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Genome-wide association study for agronomic traits in bermudagrass (Cynodon spp.)

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Bermudagrass (*Cynodon* spp.) breeding and cultivar development is hampered by limited information regarding genetic and phenotypic diversity. A germplasm collection of 206 bermudagrass accessions from 29 countries was genotyped with high-throughput genotyping-by-sequencing technique. Genomic diversity in this diverse germplasm panel was assessed with multifaceted approaches including population structure, phylogenetic analysis, principal component analysis, and genetic diversity parameters. This study revealed substantial genetic variation in the *Cynodon* accessions, demonstrating the potential of this germplasm panel for further genetic studies and cultivar development in breeding programs. Another critical issue in turfgrass breeding is the lack of information regarding the genetic architecture of traits. Four agronomic traits leaf length, leaf width, internode distance and stem diameter were evaluated in a germplasm panel of common bermudagrass accessions. Then genome-wide association study was performed to dissect the genetic basis of the traits.

DEDICATION

I would like to dedicate this work to the loving memory of my grandfather, Sardar Nachhattar Singh. His life has, and always will, serve as inspiration for my family and myself.

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CHAPTER I

LITERATURE REVIEW

Genus *Cynodon*

Cynodon L. C. Rich genus consists of warm-season grasses belonging to the tribe Cynodonteae, subfamily Chloridoideae and the family Gramineae (Poaceae). According to the revised taxonomic classification by J.R. Harlan and coworkers, the genus *Cynodon* consists of a total of nine species and ten varieties (de Wet and Harlan, 1970; Harlan et al., 1970). The eight species in the genus *Cynodon* are *C. aethiopicus* Clayton et Harlan, *C. arcuatus* J. S. Presl ex C. B. Presl, *C. barberi* Rang. Et Tad., *C. dactylon* (L.) Pers., *C. incompletes* Nees, *C. nlemfuensis* Vanderyst, *C. plectostachyus* (K. Schum.) Pilg., and *C. transvaalensis* Burtt-Davy (de Wet and Harlan 1970). A ninth species is *C.* × *magennisii*, and while it is not included in the revised system by de Wet and Harlan (1970), it is listed in "A guide to the species of *Cynodon* (Gramineae)" (Harlan et al., 1970). In fact, *C.* × *magennisii*, a naturally occurring triploid, is the interspecific hybrid between *C. dactylon* and *C. transvaalensis* (Harlan et al., 1970a). Clayton et al. (2021) also listed the aforementioned nine species of *Cynodon* on Kew's online grass database.

Cynodon dactylon ssp. *dactylon*, also called common bermudagrass, is the most important species from an economic and ecological point of view. It is a warm-season perennial grass that is mostly used for turfgrass, forage and soil stabilization (Harlan and de Wet, 1969). Bermudagrass also has medicinal properties and acquires a unique reverence in various

traditional- and ethno-medicinal practices—it is used in Ayurveda, Unani, Nepalese, and Chinese medical systems and has both antiviral and antimicrobial properties (Shendye and Gurav, 2014). *Cynodon dactylon* is a weed in agriculture (Horowitz, 1996; Nelson and Burns, 2006), as well as in various maintained turfgrass settings (McElory and Breeden, 2006; Uddin et al., 2010).

The distribution of *C. dactylon* ssp. *dactylon* is cosmopolitan in nature (Harlan and de Wet, 1969). All continents and most islands accommodate this species, ranging between 45°N to 45°S latitudes, and up to 53°N in Europe. It is also found at different elevations (Taliaferro, 1995) and has been observed at 3,000 m elevation in Nepal (South Asia) and below sea level in North Africa, West Asia, and California (Harlan and de Wet, 1969). The remaining eight species can be divided into three groups based upon geographical distribution. The first group is common in South Asia and the Indian Ocean-South Pacific Islands and includes *C. arcuatus* J. S. Presl. ex C. B. Presl. and *C. barberi* Rang. et Tad. species. The second group is present in East Africa and is comprised of *C. plectostachyus* (K. Schum.) Pilger, *C. aethiopicus* Clayton et Harlan, as well as *C. nlemfuensis* Vanderyst ssp. *nlemfuensis* and ssp. *robustus* Clayton et Harlan. The third group present in southern Africa consists of *C. incompletus* Nees ssp. *incompletus*, ssp. *hirsutus* (Stent) de Wet et Harlan, *C. transvaalensis* Burtt-Davy, and *C.* × *magennisii* Hurcombe (Harlan et al., 1970b).

Ploidy levels and chromosome numbers in *Cynodon* vary widely. Hurcombe (1947) studied the chromosome number of various species of *Cynodon* present in South Africa using root tip sections. Hurcombe reported 10 as the base chromosome number for *Cynodon* and that *C. transvaalensis* is diploid, *C.* × *magennisii* is triploid, and *C. dactylon* is a tetraploid having 20, 30, and 40 chromosomes, respectively. According to her understanding, 18 chromosomes in

species *C. bradleyi* is due to aneuploidy (Hurcombe, 1947). Moffett and Hurcombe (1949) reported 36 somatic chromosomes for *C. dactylon* and 18 or 54 for *C. plectostachyum.* They reported the base chromosome number as x = 9. Finally, Forbes and Burton (1963) confirmed the base chromosome number as 9, not 10, by studying root tip smears of six *Cynodon* species. They reported *C. dactylon* as both diploid and tetraploid cytotypes, having 18 and 36 chromosomes, respectively. They also reported the chromosome numbers of five other *Cynodon* species as follows: *C. bradleyi* (2x = 18), *C. incompletes* (2x = 18), *C. plectostachyus* (2x = 18), *C. transvaalensis* (2x = 18), and *C.* × *magennisii* (3x = 27).

Harlan et al. (1970) released the revision of the taxonomy of *Cynodon* genus based on cytotaxonomic examinations, which is widely accepted. According to the revised taxonomy, diploid (2n=2x=18) and tetraploid (2n=4x=36) are common, but hexaploid (2n=6x=54) plants are rare. Species including *C. barberi*, *C. dactylon* ssp. *aridus*, *C. incompletus* ssp. *incompletus*, *C. plectostachyus*, and *C. transvaalensis* are predominantly diploids (2n=2x=18). Species including *C. arcuatus*, *C. dactylon* ssp. *dactylon*, *C. dactylon* ssp. *coursii*, *C. dactylon* ssp. *elegans*, and *C. dactylon* ssp. *polevansii* are largely tetraploids (2n=4x=36). Other species with both diploid and tetraploid formats are *C. aethiopicus*, *C. dactylon* ssp. *afghanicus*, *C. incompletus* ssp. *hirsutus*, *C. nlemfuensis* ssp. *nlemfuensis*, and *C. nlemfuensis* ssp. *robustus* (Harlan et al., 1970c). Powell et al. (1968) found an unexpected plant in their breeding nursery that was reported as hexaploid—the progeny of tetraploid *C. dactylon* ssp. *dactylon* × diploid *C. transvaalensis*. A natural hexaploid plant is ‘Tifton 10’ that was collected by G.W. Burton from Shanghai, China in 1974 and then introduced to the USA (Hanna et al., 1990). In 1967, another hexaploid plant was reported in the progeny of tetraploid *C. dactylon* (Felder, 1967). A famous known sterile pentaploid is the released cultivar ‘Tifton 85’, an interspecific hybrid between *C.*

dactylon and *C. nlemfuensis* (Burton et al., 1993). Three other pentaploid plants were reported in progeny of a hexaploid plant (an interspecific hybrid between *C. barberi* and *C. dactylon*) crossed with the tetraploid *C. dactylon* (Johnston, 1975).

Among all the species, *C. dactylon* possesses the highest genetic diversity. According to Harlan et al. (1970), *C. dactylon* species consists of the six subspecies as follows: ssp. *afghanicus* Harlan et de Wet, ssp. *aridus* Harlan et de Wet, ssp. *coursii* Harlan et de Wet, ssp. *dactylon* (L.) Pers., ssp. *elegans* Rendle, and ssp. *polevansii* (Stent) Harlan et de Wet. This subspecies classification was based on data collected for characteristics including natural distribution, morphological distinctness, cytogenetic behavior, and ecology (Harlan and de Wet, 1969; Harlan, 1970d). Among these six subspecies, ssp. *dactylon* is widely distributed in both warm and temperate climates, others have relatively narrow endemics.

In newer studies, Taliaferro et al. (1997) used flow cytometry to determine the DNA content and nuclear genome size in *Cynodon* species. Wu et al. (2006) conducted a flow cytometry study on 132 bermudagrass accessions and reported four ploidy levels among these accessions, of which the tetraploid cytotype (88%) was the most common. Among the 132 accessions, 116 were tetraploid, seven were hexaploid, three were pentaploid, six were triploid, and no diploids were found. In fact, this was the first report of naturally occurring pentaploid plants in *Cynodon*. Kang et al. (2008) studied 43 Korean *Cynodon* accessions and reported triploid, tetraploid, pentaploid and hexaploid cytotypes using flow cytometry. They also suggested that no one has ever reported diploids in Korea, which means triploids must have been introduced into the country. The ploidy level of 182 naturally occurring *Cynodon* accessions in southern Türkiye was reported by Gulsen et al. (2009). They reported diploids, triploids,

tetraploids, pentaploids, and hexaploids, and they further added that diploids are indigenous in three provinces of Türkiye. Pang et al. (2010) reported one diploid (2%), 19 triploid (40%), 24 tetraploid (50%), one pentaploid (2%), and three hexaploid (6%) accessions for a total of 48 accessions. Jewell et al. (2012) conducted a study on 690 *Cynodon* accessions in Australia and reported ploidy level as: tetraploids (61%), triploids (14%), diploids (11%), pentaploids (0.003%), and hexaploids (0.01%). Zhang et al. (2020) reported 16 diploids (7.4%), 36 triploids (16.6%), 97 tetraploids (44.9%), 21 pentaploids (9.7%), 32 hexaploids (14.8%), and 14 aneuploids (6.4%) out of 216 accessions sampled from 16 geographical sites across China. Recently, Grossman et al. (2021) performed flow cytometry on 288 accessions from United States Department of Agriculture National Plant Germplasm System bermudagrass germplasm and reported 38 diploids, 63 triploids, 181 tetraploids, four pentaploids, and two hexaploids. The genome size of *Cynodon* accessions reported in the aforementioned studies are summarized in in Table 1.1.

Table 1.1 Genome size and ploidy level in bermudagrass (*Cynodon* spp.) accessions reported in previous literature. These studies mainly used flow cytometry to determine the genome size and it is presented in pg/2c. Ploidy levels are reported from diploidy to hexaploidy in *Cynodon* accessions in different countries.

References	Diploid (18)	Triploid (27)	Tetraploid (36)	Pentaploid (45)	Hexaploid (54)
Taliaferro et al., 1997	1.11	1.6	2.25	N/A	2.8
Arumuganathan et al., 1999	1.03	1.61, 1.37	1.95	N/A	N/A
Wu et al., 2006	N/A	1.55-1.65	1.96-2.30	2.37-2.49	2.90-3.13
Kang et al., 2008	N/A	1.42-1.56	1.94-2.19	2.54	2.77-2.85
Gulsen et al., 2009	1.03-1.14	1.44-1.62	1.95-2.36	2.56-2.75	3.13-3.44
Pang et al., 2010	1.17	2.19	3.06	2.47	4.02
Chiavegatto et al., 2016	1.08-1.17	1.63	1.88-2.10	2.55	N/A
Zhang et al., 2020	2.38	2.41	2.43	2.87	3.28
Grossman et al., 2021	1.26	1.69	2.11	2.96	3.94

Note: Numbers in parentheses are somatic chromosome numbers; genome size was measured in pg/2c.

Molecular markers

The discovery and first-time use of molecular markers was by Hunter and Market (1957); these were enzyme-based markers called allozymes. Molecular markers have advanced and are now widely employed less than a half century after their discovery (Schöltterer, 2004). Allozymes are not well suited for mapping and association studies due to few numbers of useful and relevant marker loci and insensitivity for identifying DNA variants (Lewontin and Hubby, 1966; Schlötterer, 2004). The discovery of restriction endonucleases in the 1960s, shortly after the application of allozymes, was a watershed moment in the history of DNA manipulation techniques, paving the way for development of DNA based markers (Arber and Linn, 1969; Danna and Nathans, 1971; Hamilton and Wilcox, 1970). Based on detection methods and throughput, DNA markers are currently divided into three categories:

1. Hybridization-based markers: Restriction fragment length polymorphisms (RFLPs) (Botstein et al., 1980) and variable number tandem repeats (VNTR) (Nakamura et al., 1987).

2. Polymerase chain reaction (PCR)-based markers: Random amplification of polymorphic DNA (RAPD) (Williams et al., 1990), sequence characterized amplified regions (SCARs) (Paran and Michelmore, 1993), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), and microsatellites or simple sequence repeat (SSR) (Hamada et al., 1982; Jacob et al., 1991).

3. DNA sequence-based markers: Single nucleotide polymorphism (SNPs) (Berger et al., 2001).

From the above-mentioned markers, SSRs and SNPs are currently the most used in genetic analysis (Duran et al., 2009).

Plant breeding has been greatly accelerated by the use of genetic markers. DNA amplification fingerprinting (DAF), or simply DNA fingerprinting, has been widely used in characterizing genetic diversity, determining individual identity, genetic mapping, and genome-wide association studies to elucidate the genetic basis of traits. Genetic mapping in plant breeding is the act of assigning genetic markers to different linkage groups and identifying their order and recombination distances between markers (Jones et al., 1997). The technique of quantitative trait loci (QTL) mapping, which involves creating linkage maps, has been widely used to discover genomic regions (i.e., QTL) responsible for the traits of interest. Plant breeders were able to achieve tremendous progress in boosting crop yields and improving quality by combining molecular genetic technologies like QTL mapping with traditional breeding, a process

known as marker-assisted selection (MAS) (Bernardo and Charcosset, 2006; Holland, 2001; Lande and Thompson, 1990; Xu and Crouch, 2007).

Genetic Diversity

Plant genetic diversity is an essential asset for plant breeding. Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desired characteristics. Genetic diversity is defined as the variety of alleles and genotypes present in a population and it is reflected in morphological, physiological, and behavioral differences between individuals and populations (Frankham et al., 2002).

In the 1990s, different DNA molecular techniques were used in *Cynodon* to study genetic diversity, relatedness, and phylogeny, as well as to detect cultivar off-types (Caetano-Anolles, 1998). Caetano-Anolles et al. (1995) studied the genetic variation of 13 bermudagrass cultivars by grouping them into several clusters and separating ‘Tifway’ from the irradiation-induced mutant ‘Tifway II’ using DAF analysis in conjunction with phylogenetic analysis. The DAF analysis was able to classify 18 *Cynodon* cultivars into two groups, *C. dactylon* × *C. transvaalensis* hybrids and Australian bermudagrasses (Ho et al., 1997). The DAF was also used to determine genetic instability in ‘Tifgreen’ and ‘Tifdwarf’ (Caetano-Anolles, 1998a). In ‘Tifway’ and ‘Tifdwarf’ bermudagrass, DNA fingerprinting, chromosomal number, and morphology were effectively used to differentiate seven off-types (Busey et al., 1996). Using the DAF approach, Assefa et al. (1998) determined genetic similarities among 62 *Cynodon* accessions from eight different species. RAPD analyses were used to differentiate popular *Cynodon* cultivars grown in South Africa, such as ‘Bayview’, ‘Cape Royal’, ‘Florida’, ‘Harrismith’, ‘Silverton Blue’, ‘Skaaplaas’, ‘Tifdwarf’, and ten new varieties, and genetic

distance calculations were used to quantify the genetic variation in the populations (Roodt et al., 2002).

Zhang et al. (1999) used AFLP approach, which integrates the RFLP's reliability with the power and simplicity of the PCR technology, and they found enough polymorphisms to distinguish all 27 bermudagrass genotypes, including those that were closely related. Wu et al. (2004) evaluated genetic diversity and similarities among 28 *C. dactylon* ssp. *dactylon* accessions from 11 countries (Australia, Bulgaria, China, Germany, France, Italy, Japan, South Africa, Spain, Zimbabwe, and the United Arab Emirates) across four continents by employing AFLP markers. From the 590 bands tested, 443 (75%) were polymorphic. Wu et al. (2006) conducted research using AFLP markers that was carried out on a collection of 119 *C. dactylon* ssp. *dactylon* accessions from 11 different Chinese provinces. From total 763 scored AFLP bands, 466 (61.1%) were found to be polymorphic, produced by 13 primer combinations. Their findings indicated Chinese tetraploid accessions had far more genetic diversity than hexaploid accessions, but pentaploids had very little genetic variation. The AFLP marker method was used to examine the genetic diversity of 40 Korean bermudagrass accessions (Kang et al., 2008). The authors scored 2,256 bands using 29 selected primer combinations in PCR experiments, and 87.8% (1,982) of the AFLP markers were found to be polymorphic.

Gulsen et al. (2009) studied 182 Turkish *Cynodon* accessions for genetic diversity and variance partitioning by ploidy, geographic location, and province. These accessions have a wide range of genetic similarity coefficients, ranging from 0.50 to 0.98. Chloroplast-specific SSRs, AFLP, RAPD, and directed amplification of minisatellite-region DNA were used by Karaca et al. (2002) to examine genetic diversity for several released forage bermudagrass varieties and

related selections. From total 1,423 fragments evaluated, 472 (33%) were polymorphic, showing minimal genetic diversity in forage varieties of bermudagrass. Karaca and Ince (2008) used minisatellites to classify bermudagrasses. All ten primers employed in this study amplified bermudagrass DNA with high reproducibility. Li et al. (2011) and Farsani et al. (2012) used inter-simple sequence repeat (ISSR) markers for evaluation of genetic diversity in bermudagrass accessions. Twenty-seven bermudagrass accessions and introductions were evaluated in Iran where 14 ISSR primers were used and 313 out of 389 fragments (80.5%) found to be polymorphic (Farsani et al., 2012). In China, cultivar 'Tift3' and 95 bermudagrass accessions were evaluated by 29 ISSR primers by Li et al. (2011). From total of 248 bands, 242 (97.6%) were polymorphic. The average genetic similarity coefficient across accessions was 0.74, with values ranging from 0.51 to 0.97. Accessions clustered into 11 different groups by the unweighted pair group method with arithmetic mean (Li et al., 2011). In 2017, sequence-related amplified polymorphism (SRAP) was used to determine genetic diversity and population structure of 157 bermudagrass genotypes. From total 340 bands, 328 (96.5%) were polymorphic resulted from 26 SRAP primer pairs. With a mean of 0.44, the polymorphic information content (PIC) fluctuated from 0.36 to 0.49 (Zheng et al., 2017). Sugarcane expressed sequence tag-simple sequence repeat (EST-SSR) were used for cross-taxon application to assess genetic diversity in bermudagrass. Ten 'Tif' series cultivars along with their parental species were used, and 70% (63) primer pairs were polymorphic for members of Tif diversity panel (Khanal et al., 2017).

Most recently, Fang et al. (2020) used genotyping-by-sequencing (GBS) in a *C. dactylon* Pers first generation selfed population, and with 3,544 SNP markers, a high-density genetic map of 18 linkage groups (LGs) was created.

Genome-Wide association Study (GWAS)

Genome-wide association studies use data from a diverse panel of individuals, with varying degrees of relatedness or geographical origin, to identify associations between markers and traits of interest (Lipka et al., 2015). The approach captures more historical recombination events, in contrast to bi-parental mapping, which has only one or two generations to generate the recombinations needed for mapping. In contrast, diverse GWAS panels may catch a greater number of historical recombination events that occurred during the evolution of the sampled individuals.

In GWAS panels, population structure and cryptic relatedness among individuals could lead to spurious marker-trait associations (Yu et al., 2006). Individuals in these panels often have a complicated relatedness, which can evolve naturally in the form of herds, colonies, ethnic groupings, or other sorts of aggregations, unlike bi-parental populations, which have a well-defined population structure dictated by the mating scheme. The basic statistical model used in QTL mapping is generally supplemented in GWAS with covariates for population structure and kinship to reduce false positive results and to boost statistical analysis power (Zhu et al., 2008). A plethora of analysis software and analysis tools, such as STRUCTURE (Pritchard et al., 2000), principal component analysis (PCA) (Price et al., 2006), and discriminant analysis of principal components (DAPC) (Jombart et al., 2010) are some common examples of approaches that employ genetic markers to assess population structure. Model selection is recommended to be carried out to find the appropriate number of fixed effect covariates in the GWAS model (Lipka et al., 2012).

In a diverse population, cryptic relatedness among individuals might lead to false associations. Cryptic relatedness refers to recent shared ancestry among smaller groups, whereas population structure relates to remote common ancestry of larger groups of individuals. According to Devlin and Roeder (1999), cryptic relatedness could be a more important confusing issue than population structure. As a result, using a kinship matrix as a random effect in a GWAS model is often beneficial (Kang et al., 2010; Yu et al., 2006).

There are numerous methods for calculating pairwise kinship coefficients among individuals in a diversity population. Thompson (1975) presented maximum likelihood estimates (MLEs) of the Cotterman coefficients, while Milligan (2003) studied MLEs under the Jacquard model in depth. When the number of markers is limited, these MLEs are prone to bias and can be computationally demanding to obtain, especially from genome-wide datasets (Ritland, 1996). To estimate identity-by-decent (IBD, i.e., the probability that alleles are exact copies of an ancestral allele), Loiselle et al. (1995) advocated using identity-by-state (i.e., alleles that are the same, irrespective of whether they are inherited from a recent ancestor or not), which is preferable since it has a biologically linked meaning (Lipka et al., 2015).

The statistical models are primarily based on the unified mixed, linear-model to find genotype-phenotype relationships in GWAS (Yu et al., 2006). Many different approaches for efficiently estimating variance components have been developed to lessen the computing load (Lippert et al., 2011; Wang et al., 2014). Efficient mixed-model association (EMMA) (Kang et al., 2008a), EMMA eXpedited (EMMAX) (Kang et al., 2010), population parameters previously determined (P3D) (Zhang et al., 2010), and compressed mixed linear model (Li et al., 2014; Zhang et al., 2010) are the most commonly used methods for GWAS of plant populations.

Although mixed models have been demonstrated to effectively handle the confounding effects of a large diffuse background of loci (with small effect), but they may not always account for loci with greater effects (Segura et al., 2012). Traditional linkage mapping uses multiple cofactors explicitly in the statistical model, but both composite interval mapping and multiple-QTL mapping outperform simple interval mapping. Segura et al. (2012) created the multi-locus mixed-model (MLMM) technique for genome-wide association studies based on this argument. The Fixed and random model circulating probability unification (FarmCPU) approach, that was recently introduced, allows for efficient computing, eliminates confounding, reduces model overfitting, and suppresses false positives (Liu et al., 2016). PLINK (Purcell et al., 2007), rrBLUP (Endelman, 2011), GWASTools (Gogarten et al., 2012), GAPIT (Lipka et al., 2012), MLMM (Segura et al., 2012), GAPIT Version2 (Tang et al., 2016), and TASSEL5 (Bradbury et al., 2007) are the most commonly used software for GWAS.

Genotyping-by-Sequencing

Genotyping-by-sequencing a is next-generation sequencing technology that is robust, cost-effective, and applicable to a wide range of species (Elshire et al., 2011; He et al., 2014). Costs of whole-genome sequencing have fallen considerably in recent years, and GBS can be used for high diversity and large genome species (Elshire et al., 2011). Restriction enzymes are used to reduce genome complexity, resulting in digested DNA, which is subsequently ligated with unique barcoded adaptors of four to ten base pairs in length. Then samples are pooled together and amplified through PCR. GBS libraries are sequenced on various platforms such as Illumina (San Diego, CA) and generate millions of reads. Downstream bioinformatic analysis and scientific programming are needed to mine high-quality SNPs from millions of raw sequences reads.

Plant breeders and geneticists can use GBS to genotype breeding populations quickly and cheaply, allowing them to conduct GWAS, genomic diversity studies, genetic linkage analysis, molecular marker identification, and genomic selection (GS) on a large scale. The GBS approach is robust across a range of species. SNP identification and genotyping are conducted simultaneously, and no prior knowledge of the species genomes is required (Narum et al., 2013; Poland and Rife, 2012). GBS, on the other hand, requires skills in big data processing. For the unprecedented volume of DNA data, complex bioinformatics analysis often necessitates high-performance computation (Bodi, 2011).

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CHAPTER II

GENOMIC DIVERSITY OF BERMUDAGRASS (*CYNODON* SPP.) REVEALED BY GENOTYPING-BY-SEQUENCING

Abstract

Bermudagrass (*Cynodon* spp.) breeding and cultivar development is hampered by limited information regarding its genetic and phenotypic diversity. To explore diversity in bermudagrass, a total of 206 *Cynodon* accessions consisting of 193 common bermudagrass (*C. dactylon* ssp. *dactylon*) and 13 African bermudagrass (*C. transvaalensis*) accessions of worldwide origin were assembled for genetic characterization. Genotyping-by-sequencing was employed for genetic marker development. With a minor allele frequency of 0.05 and a minimum call rate of 0.5, a total of 37,496 raw single nucleotide polymorphisms (SNPs) were called *de novo* and were used in the genetic diversity characterization. Population structure analysis using ADMIXTURE revealed four subpopulations in this germplasm panel, which was consistent with principal component analysis and phylogenetic analysis results. The first three principal components explained 15.6, 10.1, and 3.8 % of the variance in the germplasm panel, respectively. The first subpopulation consisted of *C. dactylon* accessions from various continents; the second subpopulation was comprised mainly of *C. transvaalensis* accessions; the third subpopulation contained *C. dactylon* accessions primarily of African origin; and the fourth subpopulation represented *C. dactylon* accessions obtained from the Oklahoma State University bermudagrass breeding program. Genetic diversity parameters including nucleotide diversity or average

pairwise divergence (π), estimated mutation rate or expected nucleotide diversity (θ), Tajima's D statistic, and Fst statistic revealed substantial genetic variation in the *Cynodon* accessions, demonstrating the potential of this germplasm panel for further genetic studies and cultivar development in breeding programs.

Introduction

Cynodon L. C. Rich. is a genus consisting of nine warm-season grass species in the Cynodonteae tribe, Chloridoideae subfamily, and grass family (Poaceae) (Harlan et al., 1970; Wu, 2011). *Cynodon* is characterized by a globally extensive distribution and harbors rich genetic diversity. Among these species, common bermudagrass (*C. dactylon* ssp. *dactylon*) is the most important species economically and ecologically. *Cynodon dactylon* ssp. *dactylon* is a C_4 perennial grass used as a turfgrass, forage, and soil stabilizer (Harlan and Wet, 1969). It has also been reported to be used in ethno-medicinal and traditional medical practices (Shendye and Gurav, 2014). Another important species in the *Cynodon* genus is *Cynodon transvaalensis* Burtt-Davy, commonly known as African bermudagrass. African bermudagrass is not widely used for turfgrass due to high water and fertilizer input requirements and poor performance under extreme temperature conditions (Taliaferro, 1992). African bermudagrass does, however, contain useful traits (i.e., fine texture, high density, and tolerance to low mowing) for turf cultivar development, and it can readily cross with common bermudagrass to generate interspecific hybrids; thus, it is often used in turfgrass breeding programs.

Ploidy levels and chromosome numbers of the genus *Cynodon* vary widely. Flow cytometry studies indicated diploid to hexaploid levels in *Cynodon* (Gulsen et al. 2009; Grossman et al., 2021; Jewell et al. 2012; Kang et al., 2008; Wu et al., 2006). Forbes and Burton (1963) confirmed the base chromosome number as x = 9 in *Cynodon* species. According to the

widely accepted revised taxonomy of *Cynodon* (Harlan et al., 1970a), diploidy (2n = 2x = 18) and tetraploidy (2n = 4x = 36) are common but hexaploidy (2n = 6x = 54) is rare. Species including *C. barberi, C. dactylon* ssp. *aridus, C. incompletus* ssp. *incompletus, C. plectostachyus,* and *C. transvaalensis* are predominantly diploid (2n = 2x = 18). Species including *C. arcuatus, C. dactylon* ssp. *dactylon, C. dactylon* ssp. *coursii, C. dactylon* ssp. *elegans,* and *C. dactylon* ssp. *polevansii* are largely tetraploid (2n = 4x = 36). Other species with both diploid and tetraploid cytotypes are *C. aethiopicus, C. dactylon* ssp. *afghanicus, C. incompletus* ssp. *hirsutus, C. nlemfuensis* ssp. *nlemfuensis*, and *C. nlemfuensis* ssp. *robustus* (Harlan et al., 1970a). A majority of the common bermudagrass germplasm in breeding programs are tetraploids (Mutlu et al., 2014). Although the classification of *C. dactylon* species as allotetraploid versus autotetraploid is debated (Bethel et al., 2006; Chaves et al., 2022; Fang et al., 2020; Guo et al., 2015; Harlan and de Wet, 1969; Harris-Shultz et al., 2010; Khanal et al., 2017), a recent high-density genetic map developed using genotyping-by-sequencing (GBS) provides a clearer interpretation of the *C. dactylon* allotetraploid structure (Fang et al., 2020).

Polyploidy creates higher genetic diversity in bermudagrass, and it has been reported that genetic diversity within-population was highest at low latitudes (Zhang et al., 2019). Population diversity, structure, and relationships can be studied using DNA markers, which are increasingly used in basic genomic studies and plant breeding programs. In previous studies, genetic diversity in *Cynodon* was largely assessed using traditional molecular markers like amplified length fragment polymorphism (AFLP) (Wu et al., 2006; Zhang et al., 1999), simple sequence repeat (SSR) (Ling et al., 2012; Wang et al., 2013), random amplified polymorphic DNA (RAPD) (Al-Humaid and Motawei., 2004), inter-simple sequence repeat (ISSR) (Farsani et al., 2011; Li et al., 2011), and sequence related amplified polymorphism (SRAP) markers (Huang et al., 2014;

Wang et al., 2009; Zheng et al., 2017). Molecular markers have been used to study variation among *Cynodon* species, bermudagrass genotypes, cultivar identification, and off-type confirmation (Assefa et al., 1999; Caetano-Anolles et al., 1995; Dong et al., 2022; Farsani et al., 2011; Karaca et al., 2002; Roodt et al., 2002; Wang et al., 2010; Zhang et al., 1999). The use of GBS to evaluate off-type grasses in hybrid bermudagrass has also been proposed (Reasor et al., 2018).

Genetic mapping studies are conducted to delineate the framework of chromosomes and provide resources to identify genomic regions that underly traits of interest in bermudagrass. The F_1 progeny population derived from *C. dactylon* 'T89' (4x = 36) × *C. transvaalensis* 'T574' (2x = 18) was analyzed to construct genetic maps for each parent (Bethel et al., 2006; Harris-Shultz et al., 2010; Khanal et al., 2017). Using the same population, early results regarding the genetic architecture of canopy height, stolon internode length, length of the longest stolon, and leaf traits (leaf length and leaf blade) were reported (Khanal et al., 2019). Recently, GBS was used in a first-generation, selfed common bermudagrass population, and a high-density genetic map with 3,544 single nucleotide polymorphism (SNP) markers was reported (Fang et al., 2020). More recently, a high-density genetic map for African bermudagrass was created using a GBS approach and quantitative trait loci (QTLs) for sod establishment rate were identified (Yu et al., 2021).

Although previous studies provided valuable insights into the genetic diversity, population structure, and genetic architecture of traits in *Cynodon*, most focused on small germplasm collections and accessions that were location or country specific, with low numbers of markers. Individual bi-parental or selfed populations only sample limited allelic diversity of selected parents. Therefore, a *Cynodon* germplasm panel encompassing accessions of worldwide origins

evaluated with a large volume of molecular markers would provide novel insights into the genomic diversity of this important genus. As such, a *Cynodon* germplasm panel of 206 accessions consisting of 193 common bermudagrass and 13 African bermudagrass accessions was assembled to study the genetic diversity at the genus level using the GBS approach. The objectives were to obtain high quality SNP markers and to assess the population structure, genetic relatedness, and evolutionary relationship among accessions of the germplasm panel, and to explore their potential for genome-wide association studies.

Materials and methods

Plant Materials

A total of 206 bermudagrass accessions (193 common bermudagrass and 13 African bermudagrass genotypes) were studied (Table A.1), among which 145 accessions were procured from the United States Department of Agriculture National Plant Germplasm System (USDA NPGS), 40 accessions were obtained from the Oklahoma State University (OSU) bermudagrass breeding program, and 21 accessions were from the Mississippi State University (MSU) germplasm collection. The ploidy level of accessions ranged from diploid to tetraploid. These accessions are of worldwide origin (covering five continents) representing different geographical locations (29 countries) and genetic diversity (Figure 2.1).

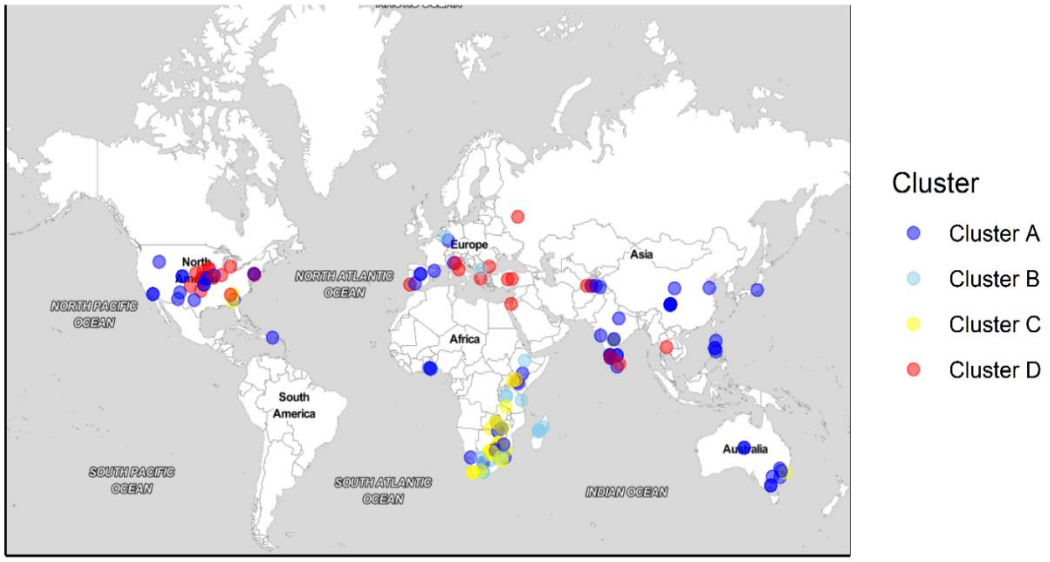

Figure 2.1 Geographic map of 183 accessions showing their worldwide location from five continents of the world and from where they have been collected. The accessions have been colored according to four subpopulations revealed by ADMIXTURE analysis.

DNA extraction, library construction, and genotyping-by-sequencing

The germplasm was cultured in separate containers under greenhouse conditions at Mississippi State University. Plant leaf material was harvested and freeze-dried. Leaf samples were then shipped to the University of Minnesota Genomic Center (UMGC), where DNA extraction, library preparation, and sequencing were performed. Genomic sequencing libraries were prepared following the GBS protocol with *Ape*KI enzyme (Elshire et al., 2011). A total of 206 unique barcodes (*i.e.*, corresponding to 206 bermudagrass accessions) were ligated to fragmented DNA sequences. DNA libraries were sequenced with 101 bp single end reads on an Illumina Novaseq SP platform at UMGC.

SNP calling

Raw sequence data were investigated for base quality using FastQC v0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). All GBS libraries were sequenced with high-quality reads. The raw GBS data generated by the UMGC did not have inline barcodes, but instead contain variable length padding sequences (0–10 bp) added in front of the enzyme cut site. Therefore, a Perl script (gbstrim.pl, https://bitbucket.org/jgarbe/gbstrim/src/master/) was used to trim padding sequences. The SNPs were then called *de novo* using the UNEAK pipeline (Lu et al., 2013) of TASSEL 3 standalone. Calling parameters included an error tolerance rate of 0.03, a minor allele frequency of 0.05, and a minimum call rate of 0.5 (i.e., no more than 50% of the bermudagrass accessions in the germplasm panel should have missing data at a given data point).

Population Structure and Genetic Diversity Analysis

Data were analyzed using the software ADMIXTURE (Alexander et al., 2009), TASSEL 5.2.77 (Bradbury et al., 2007), adegenet (Jombart et al., 2010; Jombart and Ahmed, 2011), ape (Paradis et al., 2004), pegas (Paradis , 2010), and customized R scripts for plot visualization. To evaluate the hierarchical population structure, a model-based estimation of admixed ancestry with K (subpopulations) ranging from 1 to 10 was conducted in ADMIXTURE v1.3 (Alexander et al., 2009). The optimum number of subpopulations was determined based on a five-fold cross-validation (CV) at K with a minimum CV error. Pedigree relationship and ancestry coefficients (Q) from the optimum K were used for visualization of population structure. Principal component analysis (PCA) was also conducted in TASSEL 5.2.77 (Bradbury et al., 2007), and the resulting PC matrix was visualized in R with customized scripts. An identity by state (IBS) matrix was calculated in TASSEL, and a neighbor joining (NJ) tree was visualized in Interactive

Tree Of Life (iTOL) v5 (Letunic and Bork, 2021). Based on the results of population structure analyses, fixation index (*Fst*) between subpopulations were calculated using the adegenet package (Jombart et al., 2010; Jombart and Ahmed, 2011). Observed nucleotide diversity or average pairwise divergence (π), estimated mutation rate or expected nucleotide diversity (θ), and Tajima's D statistic were also calculated for all subpopulations as well as for the whole panel using TASSEL v5.2.77.

Results

SNP calling

A total of 600,380,494 reads were generated, with a mean of 2.9 million reads per sample. After trimming padding sequences, a total of 537,127,057 reads were retained. The UNEAK pipeline identified a total of 536,993,374 reads that contained an *Ape*KI enzyme cut site remnant and a barcode sequence. After variant calling, a total of 37,496 raw SNPs were obtained with minor allele frequency of 0.05 and minimum call rate of 0.5. Across these SNPs, 23,324 (62.2%) have transition substitutions, whereas 14,172 (37.7%) have transversions—a transition to transversions ratio (Ts/Tv) of 1.64:1. The frequency of transitions and transversions observed is given in Table A.2.

Population structure

One of the major objectives of this study was to assess the relationship among 206 accessions and determine the overall population structure. A total of 37,496 SNPs were analyzed in ADMIXTURE and PCA. ADMIXTURE revealed four bermudagrass subpopulations (K=4) based on cross-validations, which were designated as subpopulations 1–4 (Figure 2.2; Figure A.1). In brief, subpopulation 1 (84 accessions; 40.7% of the germplasm panel) was comprised of

C. *dactylon* accessions collected from seventeen different countries ranging across Africa, Asia, North America, Australia, and Europe (Table A.1). Two *C. transvaalensis* accessions (PI 647879 and PI 286584) were also assigned to subpopulation 1, suggesting admixture or possibly misidentification of these two accessions. Subpopulation 2 consisted of 36 (17.4%) accessions—22 of African origin, 12 from the United States, and two from Europe. In this cluster, 9 out of 13 *C. transvaalensis* accessions clustered with 27 *C. dactylon* accessions. The third cluster (subpopulation 3) contained 54 (26.2%) accessions—52 of these accessions were *C. dactylon* and two were *C. transvaalensis* (PI 289922 and PI 647878). Again, hybridization or misidentification should be considered for these two *C. transvaalensis.* A majority of the accessions in subpopulation 3 were from five African countries, with two from the U.S., one from India, and one from Australia. The fourth cluster (subpopulation 4) contained the remaining 32 (15.5%) accessions of solely *C. dactylon*. In subpopulation 4, 17 accessions collected in the United States by the Oklahoma State University turfgrass breeding program grouped with 14 accessions collected in neighboring European and Asian countries maintained at the USDA NPGS (Griffin, GA, USA).

Similar patterns were found in PCA and phylogenetic analyses (Figures 2.3, 2.4), corroborating K=4 in this *Cynodon* germplasm panel. Principal component (PC) analysis indicated that PC1, PC2, and PC3 explained 15.6, 10.1, and 3.8% of the genetic variation in the panel, respectively (Figure 2.3). Although there is admixture observed in the PCA analysis (Figure 2.3), the four clusters can still be distinctly observed. The relatedness and evolutionary relationship of *Cynodon* accessions are shown in Figure 2.4. The four groups in the phylogenetic

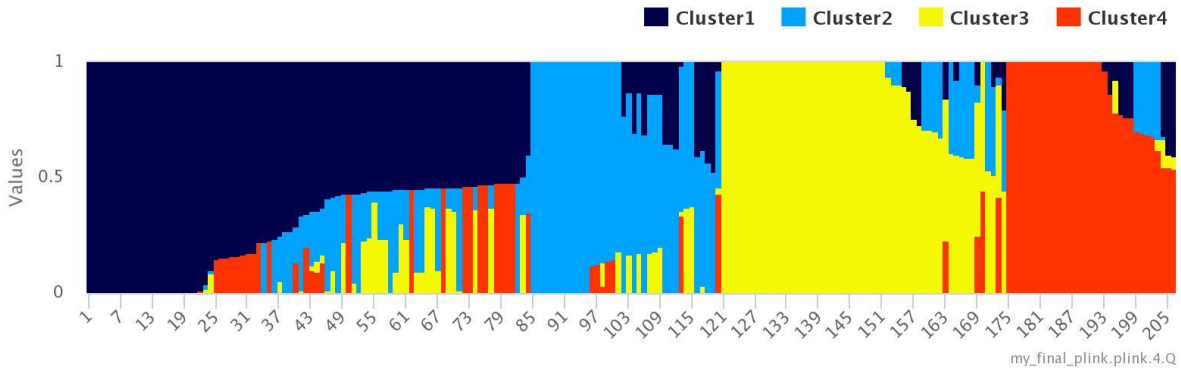

Figure 2.2 ADMIXTURE analysis determines that four subpopulations exist in the bermudagrass (*Cynodon* spp.) germplasm panel. The figure is a bar plot produced from ADMIXTURE software analysis where each bar is referring to each individual from the bermudagrass germplasm panel of 206 accessions. Analysis exhibited four subpopulations (K=4) in the panel on the basis of genome-wide SNP markers.

tree were named as A–D (note that some inconsistencies were present but that these generally correspond to 1–4 subpopulations from ADMIXTURE, in corresponding alphanumerical sequence). Detailed comparison between ADMIXTURE results and phylogenetic analysis indicated that groupings of accessions were largely consistent, except for a few differences and for subpopulation 2 (Figure 2.4). In brief, all the accessions of subpopulation 1 from ADMIXTURE also grouped together in the phylogenetic group A, together with the ten *C. dactylon* accessions from the Mississippi State University turfgrass breeding program, which were assigned to subpopulation 2 in ADMIXTURE. The group B in the phylogenetic tree corresponded to ADMIXTURE subpopulation 2 but contained three accessions from subpopulation 3. The group C of phylogenetic tree was found to be consistent with ADMIXTURE subpopulation 3, along with three accessions from subpopulation 2. The

phylogenetic group D corresponds to ADMIXTURE subpopulation 4 but also has four accessions from subpopulation 3 and seven accessions from subpopulation 2.

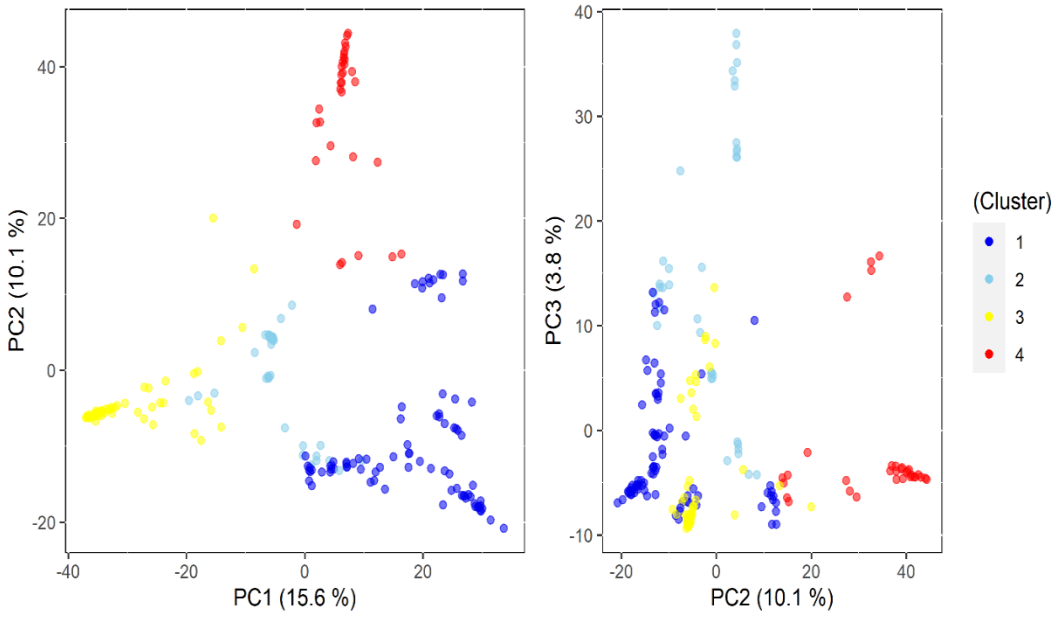

Figure 2.3 Principal component (PC) plot extracted from genome-wide SNP markers for bermudagrass (*Cynodon* spp.) germplasm panel of 206 accessions. In the figure each dot is representing a single accession. The first three principal components explained 15.6, 10.1, and 3.8 % of the variance in the germplasm panel. The results are consistent with the ADMIXTURE analysis.

Fixation index evaluates genetic differences between subgroups within a population. In plants, Fst values greater than 0.15 are considered to significantly differ while values below 0.05 are considered insignificant (Frankham et al., 2016; Hartl and Clark, 1997). Fixation index value between subpopulations 3 and 4 is 0.4553 (Table 2.2). Subpopulation 3 mainly consisted of accessions of African origin (tropical regions), while subpopulation 4 contained accessions primarily from the OSU breeding program; thus, most of the OSU accessions in this study were collected from the US Midwest (temperate regions). These high genetic differences observed between subpopulations 3 and 4 are intriguing, which may suggest that the OSU *Cynodon*

accessions could have been enriched for local selective advantages, such as cold tolerance genes, that shaped the genetic difference between these germplasm materials and other *Cynodon* accessions.

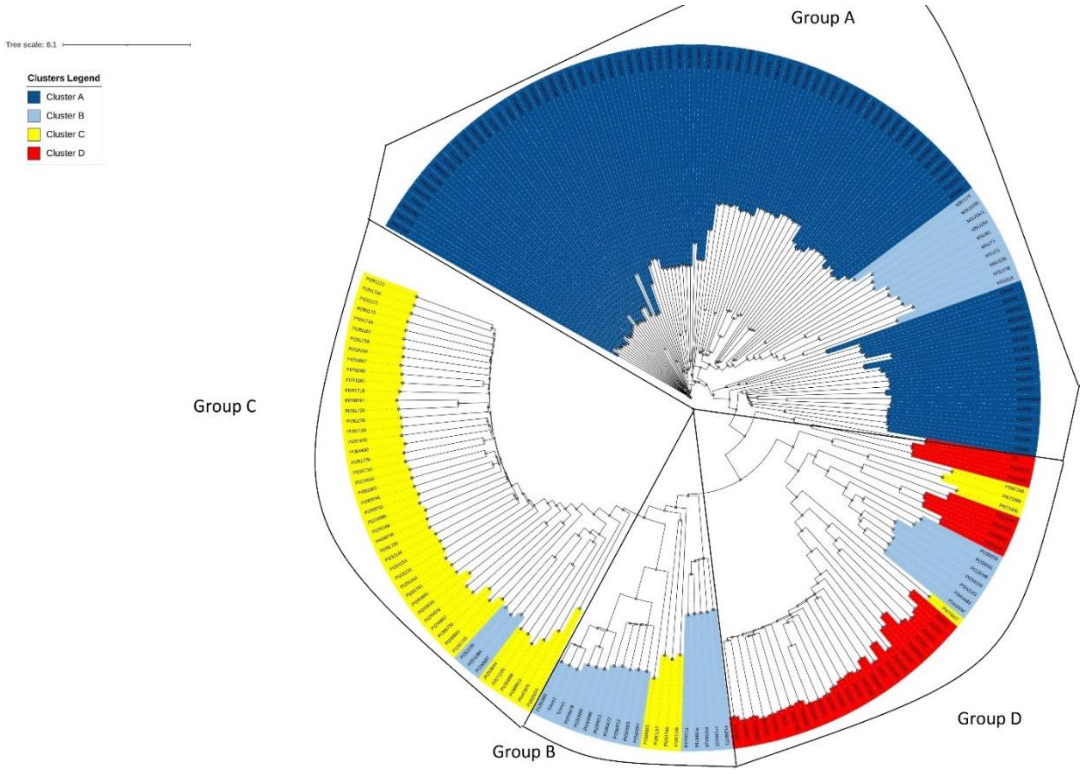

Figure 2.4 Phylogenetic tree exhibiting relatedness and evolutionary relationship of accessions in the bermudagrass (*Cynodon* spp.) germplasm panel. The *Cynodon* grasses have been colored according to four subpopulations (1–4) revealed by ADMIXTURE analysis. The four distinct groups (A–D) has been marked according to phylogenetic clusters and detailed comparison between ADMIXTURE results and phylogenetic analysis indicated that groupings of accessions were largely consistent. Each branch end represents a grass accession. Genetically similar grasses are grouped into clusters. Two grasses are more closely related if they converge with a shorter path.

Molecular variation

Plant breeding practices rely on genetic variation in order to develop new cultivars with different genetic combinations including improved traits. Molecular markers provide reliable tools to evaluate genetic variation in germplasm collections. Various parameters of genetic diversity in this germplasm collection are shown in Table 2.1. The highest number of segregating sites were found in subpopulation 1 (n = 35343), followed by subpopulation 3 (n = 28240), 2 (n = 25907) and 4 (n = 21782). Interestingly, the values of θ, π, and Tajima's D for subpopulation 1 and subpopulation 2 are similar even though they are genetically different according to ADMIXTURE results and have different numbers of segregating sites. The negative values of Tajima's D for subpopulations 3 and 4 indicate that selection might have removed variation in these subpopulations or possibly indicate a bottleneck followed by recent population expansion. On the other hand, positive values of Tajima's D for subpopulation 1, 2, and the overall panel (population) suggests that selection maintained the variation.

Table 2.1 Population structure in the bermudagrass (*Cynodon* spp.) germplasm panel and genetic diversity index of the four subpopulations revealed by ADMIXTURE analysis and for the whole germplasm panel. Genetic diversity parameters revealed substantial genetic variation in *Cynodon* accessions.

Subpopulation (K)	No. of accessions	SegSites	Theta	Pi	Tajims's D
Subpopulation 1	84	35343	0.21	0.21	0.11
Subpopulation 2	36	25907	0.22	0.22	0.11
Subpopulation 3	54	28240	0.19	0.17	-0.30
Subpopulation 4	32	21782	0.18	0.18	-0.11
Whole Panel	206	37496	0.19	0.27	1.37

Table 2.2 Pairwise fixation index (Fst) values indicating differentiation among four subpopulations in the germplasm panel. Fst values greater than 0.15 indicates that subpopulations significantly differ from each other while values below 0.05 indicates subpopulations differs insignificantly.

	Subpopulation 4	Subpopulation 1	Subpopulation 2
Subpopulation 1	0.33		
Subpopulation 2	0.34	0.20	
Subpopulation 3	0.45	0.36	0.23

Discussion

Despite recent advancements in high throughput sequencing technologies, genomic resources remain limited in bermudagrass and many other specialty crops. Most previous studies characterizing genetic variation and diversity in *Cynodon* used traditional markers such as AFLP, RAPD, ISSR, SRAP and SSRs (Farsani et al., 2011; Ling et al., 2012; Wang et al., 2013; Wu et al., 2006; Zheng et al., 2017). Albeit effective and reproducible, data collection from traditional molecular markers is time-consuming and labor-intensive. In this study, we used GBS technology and obtained a total of 37,496 high-quality SNP markers to evaluate the genomic diversity in 206 bermudagrass accessions from five continents and 29 countries of the world. GBS has identified genome-wide molecular markers at low cost, and this technology is advantageous for breeders of species like bermudagrass in which limited genomic information is available (Fang et al., 2020; Kim et al., 2016).

Previous bermudagrass characterization studies were limited due to the small size or limited geographic coverage of populations studied with small numbers of genetic loci sampled, leading to a limited scope in understanding the genetic structure and variations in *Cynodon*. For example, Wu et al. (2004) surveyed the genetic diversity of 28 accessions from 11 countries

while Jewell et al. (2012) studied 690 accessions, all collected from Australia. The present study used a large worldwide germplasm collection, presumably overcoming limitations of previous studies. Four genetic groups were identified in this *Cynodon* germplasm panel (Figures 2.2, 2.3). Similar results were obtained from ADMIXTURE analysis, PCA, and phylogenetic analysis, which corroborates the validity of the genetic structure in this study. Subpopulation 1 consisted of accessions from different geographic locations (Figure 2.2). Such grouping of genetic materials from different locations was likely due to the exchange of breeding materials between programs and common use of materials from the USDA NPGS, which were originally contributed by multiple *Cynodon* research groups. Therefore, many of the studied *Cynodon* accessions may share common ancestry at various levels.

In this germplasm panel, we included 13 African bermudagrass (*C. transvaalensis*) accessions, many of these accessions clustered in subpopulation 2 with *C. dactylon* of African origin (Figure 2.2). As *C. dactylon* plants are predominantly tetraploids and *C. transvaalensis* are diploids, mixture of *C. dactylon* and *C. transvaalensis* in this subpopulation may shed light on the polyploidization origin of *C. dactylon*. This finding is novel. We were surprised to find the common bermudagrass and African bermudagrass accessions were grouped together in the same cluster instead of forming two separate groupings relative to other common bermudagrass accessions. It is well known that common bermudagrass can readily cross with African bermudagrass. Their hybrids, however, are sterile triploids, which cannot backcross with common bermudagrass nor African bermudagrass, indicating a strong gene flow barrier between the two species, or that the two species have independent evolutionary pathways. One reasonable speculation is that ancestor(s) of modern diploid African bermudagrass may have substantially contributed to the formation of tetraploid common bermudagrass. When whole genome

sequences of the two species are available, comparative analysis may validate this hypothesis. In *Miscanthus* (Panicoideae subfamily), two major species, *M. sinensis* (largely diploid) and *M. sacchariflorus* (largely tetraploid) are used for biomass breeding. Genetic analyses in these two *Miscanthus* species revealed unidirectional gradient introgression from diploid *M. sinensis* to tetraploid *M. sacchariflorus* (Clark et al., 2019). This finding also supports the current mainstream hypothesis that Africa is the center of origin of *Cynodon* (Burton, 1948; Cui et al., 2021). In subpopulation 3, three *C. transvaalensis* accessions (PI 251108, PI 291964, and PI 290887) grouped with 46 *C. dactylon* (Figure 2.2). In addition to the possibility of close relatedness between these three *C. transvaalensis* accessions and the *C. dactylon* in this group, other reasons may include potential mislabeling of species and contamination of materials. Inevitably, further detailed investigation of accessions is needed.

In subpopulation 4 (Figure 2.2), most accessions from the Midwest and northeastern regions in the US grouped with accessions from European countries and cold/temperate areas in Asia. Bermudagrass is not native to the US (Taliaferro, 1995). This result suggests that the cold hardy, naturalized germplasm collected in OK, KS, NE, IL, MI, MO, and NJ are genetically similar to cold hardy germplasm in Europe and Asia. The genotype A12193 used in this study was collected on the campus of Michigan State University, East Lansing, MI. Bermudagrass on the campus of Michigan State University was a single clone, which was introduced by W. J. Beal in the 1880s. This bermudagrass is amongst the earliest bermudagrass introduced to the region (Gilstrap, 2002; Gilstrap 2012). The current study indicates that this bermudagrass accession most likely came from Europe or Asia, instead of Africa as originally speculated (Gilstrap, 2002).

Principal component analysis, ADMIXTURE and phylogenetic analysis all support that most of African accessions formed a unique cluster with the remaining accessions exhibiting admixture (Figures 2.2 – 2.4). The distinct grouping of African accessions indicates that unique genetic variations exist in this germplasm and that they may have important implications for *Cynodon* breeding. Due to the non-admixture of African accessions with others, they may not have been widely used in breeding programs to date. Genetic diversity within breeding programs decreases due to selection, small population size, genetic drift, and other factors (Fu, 2015; Reif et al., 2005). These accessions can be used in breeding programs to increase genetic variation. Furthermore, most of the African accessions within this PCA cluster are from four nearby countries (South Africa, Zambia, Zimbabwe, Mozambique) in southern Africa, and one accession is from Kenya (East Africa) (Table A.1). It is reasonable to hypothesize that other African countries could potentially contribute unique and diverse germplasm as well.

Results shed new light on the introduction history and origin of unknown accessions of bermudagrass. For example, in subpopulation 2, most of the African accessions clustered together except for 12 accessions from the US and two from Europe. This supports the hypothesis that bermudagrass was introduced to colonial America from Africa (Nelson and Burns, 2006). There is a possibility that many *Cynodon* accessions in North America or other places are still genetically similar to the ones from their African origin. The geographic origin of some accessions was unknown, especially the MSU accessions. Inferences from the ADMIXTURE analysis and phylogenetic tree could shed light on the origin of these accessions (Figures 2.2 and 2.4). Twelve of the MSU accessions were clustered in subpopulation 2 with African bermudagrass, indicating the origin might be in Africa. Most of the MSU accessions resulted from hybridizations made during the past 30 years, in the breeding program and are full-

or half-siblings, and thus they clustered closely together. The subpopulation 2 from ADMIXTURE is spread all over the phylogenetic tree in different groups suggests that this subpopulation is related to other subpopulations or may contain admixture from other subpopulations.

The 37,496 high-quality SNPs used in the current study allow us to survey more genomic regions of the *Cynodon* genome and enhances our understanding of this underexplored genus. Moreover, SNP data across the genome in this study assist in the clarification of *Cynodon* accessions with conflicting information and can help in clarifying the misidentification of accessions. As an example, PI 290812, PI 290813, and PI 290872 (GRIN Global) accessions are currently named as *C. nlemfuensis* by Grossman et al. (2021) while the USDA database lists them as *C. transvaalensis* Burtt Davy. In this study, these three accessions were grouped closely with *C. transvaalensis* (Figure 2.4), supporting the USDA data curation.

Conclusions

This study revealed extensive genetic diversity in the two *Cynodon* species important to breeding turfgrass cultivars by exploiting a bermudagrass germplasm panel of worldwide origin. To the best of our knowledge, this study reports the highest number of genome-wide SNP markers in a bermudagrass germplasm study. Strong and distinct genetic structure, as revealed by multiple analyses, indicates rich genetic variation for further improvement and development of new bermudagrass cultivars to reach a new adaptive peak. The grouping of African bermudagrass with common bermudagrass populations originating in Africa is a new finding and suggests significance in evolution and adaptation in the Africa continent. This study reveals that naturalized bermudagrass in the Midwest and Northeast of the US were genetically similar and likely introductions from Europe or Asia. The separate groupings of common bermudagrass

accessions provides information to breeders and geneticists aiding in parental line selection for developing breeding populations. This study provides a valuable guide for allele mining of desirable genes for the traits of interest (e.g., abiotic and biotic stress resistance), which will improve bermudagrass breeding and serve as foundation for genetic mapping of important traits.

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CHAPTER III

GENETIC DISSECTION OF MORPHOLOGICAL TRAITS IN BERMUDAGRASS GERMPLASM PANEL

Introduction

A critical issue in turfgrass breeding is the lack of information regarding the genetic architecture of traits. Common bermudagrass (*Cynodon dactylon*) and African bermudagrass (*C. transvaalensis*) are the two primary species used in developing improved bermudagrass cultivars within the genus *Cynodon*. Although bermudagrass is globally distributed between 45° S. Lat. and 53° N. Lat., and harbors enormous genetic diversity (Wu et al., 2004), previous genetic studies of bermudagrass have largely relied on single bi-parental or selfed populations (Khanal et al., 2017). In one study, an F_1 progeny population (3x) derived from *C. dactylon* 'T89' (4x = 36) × *C. transvaalensis* 'T574' (2x = 18) was analyzed to construct two genetic maps, one for each parent, with single-dose restriction fragments (Bethel et al., 2006). Two additional genetic linkage maps were published based on the same population ('T89' × 'T574') by adding more molecular markers (Harris-Shultz et al., 2010; Khanal et al., 2017). Yu et al. (2021) developed the first high-density genetic map for African bermudagrass using genotyping-by-sequencing (GBS) and identified quantitative trait loci (QTL) for sod establishment rate. In common bermudagrass, Guo et al. (2017) developed simple sequence repeats (SSR) markers, constructed a genetic linkage map, and identified genomic regions associated with establishment

rate in a first-generation selfed common bermudagrass population. Fang et al. (2020) enriched the common bermudagrass genetic map with 3,544 molecular markers by GBS.

The morphological traits of the plant are important traits in turfgrass and forage breeding. According to de deKroon et al. (1994), bermudagrass stolon morphological traits are under genetic control. The influence of these morphological traits on turfgrass quality and cold tolerance has been studied by various researchers (Anderson et al., 2007; Roche and Loch, 2005). However, most of these studies highlighting the importance of morphological traits were performed on a few commercial cultivars. Khanal et al. (2019) used QTL mapping study 110 F_1 individuals derived from a cross between *C. dactylon* ('T89') and *C. transvaalensis* ('T574') to assess genetic architecture of foliage (canopy height), stolon internode length, length of the longest stolon, and leaf traits (leaf length and leaf blade).

Despite its success, QTL mapping suffers from two fundamental limitations: 1) only allelic diversity that segregate between the parents of the particular cross can be assayed, and 2) the amount of recombination that occurs during the creation of the population places a limit on the mapping resolution (Korte and Ashley, 2013). Genome-wide association studies (GWAS) overcome these two limitations of QTL mapping. Generally, GWAS can serve as a foundation experiment by providing insights into the genetic architecture of a trait, exploring allelic diversity in the species, and suggesting candidates for mutagenesis and transgenics. To date, no GWAS has been conducted in bermudagrass.

The problems defined here are highly relevant to the game of golf and larger green industry. Better understanding of the genetics of these architectural traits in bermudagrass will

accelerate breeding cycles, improve decision-making, and enhance the competitiveness of turfgrass as a specialty crop. To the best of our knowledge, this study represents the first GWAS in bermudagrass research. Therefore, the objective of this study was to 1) evaluate leaf length, leaf width, internode distance, and stem diameter in a germplasm panel of bermudagrass accessions; 2) perform genome-wide association study to dissect the genetic basis of the traits.

Materials and Methods

Plant Materials

A germplasm panel of 206 bermudagrass accessions of worldwide origin was assembled. Some materials were recently collected in the northern U.S. (IL, NE, NJ, and MO) during the unusually harsh winter of 2020-21. Phenotypic data of 193 common bermudagrass accessions was collected in greenhouse (2021) and field (2022) conditions at Mississippi State University, Starkville, MS.

Phenotypic data collection

Bermudagrass accessions were planted in a 15.2 cm. injection molded nursery pots (Greenhouse Megastore, Danville, IL) containing Promix Potting Mix (Promix® Premier Tech Horticulture, Quebec, Canada) in 2021. Research was conducted as a completely randomized design with three replications in a climate-controlled greenhouse (33°27’11.9” N, 88°47’39.6” W) maintained at average daily temperatures of 25/18°C (day/night). Plants were irrigated as needed to prevent drought stress. Plants were fertilized once every two weeks with a water-soluble complete fertilizer at 48.8 kg nitrogen ha^{-1} (Miracle-Gro® Water-Soluble All-Purpose Plant Food; Scotts Miracle-Gro Products, Inc., Marysville, OH). Accessions were assessed in

field conditions the following year where research was conducted as a completely randomized design with three replications. Each accession was grown in 152.4 × 152.4 cm plots in a native Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquepts) soil at R. R. Foil Plant Science Farm in Starkville, MS. Irrigation was given twice a week to prevent drought stress. Plots were fertilized once a month at 48.8 kg nitrogen ha-1 with Greenview® Fertilizer (Lebanon Seaboard Corporation, Lebanon, PA). Pest management was also performed regularly to keep the plants healthy.

Four morphological traits were measured by using vernier calipers: leaf length, leaf width, internode distance, and stem diameter. Mature plants were selected for measurement (three per replication; nine measurements for every trait from each accession in 2021 year and 6 measurements for every trait in 2022 year) and measurements were made only once in June month in both years. All the traits were measured in millimeters. Then mean of every trait from those measurements were used as phenotypic data to perform GWAS.

Genetic marker development

DNA extraction and GBS was conducted at the University of Minnesota Genomics Center. Single-end 100 base pair (bp) reads were generated on an Illumina NovaSeq platform. After processing the raw data, 37,496 single nucleotide polymorphism (SNP) markers were called *de novo* using the UNEAK pipeline (Lu et al., 2013) of TASSEL 3 standalone with minor allele frequency of 0.05, minimum call rate of 0.5. Then this HapMap was used as the genotypic data for performing GWAS.

Data analysis

Phenotypic Data

Data were analyzed in R and R studio (R Core Team, 2020). Summary statistics were calculated for all four traits measured in 2021 and 2022 year. The correlations coefficients were also calculated among traits using the corrplot and Performance Analytics R package.

GWAS

GWAS was performed for four morphological traits on the 191 *C. dactylon* genotypes grown in year 2021 and on the 193 *C. dactylon* genotypes grown in year 2022 separately (two accessions failed transplantation in 2021). All four traits were analyzed using multivariate GWAS (FarmCPU model) to identify significant marker-trait associations. To manage false positives and prevent the over-fitting issue, FarmCPU uses both the fixed-effect model and the random effect model. The Genomic Association and Prediction Integrated Tool (GAPIT) R package (Lipka et al., 2012) was used to perform GWAS. Manhattan and quantile-quantile (QQ) plots were visualized to look at the results by using the qqman R package (Turner, 2018).

Results and Discussion

The summary statistics for phenotypic data recorded in both years 2021 and 2022 provides an estimate for the phenotypic diversity available in this germplasm collection for four traits (Table 3.1). The correlations between traits were recorded for both 2021 and 2022 (Table 3.2). All correlation coefficients between traits for both years 2021 and 2022 were found to be positive and were greater than 0.4, indicating every trait is at least moderately correlated to every other trait.

Table 3.1 Summary statistics for four morphological traits in common bermudagrass (*Cynodon dactylon*) germplasm diversity panel. In 2021, data were recorded in greenhouse conditions and in 2022, data were recorded under field conditions at Starkville, MS. All measurements are in units of millimeters.

Trait	Year	Mean	Standard Error	Standard Deviation	Range		Median	1st Quart (25% Percentile)	3rd Quart (75% Percentile)
					Min	Max			
Leaf Length	2021	93.03	2.11	29.24	28.85	182.30	91.44	73.43	110.02
Leaf Width	2021	02.87	0.04	00.61	01.61	006.53	02.74	2.48	3.08
Internode Distance	2021	54.28	1.01	14.02	22.67	103.63	52.56	43.57	62.59
Stem Diameter	2021	01.20	0.01	00.25	00.79	003.49	01.16	1.06	1.29
Leaf Length	2022	58.83	1.84	25.61	19.78	141.61	52.72	39.67	72.39
Leaf Width	2022	03.16	0.05	00.79	01.60	007.04	03.04	2.60	3.53
Internode Distance	2022	42.80	1.12	15.67	09.33	093.33	37.66	30.88	50.44
Stem Diameter	2022	01.61	0.02	00.34	00.86	003.05	01.55	1.35	1.88

A reference genome was not available for bermudagrass, and *de novo* alignment provided 37,496 SNP markers. It was expected that GWAS would not be as informative as in the presence of reference genome or whole genome sequence. After completion of the GWAS, Manhattan plots and QQ plots were observed to record the significant marker-trait associations (Figures 3.1, 3.2, 3.3 ,3.4). GWAS was performed separately with both phenotypic data of 2021 and 2022. Significant SNPs were recorded and explained separately for both 2021 and 2022 phenotypic

data. A total of 38 marker-trait associations were found in 2021 data (Table B1), and 55 marker-trait associations were observed with the 2022 data (Table B2) for all the four traits studied.

Table 3.2 Correlation coefficients among traits across 2021 and 2022 phenotypic data of common bermudagrass (*Cynodon dactylon*) accessions. Trait notation is as follows: leaf length (LL), leaf width (LW), internode distance (ID), stem diameter (SD).

	LL 2021	LL 2022	LW 2021	LW 2022	ID 2021	ID 2022	SD 2021	SD 2022
LL 2021								
LL 2022	0.73***							
LW 2021	0.49***	0.33***						
LW 2022	0.52***	0.66***	0.74***					
ID 2021	0.62***	0.54***	0.60***	0.60***				
ID 2022	0.54***	0.68***	0.43***	0.69***	0.64***			
SD 2021	0.14*	0.06	0.49***	0.29***	0.27***	0.10		
SD 2022	0.60***	0.71***	0.45***	0.73***	0.57***	0.66***	0.17*	

Note: Each significance level is associated to a symbol.
p-values (0, 0.001, 0.01, 0.05, 0.1, 1) <=> symbols (“***”, “**”, “*”, “.”, " “)

For stem diameter, one significant SNP was recorded with the 2021 data and three significant SNPs were recorded with the 2022 data. For internode distance, 14 significant SNPs were recorded with the 2021 data and 17 significant SNPs were recorded with the 2022 data. Three SNPs TP309116, TP740749, TP901441 were found to be common in both 2021 and 2022 years for internode distance. In both years, the highest SNPs were found to be significantly

associated with leaf width. Twenty-three SNPs were associated with leaf width in 2021 data and 32 SNPs were significant with the 2022 data. A total of 14 SNPs were found to be common for both year's GWAS for leaf width. The leaf length trait has less significant SNPs in both years. In 2021, there were no significant SNP associated with leaf length but in year 2022 three SNPs were found to be significant with the leaf length.

Interestingly, there were some SNP markers that were significant for two traits. This is a possible explanation for the correlation found between phenotypic data of different traits. As these traits are quantitative in nature and they may be controlled by similar QTLs/genes. TP 52997 SNP was found to be significant for both leaf width and internode distance with 2021 data. Three SNPs (TP 359482, TP 740749, TP 901441) were found to be significant for both leaf width and internode distance with the 2022 data. The correlation coefficient for leaf width and internode distance was 0.6 with the 2021 phenotypic data and it was 0.69 with the 2022 data. From these results, it can hypothesize that similar genomic regions could be controlling these traits.

Due to the lack of a reference genome, the location and presence of these markers on the chromosome is unknown. However, for implementing marker-assisted selection, the exact location of the marker on chromosome is not critical. The information of association between the marker and the trait, can be used to making selections to generate new cultivars. The result from this study show that non-availability of a high-quality reference genome sequence is not limiting when using SNP markers from de novo alignment. Early results from association mapping like naïve GWAS can be obtained.

Large numbers of significant SNP markers (as it was found for leaf width) will create a peak on a specific chromosome once their specific position on that chromosome is determined. When a reference genome is available and along with stringent conditions for significance, the genomic regions associated with these traits can be determined.

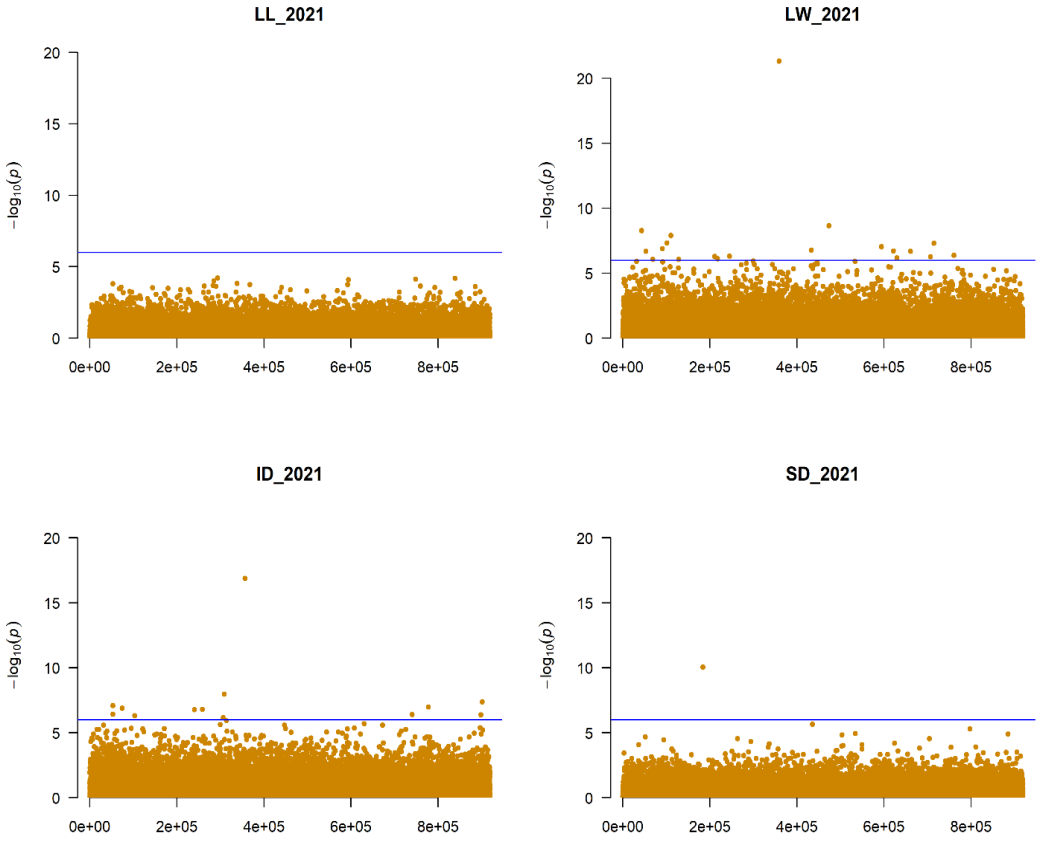

Figure 3.1 Manhattan plots of genome-wide associations for four morphological traits as per 2021 phenotypic data of common bermudagrass (*Cynodon dactylon*) accessions. Dots represent a SNP marker displayed on the x axis. The y axis is displaying negative logarithm of the p value of every SNP marker used in conducting the GWAS. The SNP markers that cross the threshold line are classified as significant. Trait notation is as follows: leaf length (LL), leaf width (LW), internode distance (ID), stem diameter (SD).

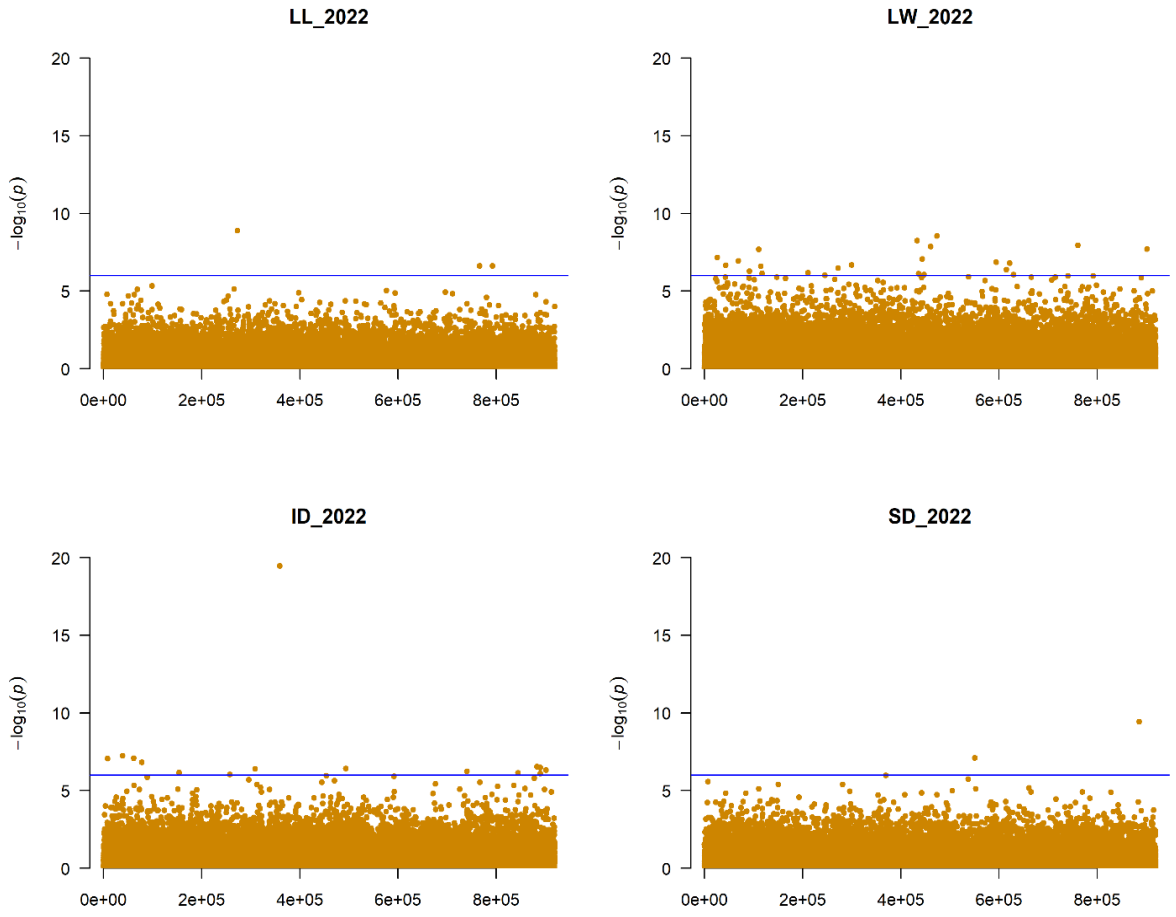

Figure 3.2 Manhattan plots of genome-wide associations for four morphological traits as per 2022 phenotypic data of common bermudagrass (*Cynodon dactylon*) accessions. Dots represent a SNP marker displayed on the x axis. The y axis is displaying negative logarithm of the p value of every SNP marker used in conducting the GWAS. The SNP markers that cross the threshold line are classified as significant. Trait notation is as follows: leaf length (LL), leaf width (LW), internode distance (ID), stem diameter (SD).

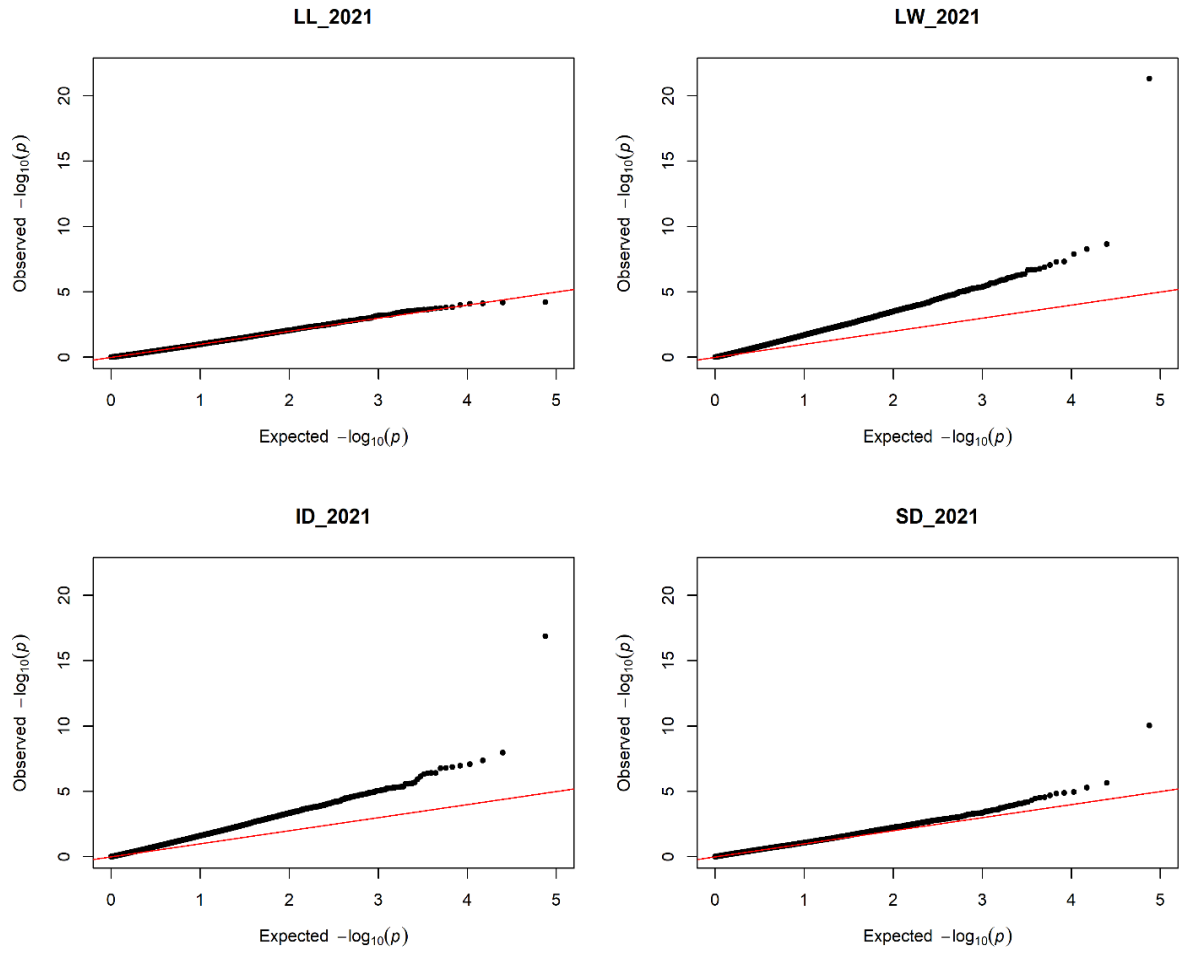

Figure 3.3 Quantile–quantile (QQ) plots of estimated −log10 (P) from genome-wide associations for four morphological traits as per 2021 phenotypic data of common bermudagrass accessions. The observed *P* values are expected to nearly follow the expected *P* values. Trait notation is as follows: leaf length (LL), leaf width (LW), internode distance (ID), stem diameter (SD).

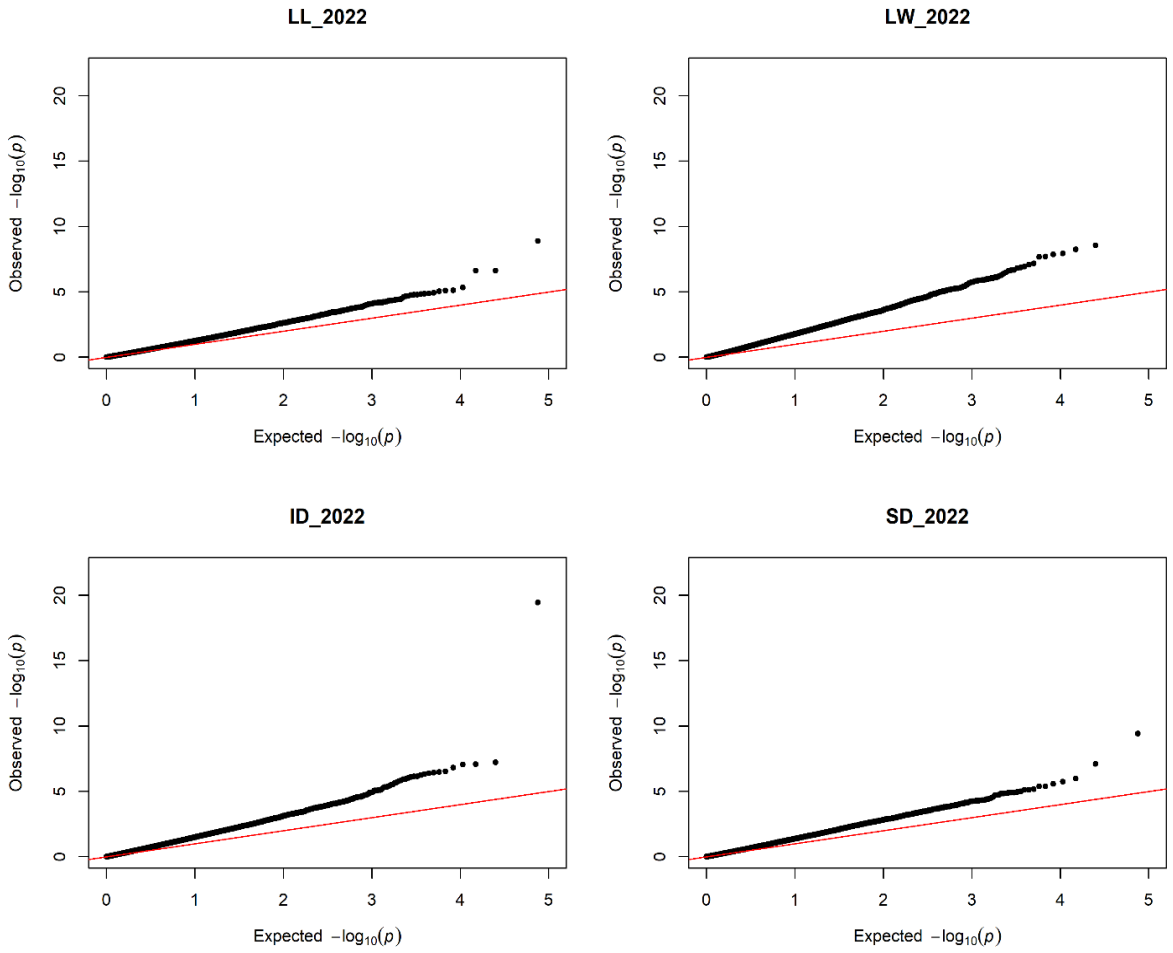

Figure 3.4 Quantile–quantile (QQ) plots of estimated −log10 (P) from genome-wide associations for four morphological traits as per 2022 phenotypic data of common bermudagrass accessions. The observed *P* values are expected to nearly follow the expected *P* values. Trait notation is as follows: leaf length (LL), leaf width (LW), internode distance (ID), stem diameter (SD).

This study was the first GWAS conducted in bermudagrass. The capacity to predict phenotypes from a genome-wide set of markers will putatively have substantial influence on bermudagrass breeding efforts. African bermudagrass is known for its fine leaves (Harlan et al.,1970). The germplasm of African bermudagrass can be explored for these traits in the future.

There is a paucity of studies on African bermudagrass, which plays an important role in commercial bermudagrass breeding efforts.

The bermudagrass germplasm panel in this study had substantial diversity in terms of traits that are important to turfgrass development. The genotypes of this panel can be phenotyped at different locations (environments) to determine the stable QTLs for specific traits. Abiotic stress tolerance is in demand for bermudagrass breeding. This panel contained accessions collected from temperate latitudes of United States, Europe, and Asia. To obtain additional insight into stress traits (i.e., cold, heat and drought tolerance) germplasm can be tested at northern locations to record the traits related to abiotic stresses. In this way, this germplasm panel can be good asset for future studies.

Conclusion

GWAS technology for morphological traits provided detailed insights into the genetic architecture of bermudagrass traits. This information can be used by breeders to exploit the available germplasm efficiently. Novel SNP markers were identified that are associated with morphological traits of common bermudagrass accessions. Positive correlations were observed among all the traits in this study. Future use of multi-environment and multi-year phenotypic data, and availability of reference genome, will determine stable QTLs across environments with their specific position on the chromosomes.

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APPENDIX A

ADDITIONAL TABLES AND FIGURES (CHAPTER 2)

Table A.1 Dataset contains source information (ID, species, ploidy, program, origin, genome size, Subpop (subpopulation)) of all 206 bermudagrass (*Cynodon* spp.) accessions used in the study. In this germplasm panel collection 145 accessions were procured from United States Department of Agriculture National Plant Germplasm System (USDA NPGS), 40 accessions are from Oklahoma State University (OSU) breeding program and 21 accessions are from Mississippi State University (MSU) germplasm collection.

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
A12193	4	*C. dactylon*		OSU	North America	Campus of Michigan State University, United States	
A12269	1	*C. dactylon*		OSU	Asia	China	2.05
A12281	1	*C. dactylon*		OSU	Asia	China	2.2
A12313	1	*C. dactylon*		OSU	Asia	China	2.04
A12342	1	*Cynodon dactylon*		OSU	Asia	China	2.06
A12347	1	*Cynodon dactylon*		OSU	Asia	China	2.12
A12367	1	*Cynodon dactylon*		OSU	Asia	China	2.09
A12378	1	*Cynodon dactylon*		OSU	Australia	Australia	
A12395	1	*Cynodon dactylon*		OSU	North America	Puerto Rico, United States	
A12397	4	*Cynodon dactylon*		OSU	North America	Kearney, NE, United States	
A12398	4	*Cynodon dactylon*		OSU	North America	Omaha, NE, United States	
A12402	4	*Cynodon dactylon*		OSU	North America	Ames, IA, United States	

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
A12405	4	*Cynodon dactylon*		OSU	North America	Ames, IA, United States	
A12406	4	*Cynodon dactylon*		OSU	North America	Kansas City, KS, United States	
A12407	4	*Cynodon dactylon*		OSU	North America	Kansas City, MO, United States	
A12408	1	*Cynodon dactylon*		OSU	North America	Denver, CO, United States	
A12422	4	*Cynodon dactylon*		OSU	North America	Urbana, IL, United States	
A12423	1	*Cynodon dactylon*		OSU	North America	Quincy, IL, United States	
A12424	4	*Cynodon dactylon*		OSU	North America	Manhattan, KS, United States	
A12425	4	*Cynodon dactylon*		OSU	North America	Manhattan, KS, United States	
A12426	1	*Cynodon dactylon*		OSU	North America	Kansas City, MO, United States	
A12427	1	*Cynodon dactylon*		OSU	North America	Kansas City, MO, United States	
A12428	4	*Cynodon dactylon*		OSU	North America	Mexico, MO, United States	
A12429	4	*Cynodon dactylon*		OSU	North America	Guymon, OK, United States	
A12430	1	*Cynodon dactylon*		OSU	North America	Denver, CO, United States	

Table A.1 (Continued)

Taxa	Subpop	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
A12431	4	*Cynodon dactylon*		OSU	North America	New Brunswick, NJ, United States	
A12432	4	*Cynodon dactylon*		OSU	North America	Adelphia, NJ, United States	
A12433	1	*Cynodon dactylon*		OSU	North America	New Brunswick, NJ, United States	
A12434	1	*Cynodon dactylon*		OSU	North America	Riverside, CA, United States	
A12435	1	*Cynodon dactylon*		OSU	North America	Riverside, CA, United States	
A12436	1	*Cynodon dactylon*		OSU	Europe	Pisa, Italy	
A12437	4	*Cynodon dactylon*		OSU	Europe	Albufeira, Portugal	
A12438	1	*Cynodon dactylon*		OSU	Europe	Barcelona, Spain	
A12439	1	*Cynodon dactylon*		OSU	Europe	Seville, Spain	
A12440	1	*Cynodon dactylon*		OSU	Europe	Madrid, Spain	
Celebration	1	*Cynodon dactylon*		MSU	Australia	Australia	
Choice	1	*Cynodon dactylon*		MSU	North America	United States	

Table A.1 (Continued)

Taxa	Subpop	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
Discovery	1	*Cynodon dactylon*		MSU	Australia	Australia	
Grif17326	4	*Cynodon dactylon* (L.) Pers.		USDA	Eurasia	Russian Federation	
MSU105	1	*Cynodon dactylon*		MSU	North America	United States	
MSU1080	2	*Cynodon dactylon*		MSU	North America	United States	
MSU109	1	*Cynodon dactylon*		MSU	North America	United States	
MSU112	1	*Cynodon dactylon*		MSU	North America	United States	
MSU116	2	*Cynodon dactylon*		MSU	North America	United States	
MSU125	2	*C. dactylon*		MSU	North America	United States	
MSU154	2	*Cynodon dactylon*		MSU	North America	United States	
MSU1541	2	*Cynodon dactylon*		MSU	North America	United States	
MSU1542	1	*Cynodon dactylon*		MSU	North America	United States	
MSU178	2	*Cynodon dactylon*		MSU	North America	United States	
MSU278	2	*Cynodon dactylon*		MSU	North America	United States	
MSU74	2	*Cynodon dactylon*		MSU	North America	United States	
MSU75	2	*Cynodon dactylon*		MSU	North America	United States	
MSU91	2	*Cynodon dactylon*		MSU	North America	United States	
MSU97	1	*Cynodon dactylon*		MSU	North America	United States	

Table A.1 (Continued)

Taxa	Subpo	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
MSU98	1	*Cynodon dactylon*		MSU	North America	United States	
NR24	4	*Cynodon dactylon*		OSU	Europe	Bulgaria	
NR28	1	*Cynodon dactylon*		OSU	Europe	Spain	
NR34	4	*Cynodon dactylon*		OSU	Europe	Italy	
PI193267	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	Asia	Afghanistan	
PI203456	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	Türkiye	2.06
PI206553	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Europe	Greece	2.41
PI206657	4	*Cynodon dactylon*		USDA	Asia	Türkiye	
PI220588	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	Afghanistan	2.01
PI223248	4	*Cynodon dactylon* (L.) Pers. ssp. afghanicus J. R. Harlan & de Wet	4	USDA	Asia	Afghanistan	2.41
PI223249	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Asia	Afghanistan	1.59
PI224314	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.79
PI224566	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Zimbabwe	0.99

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI224568	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Zimbabwe	1.93
PI224692	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Zambia	1.84
PI224694	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Zambia	2.02
PI225046	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Tanzania	1.06
PI225591	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Tanzania	1.04
PI246600	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	India	1.9
PI251108	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Europe	North Macedonia	1.87
PI251809	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Europe	Italy	2.42
PI267985	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	Pakistan	2.17
PI286584	1	*Cynodon transvaalensis* Burtt Davy		USDA	Asia	India	

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI287147	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	India	1.68
PI287149	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	India	1.67
PI287151	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	India	1.85
PI287154	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	India	1.72
PI287155	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	India	1.6
PI287156	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Asia	India	1.23
PI287157	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	India	1.84
PI287244	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	India	1.85
PI287246	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	India	1.77

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI287247	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	India	2.03
PI287256	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Asia	Sri Lanka	1.16
PI287795	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	Asia	Sri Lanka	
PI288043	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Asia	India	0.95
PI288216	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Madagascar	2.09
PI288221	2	*Cynodon dactylon* (L.) Pers. ssp. coursii (A. Camus) J. R. Harlan & de Wet	4	USDA	Africa	Madagascar	2.13
PI288676	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Madagascar	1.15
PI289714	2	*Cynodon dactylon* (L.) Pers. ssp. coursii (A. Camus) J. R. Harlan & de Wet	4	USDA	Africa	Madagascar	1.99

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI289747	3	*Cynodon dactylon* (L.) Pers. ssp. polevansii (Stent) J. R. Harlan & de Wet	3	USDA	Africa	South Africa	1.72
PI289750	3	*Cynodon dactylon* (L.) Pers. ssp. polevansii (Stent) J. R. Harlan & de Wet	4	USDA	Africa	South Africa	2.56
PI289913	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.94
PI289922	3	*Cynodon transvaalensis* Burtt Davy		USDA	Africa	South Africa	
PI289923	2	*Cynodon transvaalensis* Burtt Davy		USDA	Africa	South Africa	
PI290656	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.22
PI290657	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.36
PI290667	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.68
PI290812	2	*Cynodon transvaalensis* Burtt Davy	4	USDA	Africa	South Africa	2.16

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI290813	2	*Cynodon transvaalensis* Burtt Davy	4	USDA	Africa	South Africa	2.52
PI290872	2	*Cynodon transvaalensis* Burtt Davy	4	USDA	Africa	South Africa	2.07
PI290880	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.08
PI290881	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.15
PI290883	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.01
PI290885	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.08
PI290886	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	Africa	South Africa	
PI290887	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.24
PI290894	2	*Cynodon transvaalensis* Burtt Davy		USDA	Africa	South Africa	
PI290895	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.07

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI290901	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.52
PI290905	2	*Cynodon transvaalensis* Burtt Davy		USDA	Africa	South Africa	
PI291146	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.89
PI291148	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.18
PI291151	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.96
PI291153	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.86
PI291155	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.84
PI291157	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.94
PI291160	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.95

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI291161	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.56
PI291164	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.72
PI291166	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.62
PI291167	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.9
PI291169	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.74
PI291171	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.61
PI291172	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.38
PI291175	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.64
PI291180	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.86

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI291586	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Zimbabwe	2.07
PI291610	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.07
PI291716	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.29
PI291718	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.96
PI291719	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.85
PI291726	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.64
PI291729	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.53
PI291730	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.24

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI291733	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.64
PI291734	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.85
PI291740	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.63
PI291741	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.71
PI291745	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.63
PI291746	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.04
PI291747	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.25
PI291749	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.74
PI291750	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.86

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI291962	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Kenya	1.86
PI291964	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.17
PI291968	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	Kenya	1.67
PI291974	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Kenya	0.81
PI291977	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Kenya	1.25
PI291981	2	*Cynodon transvaalensis* Burtt Davy		USDA	Africa	Ethiopia	
PI292039	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Mozambique	2.22
PI292046	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	Zambia	1.68
PI292050	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	Zambia	1.67
PI292052	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	Zambia	1.7

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI292059	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Tanzania	0.94
PI292142	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Ghana	2
PI292143	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	Ghana	1.45
PI292231	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Ghana	2.04
PI292233	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Ghana	2.12
PI292248	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Asia	Philippines	1.9
PI292249	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Asia	Philippines	1.46
PI292250	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	Philippines	1.63
PI292252	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	Philippines	1.61

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI292509	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	Japan	1.65
PI292573	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Asia	Thailand	1.72
PI293639	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	Kenya	1.69
PI293644	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Kenya	1.91
PI295339	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Europe	Germany	2.19
PI297827	4	*Cynodon dactylon* (L.) Pers. ssp. aridus J. R. Harlan & de Wet	2	USDA	Asia	Israel	1.27
PI315902	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Europe	Germany	1.1
PI364484	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.35

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI364485	2	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Africa	South Africa	1.01
PI364490	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	South Africa	1.67
PI365499	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	1.8
PI409738	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	South Africa	2.04
PI531090	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	North America	United States	2.08
PI547108	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	North America	United States	1.72
PI547109	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	North America	United States	2.48
PI564236	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Australia	Australia	1.98
PI564237	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	2	USDA	Australia	Australia	1.58

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI564240	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	Africa	Zimbabwe	2.2
PI572233	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	Africa	Zimbabwe	1.72
PI601059	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	North America	United States	1.71
PI601157	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	4	USDA	North America	United States	2.08
PI601976	1	*Cynodon dactylon*		USDA	North America	United States	
PI606545	1	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*	3	USDA	North America	United States	1.64
PI618587	1	*Cynodon dactylon* (L.) Pers.	4	USDA	North America	United States	2.14
PI641703	1	*Cynodon dactylon* (L.) Pers.	4	USDA	Asia	China	2.01
PI647875	1	*Cynodon dactylon* (L.) Pers.	4	USDA	Australia	Australia	1.82
PI647876	1	*Cynodon dactylon* (L.) Pers.	4	USDA	Australia	Australia	1.91
PI647878	3	*Cynodon transvaalensis* Burtt Davy		USDA	Australia	Australia	

Table A.1 (Continued)

Taxa	Subpopulation	Species	Ploidy	Program	Continent	Origin	2C DNA (pg)
PI647879	1	*Cynodon transvaalensis* Burtt Davy		USDA	Australia	Australia	
PI671960	1	*Cynodon dactylon* (L.) Pers.		USDA	North America	United States	
PI673406	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	North America	United States	
PI673407	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	North America	United States	
PI673408	4	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	North America	United States	
PI673409	3	*Cynodon dactylon* (L.) Pers. ssp. *dactylon*		USDA	NA	NA	
Quickstand	1	*Cynodon dactylon*		OSU	NA	NA	
Trans1	2	*Cynodon transvaalensis*		MSU	North America	United States	
Trans2	2	*Cynodon transvaalensis*		MSU	North America	United States	
U3	4	*Cynodon dactylon*		OSU	NA	NA	

Table A.2 Frequency of transitions and transversions observed in SNP dataset. Transitions are interchanges of two ring purines adenine (A) and guanine (G), or of one ring pyrimidines cytosine (C) and Thymine (T) involving bases of similar shape. Transversions are interchanges of purine for pyrimidine bases, which therefore involve exchange of one-ring and two-ring structures.

Transitions		Transversions	
A/G	11458	A/C	3431
G/A	173	G/T	3370
C/T	11501	A/T	2809
T/C	192	G/C	76
Total	23324	C/G	4442
		T/A	44
		Total	14172

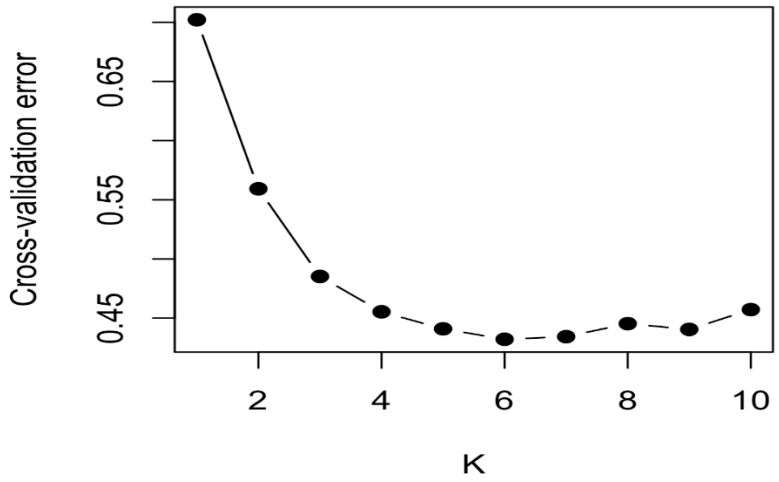

Figure A.1 Cross-validation figure from Admixture that was used to determine the number of subpopulations that exist in the bermudagrass (*Cynodon* spp.) germplasm panel. Here K is number of subpopulations. The line flats when K=4 showing four subpopulations exist in the germplasm panel.

APPENDIX B

ADDITIONAL TABLES (CHAPTER 3)

Table B.1 SNP markers that were found to be associated with the traits with the 2021 phenotypic data. The table is presenting SNP markers that were found to be significant along with p value and minor allele frequency for four traits: leaf length, leaf width, internode distance and stem diameter.

SNP	Pos	P. Value	MAF	Trait
TP32068	32068	1.23E-06	0.442408	Leaf Width
TP43323	43323	5.42E-09	0.172775	Leaf Width
TP52997	52997	2.07E-07	0.164921	Leaf Width
TP69026	69026	8.73E-07	0.201571	Leaf Width
TP91039	91039	1.33E-07	0.060209	Leaf Width
TP101306	101306	4.75E-08	0.353403	Leaf Width
TP110980	110980	1.28E-08	0.057592	Leaf Width
TP128613	128613	8.56E-07	0.371728	Leaf Width
TP211368	211368	5.12E-07	0.47644	Leaf Width
TP217832	217832	7.75E-07	0.424084	Leaf Width
TP245550	245550	4.85E-07	0.062827	Leaf Width
TP299934	299934	1.15E-06	0.112565	Leaf Width
TP359482	359482	4.88E-22	0.094241	Leaf Width
TP433480	433480	1.74E-07	0.057592	Leaf Width
TP473724	473724	2.22E-09	0.039267	Leaf Width
TP534003	534003	1.25E-06	0.04712	Leaf Width
TP594177	594177	9.11E-08	0.170157	Leaf Width
TP622000	622000	1.98E-07	0.17801	Leaf Width
TP629738	629738	6.6E-07	0.264398	Leaf Width
TP661053	661053	2.1E-07	0.308901	Leaf Width
TP706985	706985	5.6E-07	0.243455	Leaf Width
TP714870	714870	5.11E-08	0.447644	Leaf Width
TP760782	760782	4.28E-07	0.429319	Leaf Width
TP52896	52896	3.84E-07	0.434555	Internode Distance
TP52997	52997	8.29E-08	0.164921	Internode Distance
TP74913	74913	1.34E-07	0.41623	Internode Distance
TP103664	103664	4.9E-07	0.159686	Internode Distance
TP240255	240255	1.73E-07	0.45288	Internode Distance
TP259105	259105	1.64E-07	0.335079	Internode Distance

Table B.1 (Continued)

SNP	Pos	P. Value	MAF	Trait
TP306802	306802	6.99E-07	0.206806	Internode Distance
TP309116	309116	1.09E-08	0.081152	Internode Distance
TP313976	313976	1.18E-06	0.086387	Internode Distance
TP356637	356637	1.34E-17	0.073298	Internode Distance
TP740749	740749	3.94E-07	0.481675	Internode Distance
TP778173	778173	1.08E-07	0.465969	Internode Distance
TP898295	898295	4.19E-07	0.253927	Internode Distance
TP901441	901441	4.35E-08	0.468586	Internode Distance
TP183957	183957	9.11E-11	0.086387	Stem Diameter

Table B.2 SNP markers that were found to be associated with the traits with the 2022 phenotypic data. SNP markers that were found to be associated with the traits with the 2021 phenotypic data. The table is presenting SNP markers that were found to be significant along with p value and minor allele frequency for four traits: leaf length, leaf width, internode distance and stem diameter.

SNP	Pos	P.value	MAF	Trait
TP272883	272883	1.27E-09	0.282383	Leaf Length
TP766421	766421	2.39E-07	0.199482	Leaf Length
TP792453	792453	2.42E-07	0.212435	Leaf Length
TP26029	26029	6.81E-08	0.108808	Leaf Width
TP42967	42967	1.29E-06	0.259067	Leaf Width
TP43323	43323	2.25E-07	0.173575	Leaf Width
TP69026	69026	1.15E-07	0.202073	Leaf Width
TP91767	91767	5.35E-07	0.266839	Leaf Width
TP110980	110980	2.09E-08	0.056995	Leaf Width
TP114756	114756	2.55E-07	0.303109	Leaf Width
TP117368	117368	7.39E-07	0.471503	Leaf Width
TP147671	147671	1.26E-06	0.103627	Leaf Width
TP211368	211368	6.58E-07	0.476684	Leaf Width
TP245550	245550	9.8E-07	0.064767	Leaf Width
TP272079	272079	3.4E-07	0.406736	Leaf Width
TP299934	299934	2.12E-07	0.11399	Leaf Width
TP359482	359482	2.94E-28	0.093264	Leaf Width
TP433480	433480	5.75E-09	0.056995	Leaf Width
TP436308	436308	7.54E-07	0.329016	Leaf Width
TP442751	442751	1.33E-06	0.409326	Leaf Width
TP443823	443823	8.58E-08	0.163212	Leaf Width
TP447384	447384	9.01E-07	0.468912	Leaf Width
TP460714	460714	1.4E-08	0.183938	Leaf Width
TP473724	473724	2.81E-09	0.03886	Leaf Width
TP538276	538276	1.2E-06	0.11658	Leaf Width
TP594177	594177	1.36E-07	0.170984	Leaf Width
TP614831	614831	4.16E-07	0.145078	Leaf Width
TP622000	622000	1.57E-07	0.176166	Leaf Width
TP629738	629738	8.7E-07	0.264249	Leaf Width
TP666050	666050	1.27E-06	0.054404	Leaf Width
TP714870	714870	1.3E-06	0.448187	Leaf Width
TP740749	740749	1.07E-06	0.481865	Leaf Width
TP760782	760782	1.15E-08	0.427461	Leaf Width

Table B.2 (Continued)

SNP	Pos	P.value	MAF	Trait
TP792228	792228	1.07E-06	0.274611	Leaf Width
TP901441	901441	1.99E-08	0.468912	Leaf Width
TP8336	8336	8.91E-08	0.11399	Internode Distance
TP38872	38872	5.88E-08	0.15285	Internode Distance
TP61721	61721	8.42E-08	0.479275	Internode Distance
TP78205	78205	1.51E-07	0.458549	Internode Distance
TP154426	154426	7.14E-07	0.106218	Internode Distance
TP257531	257531	9.32E-07	0.103627	Internode Distance
TP309116	309116	4.03E-07	0.080311	Internode Distance
TP359482	359482	3.56E-20	0.093264	Internode Distance
TP454050	454050	1.12E-06	0.339378	Internode Distance
TP494048	494048	3.78E-07	0.085492	Internode Distance
TP592055	592055	1.2E-06	0.132124	Internode Distance
TP740749	740749	5.74E-07	0.481865	Internode Distance
TP844605	844605	7.25E-07	0.310881	Internode Distance
TP883028	883028	2.93E-07	0.082902	Internode Distance
TP889754	889754	7.91E-07	0.173575	Internode Distance
TP889896	889896	3.31E-07	0.194301	Internode Distance
TP901441	901441	4.93E-07	0.468912	Internode Distance
TP369567	369567	1.04E-06	0.411917	Stem Diameter
TP550772	550772	8.1E-08	0.243523	Stem Diameter
TP885757	885757	3.78E-10	0.253886	Stem Diameter