GENETIC ANALYSIS OF INHERITANCE AND LINKAGE OF MOLECULAR MARKERS, AND VARIABILITY OF ADAPTIVE, MORPHOLOGICAL AND REPRODUCTIVE TRAITS IN COMMON BERMUDAGRASS [*CYNODON DACTYLON* (L.) PERS.]

By

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Abstract: Common bermudagrass [*Cynodon dactylon* (L.) Pers.] is economically and environmentally the most important member among *Cynodon* species because of its extensive use for turf, forage and soil erosion control in the world. However, information regarding the inheritance, genetic linkage and cold germplasm variability analysis within this taxon is limited. Therefore, the objectives of this study were 1) to determine qualitative inheritance mode in common bermudagrass; 2) to construct a genetic map based on simple sequence repeats markers for common bermudagrass; and 3) to quantify genetic variability and determine relationships among turf performance and reproductive traits. Two tetraploid (2n=4x=36), first-generation selfed populations, 228 progenies of 'Zebra' and 273 from A12359, were analyzed for segregation with simple sequence repeat markers in the first project. It was concluded that the inheritance mode of tetraploid bermudagrass was complete or near complete disomic, and the two bermudagrass parents had an allotetraploid genome with two distinct subgenomes. Severe transmission ratio distortions occurred in the Zebra population while less so in the A12359 population, so A12359 population was used for linkage analysis and bermudagrass map construction in the second project. A total of 249 simple sequence repeat primer pairs were mapped to 18 linkage groups. The total length for the map was 1094.7 cM with an average marker interval of 4.3 cM, and length for individual linkage groups ranged from 122.3 cM for LG 18 to 12.7 cM for LG 6. Comparative mapping was conducted to compare genomes of common bermudagrass, sorghum (*Sorghum bicolor* L.) and foxtail millet (*Setaria italica* L.), and three conservative regions among these three species were discovered in the study. The findings of disomic inheritance and construction of linkage map could be beneficial for quantitative trait loci mapping, marker assisted selection and other genome study in common bermudagrass. The third project focused on quantifying the genetic variability of important adaptive, morphological and reproductive traits in cold hardy common bermudagrass populations and testing possible correlations among these traits. Substantial and significant genetic variances were observed in our germplasm, which could be utilized in bermudagrass breeding programs such as producing interspecific hybrids and synthetic cultivars.

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CHAPTER I

GENERAL INTRODUCTION

Cynodon is a genus of the family *Gramineae* (*Poaceae*), subfamily *Chloridoideae*, Tribe *Cynodonteae*, and subtribe *Chloridinae* (Clayton and Renvoize, 1986). It is taxonomically classified into nine species, *C. aethiopicus* Clayton et Harlan, *C. arcuatus* J. S. Presl ex C. B. Presl, *C. barberi* Rang. et Tad., *C. dactylon* (L.) Pers., *C. incompletus* Nees, *C. nlemfuensis* Vanderyst, *C. plectostachyus* (K. Schum.) Pilger, *C. transvaalensis* Burtt-Davy, and *C. x magennisii* Hurcombe (Harlan et al., 1970a). "Star grass" is a name usually used for the three East African tall-growing grasses (*C. aethiopicus*, *C. nlemfuensis*, and *C. plectostachyus*) which are big, and non-rhizomatous. "African bermudagrass" is for *C. transvaalensis*, and *C. dactylon* is often named "common bermudagrass" (Wu and Anderson, 2011). Among these *Cynodon* species the best known and economically most important one is *C. dactylon* because of its wide geographic distribution, enormous variability, and multiple uses, such as for turf and forage production, in soil erosion control, and as a potential bioenergy feedstock (Taliaferro, 1995). *Cynodon dactylon* is separated into six botanical varieties, which are var. *afghanicus* Harlan et de Wet, var. *aridus* Harlan et de Wet, var. *coursii* (Camus) Harlan et de Wet, var.

dactylon, var. *elegans* Rendle and var. *polevansii* (Stent) Harlan et de Wet, according to their ecology, morphology and geographic distribution (Harlan et al., 1970a). These warm-season, sod-forming, perennial grasses are widely used in tropical and warmer temperate regions of the world for soil stabilization, turf and livestock herbage (Harlan, 1970).

According to the previous research, there were some different opinions for the base chromosome number, initially, *Cynodon*'s basic chromosome number x=10 from root tips study was reported by Hurcombe (1947), but most researchers believed that the basic chromosome of *Cynodon* should be x=9 (Burton, 1947; Forbes and Burton, 1963), the reason for x=10 was because of the presence of B-chromosomes in some *Cynodon* species (Hoff, 1967; Sengupta, 1968). *Cynodon* had several ploidy levels which range from diploid to hexaploid (Harlan et al., 1970b; Forbes and Burton, 1963), and it seemed like that diploid (2n=2x=18) and tetraploid (2n=4x=36) were dominant through these ploidy levels (Harlan et al., 1970b; de Silva and Snaydon, 1995). *Cynodon. barberi*, *C. dactylon* var. *aridus*, *C. incompletes* var. *incompletes*, *C. plectostachyus*, and *C. transvaalensis* are diploids, and *C. arcuatus*, *C. dactylon* var. *dactylon*, *C. dactylon* var. *coursii*, *C. dactylon* var. *elegans*, and *C. dactylon* var. *polevansii* were tetraploids (Harlan et al., 1970b). TifEagle (*C. transvaalensis* × *C. dactylon*) was triploid (2n=3x=27), one *C. dactylon* plant from Malta was pentaploid (2n=5x=45) (Thomas and Murray, 1978), and Tifton 10 was a hexaploid (2n=6x=54) (Burton, 1991). Because tetraploid var. *dactylon* came from internally crossing within diploid progenitors, so that it should be autotetraploids since there was only one genome for crossable species (Harlan and de Wet, 1969). Also some bermudagrass plants tended to be aneuploids which might be caused by overlapping chromosomes. In meiosis, chromosomes usually form bivalents in diploids and tetraploids, but trivalent and quadrivalent forms also appear in tetraploids (Forbes and Burton, 1963). Homologous chromosome pairing is more complex in triploids. For example, Forbes and Burton (1963) found that the chromosome behavior was irregular in triploid hybrids, and the parent difference leaded to meiotic irregularity in triploids (Harlan et al., 1970b). Hanna and Burton (1977) showed the number

of bivalents was dominant for five common bermudagrass cultivars (‘Coastal ‘,’Coastcross-1’,’Midland’ and ‘Suwannee’), but univalents, quadrivalents and few trivalents were observed among them. Although the cytogenetic study during meiosis contributed to explain the paring behavior for bermudagrass, but there was still no clear conclusions from the previous study for chromosome paring behavior in tetraploid bermudagrass. Limited information is available on the inheritance of molecular markers in common bermudagrass.

Bethel et al. (2006) reported two framework linkage maps, one for tetraploid *C. dactylon* and another one for *C. transvaalensis*, using a cross population between a tetraploid parent and a diploid parent with single-dose restriction fragments (SDRFs). An SDRF was present as one single copy from one parent, and it was based on 1:1 (presence: absence) ratio in the gametes (Wu et al., 1992), so SDRF mapping could bypass the polyploids mapping difficulties and complexities (Luo et al., 2001). The tetraploid map covers 1837.3 cM with 155 SDRF markers, while the African diploid map constitutes 973.4 cM with 77 markers. Their results indicate the tetraploid bermudagrass parent (T89) has polysomic inheritance of an autotetraploid (Bethel et al., 2006). Using the same mapping population, Harris-Shultz et al. (2010) added expressed sequence tags-derived simple sequence repeat (EST-SSR) markers to the bermudagrass linkage maps. However, the later study indicated 15 SSR markers exhibited disomic segregation in the common bermudagrass parent which may be a segmental allotetraploid or allopolyploid rather than an autotetraploid reported before (Harris-Shultz et al., 2010). For our study, we will use a selfed tetraploid population to develop a detailed linkage map for bermudagrass using SSRs.

According to the previous reports, huge genetic variability exits among different bermudagrass accessions. Recently, Wu et al. (2007) found genetic variations were much larger within tetraploids than in pentaploids and hexaploids, and the large genetic variability existed in Chinese bermudagrass accessions. There were also genetic variation reports for clonally propagated bermudagrass. Yerramsetty et al. (2008) showed that Tifton 10 was genetically similar with other Chinese cultivars

using DNA fingerprinting. Anderson et al. (2009) found the integrated variability caused by genetic and phenotypic interaction could contribute to parental line selection in clonal bermudagrass breeding. Actually, the sexual propagating had been an important reproductive way for bermudagrass because seed was easy and convenient to store and carry, and Burton (1947) found that there were huge variations for reproductive ability. Wu et al. (2006) reported reproductive ability varied greatly among 114 bermudagrass accessions from China, and also the variability in tetraploids was higher than other ploidy.

The objectives of our study were to (i) determine the inheritance mode of common bermudagrass populations; (ii) construct a detailed genetic linkage map for common bermudagrass; and (iii) analyze genetic variability and relationships for important morphological, adaptive and reproducible traits in cold hardy selections of common bermudagrass.

CHAPTER II

EXPERIMENT 1. DISOMIC INHERITANCE AND SEGREGATION DISTORTION OF SSR MARKERS IN TWO POPULATIONS OF *CYNODON DACTYLON* (L.) PERS. VAR. *DACTYLON*

I. Introduction

Common bermudagrass [*C. dactylon* (L.) Pers. var. *dactylon*] is the best known and economically most important species in the genus *Cynodon* L. C. Rich. because of its widespread geographic distribution, important economic uses and enormous genetic variability (Clayton and Genvoize, 1986; Taliaferro, 1995). The warm-season, sod-forming and perennial grass has been widely used for turf installation, forage production, soil stabilization and remediation in tropical and warmer temperate regions around the world (Harlan, 1970). In the United States, turf bermudagrass, including common bermudagrass and interspecific hybrids between common bermudagrass and African bermudagrass (*C. transvaalensis* Burtt-Davy), is a major warm-season turfgrass; whereas forage bermudagrass, encompassing common bermudagrass and interspecific hybrids between common bermudagrass and *C. nlemfuensis* Vanderyst, has been planted on approximately 12 million hectares as livestock herbage (Taliaferro et al., 2004).

The knowledge of ploidy level and meiotic chromosome behavior is important for genetic research and breeding new cultivars (Liu and Wu, 2011). Although the basic chromosome number of *Cynodon* species initially was mistakenly reported to be x=10 (Hurcombe, 1947) the

confirmed basic chromosome number of *Cynodon* is x=9 (Burton, 1947; Forbes and Burton, 1963). Among a series of ploidy levels (2n=2x=18, 3x=27, 4x=36, 5x=45, and 6x=54), tetraploid common bermudagrass is the most popular and prevalent form in nature (Forbes and Burton, 1963; Harlan et al., 1970c; Wu et al., 2006; Kang et al., 2008; Gulsen et al., 2009; Jewell et al., 2012). During the meiosis of tetraploid common bermudagrass, chromosomes usually form 18 bivalents, but irregular pairing forms have been observed, including two or more univalents, or one or two quadrivalents (Forbes and Burton, 1963; Harlan and de Wet, 1969; Hanna and Burton, 1977). Observing meioses of hybrids of 50 crosses within and between *C. dactylon* var. *dactylon* geographic races (tropical, temperate, and seleucidus), Harlan and de Wet indicated that most crosses that displayed bivalent pairing were found in hybrids between parents of similar geographic origins, with exceptions (Harlan and de Wet, 1969).

The qualitative inheritance mode is essential information in a species not only because it illuminates homologous chromosome pairing behavior and transmission of alleles from parents to progenies, but also provides basic knowledge for linkage map construction, and marker-trait association like quantitative trait locus (QTL) mapping. The mode of allelic inheritance can also influence breeding procedures that are used for cultivar development. Bethel et al. (2006) reported that the tetraploid bermudagrass parent, T89, exhibited polysomic inheritance (with an autotetraploid genome) based on the ratio of 22 repulsion versus 102 coupling linkages revealed with single dose restriction fragment markers. However, with the same mapping population, Harris-Shultz et al. (2010) indicated that T89 had two alleles segregating at each locus for 15 simple sequence repeat (SSR) loci except one progeny suggesting the parent displayed disomic inheritance and may be a segmental allotetraploid or allopolyploid rather than an autotetraploid. Since tetraploid common bermudagrass is extremely diverse and widely distributed around the world (Taliaferro, 1995; Wu et al., 2006), more work is needed to examine inheritance, segregation and genomic structure of the taxon. Therefore, our hypotheses were that the

qualitative inheritance of tetraploid common bermudagrass was tetrasomic and that the species had an autopolyploid genome (i.e., two subgenomes were from the same diploid species). Accordingly, the specific objective of this study was to investigate the inheritance mode and segregation regularity in two common bermudagrass populations.

II. Materials and Methods

1. Plant materials

To facilitate codominant marker segregation analysis, two populations of first-generation selfed (S1) progenies were created and used to investigate the inheritance of common bermudagrass. The parent for one population was 'Zebra' (2n=4x=36), a variegated plant found in an F1 bermudagrass population growing on the Oklahoma State University Agronomy Research Farm at Stillwater, Oklahoma in 1971, and it was so named because its leaf blades had alternating green and chlorotic strips (Johnston and Taliaferro, 1975; Taliaferro and Lamle, 1997). Zebra plants prepared in a greenhouse were moved into a growth chamber in the OSU Controlled Environment Research Laboratory (CERL) with 16 h/32.2^{o}C light and 8 h/26.7 ^{o}C dark periods in October and S1 seeds were harvested in December 2006. Seeds were germinated in another growth chamber in the winter between 2010 and 2011. A total of 228 single plants were transplanted into 10 cm pots in the greenhouse in the spring of 2011.

The second population consisted of selfed progenies of A12359 common bermudagrass (2n=4x=36) (Wu et al, 2006). Originally, two parental plants, A12328 and A12359, were grown in alternating rows to make a full-sib hybrid population in an isolated plot on the OSU Agronomy Research Station. Mature inflorescences of A12359 were harvested from the plot in September, 2012 and February, 2013. Seeds were germinated in a growth chamber. Seedlings of A12359 were transplanted into 10 cm pots in the greenhouse. Since a small amount of seeds from A12328

were harvested, their seedlings were not included in subsequent genetic analysis. A total of 1275 progeny plants from A12359 seeds were developed and used for identification of selfed progeny with 12 polymorphic SSR primer pairs. A progeny was identified as a selfed progeny if all polymorphic bands of the 12 SSR primer pairs for the progeny were from A12359 and none from A12328. All progeny plants of the two S1 populations along with their respective parent plants were cultivated to maintain healthy growth in the greenhouse.

2. DNA isolation and PCR conditions

Genomic DNA of each plant in the two populations and their parents was extracted following the cetyltrimethylammonium bromide (CTAB) method of Doyle with minor modifications (Doyle, 1990). Approximately 1.5 g of fresh leaf tissue from each plant was collected and ground to a fine powder in a Genogrinder (Zymo Research, CA, USA), and then transferred to a tube of pre-heated 1% CTAB buffer with 2-mercaptoethanol and incubated in 65°C water bath for 60 min. Chloroform/isoamyl alcohol was added for protein removal (Liu and Wu, 2011). Iso-propanol was added to allow DNA to precipitate. The DNA pellet was washed with a washing buffer and stored in 1× TE Buffer (pH 8.0). The DNA concentration of each sample was quantified using a NanoDrop ND-1000 spectrophotometer (Nanodrop Products, DE, USA) and the DNA quality was checked by 0.5 % KB^{plus} gels (Tan et al., 2010). Each DNA sample was diluted to a concentration of 10 ng/µl for PCR (polymerase chain reaction).

Ninety-six-well PCR plates were used to perform SSR PCR reactions in a Biosystems 2720 Thermal Cycler (Applied Biosystems Inc., CA, USA). Each 10.5 µl reaction (one well) included 1.50 µl of 10 ng/ µl genomic DNA, 4.87 µl nuclease free water, 1.00 µl 1× standard *Taq* reaction buffer (10 mM Tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$) (New England Biolabs Inc., MA, USA), 0.20 µl 10 mM dNTPs (Promega Corporation, WI, USA), 0.05 µl *Taq* enzyme (0.025 Units) (New England Biolabs Inc., MA, USA), 0.20 µl 1 µM IR-700 or -800 fluorescence labeled

M 13 primer, and 1.34 µl each of 10 µM/µl forward and reverse primers. The PCR cycling was started with a denaturing step at 95°C for 5 min; followed by 14 cycles of 94°C for 20 s, 58°C 1 min, and 72°C 30 s; 28 cycles of 94°C for 20 s, 55°C 1 min, and 72°C 30 s; and a final extension at 72°C for 10 min, and then was stored at 4°C. A Blue Stop Solution (95% formamide, 25mM ethylenediaminetetraacetic acid, and 2% bromophenol blue) of 5.0 µl was added to each PCR reaction bringing a total volume to 15.5 µl, which was denatured for 3 min at 94°C (Liu and Wu, 2011). The PCR products in two plates were labeled with 700 nm and 800 nm fluorescence primers respectively, were mixed thoroughly, spun down, and loaded into wells of 6.5 % KB^{plus} gels for electrophoresis on a 4300 LI-COR DNA Analyzer (LI-COR Biosciences, Lincoln, NE). Parameters for the electrophoresis in the LI-COR 4300 DNA Analyzer were set at 1500 V, 40 mA, 40 W, 45°C and 15 min for a pre-run, and one hour and 45 min to finish rest of the run.

3. Polymorphic SSR primer pair selection, gel scoring and data analysis

For each of the two populations, a small subset, which included two replicates of the parent and six randomly selected progeny samples, was used to screen common bermudagrass SSR primer pairs developed in our lab in order to select 33 polymorphic pairs (Table 1). If two or more bands were amplified with an SSR primer pair and heritable in the subset, the SSR primer pair was judged to be polymorphic (Tan et al., 2010). For a parent with "Aa" bands, if only one upper band was amplified in a progeny sample, the band pattern was scored as "AA"; if only one lower band amplified it was scored as "aa"; and if two bands amplified it was scored as "Aa". If four bands were amplified for a parent, the band pattern was scored as "abcd", and if three bands of a progeny were amplified, it was scored as "abc", "abd ", "acd", or "bcd" depending on the size of each band ("a" > "b" > "c" > "d"). If a PCR failed or an ambiguous band was found for a sample, it was scored as "M" (i.e., missing) (Liu and Wu, 2011).

Table 1. Information for selected SSR primer pairs in two selfed populations. Marker ID, repeat motif, primer sequences and expected band size for each of 33 selected SSR primer pairs used for genotyping Zebra and A12359 first-generation selfed populations.

No.	Marker ID	Repeat Motif	Primer Sequence (5′ to 3′)	Expected Size Plus M13F (bases)
1	CDCA1-21/22	(AC)13-(AG)14	F: GGGCCTCCCCTTTTATACAT R: GGTAACCAATCAAGGCCACT	199
2	CDCA2-231/232	(CA)13	F: CTTTTACGTCGGCCTTTGAG R: GTCCTGGTCTTGTCGGTTTC	186
3	CDCA3-247/248	(GT)8	F: TACCTCGCTGGACTGAAGTG R: GCAGTAGTCCCACCAACCTT	190
4	CDCA3-313/314	(CA)23	F: GGGTCATGAGTCAAATGTGC R: CTTTTGTGAGCCAGAAGCAA	311
5	CDCA4-323/324	(TG)13	F: ACAAGCCTTCCGGACTTTTA R: CATGCAGCACTGCTCAGATA	237
6	CDCA5-491/492	(GT)12	F: CTTGGTTCTTGGGTCCTTGT R: AGCTCAAGCACCATTGTCAG	289

7	CDCA7-623/624	(AC)17-(CA)5	F: CGAGACCTAGTGAACAGCGA R: GGCCGTGCTTAAAGGAATAG	329
8	CDCA7-653/654	(TG)10-(AG)9	F: CGCTACACTTTCGAGGTCAA R: ACCAGATATGCACTCCACCA	165
9	CDCA7-693/694	(AC)11	F: CGTCATCACAACCCACTCTC R: TGTTCTTCCACGACTGCTTC	160
10	CDCA8-737/738	(TG)14-(AG)11	F: ATGTCTAAAACACGCCCACA R: GACAACCAAGAGTGGCGATA	338
11	CDGA1-783/784	(GA)14	F: CACTGTTTACCCATCCAACG R: TTTTCGTACACACCCCAGAA	240
12	CDGA1-847/848	(CT)19-(TC)5-(TG)8	F: CCGATCGCTACTGAGAAACA R: TGGCCGAAAAACAGGGTA	296
13	CDGA1-921/922	(GA)14	F: GTTGGGTGAACGTACACAGG R: TAATTGACGTCCCTTCCCTC	267
14	CDGA1-929/930	(GA)18	F: TCAAGGTACCTGATGTGGAAAC R: GACTTCCCCTTAACAGCAGC	162
15	CDGA3-1103/1104	(AG)16	F: AAGAATAATGCCCAAGGCAC R: ACCATCACTCGACACCACAT	258

16	CDGA3-1195/1196	(CT)15	F: ACCACCAATAGCACACCAGA R: CGGAACAAGGAGTGAGACAC	324
17	CDGA4-1245/1246	(GA)20	F: AAGGAAAGGTGCATACCTGG R: GGCAGGTGTGGAGAAGTACA	194
18	CDGA5-1427/1428	(CT)21	F: TAGCAGGAACCTGTGGTCTG R: TGTTCTAACTGTCGCCATCC	307
19	CDGA6-1583/1584	(AG)25	F: GTATCGTCATCGTCCTGGTG R: TCGGCCAGAAAACCTCTATT	349
20	CDGA7-1601/1602	(GA)13	F: CCTGCTGGTCAGAACTCAAC R: TATTGGTTGCACCTTCCAGA	257
21	CDGA7-1611/1612	(AGAT)6-(AG)15	F: TCCTTCTTGTCCTGAAGCCT R: ACAGTCCATGCGACTCAGAA	246
22	CDGA8-1765/1766	(TC)16	F: GGGCTTTTGGAATGACTTGT R: CGAAGAGCGAGGAGAGATTT	198
23	CDGA8-1795/1796	(AC)5-(AG)36	F: TTCGTGGACTCTGGCTATTG R: GCCCAGGTAACGTGTTCTTT	364
24	CDGA8-1807/1808	(GA)14	F: CCTCAACTCCAGTGCTGAAA R: TGTTAACCGGGGTTCAGATT	226

25	CDATG1-1889/1890	(GCT)7-(GAT)7	F: AAACGTGAGAGGCTCTTGCT R: GTATGACACACGGAAGGACG	309
26	CDATG3-1999/2000	(ATG)7	F: CCAGGTTCGCATCAGATA R: TGCATATCATGAACACGACG	278
27	CDATG6-2123/2124	(CAT)5	F: AATGGAACCTTGGCACTTTC R: GGTGGGTGTTACTGCTCCTT	197
28	CDATG6-2143/2144	(TCT)6	F: ATCCTTCCCCTCCTCTTTGT R: TTGTACGATATCAACCCGGA	355
29	CDAAC5-2523/2524	(TGT)9	F: AAGGCCTAACCCAATTTGC R: ACAATGCTTTTCATCCTCCC	214
30	CDAAC7-2675/2676	(TGT)8	F: TAGCCTACCCCAACTTGCTT R: GTATACTGGCTTCATGGGCA	206
31	CDAAC7-2693/2694	(AAC)7	F: TTGCCTACCAAACACGAAAG R: TCCAAACTCGTGTAATTGCC	321
32	CDAAC7-2703/2704	(ACA)10	F: CTATTGCACATTGGATTCCG R: AGGAGTGGGAGGGTTTCTTT	359
33	CDCAG3-2897/2898	(CTG)7	F: TTGCCACTTTTGCAGGTAAC R: AAGTAGTGCCATGCGATCAG	174

Chi-square test was used to examine inheritance modes of SSR markers in the two populations. Segregation ratios of SSR markers in each of the two populations were tested to determine segregation modes, and disomic versus tetrasomic inheritance. For a polymorphic SSR locus under disomic inheritance, if a parental genotype was *Aa* which would have an "Aa" band pattern, then the progeny segregation ratio would be 1AA: 2Aa: 1aa (Table 2). Under tetrasomic inheritance, if the parent had "Aa" bands, there were three possible genotypes, *AAAa*, *AAaa*, or *Aaaa*. If parental genotype was *AAAa*, the expected phenotype segregation ratio in the progenies would be 1 upper band (AAAA): 3 double bands (2AAAa + 1AAaa); and parental genotype *AAaa* would result in progeny ratio of 1 upper band (AAAA): 34 double bands (8AAAa + 16AAaa + 2AAaa + 8Aaaa): 1 lower band (aaaa); and 3 double bands (1AAaa + 2Aaaa):1 lower band (aaaa) ratio would occur if parental genotype was *Aaaa*, respectively. If one SSR primer pair amplified four bands, under tetrasomic inheritance, the parent would have a genotype *ABCD*, and the expected progeny band patterns and segregation ratio are given in Table 3. The software "Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence" was used to perform chi-square tests and calculate actual P-values (Preacher, 2001).

Table 2. Twenty-one SSR markers and their segregation data in 228 selfed progenies of 'Zebra' common bermudagrass.

SSR marker information		Chi-square testing in 228 selfed progenies							
		Observed progeny bands				For 1:2:1 ratio (disomic)		For 1:34:1 ratio (tetrasomic)	
No.	Primer pair ID	AA	Aa	aa	Missing	χ^2	*P*	χ^2	*P*

1	CDCA1-21/22	16	183	28	1	86.38	<0.01	94.28	<0.01
2	CDCA2-231/232	39	111	77	1	12.38	<0.01	1013.01	<0.01
3	CDCA3-247/248	50	147	28	3	25.46	<0.01	402.13	<0.01
4	CDCA3-313/314	10	132	86	0	56.35	<0.01	1042.73	<0.01
5	CDCA4-323/324	16	156	47	9	48.27	<0.01	309.40	<0.01
6	CDCA7-653/654	39	110	77	2	12.94	<0.01	1013.24	<0.01
7	CDCA7-693/694	13	120	93	2	57.50	<0.01	1241.16	<0.01
8	CDCA8-737/738	31	146	47	4	22.93	<0.01	388.03	<0.01
9	CDGA1-847/848	37	135	56	0	10.90	<0.01	571.69	<0.01
10	CDGA1-921/922	52	100	73	3	6.70	0.04	1107.34	<0.01
11	CDGA1-929/930	8	161	52	7	63.68	<0.01	356.91	<0.01
12	CDGA3-1103/1104	10	173	42	3	74.17	<0.01	214.08	<0.01
13	CDGA4-1245/1246	36	184	7	1	94.99	<0.01	144.40	<0.01
14	CDGA5-1427/1428	60	153	15	0	44.45	<0.01	487.82	<0.01
15	CDGA7-1611/1612	0	172	56	0	86.53	<0.01	407.12	<0.01
16	CDGA8-1765/1766	48	117	61	2	1.78	0.41	794.50	<0.01
17	CDGA8-1807/1808	44	117	61	6	3.25	0.19	755.73	<0.01
18	CDATG6-2123/2124	9	145	71	3	52.95	<0.01	693.46	<0.01
19	CDATG6-2143/2144	57	154	15	2	45.36	<0.01	436.56	<0.01

20	CDAAC7-2703/2704	37	161	28	2	41.50	<0.01	237.21	<0.01
21	CDCAG3-2897/2898	1	174	51	2	87.98	<0.01	328.89	<0.01

Table 3. Counts of observed and expected phenotypes amplified with SSR CDAAC7-2693/2694 in 272 S1 progenies of A12359 common bermudagrass under tetrasomic and disomic inheritance.

Observed		Expected genotypes and phenotypes of 272 progenies if			
		Parent genotype *ABCD* at one locus under tetrasomic inheritance		Parent genotype of two independent loci *AB* & *CD* under disomic inheritance§	
Phenotypes	Counts	Genotypes	Counts	Genotypes	Counts
ab	0	*AABB*	7.5	*AABB*	0
abc	3	*AABC, ABBC, ABCC*	45.4	*ABCC*	34
abd	45	*AABD, ABDD, ABDD*	45.4	*ABDD*	34
abcd	77	*ABCD*	45.4	*ABCD*	68
ac	9	*AACC*	7.5	*AACC*	17
acd	69	*AACD, ACCD, ACDD*	45.4	*AACD*	34
ad	26	*AADD*	7.5	*AADD*	17
bc	2	*BBCC*	7.5	*BBCC*	17

bcd	21	*BBCD, BCCD, BCDD*	45.4	*BBCD*	34
bd	20	*BBDD*	7.5	*BBDD*	17
cd	0	*CCDD*	7.5	*CCDD*	0

§: AB and CD were considered respective genotypes at two separate loci based on the appearance of "ac", "ad", "bc" and "bd" phenotypes in the progeny population.

III. Results

1. Selection of polymorphic SSR markers and verification of selfed progeny

Fifty-one common bermudagrass SSR PPs were initially screened for polymorphism in the subset of Zebra population, resulting in selection of 21 SSRs for further genotyping work (Table 2). Each of the 21 selected SSR primer pairs produced polymorphic, heritable bands, while the other 30 amplified single bands or unclear bands and were discarded. Similarly, 12 polymorphic SSRs were selected to genotype the A12359 S1 population (Table 1 and Table 4). All 228 progeny plants derived from Zebra were selfed progenies while 273 progeny plants derived from A12359 were selfed as identified using the respective polymorphic SSR markers. Hence, the selfed plants formed two separate S1 populations, one from Zebra and the other from A12359 used in subsequent analyses.

Table 4. Twelve SSR markers and their segregation in 273 selfed progenies of parent A12359 common bermudagrass.

SSR marker		Chi-square testing in 273 selfed progenies							
		Progeny genotypes				For 1:2:1 (disomic)		For 1:34:1 (tetrasomic)	
No.	Primer pair ID	AA	Aa	aa	Missing	χ^2	P	χ^2	P
1	CDCA5-491/492	67	126	73	7	1.01	0.6	1106.32	<0.01
2	CDCA7-623/624	60	154	54	5	6.24	0.04	694.54	<0.01
3	CDGA1-783/784	57	152	62	2	4.2	0.12	764.98	<0.01
4	CDGA3-1195/1196	62	125	70	16	0.69	0.71	1021.85	<0.01
5	CDGA6-1583/1584	47	152	65	9	8.51	0.01	686.65	<0.01
6	CDGA7-1601/1602	4	195	66	8	87.97	<0.01	470.03	<0.01
7	CDGA8-1795/1796	70	143	58	2	1.89	0.39	910.75	<0.01
8	CDATG1-1889/1890	66	117	83	7	6.02	0.05	1287.87	<0.01
9	CDATG3-1999/2000	59	145	59	10	2.77	0.25	750.05	<0.01
10	CDAAC5-2523/2524	66	139	67	1	0.14	0.93	982.51	<0.01
11	CDAAC7-2675/2676	67	141	59	6	1.32	0.52	874.56	<0.01
12	CDAAC7-2693/2694 (1)	103	117	43	10	30.57	<0.01	1453.26	<0.01

CDAAC7-2693/2694 (2)	14	168	90	1	57.53	<0.01	943.95	<0.01

2. Genotyping of selected SSR markers

The Zebra S1 progenies were genotyped with 21 selected polymorphic SSRs (Table 2), and all of them consistently amplified two segregating bands (Figure 1 and Table 2). This result suggests the genotypes of Zebra are likely *Aa* following disomic inheritance, which was based on the assumption if each SSR only amplified one locus in one subgenome, rather than in both subgenomes. Another assumption for an SSR with two segregating bands was that the genotype of Zebra was either of *Aaaa, AAaa* or *AAAa* under tetrasomic inheritance, which was derived from amplifying one locus by one SSR. Three or four segregating bands per SSR locus were not observed although they were expected in some SSR loci in tetrasomic inheritance. Among the 12 polymorphic SSR markers genotyped in the A12359 S1 population, 11 produced two segregating bands while one (CDAAC7-2693/2694) amplified four segregating bands (Figure 2 and Table 4). Under disomic inheritance, the four bands of A12359 would represent two independent loci (i.e., *AB* genotype at one locus, and *CD* at the other), while the genotype would be *ABCD* at one locus under tetrasomic inheritance (Table 5 and Table 6).

Figure 1. A gel image of 'Zebra' common bermudagrass and its 60 progenies amplified with SSR CDGA8-1765/1766. The band pattern of Zebra (P) was coded as "Aa", and a progeny band pattern coded as "AA" if one upper band, "Aa" if two bands, and "aa" if one lower band. A missing lane was labeled as 'M'. Standard size markers were loaded on first and last lanes.

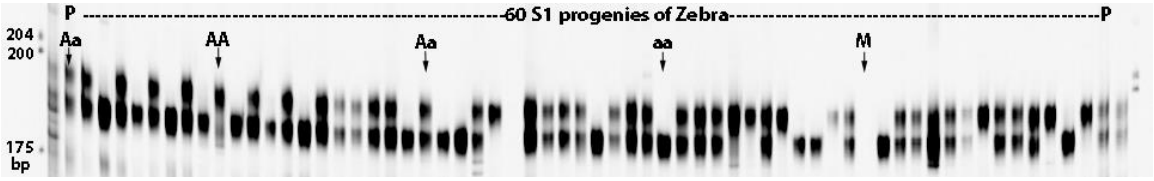


Figure 2. A gel image of A12359 and its 60 progenies amplified with an SSR CDAAC7-2693/2694. Four bands of A12359 segregated in two pairs labeled on the right side of the gel, "Pair 1" for bands in the range of 300 and 325 bp, and "Pair 2" for the other pair smaller than 300 bp. P represents the parent A12359 common bermudagrass. Size markers are labeled for the first lane on the left side.

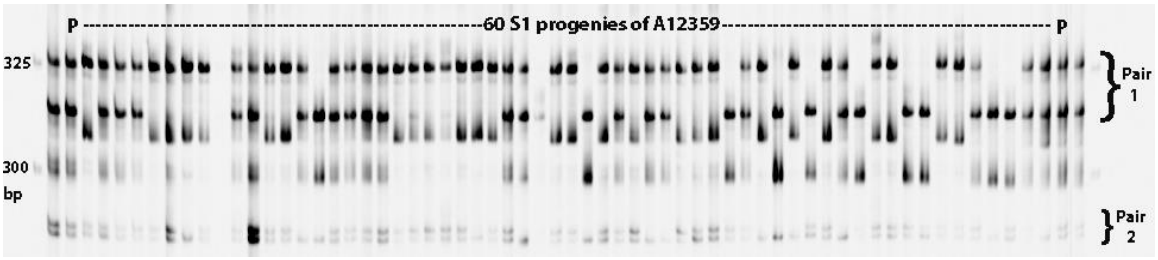


Table 5. Possible genotypes of gametes and zygotes under tetrasomic inheritance if the parental genotype is *ABCD* at one locus.

	AB	*AC*	*AD*	*BC*	*BD*	*CD*
AB	*AABB*	*AABC*	*AABD*	*ABBC*	*ABBD*	*ABCD*
AC	*AABC*	*AACC*	*AACD*	*ABCC*	*ABCD*	*ACCD*
AD	*AABD*	*AACD*	*AADD*	*ABCD*	*ABDD*	*ACDD*
BC	*ABBC*	*ABCC*	*ABCD*	*BBCC*	*BBCD*	*BCCD*
BD	*ABBD*	*ABCD*	*ABDD*	*BBCD*	*BBDD*	*BCDD*
CD	*ABCD*	*ACCD*	*ACDD*	*BCCD*	*BCDD*	*CCDD*

Table 6. Possible genotypes of gametes and zygotes under disomic inheritance if the parental genotype is *AB* & *CD* at two independent loci

	AC	*AD*	*BC*	*BD*
AC	*AACC*	*AACD*	*ABCC*	*ABCD*
AD	*AACD*	*AADD*	*ABCD*	*ABDD*
BC	*ABCC*	*ABCD*	*BBCC*	*BBCD*
BD	*ABCD*	*ABDD*	*BBCD*	*BBDD*

3. Segregation analysis

In the Zebra S1 population, chi-square tests indicated that the segregation of two SSRs (No. 16 and 17) was consistent with the 1:2:1 Mendelian segregation ratio (P=0.41 and 0.19) for disomic inheritance (Table 2). One SSR (No. 15) produced a segregation pattern following a 3Aa: 1aa (P=0.88) that was expected for the segregation of an *Aaaa* genotype under tetrasomic inheritance. The segregation patterns of the 18 other SSRs were much closer to the 1:2:1 for disomic inheritance ratio than to 1:34:1 for tetrasomic inheritance based on P values in chi-square testing, although their patterns did not fit either of them at $p<0.001$ (Table 2).

For the A12359 S1 population, all 12 polymorphic SSRs except CDAAC7-2693/2694 amplified two bands in the parent, and segregated in its selfed progenies (Table 4). Segregation of eight SSRs in the population followed the 1:2:1 disomic segregation ratio ($P\geq0.05$) and two SSRs had a segregation ratio close to 1:2:1 ($0.05>P\geq0.01$), while the segregation of CDGA7-1601/1602

deviated significantly from the disomic ratio (P<0.01). It is worth noting that none of these 11 SSR markers exhibited segregation approaching the 1:34:1 theoretical ratio (Table 4) for selfing a tetrasomic *AAaa* genotype.

IV. Discussion

Under disomic inheritance, there would be possibilities that one SSR amplified one locus on subgenome *A* and another locus on subgenome *B*, resulting in two bands for the parent if the genotypes of the parent were *AA/Bb*, *AA/bb*, *Aa/BB*, *Aa*/*Bb*, *Aa*/*bb*, *aa*/*BB*, or *aa*/*Bb* (Burnham, 1962) and if both A and B alleles have the same band size (i.e., A=B) and both a and b alleles have the same band size (a=b) (Table 7). The expected segregation ratios in S1 progenies would be 1 upper band: 3 double bands for *AA/Bb* and *Aa/BB*; all double bands (i.e., no segregation) for *AA/bb* and *aa*/*BB*; 1 upper band: 14 double bands: 1 lower band for *Aa*/*Bb*; 3 double bands: 1 lower band for *Aa*/*bb* and *aa*/*Bb*, respectively. The observed segregation patterns in the two populations (Tables 2 and 4) were not consistent with the expected ratios of tetrasomic inheritance except 3:1. In addition, the theoretical frequency of the 3:1 ratio would be 4 of 7 (58%), but we only observed 1 out of 33 (3%) SSRs in the two populations. Therefore, there was no possibility that one locus was amplified in each subgenome, and band size of each allele was the same for any observed SSRs in our common bermudagrass populations.

Table 7. Genotypes of gametes and zygotes for possible parental genotypes under disomic inheritance if one SSR primer pair simultaneously amplifies one locus in both subgenomes

Parental genotype: *AA/Bb*

	AB	*Ab*
AB	*AABB*	*AABb*
Ab	*AABb*	*AAbb*

Parental genotype: *AA/bb*

	Ab
Ab	*AAbb*

Parental genotype: *Aa/BB*

	AB	*aB*
AB	*AABB*	*AaBB*
aB	*AaBB*	*aaBB*

Parental genotype: *Aa/Bb*

	AB	*Ab*	*aB*	*ab*
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AABb*	*AAbb*	*AaBb*	*Aabb*
aB	*AaBB*	*AaBb*	*aaBB*	*aaBb*
ab	*AaBb*	*Aabb*	*aaBb*	*aabb*

Parental genotype: *Aa/bb*

	Ab	*ab*
Ab	*AAbb*	*Aabb*
ab	*Aabb*	*aabb*

Parental genotype: *aa/BB*

	aB
aB	*aaBB*

Parental genotype: aa/Bb

	aB	*ab*
aB	*aaBB*	*aaBb*
ab	*aaBb*	*aabb*

Interestingly, the SSR CDAAC7-2693/2694 amplified four bands, scored as "abcd" in A12359 and segregated in the S1 population (Figure 2 and Table 3). Under tetrasomic inheritance, a parent with the "abcd" band pattern would have *ABCD* genotype and produce 19 genotypes resulting in 11 phenotypes in its progenies (Table 3). In contrast, under disomic inheritance, two independent loci of *AB* at one locus and *CD* at the other would produce an S1 population of nine phenotypes (Table 3). The nine phenotypes observed in the A12359 S1 population were consistent with that of expected phenotypes if the two loci segregated independently in disomic

inheritance, and the other two phenotypes with band patterns “ab” and “cd” which were expected under tetrasomic inheritance were not observed in the S1 progenies for CDAAC7-2693/2694. Using the pooled data in Table 3, Chi-square testing indicated that the segregation of alleles of the SSR in A12359 S1 population significantly differed from tetrasomic inheritance (χ^2=172.8, P<0.0001) and also deviated from disomic inheritance (χ^2=96.3, P<0.0001). Further chi-square testing for segregation data at each of the two loci revealed that neither followed the typical 1:2:1 ratio (Table 4).

The inheritance mode of two tetraploid common bermudagrasses was analyzed with 33 SSRs amplifying 34 loci in two S1 populations. Transmission of two target bands at each locus and segregation ratios are consistent with preferential pairing at 33 loci exhibiting disomic inheritance. The segregation pattern of two alleles at the locus amplified by SSR CDGA3-1611/1612 was not different from a 3Aa: 1aa ratio, suggesting either disomic or tetrasomic inheritance. For tetrasomic inheritance, the cytological observations in the past indicated that four chromosomes pairing formed one or two quadrivalents in some genotypes of common bermudagrass (Forbes and Burton, 1963; Harlan and de Wet, 1969; Hanna and Burton, 1977). However, the 3Aa: 1aa segregation could be a result of segregation ratio distortion if all homozygous “*AA*” plants were dead.

Of the 33 SSRs tested in the two S1 populations, only one amplified four alleles, which segregated at two separate loci. The results indicated substantial subgenome differentiation in common bermudagrass, revealing common bermudagrass is an allotetraploid. However, the finding was not expected as it is opposite to the previously proposed hypothesis that common bermudagrass was an autotetraploid (Harlan and de Wet, 1969; Harlan et al., 1969). Harlan and de Wet (1969) indicated that diploid *C. dactylon* var. *aridus* Harlan et de Wet (2n=2x=18) bermudagrass was the only likely source of tetraploid common bermudagrass. Var. *aridus* is the

only diploid bermudagrass which has rhizomes and is morphologically similar to tetraploid common bermudagrass. It is warranted to confirm this finding by genotyping a larger number of codominant markers (i.e., SSRs) that cover the whole genome in the cosmopolitan common bermudagrass.

Segregation distortions were evident in the two S1 populations. Nineteen of 21 (90%) loci in the Zebra S1 population and four of 13 (31%) loci in the A12359 population had distorted segregation ratios. Segregation distortion is common in allogamous species, especially when they are selfed. Liu et al. reported segregation ratio distortion was identified for approximately 19% of loci in an S1 population of switchgrass (*Panicum virgatum* L.) (Liu et al., 2012), while Okada et al. (2010) observed segregation ratio distortion for 14% of loci in a hybrid population in the species. Selfed populations tend to have a higher segregation ratio distortion because of inbreeding depression (Charlesworth and Willis, 2009; Andru et al., 2011). Segregation ratio distortion occurs when a molecular marker locus links to a distorter, such as recessive lethal genes (Lyttle, 1991). When the recessive lethal genes become homozygous under selfing or inbreeding, the plant is weak or dead.

Segregation distortion can also cause spurious linkages and biased recombination fractions leading to inaccurate genetic distances between markers and incorrect marker order of linkage maps (Liu et al., 2012). Subsequently, quantitative trait locus mapping is negatively affected if an inaccurate linkage map is used. One strategy commonly employed in construction of linkage maps is to use regularly segregated markers to establish a frame map before attaching segregation distorted markers (Okada et al., 2010; Liu et al., 2012; Liu et al., 2013). Another strategy is to use a population which has fewer segregation distortions for linkage mapping (in this case using A12359 S1 population rather than the Zebra S1 population).

V. Conclusions

In summary, we report two common bermudagrass populations exhibiting complete or near complete disomic inheritance and provide evidence supporting two distinct subgenomes constituting an allotetraploid genome in two tetraploid genotypes (Zebra and A12359). Severe to moderate segregation distortion occurred in the two S1 populations. The findings add to the knowledge pool of genetics and will benefit genetic map construction, quantitative trait locus mapping and breeding efforts in the taxon, *C. dactylon* var. *dactylon*.

CHAPTER III

EXPERIMENT 2. A LINKAGE MAP OF COMMON BERMUDAGRASS [*CYNODON DACTYLON* (L.) PERS.] BASED ON SIMPLE SEQUENCE REPEAT MARKERS

I. Introduction

Bermudagrass (*Cynodon* spp.), a type of warm-season, sod forming and perennial grasses, is widely used for recreational turf, livestock feed and soil stabilization (Taliaferro, 1995). *Cynodon* Rich. is taxonomically classified into nine species, and among them, common bermudagrass (*C. dactylon* Pers.) is the most important because of its wide distribution, enormous variability, and extensive use in the world (Taliaferro, 1995). *C. dactylon* was first introduced into Americas during colonial times from Africa or India (Kim et al., 2008). Ploidy levels of bermudagrass range from diploid (2n=2x=18 somatic chromosomes) to hexaploid (2n=2x=54) (Harlan et al., 1970c; Forbes and Burton, 1963), and tetraploidy (2n=4x=36) is the prevalent cytological form (Wu and Anderson, 2011).

Simple sequence repeats (SSRs) or microsatellites are tandemly repeated sequences with 1 to 6 nucleotides long for one repeating unit and variable repeating times. SSRs have been extensively used for identifying inheritance pattern, linkage analysis and genetic map construction in multiple crops like sorghum (*Sorghum bicolor* L.), wheat (*Triticum aestivum* L.), and pear (*Pyrus* spp.) as

they are co-dominant, multi-allelic, based on polymerase chain reaction (PCR) (Diwan et al., 2000; Wu and Huang, 2006; Peleg et al., 2008; Wu et al., 2014). 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, simple sequence repeat markers are useful for developing genetic maps in common bermudagrass.

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 (i.e., quantitative trait locus (QTL) mapping) and to indirectly select economically important traits (i.e., marker assisted selection). High density genetic maps are essential tools for reference whole genome sequence projects, and they will make subsequent marker assisted selection and breeding more efficient (Koning-Boucoiran, 2012; Liu and Wu, 2012). Linkage maps developed from different populations or resources are necessary to understand genome information and species inheritance (Semagn et al., 2010).

However, limited efforts have been made to construct genetic maps in common bermudagrass. Using a cross population between a tetraploid common bermudagrass and a diploid African bermudagrass (*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 constitutes 973.4 cM with 77 markers. The map of common bermudagrass is estimated to cover about 60% of its whole genome (Bethel et al., 2006). Harris-Shultz et al. (2010) mapped 35 alleles of SSR markers onto the map of Bethel et al. (2006). But the latter map spans just 1055 cM (Harris-Shultz et al., 2010). They also reported a revised linkage map of diploid African bermudagrass covering 311.1 cM with 36 SDAFs including four SSR markers. 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, which is rarely used now. We have developed a first-generation inbred population from a tetraploid common bermudagrass (Guo et al., 2015) as described in last chapter. The common bermudagrass exhibited disomic inheritance. Accordingly, the objective of this experiment was to construct a high density map for common bermudagrass based on SSR markers.

II. Materials and Methods

1. Plant materials

A first-generation selfed progeny population from one common bermudagrass genotype A12359 (2n=4x=36) was used in this experiment. This population was thoroughly described in the previous chapter. Within the population, 130 selfed progenies of A12359 were randomly selected and used for SSR marker genotyping, and subsequent segregation analysis and genetic map construction.

2. DNA isolation and PCR protocol

Genomic DNA of A12359 and its progeny plants was extracted following the CTAB method of Doyle with minor modifications (Doyle, 1990). The DNA concentration of each sample was quantified using a NanoDrop ND-1000 spectrophotometer (Nanodrop Products, DE, USA), and each DNA sample was diluted to a concentration of 10 ng/µl which was prepared for polymerase chain reaction (PCR). Then PCR was used to amplify SSR markers in Biosystems 2720 Thermal Cyclers (Applied Biosystems Inc., CA, USA) with the reaction conditions as described in the previous chapter. The PCR products were separated on a 4300 LI-COR DNA Analyzer (LI-COR Biosciences, Lincoln, NE) for collecting genotypic data of SSR markers as described in chapter II.

3. SSR markers and genotyping analysis

A total of 810 SSR primer pairs developed from small-insert genomic DNA libraries in our lab were screened for polymorphism using a small panel consisting of two replicates of A12359 and six progeny samples. Polymorphic markers were tested to amplify one DNA plate with 64 samples (two replicates of parent 118 and 62 progenies), and those producing stable and heritable bands were used to genotype the other DNA plate encompassing two parent 118 replicates and another 62 progeny samples.

SSR allelic data for each polymorphic primer pair 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 one locus 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 two bands amplified it was scored as "hk"; missing genotypes were encoded as "--". As for those SSR PPs producing stable but segregating four bands with one SSR PP, termed multi-allele markers, two sets of loci were recorded and analyzed respectively on the basis of their distinguishing allele sizes and segregation patterns (Liu and Wu, 2012).

4. Data analysis and linkage map construction

Chi-square test was performed to examine the Mendelian segregation ratio (i.e.1: 2: 1) using Joinmap 4.0 (Van Ooijen, 2006). Markers distorted from the expected segregation ratio were marked as *, **, and *** 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 pollinators" (CP) was used as type code for the selfed population (Van Ooijen,

2006). According to Van Onijen (2006), 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 linkage groups (LGs) than the actual number, and then LOD value with reduced stringency was decreased to re-group 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 rec. freq. estimates are larger than 0.6; Number of maximum linages 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 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 re-group the map, which allowed us to link two independent linkage groups if one marker on one group could show a cross linkage with another marker on the other linkage group (Liu et al., 2012). Approximate genetic length of the common bermudagrass genome was estimated using the method of Hulbert et al. (1988), and genome coverage of this linkage map was calculated using the formula used by Okada et al. (2010).

5. Comparative mapping

Reverse Complement (http://www.bioinformatics.org/sms/rev_comp.html) was used for converting the reverse primer sequence into its reverse-complement counterpart, along with the forward primer sequence to find the query sequence from our common bermudagrass sequence

data source. Phytozome v10.3 (http://phytozome.jgi.doe.gov/pz/portal.html#!search) was used for comparative genomic analysis , and the Basic Local Alignment Search Tool for Nucleotide (BLASTN) program was used to search corresponding locations for mapped markers of common bermudagrass in chromosomes of sorghum and foxtail millet based on sequence similarity. For example, *Sorghum bicolor* or *Setaria italic* was selected as target species, then a specific common bermudagrass sequence was entered in BLASTN program as a searching query, and parameters were set as: 10 for Expect (E) threshold, BLOSUM62 for Comparison matrix, default for Word (W) length, 100 for # of alignments to show, Yes for Allow gaps and Yes for Filter query (Liu et al., 2012).

III. Results

1. 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 SSR PPS 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 of one locus each. A gel image of the parent (A12359) and 62 S1 progeny amplified with one SSR PP amplifying two alleles is given in Figure 3.

Two hundred fifty loci were assigned to 18 LGs that constituted a genetic map for the common bermudagrass plant A12359 while 14 loci were unlinked (Figure 4). The total length of the map was 1094.7 cM, and two adjacent markers average distance was 4.3 cM. The largest linkage group (LG 18) and shortest linkage group (LG 6) was 122.3 cM and 12.7 cM in length and contained 38 and 4 loci, respectively (Table 8). The average linkage group length was 60.8 cM, and mean number of loci each group was 14. A total of 39 gaps (greater than 10 cM between two

adjacent loci) were found in the map. Except LGs 1 and 2, the number of gaps in each LG ranged from 1 to 5.

Figure 3. 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 "--".

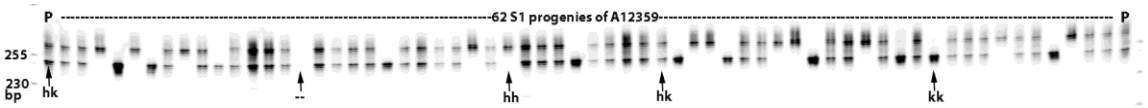
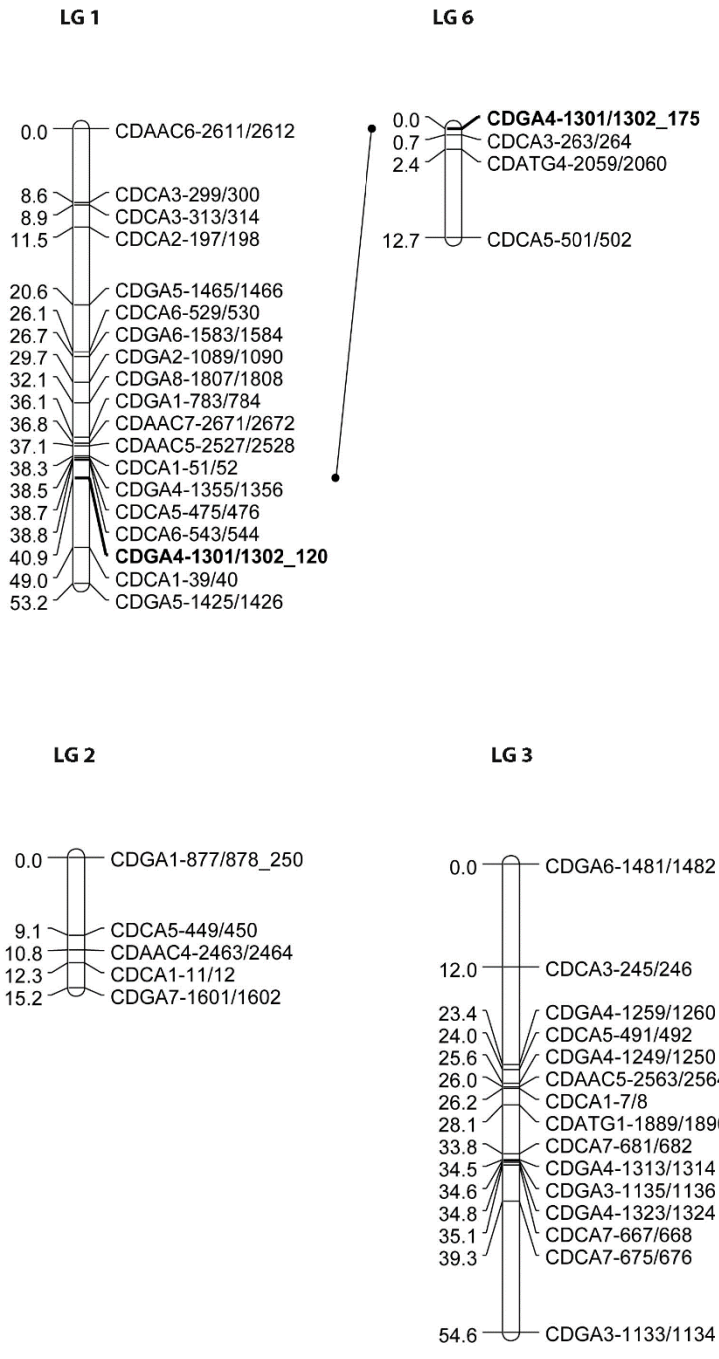
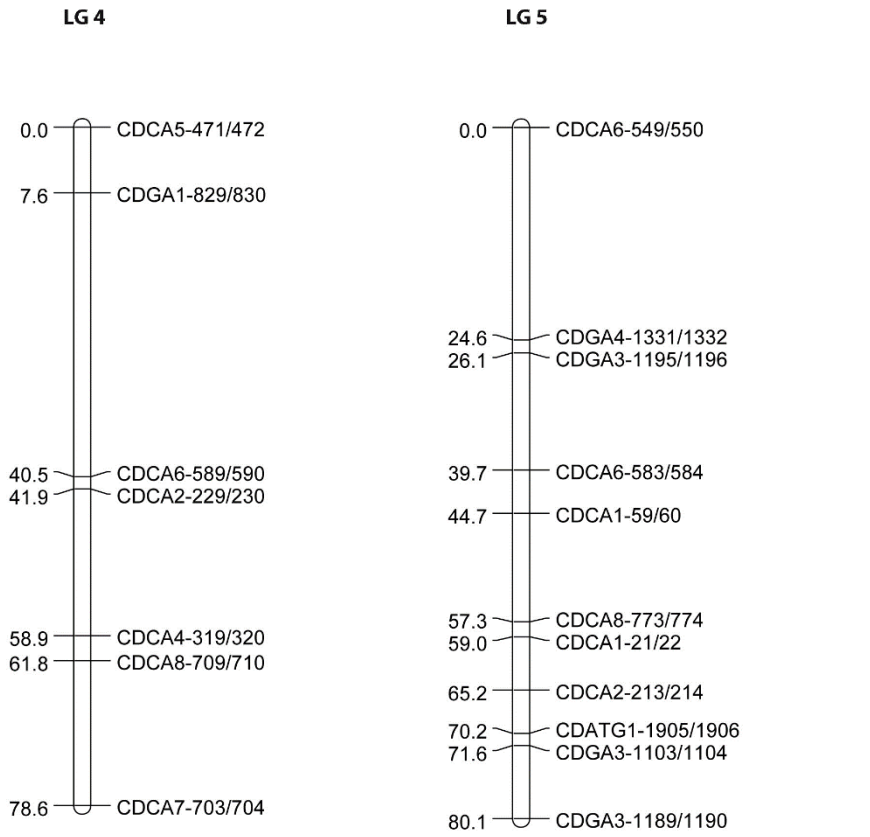
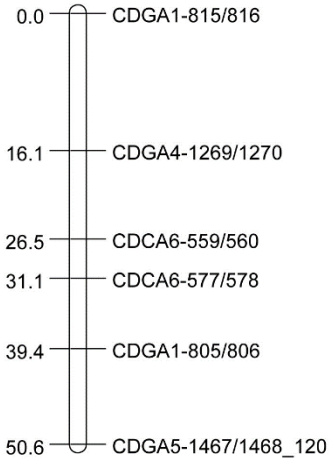


Figure 4. A linkage map developed from 130 selfed progenies of common bermudagrass A12359. The left side of each linkage group showed map distance (cM), and right side presented SSR names. Homeologous groups were determined by multi-allele markers (in bold) and connected by solid lines.


LG 1
0.0 CDAAC6-2611/2612
8.6 CDCA3-299/300
8.9 CDCA3-313/314
11.5 CDCA2-197/198
20.6 CDGA5-1465/1466
26.1 CDCA6-529/530
26.7 CDGA6-1583/1584
29.7 CDGA2-1089/1090
32.1 CDGA8-1807/1808
36.1 CDGA1-783/784
36.8 CDAAC7-2671/2672
37.1 CDAAC5-2527/2528
38.3 CDCA1-51/52
38.5 CDGA4-1355/1356
38.7 CDCA5-475/476
38.8 CDCA6-543/544
40.9 CDGA4-1301/1302_120
49.0 CDCA1-39/40
53.2 CDGA5-1425/1426
LG 6
0.0 CDGA4-1301/1302_175
0.7 CDCA3-263/264
2.4 CDATG4-2059/2060
12.7 CDCA5-501/502
LG 2
0.0 CDGA1-877/878_250
9.1 CDCA5-449/450
10.8 CDAAC4-2463/2464
12.3 CDCA1-11/12
15.2 CDGA7-1601/1602
LG 3
0.0 CDGA6-1481/1482
12.0 CDCA3-245/246
23.4 CDGA4-1259/1260
24.0 CDCA5-491/492
25.6 CDGA4-1249/1250
26.0 CDAAC5-2563/2564
26.2 CDCA1-7/8
28.1 CDATG1-1889/1890
33.8 CDCA7-681/682
34.5 CDGA4-1313/1314
34.6 CDGA3-1135/1136
34.8 CDGA4-1323/1324
35.1 CDCA7-667/668
39.3 CDCA7-675/676
54.6 CDGA3-1133/1134


LG 4
0.0 CDCA5-471/472
7.6 CDGA1-829/830
40.5 CDCA6-589/590
41.9 CDCA2-229/230
58.9 CDCA4-319/320
61.8 CDCA8-709/710
78.6 CDCA7-703/704
LG 5
0.0 CDCA6-549/550
24.6 CDGA4-1331/1332
26.1 CDGA3-1195/1196
39.7 CDCA6-583/584
44.7 CDCA1-59/60
57.3 CDCA8-773/774
59.0 CDCA1-21/22
65.2 CDCA2-213/214
70.2 CDATG1-1905/1906
71.6 CDGA3-1103/1104
80.1 CDGA3-1189/1190
LG 7
0.0 CDCA2-181/182
0.6 CDCA8-725/726
12.1 CDCA5-465/466
12.5 CDCA5-469/470_230
13.3 CDCA7-671/672
15.0 CDGA2-991/992
15.1 CDGA5-1455/1456_180 CDGA1-785/786
15.8 CDGA3-1119/1120
16.8 CDCA5-505/506
17.3 CDCA5-503/504
19.4 CDGA4-1295/1296
20.7 CDGA1-791/792
21.6 CDCA5-409/410
22.5 CDGA2-1027/1028
24.9 CDGA1-787/788
26.2 CDGA8-1795/1796
28.5 CDGA2-965/966
33.0 CDCA7-623/624
33.8 CDCA1-115/116
34.2 CDCA7-695/696
39.9 CDCA6-565/566
41.6 CDATG3-1999/2000
43.7 CDGA4-1307/1308
46.9 CDGA5-1399/1400
48.7 CDCA3-237/238
LG 8
0.0 CDGA2-1037/1038
2.3 CDGA4-1335/1336
4.9 CDAAC3-2439/2440
16.2 CDCA6-563/564
16.6 CDCA3-269/270
16.9 CDCA8-717/718
18.1 CDGA5-1439/1440
18.8 CDAAC7-2675/2676
27.6 CDCA5-469/470_260
31.2 CDGA1-921/922
32.2 CDGA5-1455/1456_150
35.5 CDGA2-1011/1012
37.3 CDCA6-571/572
40.9 CDGA1-865/866

LG 9



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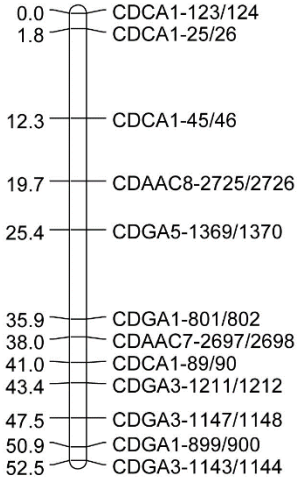

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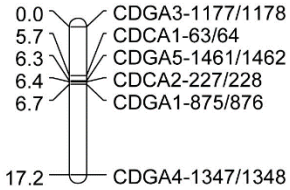
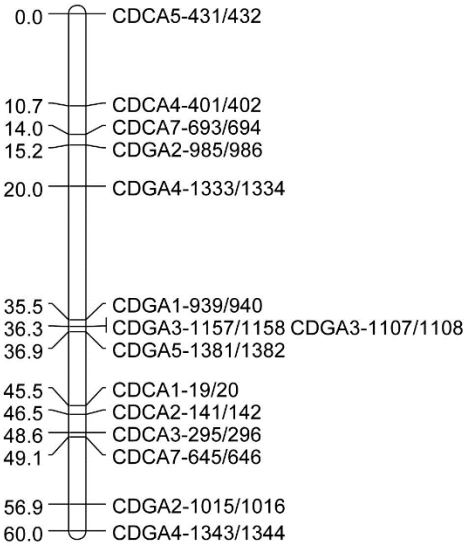
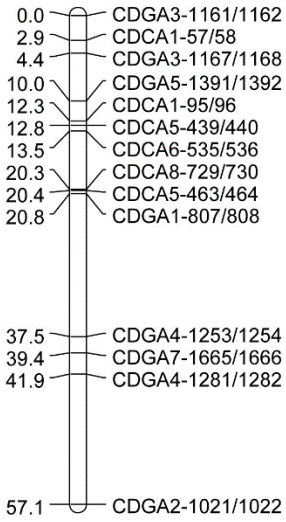


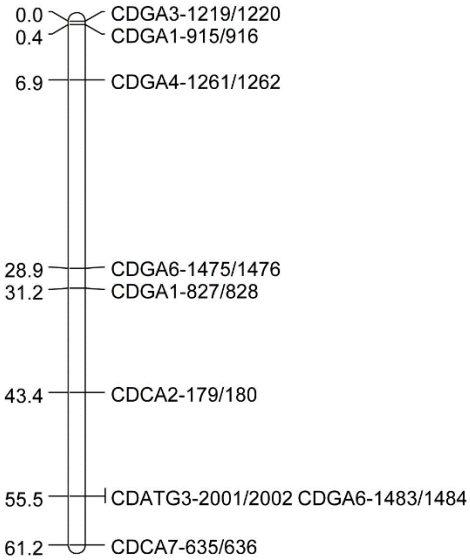
LG 12

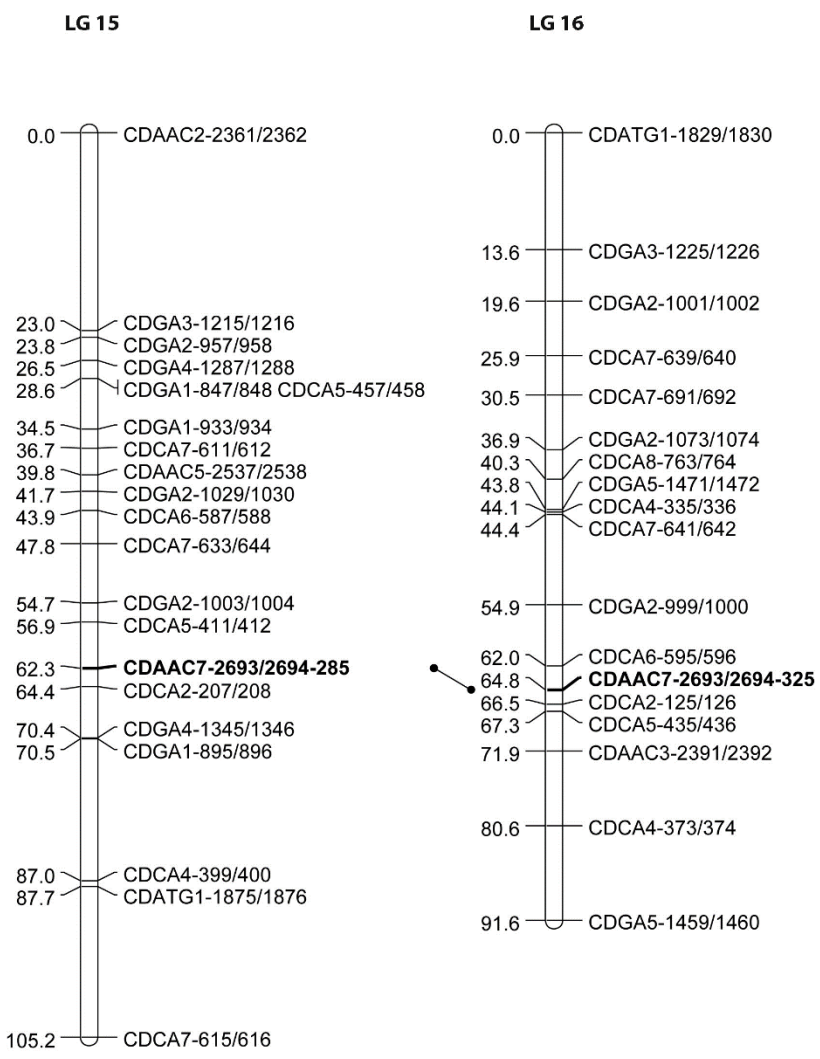


LG 13


0.0 CDGA3-1161/1162
2.9 CDCA1-57/58
4.4 CDGA3-1167/1168
10.0 CDGA5-1391/1392
12.3 CDCA1-95/96
12.8 CDCA5-439/440
13.5 CDCA6-535/536
20.3 CDCA8-729/730
20.4 CDCA5-463/464
20.8 CDGA1-807/808
37.5 CDGA4-1253/1254
39.4 CDGA7-1665/1666
41.9 CDGA4-1281/1282
57.1 CDGA2-1021/1022

LG 14


0.0 CDGA3-1219/1220
0.4 CDGA1-915/916
6.9 CDGA4-1261/1262
28.9 CDGA6-1475/1476
31.2 CDGA1-827/828
43.4 CDCA2-179/180
55.5 CDATG3-2001/2002 CDGA6-1483/1484
61.2 CDCA7-635/636


LG 15
0.0 CDAAC2-2361/2362
23.0 CDGA3-1215/1216
23.8 CDGA2-957/958
26.5 CDGA4-1287/1288
28.6 CDGA1-847/848 CDCA5-457/458
34.5 CDGA1-933/934
36.7 CDCA7-611/612
39.8 CDAAC5-2537/2538
41.7 CDGA2-1029/1030
43.9 CDCA6-587/588
47.8 CDCA7-633/644
54.7 CDGA2-1003/1004
56.9 CDCA5-411/412
62.3 CDAAC7-2693/2694-285
64.4 CDCA2-207/208
70.4 CDGA4-1345/1346
70.5 CDGA1-895/896
87.0 CDCA4-399/400
87.7 CDATG1-1875/1876
105.2 CDCA7-615/616
LG 16
0.0 CDATG1-1829/1830
13.6 CDGA3-1225/1226
19.6 CDGA2-1001/1002
25.9 CDCA7-639/640
30.5 CDCA7-691/692
36.9 CDGA2-1073/1074
40.3 CDCA8-763/764
43.8 CDGA5-1471/1472
44.1 CDCA4-335/336
44.4 CDCA7-641/642
54.9 CDGA2-999/1000
62.0 CDCA6-595/596
64.8 CDAAC7-2693/2694-325
66.5 CDCA2-125/126
67.3 CDCA5-435/436
71.9 CDAAC3-2391/2392
80.6 CDCA4-373/374
91.6 CDGA5-1459/1460

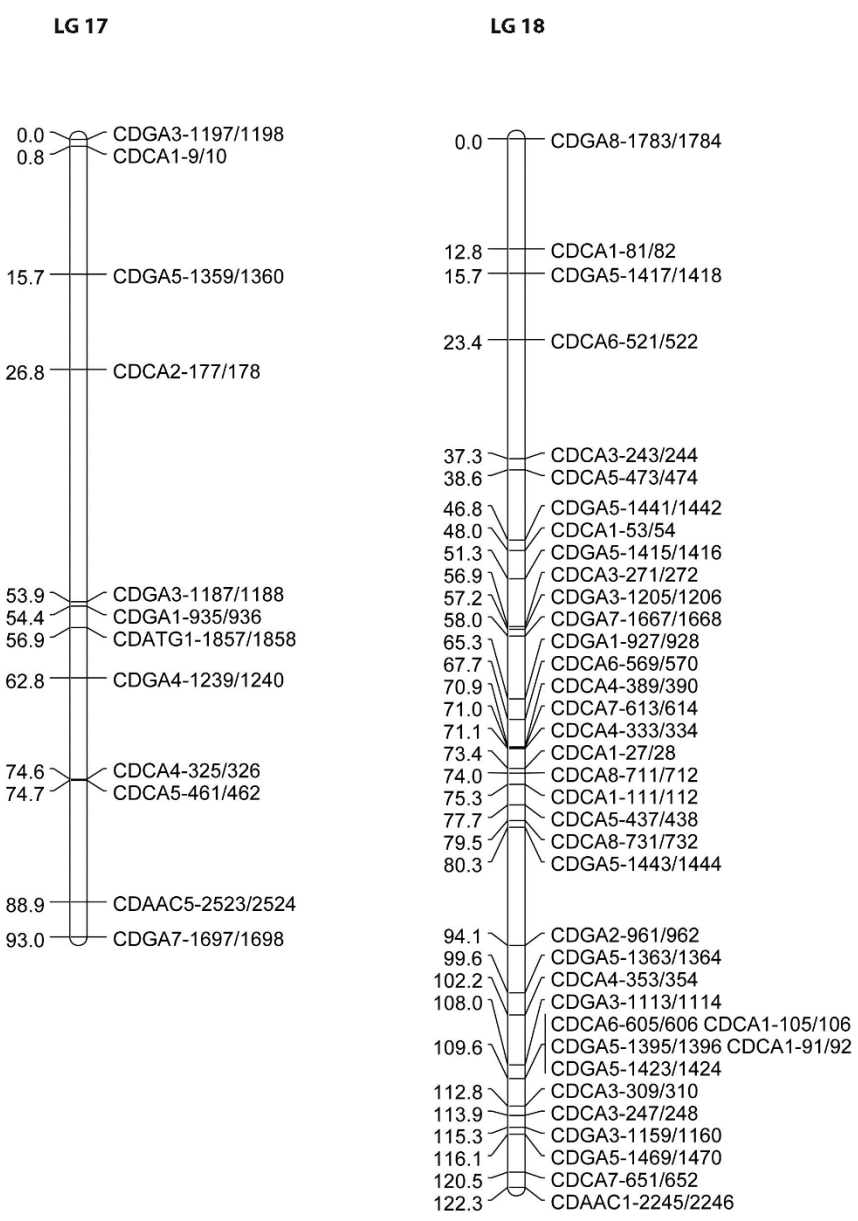

LG 17
0.0 CDGA3-1197/1198
0.8 CDCA1-9/10
15.7 CDGA5-1359/1360
26.8 CDCA2-177/178
53.9 CDGA3-1187/1188
54.4 CDGA1-935/936
56.9 CDATG1-1857/1858
62.8 CDGA4-1239/1240
74.6 CDCA4-325/326
74.7 CDCA5-461/462
88.9 CDAAC5-2523/2524
93.0 CDGA7-1697/1698
LG 18
0.0 CDGA8-1783/1784
12.8 CDCA1-81/82
15.7 CDGA5-1417/1418
23.4 CDCA6-521/522
37.3 CDCA3-243/244
38.6 CDCA5-473/474
46.8 CDGA5-1441/1442
48.0 CDCA1-53/54
51.3 CDGA5-1415/1416
56.9 CDCA3-271/272
57.2 CDGA3-1205/1206
58.0 CDGA7-1667/1668
65.3 CDGA1-927/928
67.7 CDCA6-569/570
70.9 CDCA4-389/390
71.0 CDCA7-613/614
71.1 CDCA4-333/334
73.4 CDCA1-27/28
74.0 CDCA8-711/712
75.3 CDCA1-111/112
77.7 CDCA5-437/438
79.5 CDCA8-731/732
80.3 CDGA5-1443/1444
94.1 CDGA2-961/962
99.6 CDGA5-1363/1364
102.2 CDCA4-353/354
108.0 CDGA3-1113/1114
CDCA6-605/606 CDCA1-105/106
109.6 CDGA5-1395/1396 CDCA1-91/92
CDGA5-1423/1424
112.8 CDCA3-309/310
113.9 CDCA3-247/248
115.3 CDGA3-1159/1160
116.1 CDGA5-1469/1470
120.5 CDCA7-651/652
122.3 CDAAC1-2245/2246

Table 8. Linkage groups, marker number, length, and marker density in our common bermudagrass genetic map

Linkage group	Total markers	Length (cM)	cM/marker	Gaps(>10 cM)
1	19	53.2	2.8	0
2	5	15.2	3.0	0
3	15	54.6	3.6	3
4	7	78.6	11.2	3
5	11	80.1	7.3	3
6	4	12.7	3.2	1
7	26	48.7	1.9	1
8	14	40.9	2.9	1
9	6	50.6	8.4	3
10	12	52.5	4.4	2
11	6	17.2	2.9	1
12	15	60.0	4	2
13	14	57.1	4.1	2
14	9	61.2	6.8	3
15	21	105.2	5.0	3
16	18	91.6	5.1	3

17	12	93.0	7.8	5
18	38	122.3	3.2	3

Six multi-allele PPs which simultaneously amplified one locus on each subgenome were detected during the gel scoring process. Among the six multi-allele PPs, four were identified to link three pairs of homeologous linkage groups while the loci amplified by other two PPs were not linked to other markers on the map. SSR PP CDGA4-1301/1302 was found to bridge LG 1 and LG 6, CDCA5-469/470 and CDGA5-1455/1456 were mapped to associate LG 7 and LG 8, and CDAAC7-2693/2694 to connect LGs 15 and 16 as homeologous pairs.

Whole genome length and markers distance in the linkage map are critical for gene effects study, region location of interest trait, and map coverage estimation (Liu et al., 2012). The estimated genetic length of the whole genome for the common bermudagrass genotype was 1207.6 cM based on the method of Hulbert et al. (1988), and the genome coverage was 98.4% within 10 cM for our map according to the formula used by Okada et al. (2010).

2. Marker clustering, segregation distortion and linkage phase ratio

SSR markers were distributed non-evenly across the 18 LGs in the common bermudagrass genome, and markers distribution with greater than 5 loci per 10 cM was identified as marker clustering. Clustering of SSR markers were observed in nine linkage groups including LGs 1, 3, 7, 8, 11, 13, 15, 16 and 18 while the phenomenon was not apparent on other linkage groups (Figure 4). 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 non-distorted 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).

3. Comparative mapping

The genetic map of common bermudagrass was compared to those of sorghum and foxtail millet, respectively. When compared with sorghum genome, 201 out of 266 genotyped loci had hits based on the BLASTN analysis (Table 9). Bermudagrass LGs 3 and 4 were anchored to sorghum chromosome Sb 9 with 35.7% and 50.0% of loci, and LGs 1, 8 and 18 were matched with chromosome Sb 10 with 30.8%, 30.8% and 32.3% synteny (Table 9). Bermudagrass LGs 5, 9, 11, and 14 were anchored on chromosome Sb 5, Sb 4, Sb 8, Sb 6 with 57.1%, 66.7%, 80.0%, and 57.1% synteny (Table 9). As compared with foxtail millet genome, bermudagrass LGs 7 and 11 were anchored to foxtail millet chromosome Si 9 with 50.0% and 83.3% (Table 10). LG 3, 5, 8, 9 and 18 were anchored to Si 3 (42.9%), Si 7 (50.0%), Si 6 (50.0%), Si 1 (66.7%), Si 4 (36.4%) (Table 10). According to the percentage of syntenic rate, common bermudagrass LGs and corresponding chromosomes in sorghum and foxtail millet were listed in Table 11.

Table 9 Assignment of common bermudagrass mapped loci in sorghum chromosomes

	Sorghum Chromosome											
Common bermudagrass linkage group	**Sb 1**	**Sb 2**	**Sb 3**	**Sb 4**	**Sb 5**	**Sb 6**	**Sb 7**	**Sb 8**	**Sb 9**	**Sb 10**	**Total**	**%**
1	1	1	2	1	1	2			1	4	13	30.8
2	1		1			1			1		4	
3	1		1	1	3	1	2		5		14	35.7
4			2	1					3		6	50.0
5	1		1		4			1			7	57.1
6			2							1	3	
7	4	1	3	2	1	1	1	2	3	2	20	
8	1	2	2			2		1	1	4	13	
9				4				1		1	6	66.7
10	3	1	2							3	9	
11		1						4			5	80.0
12		2	1			1	1	1	3	2	11	
13		2	1	1		3	1			4	12	
14			1			4	1	1			7	57.1
15	1	1	1		1	4	1	3	2	1	15	

16	2	3		1	3	3	1	1	1		15	
17	1	3		1			3		2		10	
18	1	2	3	4	4		2	4	1	10	31	32.3
Total	17	19	23	16	17	22	13	19	23	32	201	

Table 10 Assignment of common bermudagrass mapped loci in foxtail millet chromosomes

	Foxtail Millet Chromosome										
Common bermudagrass linkage group	**Si 1**	**Si 2**	**Si 3**	**Si 4**	**Si 5**	**Si 6**	**Si 7**	**Si 8**	**Si 9**	Total	%
1	1	2	2		2	1	1	3	3	15	
2		1					2		1	4	
3	1		6	1	1			2	3	14	42.9
4		2	2	1						5	
5	2			1			4		1	8	50.0
6		1					1		1	3	
7	2	2		2	3		1		10	20	50.0

8	1	1		3	1	7			1	14	50.0
9	4					1	1			6	66.7
10	1	1		2	2				3	9	
11			1						5	6	83.3
12	1	3			2		1	2	2	11	
13	3	1		3		1	2	2		12	
14				2	2		3			7	
15	2		3	2	2		4	1	2	16	
16		1	2	1	3	3	2	2	1	15	
17					3	3	2	1	2	11	
18		2	2	12	6	2	5	2	2	33	36.4
Total	18	17	18	30	27	18	29	15	37	209	

Table 11 Corresponding chromosomes of common bermudagrass in sorghum and foxtail millet

Bermudagrass	Sorghum	Foxtail Millet
LG 1	Sb 10	
LG 2		
LG 3	Sb 9	Si 3
LG 4	Sb 9	

LG 5	Sb 5	Si 7
LG 6		
LG 7		Si 9
LG 8	Sb 10	Si 6
LG 9	Sb 4	Si 1
LG 10		
LG 11	Sb 8	Si 9
LG 12		
LG 13		
LG 14	Sb 6	
LG 15		
LG 16		
LG 17		
LG 18	Sb 10	Si 4

IV. Discussion

1. Genetic linkage map

Whole genome length and marker distances in the linkage map are critical for gene effects studies, region location of traits of interest, and map coverage estimation (Liu et al., 2012). The mapping population was for this investigation was selected from one of the two populations used for our previous inheritance mode study of common bermudagrass. Since the S1 progenies from 'Zebra' (2n=4x=36) had severe segregation distortion problems that would negatively affect linkage analysis by biased recombination fractions and spurious linkages (Liu et al., 2012), we selected S1 progenies from A12359 (2n=4x=36), which had less segregation distortion for 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 and hypervariability, and most importantly, SSRs could be randomly dispersed throughout most genomes indicating they are transferable to other species or other labs (Xu, 2010), which makes our common bermudagrass linkage map valuable not only for bermudagrass genome study but also for comparative study with other grasses or crops. In figure 4, 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. Firstly, population size was one of the most important factors affecting genetic map 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). Secondly, allelic dropout (ADO: one allele of a heterozygous locus could not be amplified during a polymerase chain reaction) will increase genotyping error rate which could not be avoided by laboratory procedures (Broquet and Petit, 2004), so fewer recording alleles will help fewer ADO occur. 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). As described above, population selection was also considered for this map construction. A population with severe segregation distortion is not suitable for linkage analysis (Knoing-Boucoiran et al., 2012). At the very beginning of our experiment, we tried to use a selfed population from a variegated common bermudagrass parent ‘Zebra’, but severe distortion was found in the population (18 out of 21 markers were deviated from the 1:2:1 Mendel segregation law). The mapping population in this

study showed less segregation distortion as compared with the Zebra S1 population described in the previous chapter According to Pawlowsky-Glahn and Buccianti (2011), missing value less than 10% was considered to be acceptable, and the highest missing value for each SSR PP was 8 (less than 13) in this study, indicating the high map accuracy.

2. Marker clustering and segregation distortion

Segregation distortion phenomenon is common and has been reported in both outcrossing and selfing crops, including rice (*Oryza sativa*) (Xu et al., 1997; Habu et al., 2015), maize (*Zea mays*) (Lu et al., 2002; Xu et al., 2013), soybean (*Glycine max*) (Baumbach et al., 2012), barley (*Hordeum vulgare*) (Cistue et al., 2005), and also some perennial herbages like alfalfa (*Medicago sativa*) (Li et al., 2011), ryegrass (*Lolium perenne*) (Bert et al., 1999), and switchgrass (*Panicum virgatum*) (Missaoui, 2005; Liu and Wu, 2012). As for bermudagrass, 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 populations have a higher tendency for segregation distortion (Liu et al., 2012), a higher rate (39%) of segregation distortion was observed in our population as compared with the previous study of bermudagrass. Segregation distortion will result in linkage disequilibrium, and the enhancer alleles will be found in coupling phase and suppressor alleles in repulsion phase (Lyttle, 1991). However, we still preferred to keep those distorted markers, as suggested by Van Ooijen (2006) that distorted markers could be better understood after map construction.

3. Allopolyploid origin for common bermudagrass

According to Wu et al. (1992), the ratio of coupling to repulsion linkage phase was used to detect allopolyploid or autopolyploid, in which a ratio of 1:1 was expected for an allopolyploid and 1: 0.25 or 1:0 for an autopolyploid. We observed a ratio of 88:66 for coupling versus repulsion

phases, and the chi-square value for the observed and expected 1:1 ratio was 3.143, which was 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). In addition to comparing with switchgrass, which is another allopolyploid grass species, in which 12 multi-allele SSR markers were mapped in both subgenomes by Liu et al. (2012), we only observed that six out of 260 SSRs (2%) were multi-allelic, indicating the allopolyploid origin rather than autopolyploid.

4. Comparative mapping with Panicoideae and genome evolution for Chloridoideae

Cynodon genus belongs to the Cynodonteae tribe, *Chloridoidea*e sub-family in the Poaceae family (Clayton and Renvoize, 1986). Large differences in genome size, ploidy level and chromosome numbers exist in grass genomes in *Poeceae*. Although many species are economically and ecologically important *Chloridoideae* grasses are among the least explored in developing genomic resources as compared with *Pooideae*, *Panicoideae* and *Ehrhartoideae* subfamilies containing economically important species, rice, wheat, maize, millet and sorghum (Kellogg, 1998; Feuillet and Keller, 2002; Bethel et al., 2006). Although rice had been recommended as a model plant for grasses with respect to comparative mapping (Devos and Gale, 2000; Feuillet and Keller, 2002), sorghum and foxtail millet were thought to be better choices for grasses in *Chloridoideae* because they are closer to *Chloridoidea*e in grass phylogeny (Kellogg, 1998). *Cynodonteae* is one important tribe in Chloridoideae because it contains several widely used turf and forage grasses such as bermudagrass, buffalograss (*Bouteloua dactyloides*) and zoysiagrass (*Zoysia* spp.), but no reference whole genome sequence is available in this tribe.

According to the syntenic blocks between sorghum and foxtail millet genomes (Zhang et al., 2012), sorghum chromosome Sb 9 was conservative to foxtail millet chromosome Si 3, and our

comparative mapping results showed common bermudagrass LG 3 was anchored to sorghum Sb 9 and foxtail millet chromosome Si 3, which confirmed that this was one of the conservative regions among these three species. Bermudagrass LG 9 was anchored to sorghum Sb 4 and foxtail millet Si 1, indicating another conservative region among the species. The third synteny was identified between common bermudagrass LG 18, sorghum chromosome Sb 10 and foxtail millet chromosome Si 4, which showed highly conserved synteny according to Zhang et al. (2012) as well. Intergenomic analysis also revealed high synteny when comparing sorghum and foxtail millet with Zoysiagrass, another grass species belonging to the subfamily of *Chloridoideae*. (Wang et al., 2015). *Chloridoideae* has a close phylogenetic relationship with *Panicoideae* (Kellogg, 1998), which diverged from each other around 34.6-38.5 Ma (Kim et al., 2009). Despite the fact that several conservative regions were observed among these grasses, various genome rearrangements during evolution, involving insertions, deletions, duplications, inversions and translocations, could result in chromosome changes and differences among different grass genomes. For instance, many markers linked in common bermudagrass linkage groups but dispersed on sorghum or foxtail millet chromosomes (Tables 9 & 10).

It has been proposed that all grasses were derived from a common ancestor with a basic chromosome number seven, and after whole genome duplication events, the basic chromosome number increased to n=14, then this number was reduced to n=12 through two chromosomes fusions (Wang et al., 2015). Other grass genomes evolved from this paleo-ancestral form through different genome rearrangements, with a most important one being insertional or nested chromosome fusion (NCF), which was supposed to be a prominent factor for descending dysploidy (Luo et al., 2009). In NCF, one complete chromosome was inserted into the centrometric area of another chromosome (Murat et al., 2010; Wang et al., 2015). As for grasses including common bermudagrass in *Chloridoideae*, their basic number of chromosomes is nine possibly due to three NCFs, which were revealed in genome changes of *Panidoideae* and

Pooideae (Murat et al., 2010; Wang et al., 2015). Therefore, though there were many events like genome rearrangements undergoing during the process of evolution, great collinearity and a high conservative rate exits among different species in the Plantea Kingdom (Wang et al., 2015).

V. Conclusions

Using an S1 progeny population, we reported the first complete or near complete genetic map of 18 linkage groups based on codominant SSR markers for common bermudagrass with a map length of 1094.7 cM. The mapping phase ratio of 1 coupling: 1 repulsion in this population firmly indicated the allopolyploid origin of the tetraploid bermudagrass and six of 260 polymorphic SSR PPs generating alleles across two subgenomes indicated high divergence between the two subgenomes. Compared with the reference genomes of sorghum and foxtail millet, and three conservative regions among these three species were found. This map will be used in quantitative trait analyses for important agronomic traits, which could be used for subsequent marker-assisted selection, map-based cloning, evolutionary genomics and comparative genomics.

CHAPTER IV

EXPERIMENT 3. GENETIC ANALYSIS OF COLD HARDY SELECTIONS IN BERMUDAGRASS

I. Introduction

Bermudagrass (*Cynodon* spp.) is a commonly used warm-season turfgrass in the world (Shearman, 2006). Among the nine species included in *Cynodon*, common bermudagrass (*C. dactylon*) is the best known and most economically important because of its wide geographic distribution, enormous variability, and multiple uses, such as for turf and forage production, in soil erosion control, and as a potential bioenergy feedstock (Taliaferro, 1995). Numerous vegetatively propagated and seeded cultivars in this species have been selected, developed and released for commercial production since the beginning of the 20th century, and selected plants in the species have been successfully used in interspecific crosses with African bermudagrass (*C. transvaalensis*) plants to produce hybrids of high turf quality since the 1940s (Burton, 1991).

In the United States, most prior bemudagrass breeding projects were aimed at developing vegetatively-propagated cultivars in the southern part where temperature is relatively warm and suitable for bermudagrass survival and growth (Taliaferro et al., 2004), however bermudagrass

will suffer severe winter-kill when grown in a region beyond its adaptation range (i.e., tropical and subtropical climates) (Anderson and Taliaferro, 2002). In addition, most of bermudagrass cold hardiness improvement programs were focused on first generation (F1) sterile triploid hybrids. However, developing cold hardy populations for selecting elite parents is possible for bermudagrass improvement (Anderson and Taliaferro, 2002). Breeding seeded varieties is a complement for clonal bermudagrass varieties (Taliaferro et al., 2004) due to seed advantages like easy transportability, convenient storage and lower cost compared with sod or vegetative materials. Therefore, breeding turf use, cold tolerant, seed-propagated, market acceptable cultivars has been one of the major goals in common bermudagrass (Taliaferro et al., 2004).

Enormous success has been achieved in breeding cold tolerant turf bermudagrass cultivars through germplasm enhancement, inter- and intra-specific hybridization, and selection in the U.S. At first, traditional breeding had been used to enhance cold hardiness and reduce winterkill risk for clonal bermudagrass, for example, 'Midlawn' 'Midiron', and 'Midfield' being F1 hybrids derived from interspecific crossing between *C. dactylon* and *C. transvaalensis*. Then mutagenic agents were utilized to increase turf quality in some cold tolerant bermudagrass cultivars, and cold acclimation pathways had been studied to discover molecular mechanisms related to cold tolerance for bermudagrass (Hanna, 1990; Baird et al., 1998).

Recently we reported 48 clonal plants were selected from a 'Riviera' population and 50 plants were selected from a 'Yukon' population after two winters when they were grown in nurseries at the Landscape and Horticulture Center of the University of Illinois, Urbana-Champaign (Wu et al., 2013). 'Yukon' is a turf-type, seed-propagated, and synthetic cultivar, and it was released by Oklahoma Agricultural Experiment Station in 1997 because of its improved strength and better cold tolerance compared with other seed-propagated turf bermudagrass (Taliaferro et al., 2003), but turf quality and seed yield of 'Yukon' are relatively low (Taliaferro et al., 2004). 'Rivera' was another synthetic cultivar released by Oklahoma Agricultural Experiment Station in 2000, and it

performed well with improved turf quality and higher seed yield (Taliaferro et al., 2004). However, genetic variability of the winter hardy germplasm for turf performance traits and seed yield components is unknown. The information, if available, will help to develop new breeding populations and use of the germplasm in creating new interspecific hybrids and synthetic cultivars. The objective of this experiment was to quantify genetic variability and determine relationships among turf performance and reproductive traits.

II. Materials and Methods

1. Materials

This experiment included two seed-propagated bermudagrass populations that were composed of a total of 420 plants from Rivera and Yukon bermudagrass seed (210 from Rivera and 210 from Yukon respectively) (Wu et al., 2013). Initially, seeds from both populations were germinated in a greenhouse on the Agronomy Research Station at Oklahoma State University, Stillwater, OK, in the spring of 2009, then plants were transplanted into a nursery at Landscape and Horticulture Center of the University of Illinois, Urbana-Champaign (UIUC), IL, in the summer of the same year (Wu et al., 2013). After implementing proper field management and careful selection procedures described by Wu et al. (2013), the best surviving plants were taken back and transplanted into a nursery at the OSU Agronomy Research Station.

2. Experimental design and field management

Ninety eight bermudagrass plants selected from 2009 UIUC nurseries with two replications were transplanted to an experimental field on the Agronomy Farm, Stillwater, OK, which was in the south end of #8100W on July 08, 2011. The experimental design was a randomized complete block. The area dimensions were 40 m in width by 50 m in length. Plot size was 1.5 m × 1.5 m

with 1 m alleys between neighboring plots. The soil type was loam or clay loam with a pH of 6.8. Based on soil test report (Soil, Water & Forage Analytical Laboratory, Oklahoma State Univesity), nitrogen was applied at a rate of 112 kg N/ha in 2012 to increase plant growth and the alleys were cleaned with periodic applications of Roundup herbicide in order to separate the plots in early spring. In the years post establishment, 2012, 2013 and 2014, plant residues in the plots were mowed off at 2 inches height by the end of February or early March. Before the initiation of bermudagrass spring green up, Roundup (glyphosate, N-phosphonomethyl glycine), 2, 4-D [(2,4-dichlorophrnoxy) acetic acid] and Barricade (Prodiamine) were applied in early March according to the labels of each herbicide. Nitrogen fertilizer was applied at a rate of 60 lb per acre (68 kg N/ha) in early May after a mowing at 2 inch height in the first week of that month. During the growing season, Roundup herbicide with surfactant and ammonia sulfate [$(NH_4)_2SO_4$] was applied to control weeds in alleys of 0.6 m wide when needed on the basis of weekly observations in the field.

3. Important traits for turf bermudagrass

Response traits for turf bermudagrass were listed in Table 12. The traits included establishment rate (ER), greenup (GU), leafspot (LS), sod density (SD), fall color retention (FCR) and genetic color (GC), were evaluated with a rating scale from 1 to 9 visually in field plots (Wu et al., 2007). In August of 2013 and 2014, five randomly collected stems from each plot were collected for measuring morphological traits. Collected stems were placed immediately on ice to keep leaves fresh, and then transported to the lab and stored in a -20^0C freezer for subsequent morphological measurements consisting of leaf blade width (LW), leaf blade length (LL), internode length (IL) and internode diameter (ID) (Wu et al., 2007). As for reproductive traits, inflorescence prolificacy (IP) was assessed by visual ratings in each plot, and 10 randomly collected mature inflorescences were used for measurement of other reproducible traits (Wu et al., 2006). Raceme length (RL)

was the length sum of racemes in a bermudagrass inflorescence, and seed number (SN) per inflorescence was measured by slightly modifying the method of Wu et al. (2006). Each inflorescence was soaked in a 40% (v/v) bleach solution for 4-6 h, then spikelets containing seeds were easily counted using a microscope with 10× magnification. However, the total spikelet number per inflorescence was estimated using a linear formula: Y=8.4+0.79X (where X indicated raceme length inflorescence^{-1}, and Y represented the estimated number of spikelets inflorescence^{-1}), which was described by Wu et al. (2006). Percent seed set (PSS) was the percentage of seed number (SN) over spikelet number per inflorescence, which was calculated by dividing the number of seeds by number of spikelets in the same inflorescence and multiplying 100 to get the final percent seed set.

Table 12. Dates and methods of trait evaluations on 98 bermudagrass plants.

Date	Trait descriptor (abbreviation)	Method (per plot)
Sep. 2011	Establishment rate (ER)	1-9 scale, 1 indicating least in size, 9 biggest in size
Mar. 2012-Mar. 2014	Greenup (GU)	1-9 scale, 1 indicating brown, 9 completely green
Apr. 2012	Leaf spot disease (LS)	1-9 scale, 1 equaling no resistance, 9 complete resistance
May. 2014	Sod density (SD)	1-9 scale, 1 indicating least dense, 9 most dense
Oct. 2013	Fall color retention (FCR)	Same scale as for GU
Apr. 2014	Genetic color (GC)	1-9 scale, 1 indicating light green, 9 dark green
Aug. 2013-Aug. 2014	Fourth leaf blade width (FLW)	Using five random stems to measure (mm)
Aug. 2013-Aug. 2014	Fourth leaf blade length (FLL)	Using five random stems to measure (mm)
Aug. 2013-Aug. 2014	First internode length (FIL)	Using five random stems to measure (mm)
Aug. 2013-Aug. 2014	First internode diameter (FID)	Using five random stems to measure (mm)

Aug. 2012-Aug. 2014	Inflorescence prolificacy (IP)	1-9 scale, 1 indicating no inflorescences and 9 most abundant inflorescence
Aug. 2012-Aug. 2014	Raceme length (RL)	Using 10 random mature inflorescence from each plot to measure (mm)
Aug. 2012-Aug. 2014	Seed number (SN)	Using 10 random mature inflorescence from each plot after 40% (v/v) bleach treatment for 4-6 h, then using a 10 × magnification to count
Aug. 2012-Aug. 2014	Spikelet number	Using $Y= 8.4 + 0.79X$ ($r^2=0.68$, $P<0.01$) to estimate
Aug. 2012-Aug. 2014	Percent seed set (PSS)	Using 100 (number of caryopses /number of spikelets) (%)

III. Data analysis

For repetitive values, including morphological variables measured using 5 random stems and some response variables of reproductive traits based on 10 random inflorescences in each plot, means of each variable from 98 individual genotypes and phenotypic standard deviations were determined by PROC MEANS procedure in SAS Version 9.4 (SAS Institute) and were used to represent individual plot values for subsequent data analyses (Cortese et al., 2010). Randomized complete block design (RCBD) was utilized in this experiment with plant ID, year, block, and Plant ID × year as random effects. Analysis of variance (ANOVA) and restricted maximum likelihood (REML) estimates of variance components for each trait was tested by PROC MIXED procedure, and histograms were created through PROC UNIVARIATE for each response variable. PROC CORR procedure was utilized to perform phenotypic correlation analysis with data of the traits collected in the same year (Wu et al., 2006; Wu et al., 2007).

IV. Results and Discussion

1. Variability of 13 adaptive, morphological and reproductive traits

According to the analysis of variance (ANOVA) results in Table 13, effects of genotype (Plant ID) was significant (P<0.01 or 0.05) for all traits except first internode length (FIL). The effect of Year was significantly (P<0.01) different for 7 of 8 traits with two or three year data (Table 13). The interaction between Plant ID and Year was significantly (P<0.01) different for greenup (GU), fourth leaf blade width (FLW), fourth leaf blade length (FLL), first internode diameter (FID), inflorescence prolificacy (IP) and raceme length (RL) (Table 13), which indicated genotypes did not respond similarly and constantly over years for these traits (Wu et al., 2007). Significant (P<0.01 or 0.05) differences among block were observed in 10 traits (Table 13).

Spring greenup (GU) is grass recovery and new shoot production from dormant rhizomes and stolons in the spring season, and is considered to be an important indicator for winter hardy evaluation. Due to the large Year effect difference, selection of GU should base on multiple years data rather than just one year. Leaf spot disease showed large Block effect, which may because the bermudagrass plants were unevenly infected naturally. First internode length (FIL) could not be selected among these plants because the genetic variability was not significantly different according to Table 13.

Table 13. Mean Square results for tested traits in selected bermudagrasses plants

	Source	Plant ID (G)	Year (Y)	Block (B)	G*Y	Residual
Adaptive	ER	4.47**		8.68		2.51
	LS	6.40**		22.31**		2.47
	SD	4.36**		0.42		0.94
	FCR	2.63**		1.39		0.42

	GU	5.21**	859.78**	3.36*	1.21**	0.77
	GC	1.78*		4.70*		1.17
	FLW	0.49**	26.92**	1.28**	0.23**	0.12
Morphological	FLL	46.59**	245.93*	14.80*	8.04**	3.25
	FIL	3.79	94.51**	16.12*	3.47	3.02
	FID	0.32**	2.17**	7.97**	0.14**	0.05
	IP	10.60**	235.08**	6.68**	2.20**	0.84
Reproductive	RL	30297.00**	2.92	42305.00**	5111.55**	1321.88
	PSS	2039.47**	6476.27**	18447.00**	182.97	263.60

* Significance at the probability level of 0.05.

**Significance at the probability level of 0.01.

[l]ER, establishment rate; SD, sod density; FCR, fall color retention; GU, greenup; GC, genetic color; FLW, fourth leaf blade width; FLL, fourth leaf blade length; FIL, first internode length; FID, first internode diameter; IP, inflorescence prolificacy; RL, raceme length; PSS, percent seed set.

Means, associated standard deviations, and ranges from minimum to maximum for 13 traits are listed in Table 14. The data indicated the phenotypic differences for traits measured on the tested bermudagrasses. For spring greenup (GU), the mean value in 2012 was much higher than in 2013 and 2014 as well as the range values, which indicated a better spring greenup performance for the bermudagrass plants in 2012 (Table 14).

Table 14. Means, standard deviations (SD), and ranges for tested traits by year in selected bermudagrass plants

	2011		2012		2013		2014	
	Mean ± SD	Minimum-Maximum	Mean ± SD	Minimum-Maximum	Mean ± SD	Minimum-Maximum	Mean ± SD	Minimum-Maximum

	ER	3.5 ± 1.9	1.0-9.0						
	LS	4.7 ± 2.1	1.0-9.0						
Adaptive	SD							4.9 1.6	1.0-9.0
	FCR					6.1 ± 1.2	1.0-9.0		
	GU			7.4 ± 1.1	4.0-9.0	3.5 ± 1.3	1.0-7.0	4.0 ± 1.4	1.0-8.0
	GC							5.2 ± 1.2	1.0-9.0
	FLW (mm)					2.0 ± 0.3	1.1-3.0	2.2 ± 0.4	1.0-7.9
Morphological	FLL (cm)					7.1 ± 2.4	1.9-18.2	6.4 ± 2.4	1.0-14.5
	FIL (cm)					0.9 ± 0.3	0.3-2.1	1.4 ± 0.6	0.4-4.5
	FID (mm)					1.0 ± 0.2	0.7-1.7	1.1 ± 0.3	0.2-2.4
	IP			4.7 ± 1.6	1.0-9.0	6.3 ± 1.6	1.0-9.0	6.9 ± 1.9	1.0-9.0
Reproductive	RL (mm)			152.3 ± 47.2	29.0-383.0	180.7 ± 50.4	58.0-413.0**	181.0 ± 48.2	55.0-401.0
	PSS (%)			41.6 ± 29.3	0.5-99.3	8.0 ± 10.5	0.3-99.3	3.8 ± 4.5	0.3-36.1

* Significance at the probability level of 0.05.

**Significance at the probability level of 0.01.

[l]ER, establishment rate; SD, sod density; FCR, fall color retention; GU, greenup; GC, genetic color; FLW, fourth leaf blade width; FLL, fourth leaf blade length; FIL, first internode length; FID, first internode diameter; IP, inflorescence prolificacy; RL, raceme length; PSS, percent seed set.

Most stem morphological traits including fourth leaf blade width (FLW), first internode length (FIL) and First internode diameter (FID) were relatively higher in the year of 2014 compared to 2013, with only fourth leaf blade length (FLL) being the exception and having a higher value in 2013 (Table 14). For turf use, smaller leaf blades and shorter internode are desirable. Among the plants tested, large ranges for the morphological traits indicated the possibility to select finer plants for breeding. In this study, mean value of fourth leaf blade width (FLW) was 2.0mm,

which was much lower than the previous report of Wu et al. (2007), which may result from the reason our fourth leaf blade was less expanded than the leaves on third node they measured.

For reproducible traits, although means of inflorescence prolificacy (IP) and raceme length (RL) increased from 2012 to 2014, percent seed set (PSS) dropped from 41.6% (2012) to 3.8% (2014) based on Table 14, but the range values for reproductive traits among the three years were not as much different as means. Significant variances among bermudagrass genotypes for percent seed set were also reported by Richardson et al. (1978), Kenna et al. (1983) and Wu et al. (2006). However, the studies from Richardson et al. (1978) and Kenna et al. (1983) indicated the percentage ranging from 0 to 78.8% and from 4.7 to 44.6 % for open-pollinated (OP) seed set of bermudagrass, which was narrower than our study. Our findings were relatively closer to the results from Wu et al. (2006) with a seed set percentage ranging from 0.1 to 96.1%. According to Wu et al. (2006), two reproductive traits including inflorescence prolificacy and percent seed set were found to be significantly and positively related with seed yield, and the effect of raceme length per inflorescence for seed yield was ignorable. Therefore inflorescence prolificacy and percent seed set could be used as important indicators for selecting high seed yield cultivars.

Variance components estimates and their associated standard errors for 13 bemudagrass adaptive, morphological and reproductive traits are shown in Table 15. Significant (P<0.01 or 0.05) differences existed for Plant ID (genotypic) variance estimates (σ^2_G) in all traits except genetic color (GC) and first internode length (FIL), and year variance estimates (σ^2_Y) were not significant for all traits with two or three year data, block variance estimates (σ^2_B) were also found not significant for all traits (Table 15). Plant ID × year interaction variance estimates ($\sigma^2_{G\times Y}$) were only significant (P<0.01) for spring greenup (GU), fourth leaf blade length (FLL), first internode diameter (FID) and inflorescence prolificacy (IP).

Table 15. Variance components estimates and associated standard errors for tested traits in selected bermudagrass plants

		σ^2_G	σ^2_Y	σ^2_B	$\sigma^2_{G\times Y}$	$\sigma^2_{Residual}$
Adaptive	ER	1.01 ± 0.37**		0.05 ± 0.11		2.49 ± 0.36**
	LS	1.99 ± 0.50**		0.22 ± 0.34		2.46 ± 0.35**
	SD	1.73 ± 0.32**		0.00 ± 0.00		0.93 ± 0.13**
	FCR	1.10 ± 0.19**		0.01 ± 0.02		0.42 ± 0.06**
	GU	0.67 ± 0.13**	4.48 ± 4.49	0.01 ± 0.02	0.22 ± 0.07**	0.77 ± 0.06**
	GC	0.23 ± 0.16		0.02 ± 0.04		1.25 ± 0.1992
Morphological	FLW (mm)	0.01 ± 0.00**	0.03 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.00**
	FLL (cm)	1.92 ± 0.34**	0.25 ± 0.36	0.01 ± 0.02	0.48 ± 0.12**	3.24 ± 0.11**
	FIL (cm)	0.02 ± 0.04	0.09 ± 0.14	0.01 ± 0.02	0.04 ± 0.05	3.02 ± 0.10**
	FID (mm)	0.01 ± 0.00**	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00**	0.05 ± 0.00 **
Reproductive	IP	1.468 ± 0.27**	1.23 ± 1.24	0.06 ± 0.08	0.6829 ± 0.12**	0.8597 ± 0.071**
	RL (mm)	894.56 ± 114.11**	246.77 ± 261.92	14.18.87 ± 20.71	193.49 ± 37.77	1322.31 ± 25.34**
	PSS (%)	110.52 ± 13.26**	393.43 ± 395.18	10.41 ± 14.93	0.00 ± 0.00	261.9 ± 6.08**

* Significance at the probability level of 0.05.

**Significance at the probability level of 0.01.

[l]ER, establishment rate; SD, sod density; FCR, fall color retention; GU, greenup; GC, genetic color; FLW, fourth leaf blade width; FLL, fourth leaf blade length; FIL, first internode length; FID, first internode diameter; IP, inflorescence prolificacy; RL, raceme length; PSS, percent seed set.

According to the results of ANOVA test, means and ranges, and estimates of variance components and associated standard errors, substantial genetic variation was found for 12 of 13 turf adaptive, morphological and reproductive traits in the two bermudagrass populations. *Cynodon dactylon* had been reported to be highly variable in several previous publications as well (Harlan and de Wet, 1969; Harlan, 1970; Wu et al., 2006; Wu et al., 2007), and substantial variability was supposed to be generated by genetic interaction of varieties within this species, or

fragmentation of gene pool (Harlan and de Wet, 1969). Genotypes 1*9 and 20*7 showed significant improvement in the field study of drought performance (Liu, 2013), and they also appeared to perform better in our study for most of the tested traits including establishment rate (ER), sod density (SD), fall color retention (FCR), greenup (GU) and inflorescence prolificacy (IP), and also they were less affected by leaf spot (LS) disease.

2. Trait correlation analysis

Phenotypic relationships with data of the traits collected in the same year were presented in Table 16. As for relatively high positive relationships, a correlation coefficient of 0.66 (r=0.66, $P<0.01$) was detected between sod density (SD) and greenup (GU), which reflected bermudagrass with higher sod density had earlier spring greenup in the populations (Table 16). Fall color retention (FCR) and greenup (GU) had a correlation value of 0.51, implying that better fall color retention ability was significantly ($P<0.01$) associated with earlier spring greenup in the selected bemudagrass plants (Table 16). For morphological traits, fourth leaf blade width (FLW) (r=0.58, $P<0.01$) was positively related with first internode diameter (FID), which indicated leaves with wider blade width usually had wider internodes (Table 16). Significant correlation coefficients were -0.4 ($P<0.01$) when comparing leaf spot (LS) and greenup (GU), and r=-0.26 ($P<0.01$) between leaf spot (LS) and inflorescence prolificacy (IP), which indicated bermudagrass with less leaf spot problems had the ability to perform better in spring greenup and have higher inflorescence prolificacy. Percent seed set (PSS) was negatively associated (r=-0.23, $P<0.01$) with raceme length (RL), suggesting the possibility of choosing shorter raceme length to select bemudagrass plants with higher seed set percentage.

Table 16. Phenotypic correlations between tested traits in selected bermudagrass plants

	Adaptive					Morphological				Reproductive		
	LS	SD	FCR	GU	GC	FLW	FLL	FIL	FID	IP	RL	PSS

ER	-	-	-	-	-	-	-	-	-	-	-	-
LS		-	-	-0.40**	-	-	-	-	-	-0.26**	-	-
SD			-	0.66**	0.14*	-	-	-	-	0.45**	-	-
FCR				0.51**	-	-	-	-	-	0.39**	-	-
GU					0.08	-	-	-	-	-0.19**	-	-
GC						-	-	-	-	0.26**	-	-
FLW							-0.07	0.03	0.58**	-	-	-
FLL								0.11*	-0.22**	-	-	-
FIL									-0.14**	-	-	-
FID										-	-	-
IP											-	-
RL												-0.23**

* Significance at the probability level of 0.05.

**Significance at the probability level of 0.01.

[l]ER, establishment rate; SD, sod density; FCR, fall color retention; GU, greenup; GC, genetic color; FLW, fourth leaf blade width; FLL, fourth leaf blade length; FIL, first internode length; FID, first internode diameter; IP, inflorescence prolificacy; RL, raceme length; PSS, percent seed set.

V. Conclusions

Based on results of our analysis of variance (ANOVA), substantial genetic variability existed for the 12 adaptive, morphological and reproductive traits among the 98 cold hardy bermudagrass plants. Spring greenup was found to be highly and positively correlated with sod density and fall color retention in our study. Leaf spot had negative correlations with greenup and inflorescence prolificacy, and percent seed set was negatively associated with raceme length as well. This germplasm will be valuable in breeding new cultivars through selection of superior plants as

parents for targeted traits, and also be utilized in conventional breeding such as producing interspecific hybrids and synthetic cultivars.

CHAPTER V

CONCLUSIONS

In conclusion, we studied the inheritance mode, linkage analysis and genetic variability for common bermudagrass. According to our first experiment, two common bermudagrass populations exhibited complete or near complete disomic inheritance, which provided evidence supporting an allotetraploid genome origin with two distinct subgenomes in two tetraploid genotypes (Zebra and A12359). Severe to moderate segregation distortion occurred in the two first-generation selfed (S1) populations. This finding has benefited genetic map construction of common bermudagrass and added knowledge to the pool of common bermudagrass genetics.

A genetic map was constructed based on our second experiment. This linkage map was the first map completely based on codominant simple sequence repeat (SSR) markers for common bermudagrass using a selfing population of common bermudagrass. A total number of 252 loci were assigned into 18 common bermudagrass linkage groups (LGs) with a whole map length of 1094.7 cM. Our genetic map was compared with the genomes of sorghum and foxtail millet, and three conservative regions among these three species were identified according to the comparative mapping results. This map will be used in marker-trait association analysis study like agronomic important quantitative trait loci

(QTL) mapping and subsequent marker-assisted selection, and also be beneficial for map-based cloning, evolutionary genomics and comparative genomics with other well studied crops.

A total number of 13 adaptive, morphological and reproductive traits were evaluated among the 98 winter hardy bermudagrass plants, and large genetic variability was detected based on results of analysis of variance (ANOVA). Correlation tests were also performed in our study, and spring greenup was found to be highly and positively correlated with sod density and fall color retention. Leaf spot had negative correlations with greenup and inflorescence prolificacy, and percent seed set was negatively associated with raceme length as well. This winter hardy germplasm will be valuable in breeding new cultivars through selection of superior plants as parents for targeted traits in conventional breeding of new cultivars.

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