regression mapping algorithm was used with parameters: Use linkages with an RF smaller than 0.49, and a LOD value larger than 0.1; Goodness-of-fit jump threshold for removal of loci = 5; Number of added loci after which to perform a ripple = 1. Kosambi’s mapping function was performed, and yes for third round, and map was printed for each round. A minimum LOD value of 3 was considered as appropriate for the acceptance of two loci linkage (Bandaranayake and Kearsey, 2005). Accordingly, the lowest LOD value was set to 3.0 to regroup the map, which allowed us to link two independent LGs if one marker on one group could show a cross linkage with another marker on the other LG (Liu et al., 2012).

**Phenotypic Data Collection and QTL Analysis**

Digital photographs were taken on 3 July for the trial in STW and 27 Aug. 2014 for the trial in PKS with a digital camera mounted on a light box. Bermudagrass GCR was determined using Assess 2.0 (Image Analysis Software for Plant Disease Quantification) for each plot. Phenotypic data was analyzed using SAS 9.4 (SAS Institute, 2014). SAS/MEANS procedure was conducted for mean values and associated standard deviations for the parent and selfed population in each location, and SAS/MIXED was used for ANOVA.

Software MapQTL 6.0 (Van Ooijen, 2006b) was used to perform QTL analysis for GCR with three text files including locus genotype, quantitative data, and genetic map files. Mean values of GCR over three replications for each genotype were used for separate QTL mapping analyses by each location (Dong et al., 2015), and one joint QTL mapping was analyzed using mean values of GCR across two locations (Zhang et al., 2014). Regression algorithms were used in QTL analysis, interval mapping was performed initially to determine the presence of a segregating QTL with mapping step size 1.0, and a permutation test was used to decide genome-wide significance LOD threshold at 95% confidence level when the number of permutation was set as 1000. Using selective cofactors which were QTLs found in the initial analysis, multiple-QTL model mapping was reiterated until stable cofactors were obtained (Makaju, 2014).

**Results**

**SSR Marker Development**

For each of the five SSR enriched libraries, 766 clones were sequenced, resulting in collectively 3830 sequences available for SSR marker development (Table 1). SSR Locator identified 3842 SSRs in the 3830 sequences since some sequences containing more than one SSR motif. For example, 951 SSR fragments were identified from 766 sequences in Library B. Similarly, 947 SSR sequences were recognized.
in Library D. From the SSR containing sequences, 3096 SSR PPs were designed. Excluding redundant sequences, 1311 SSR PPs were unique. Higher redundant SSRs were identified in ATG- and AAC-enriched libraries than in AC-, AG-, and CAG-enriched libraries (Table 1). Library B, enriched for (GA)n repeat had the highest nonredundant rate of 67%. The PCR amplifications of the 1311 SSR PPs on Zebra DNA showed that 1003 PPs were effective in the amplification of target bands (Table 1). Among the five libraries, efficiency was different in the development of effective SSR markers. Library B was the most efficient and contributed 385 effective SSRs, while Library C was the least, contributing 74 SSR PPs.

Among the 1003 unique and effective SSR markers (shown in Supplemental Table S1), 731 were of perfect types, and the remaining 272 were compound types based on the repeat type and structure. The largest number (278) of a single perfect type, (AG/TC)n or (GA/CT)n, was observed in Library B, which was expected as the library was enriched for the perfect core sequence. Following the number of (AG/TC)n or (GA/CT)n SSR repeat motifs, there were 145 (CA/GT)n or (AC/TG)n motifs found in Library A. Collectively, most (712 of 731) of the perfect SSRs in the five libraries belonged to the expected target repeat types. However, 98 SSRs of AAC/TTG or GTT/CAA or ACA/TGT were developed in Library E, enriched for CAG repeats; while only 20 markers were in Library D, enriched for AAC core sequence. Among the compound SSR markers, majority sequences contained the target repeats. Of the five libraries, the predominant motifs of SSR markers were dinucleotide repeats (AC/TG, CA/GT, GA/CT, AG/TC, and related compounds).

Genetic Linkage Map

Out of 810 SSR PPs screened in the small panel of two parent replicates and six progenies, 260 were polymorphic and then selected for genotyping the whole progeny population. The 260 polymorphic SSRs amplified a total of 266 loci, since six SSR PPs amplified four alleles of two loci each, and the other 254 PPs generated two alleles each. A gel image of the parent (A12359) and 62 selfed progenies amplified one upper band, one lower band, or two bands were amplified, respectively, and a missing lane was labeled as “-”.

Fig. 1. A gel image of genotyping an SSR marker CDGA5-1465/1466 in the parent A12359 and its 62 selfed progenies. The band pattern of A12359 (P) was scored as “hk,” and an individual progeny band pattern was coded as “hh,” “kk,” or “hk” if one upper band, one lower band, or two bands were amplified, respectively, and a missing lane was labeled as “-”.

The SSR markers were distributed nonevenly across the 18 LGs in the common bermudagrass genome, and marker distribution with >5 loci per 10 cM was identified as marker clustering. Clustering of SSR markers was observed in many LGs, including LG 1, 3, 7, 8, 11, 13, 15, 16, and 18, while the phenomenon was not apparent on other LGs (Fig. 2). Severe segregation distortion was observed in 98 out of 252 (39%) mapped loci in the selfed population. Distorted markers were clustered in LGs 2, 5, 6, 10, 15, 16, and 18, and more severely, all markers on LGs 2 and 6 were distorted. Among the 154 nondistorted markers, the number of SSRs with coupling linkage phase and repulsion phase was 88 and 66, respectively. Therefore, the ratio of coupling to repulsion phase SSRs was consistent with 1:1 ratio (P = 0.076), which confirmed the allopolyploid origin for the common bermudagrass genotype (Guo et al., 2015).

ANOVA Results and QTLs Detection

There was substantial variation in GCR among genotypes within the selfed population. Genotype, location, and block had significant effects for the trait with the probability values of <0.0001, 0.0033, and <0.0001, respectively, except for genotype by location interaction with a probability of 0.1415. Means values (%) and associated standard deviations for GCR in STW were 43.27 ± 6.97 (parent) and 38.48 ± 17.39 (selfed population), while in PKS the values (%) were 63.66 ± 6.14 and 60.00 ± 28.07, respectively. For the trait with genotype by location interaction, and block had significant effects for the trait with a probability of 0.1415. Means values (%) and associated standard deviations for GCR in STW were 43.27 ± 6.97 (parent) and 38.48 ± 17.39 (selfed population), while in PKS the values (%) were 63.66 ± 6.14 and 60.00 ± 28.07, respectively. Therefore, the ratio of coupling to repulsion phase SSRs was consistent with 1:1 ratio (P = 0.076), which confirmed the allopolyploid origin for the common bermudagrass genotype (Guo et al., 2015).
CDCA5-469/470_260 on LG 8 explained 7.7% of GCR variation. One region located between CDCA5-463/464 and CDGA1-807/808 on LG 13 accounting for 14.9% of the variation. The QTL around SSR CDGA5-1459/1460 localized on LG 16 explained 7.0% of the phenotypic variance. The fourth QTL were detected on LG 18 near marker CDGA8-1783/1784, accounting for 6.5% of the variation. In PKS, one peak was identified on LG 18 close to SSR CDGA8-1783/1784, explaining 8.7% of the phenotypic variation. This QTL was located in a similar interval as the QTL found in the trial at STW. Across the two locations, joint data analysis recognized three QTLs, including one close to CDGA8-1807/1807 on LG 1, one between CDGA5-1391/1392 and CDGA1-807/808 on LG 13, and one near SSR CDGA8-1783/1784 on LG 18, with each explaining 8.1, 9.9, and 11.6% of the phenotypic variation, respectively. Among the five identified QTLs, four had positive values of additive effects, and one had...
negative value of additive effects (Table 3). LOD profiles of major QTLs detected are shown in Fig. 3.

Discussion

Development and Characterization of SSR Markers

Heterozygous loci of common bermudagrass necessitate codominant markers to fully reveal their genotypes. This research project generated the largest set of more than 1,000 validated SSR PPs in common bermudagrass. Tan et al. (2010) developed 230 SSR PPs from bermudagrass EST sequences and demonstrated 65 SSR PPs were transferable from sorghum to bermudagrass. Harris-Shultz et al. (2010, 2011), Jewell et al. (2010), and Kamps et al. (2011) also reported the development of EST and genomic SSR PPs. The SSR PPs should be highly valuable for genotyping clonal cultivars (Wang et al., 2011; Harris-Shultz et al., 2011; Fang et al. 2015), constructing genetic maps (Harris-Shultz et al., 2010; and this report), mapping QTL for important agronomic traits, and more in-depth genomic research, including full genome sequencing, in the future.

In the technical aspects for the development of SSR markers, the redundancy rate in the five libraries ranged from 33 to 84%, which were higher than other crop species, including perennial ryegrass (81.0%), and zoysiagrass (91%; Jones et al., 2001; Tsuruta et al., 2005). Our results of core sequence types were consistent with the report of Cardle et al. (2000), indicating (AT/TA)n was the most common dinucleotide repeat motif among plant genomic sequences, followed by GA/CT and CA/GT. However, AT/TA was not usually used in SSR markers due to its self-complementary nature (Cai et al., 2005).

Genetic Linkage Map

The mapping population for this investigation was selected from one of two populations used for our previous inheritance mode study in common bermudagrass (Guo et al., 2015). Since the S1 progenies from Zebra had severe segregation distortion that would negatively affect linkage analysis by biased recombination fractions and spurious linkages (Liu et al., 2012), we selected S1 progenies of A12359 (2n = 4x = 36) with less segregation distortion for the genetic map construction. This is the first common bermudagrass genetic map completely based on SSR markers. Because of the various advantages of SSR markers including reproducibility, locus specificity, hypervariability, and especially transferability among different species (Xu, 2010), our common bermudagrass linkage map will be valuable for comparative genomic study with other grasses or crops. In Fig. 2a, LG 1 was much longer than its homeologous pair LG 6, indicating the latter LG may contain homozygous regions which cannot be mapped. Likely, the genetic length of the whole genome is underestimated as a result of homozygous regions in the genome.

In order to improve the accuracy of our linkage map, several considerations were made in the process of developing the linkage map. First, population size was one of the most important factors affecting genetic map

---

### Table 2. Linkage group (LG) assignment, marker numbers, LG length, and marker density in a common bermudagrass genetic map.

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Total markers</th>
<th>Length (cM)</th>
<th>cM/marker</th>
<th>Gaps (&gt;10 cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG 1</td>
<td>19</td>
<td>53.2</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>LG 2</td>
<td>5</td>
<td>15.2</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td>LG 3</td>
<td>15</td>
<td>54.6</td>
<td>3.6</td>
<td>3</td>
</tr>
<tr>
<td>LG 4</td>
<td>7</td>
<td>78.6</td>
<td>11.2</td>
<td>3</td>
</tr>
<tr>
<td>LG 5</td>
<td>11</td>
<td>80.1</td>
<td>7.3</td>
<td>3</td>
</tr>
<tr>
<td>LG 6</td>
<td>4</td>
<td>12.7</td>
<td>3.2</td>
<td>1</td>
</tr>
<tr>
<td>LG 7</td>
<td>26</td>
<td>48.7</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>LG 8</td>
<td>14</td>
<td>40.9</td>
<td>2.9</td>
<td>1</td>
</tr>
<tr>
<td>LG 9</td>
<td>6</td>
<td>50.6</td>
<td>8.4</td>
<td>3</td>
</tr>
<tr>
<td>LG 10</td>
<td>12</td>
<td>52.5</td>
<td>4.4</td>
<td>2</td>
</tr>
<tr>
<td>LG 11</td>
<td>6</td>
<td>17.2</td>
<td>2.9</td>
<td>1</td>
</tr>
<tr>
<td>LG 12</td>
<td>15</td>
<td>60.0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>LG 13</td>
<td>14</td>
<td>57.1</td>
<td>4.1</td>
<td>2</td>
</tr>
<tr>
<td>LG 14</td>
<td>9</td>
<td>61.2</td>
<td>6.8</td>
<td>3</td>
</tr>
<tr>
<td>LG 15</td>
<td>21</td>
<td>105.2</td>
<td>5.0</td>
<td>3</td>
</tr>
<tr>
<td>LG 16</td>
<td>18</td>
<td>91.6</td>
<td>5.1</td>
<td>3</td>
</tr>
<tr>
<td>LG 17</td>
<td>12</td>
<td>93.0</td>
<td>7.8</td>
<td>5</td>
</tr>
<tr>
<td>LG 18</td>
<td>38</td>
<td>122.3</td>
<td>3.2</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 3. Detected quantitative trait loci (QTLs) associated with ground coverage in a common bermudagrass population.

<table>
<thead>
<tr>
<th>Location†</th>
<th>LG</th>
<th>LOD peak</th>
<th>Position of LOD peak</th>
<th>Marker region</th>
<th>PVE_A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>STW</td>
<td>8</td>
<td>27.99</td>
<td>CDCA7-2575/2576 to CDCA5-469/470_260</td>
<td>7.7</td>
<td>3.12</td>
<td></td>
</tr>
<tr>
<td>STW</td>
<td>13</td>
<td>20.431</td>
<td>CDGA5-463/464 to CDGA8-807/808</td>
<td>14.9</td>
<td>7.04</td>
<td></td>
</tr>
<tr>
<td>STW</td>
<td>16</td>
<td>91.638</td>
<td>CDGA5-463/464 to CDGA8-807/808</td>
<td>7</td>
<td>2.78</td>
<td></td>
</tr>
<tr>
<td>STW</td>
<td>18</td>
<td>7.70</td>
<td>CDGA8-1783/1784</td>
<td>6.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>PKS</td>
<td>18</td>
<td>2.66</td>
<td>CDGA8-1783/1784</td>
<td>8.7</td>
<td>4.94</td>
<td></td>
</tr>
<tr>
<td>STW + PKS</td>
<td>1</td>
<td>32.12</td>
<td>CDGA8-1807/1808</td>
<td>8.1</td>
<td>3.64</td>
<td></td>
</tr>
<tr>
<td>STW + PKS</td>
<td>13</td>
<td>12.307</td>
<td>CDGA5-1391/1392 to CDGA8-807/808</td>
<td>9.9</td>
<td>4.73</td>
<td></td>
</tr>
<tr>
<td>STW + PKS</td>
<td>18</td>
<td>3.6</td>
<td>CDGA8-1783/1784</td>
<td>11.6</td>
<td>4.69</td>
<td></td>
</tr>
</tbody>
</table>

† A, the additive effects contributed by QTL; LG, linkage group; LOD, log-likelihood of the odds; PVE, phenotypic variance explained.
‡ PKS, Cimarron Valley Research Station, Perkins, OK; STW, Cow Creek Bottom, Stillwater, OK.
construction, and a total number of 260 gametes were included in our data analysis, which should lead to a higher quality as compared with the previous bermudagrass maps (113 gametes in a bermudagrass framework linkage map of Bethel et al., 2006, and 118 gametes for linkage mapping by Harris-Shultz et al., 2010). Second, allelic dropout (ADO: one allele of a heterozygous locus could not be amplified during a PCR) will increase genotyping error rate which could not be avoided by laboratory procedures (Broquet and Petit, 2004); two alleles at each locus should reduce ADO. In our study, only coding type <hk × hk> was used to genotype the progeny individuals instead of other patterns (<ab × cd>, <ef × eg>, <lm × ll>, and <nn × np>) which could increase map accuracy because of only two coding alleles at each locus (Liu et al., 2012). According to Pawlowsky-Glahn and
Segregation Distortion

Segregation distortion, a common genetic phenomenon, has been reported in both outcrossing and selfing crops, including rice (*Oryza sativa* L.; Xu et al., 1997; Habu et al., 2015), maize (*Zea mays* L.; Lu et al., 2002; Xu et al., 2013), soybean (*Glycine max* (L.) Merr.; Baumbach et al., 2012), barley (*Hordeum vulgare* L.; Cistue et al., 2005), and also some perennial herbage like alfalfa (*Medicago sativa* L.; Li et al., 2011), ryegrass (Bert et al., 1999), and switchgrass (*Panicum virgatum* L.; Missaoui et al., 2005; Liu et al., 2012). Harris-Shultz et al. (2010) reported severe segregation distortion in 11 out of 75 (15%) EST-SSR alleles in a triploid bermudagrass population derived from a cross of T89 (2n = 4x = 36) and T574 (2n = 2x = 18). Because a selfed population has a higher tendency for segregation distortion (Liu et al., 2012), a higher rate (39%) of segregation distortion was observed in this population as compared with the previous study by Harris-Shultz et al. (2010). Segregation distortion will result in linkage disequilibrium, and the enhancer alleles will be found in coupling phase and suppressor alleles in repulsion phase (Lytte, 1991). However, we still preferred to keep those distorted markers as suggested by Van Ooijen (2006a) that distorted markers could be better understood if used in map construction.

Allopolyploid Origin of Common Bermudagrass

According to Wu et al. (1992), the ratio of coupling to repulsion linkage phase was used to detect allopolyploid or autoploidy, in which a ratio of 1:1 was expected for an allopolyploid and 1:0.25 or 1:0 for an autoploid. We observed a ratio of 88:66 (coupling versus repulsion), and the chi-square value for the observed and expected 1:1 ratio was 3.143, smaller than the critical value 3.841 (df = 1, α = 0.05). This chi-square test confirmed the bivalent preferential chromosome pairing behavior during meiosis (Wu et al., 1992), which further confirmed disomic inheritance pattern for common bermudagrass reported by Guo et al. (2015). Compared with switchgrass, another allopolyploid grass species, in which 12 multiallele markers of 499 SSRs tested (2.4%) were mapped in both subgenomes by Liu et al. (2012), we observed that six out of 260 SSRs (2.3%) were multiallelic, further indicating the allopolyploid origin rather than autoploidy in the common bermudagrass genotype.

Detection of QTLs for Ground Coverage in Establishment

Establishment rate (measured as GCR) during the turf forming stages or post damage recovery is an important trait for sod production or divot repair on golf courses, or damage recovery on sports fields. Our study identified common bermudagrass genomic regions associated with GCR during establishment, and clearly indicated common bermudagrass GCR was controlled by multiple genetic loci and was significantly affected by environments. Among the five QTLs affecting GCR detected in this study, individual QTLs appeared to show different sensitivities to environments (Paterson et al., 1991). One close to CDGA8-1783/1784 SSR on LG 18 were identified in all three analyses using data from STW, PKS, and combined data of the two locations, and this QTL explained 6.5 to 11.6% of the phenotypic variation in the two field trials. One QTL between CDCAS-463/464 and CDGA1-807/808 on LG13 were detected in STW and then confirmed in the joint analysis, but not in the trial at PKS. Three other QTLs were more sensitive than others, including two on LGs 8 and 16 only found in the trial at STW, and one genomic region in LG 1 only identified in the joint analysis. These unstable QTLs likely result from QTL × micro-environment interactions, wherein QTL phenotypic effect is affected by environmental factors including soil type, water, light, and temperature (Johnson et al., 2012).

The five QTLs identified in this study together explained nearly half of the observed phenotypic variation of GCR. The remaining variation could be derived from other sources, including environment plus error term, QTLs with effects too small to be detected, QTL × QTL interactions, and interaction of progeny individuals with environmental variation (Paterson et al., 1991).

Conclusions

Common bermudagrass is an important perennial grass. More than 1000 SSR PPs, the largest set of codominant DNA markers, were developed in this study. Using an SI progeny population, we reported the first genetic map of 18 LGs based on codominant SSR markers in common bermudagrass. The mapping phase ratio of one coupling to one repulsion in this population firmly indicated the allopolyploid origin of the tetraploid bermudagrass. This study identified five QTLs associated with GCR in the establishment stage in two field trials. One genomic region on LG 18 was stable in the testing environments. The developed SSR PPs, genetic map, and QTLs associated with GCR have high potential to be used for various genetic applications and hopefully in accelerating bermudagrass breeding.

Supplemental Information Available

Supplemental information is included with this article. Supplemental Table S1. Marker ID, repeat motif, forward and reverse primer sequence, and predicted size information for the effective 1003 SSRs.

Acknowledgments

We thank Drs. Tim Samuels and Tilin Fang for technical support and Mrs. Pu Feng for greenhouse assistance, and we appreciate the help from our previous and current lab members (Linglong Liu, Lie Yang, Chengcheng Tan, Shuiyi Lu, Laxman Adhikari, Hongxu Dong, Shiva Makaju, Dan Chang, and John Baker). The research was funded by USDA NIFA SCRI Award 2010-51811-21064, US Golf Association, China Natural Science Foundation Grant 31428021, Oklahoma Agricultural Experiment Station, and China Scholarship Council.
References


