BREEDING AND GENETIC CHARACTERIZATION OF FORAGE BERMUDAGRASS FOR THE TRANSITION ZONE OF THE UNITED STATES

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BREEDING AND GENETIC CHARACTERIZATION

OF FORAGE BERMUDAGRASS FOR THE

TRANSITION ZONE OF THE UNITED STATES

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This work is dedicated to my family – my mom Amy, brother Tyler, grandpa David, and grandma Diane. Thank you for all of your love and support throughout the years. I wouldn't be where I am today without all that you have done and sacrificed for me.

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Abstract: Bermudagrass is a robust forage option for livestock producers as both a grazed and stockpiled herbage. Breeding efforts focused on improved cold tolerance have expanded the geographic range of bermudagrass into the transition zone of the United States. However, many cold tolerant clonal hybrids experience a gap in yield potential and quality compared to southern adapted cultivars. Additionally, producers experience a limited option of seeded commercial cultivars that have the necessary adaptation for production within the transition zone. This study was conducted to genetically characterize collections of germplasm for the improvement of forage bermudagrass in the transition zone of the United States. A collection of 215 *Cynodon dactylon* SSR markers were identified as transferable to *C. nlemfuensis*, with confirmed effectiveness through a genetic diversity analysis. Transferable markers were used to identify interspecific hybrids from a cross between P3 1x7 and Tifton 68 that employed to develop a population with improved cold tolerance, yield potential, and forage quality in Stillwater and Perkins, OK. Population evaluations of 100 seeded genotypes were conducted in Goodwell, OK to characterize the genetic variation of biomass and reproductive traits, in addition to identifying elite germplasm for synthetic seeded cultivar development. Furthermore, a collection of 31 commercial cultivars and experimental accessions were characterized with SSR markers for molecular genetic diversity. Ten seeded genotypes and 25 interspecific hybrids were selected for further testing. Several trait associations were identified for indirect selection of seed and biomass yield. Furthermore, broad sense heritability estimates of interspecific hybrids displayed a significant genetic influence to adaptive trait performance. Molecular characterization confirmed a relatively narrow genetic base within current commercial cultivars. The genetic information, selections and SSR markers developed in the investigation will further enhance the capabilities of forage bermudagrass breeding, as we seek to broaden the genetic base and improve key traits for transition zone performance.

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CHAPTER I

INTRODUCTION

Bermudagrass (*Cynodon* spp.) occupies substantial acreage throughout the southern half of the United States. In addition to its employments in turf and soil stabilization, bermudagrass offers reliable forage production in pastures and hay fields. Bermudagrass contains a wide range of natural disease and insect tolerance (Quisenberry, 1990; Smiley et al., 1992), in addition to substantial heat and drought tolerance (Burton et al., 1957; Taliaferro et al., 2004). A defining characteristic of bermudagrass is its ability to withstand close grazing and heavy livestock traffic (Taliaferro et al., 2004), allowing for productive stands throughout the growing season. As a perennial grass, bermudagrass pasture translates into a long term investment for producers, as some stands have persisted for more than 25 years (Rouquette et al., 1997; Rouquette et al., 1998).

Within the *Cynodon* genus, there are 9 species in which some are readily employed as turf and/or forage biotypes (Taliaferro, 1995). Of these 9 species, *C. dactylon* and *C. nlemfuensis* are the predominant germplasm sources, with limited use of *C. polevansii* and *C. plectostachyus* (Anderson et al., 2009). Various characteristics are

associated with each of the species, where *C. nlemfuensis* is known for forage yield and quality, while *C. dactylon* is predominantly associated with elevated levels of cold tolerance and adaptation. Hybridization between species is a further source of genetic diversity offering substantial opportunity for genetic gain (Harlan et al., 1970a).

As a warm season grass, bermudagrass has readily established itself in the southern United States, however, cold tolerant germplasm has extended as far north as 53ºN latitude (Harlan et al., 1970b). Despite this, much of the territorial expansion of bermudagrass within the United States is limited to 36ºN latitude due to lack of substantial cold tolerance in several commercialized cultivars (Taliaferro et al., 2004). Locations north of 36ºN latitude are known as the transition zone, which features the adoption of several cold tolerant varieties and hybrids that have demonstrated successful performance in this swath of the United States. Throughout the United States, bermudagrass is estimated to cover 10 to 12 million hectares of land for forage production (Taliaferro et al., 2004). Developing varieties that tolerate harsh winters is a direct method to increasing the territorial range of forage bermudagrass (Burton and Monson, 1978). Further objectives of forage breeding programs have long been centered upon biomass yield (Burton, 1943; Burton et al., 1993), forage digestibility (Burton, 1972; Burton and Monson, 1984), and disease tolerance (Burton and Monson, 1988). Significant advancement has been made in improving this once noxious weed into a productive forage that has revolutionized the livestock industry. Traditional breeding practices have paved a path for heightened success with molecular methods in ensuring the continued development and commercialization of elite bermudagrass germplasm.

Cytological Profile

The base chromosome number of bermudagrass is nine, predominantly found in the diploid (2n=2x=18) and tetraploid (2n=4x=36) forms (Forbes and Burton, 1963; Harlan et al., 1970c; de Wet and Harlan, 1971; de Silva and Snaydon, 1995). Wu et al. (2006) reported that triploid (2n=3x=27) plants are relatively common, arising from natural and artificial hybridization events within and between species. Additionally, Johnston (1975) and Burton et al. (1993) have documented the rare occurrence of pentaploid (2n=5x=45) bermudagrass. Hexaploid (2n=6x=54) genotypes have been the highest observed ploidy level in bermudagrass, however, their occurrence is relatively limited (Hurcombe, 1947; Moffett and Hurcombe, 1949; Powell et al., 1968; Felder, 1967; Malik and Tripathi, 1968; Johnston, 1975). Wu et al. (2006) conducted work in determining the ploidy level of 132 *Cynodon* accessions collected from China. In total, their work identified a tetraploid rate of 88%, followed by 7 hexaploids, 3 pentaploids, and 6 triploids within the pool of accessions. Although a small sample size in comparison to the total naturalized germplasm found in China, the observed data is useful in providing key insights towards the genetic profiles and variations present within the *Cynodon* genus. From this study, the strongest genetic variation was found within the tetraploid germplasm, with pentaploids displaying the least variation.

Genetic Diversity

Genetic variation is critical to successful breeding programs. A wide range in heritable traits is essential to capturing, incorporating, and transforming useful traits into elite germplasm and commercialized cultivars. Understanding the diversity of available germplasm is crucial in determining opportunities for hybridization. Many studies have been

deployed in evaluating diversity of core collections and understanding the relationships of diverse accessions using a variety of molecular methods. Amplified fragment length polymorphism (AFLP) is one of most commonly used methods in evaluating diversity (Anderson et al., 2009; Kang et al., 2008; Karaca et al., 2002). Jewell et al. (2012) evaluated the genetic diversity of Australian accessions of bermudagrass with expressed sequence tag (EST)-simple sequence repeat (SSR) markers. Furthermore, Karaca et al. (2002) utilized additional methods such as chloroplast-specific simple sequence repeat length polymorphism (CpSSRLP), random amplified polymorphic DNA (RAPD), and directed amplification of minisatellite region DNA (DAMD). In addition to employing RAPD, Gulsen et al. (2009) utilized sequence related amplified polymorphism (SRAP), peroxidase gene polymorphism (POGP), and inter-simple sequence repeat markers (ISSR) in evaluating genetic diversity of *Cynodon* accessions. From Gulsen et al. (2009), the observed genetic diversity was that diversity was strongly influenced by ploidy level and geographic location in Turkey.

As is evident from recent work, molecular techniques have become common place in studying the genetic diversity of bermudagrass germplasm. New methods and reduced costs of established protocols have led to enhanced implementation and ease of access to these laboratory methods for many breeding programs. Genetic diversity of germplasm is the backbone to breeding programs, and it is through this diversity that many elite cultivars have been developed and released within the United States.

Commercial History of Forage Bermudagrass

Early works by G.W. Burton led to the transition of bermudagrass from a noxious weed to a productive forage, which revolutionized the livestock industry (Burton, 1947).

Coastal (*C. dactylon*) was one of the first forage bermudagrass intraspecific hybrids, as it was developed from an intra-specific cross between Tift bermudagrass and a South African accession (Burton, 1943). Despite its high yields, low winter temperatures result in complete winter kill of Coastal stands. Crossing Coastal with a local strain of cold tolerant bermudagrass from Indiana led to the selection of a productive, cold tolerant F_1 hybrid named Midland (Hein, 1953). Coastal continued to serve as the genetic backbone for future breeding initiatives. It was used in the development of Coastcross-1, a sterile F_1 hybrid with superior digestibility yet less cold tolerance than Coastal (Burton, 1972). Burton and Monson (1978) crossed Coastal with a German accession, resulting in the release of Tifton 44. This hybrid features superior digestibility and yield potential in comparison to Coastal, and possesses cold tolerance on par with Midland. Further work by Burton and Monson (1984) led to the release of Tifton 68 (*C. nlemfuensis*). Primary features of Tifton 68 include its superior digestibility compared to Coastal and Coastcross-1, in addition to it being fertile. Fertility of Tifton 68 played a major role in its use as a parent in the development of Tifton 85, possessing enhanced digestibility and yield, yet limited cold tolerance (Burton et al., 1993). Other prominent cultivars released by the USDA-ARS and Georgia Agricultural Experiment Station include Suwanee and Tifton 78. Suwanee is superior to Coastal when grown on deep, sandy soils as a result of its heightened drought tolerance (Burton, 1962). Tifton 78 excels when compared to Coastal, with the exception of being less cold tolerant (Burton and Monson, 1988).

Work by C.M. Taliaferro featured a sincere focus on incorporating cold tolerance in commercial cultivars for pasture use in the transition zone of the Southern Great Plains. ‘Hardie’ was released in 1974, yielding more than Midland, yet possessing issues with stand

persistence, low pH tolerance, and foliar disease (Taliaferro and Richardson, 1980). Employing a cross between accessions of *C. dactylon* and *C. nlemfuensis*, Taliaferro et al. (2002) released a hybrid with improved cold tolerance and forage production when compared to Midland and Tifton 44. This hybrid was coined Midland 99. Richardson and Taliaferro (2005) collaborated in the release of Ozark. This F_1 hybrid resulted from a cross of a cold tolerant, Yugoslavian bermudagrass accession and Coastal. Wu et al. (2009) continued in improving the cold tolerance of forage bermudagrass for the transition zone with the release of Goodwell. This sterile hybrid is well adapted to irrigated conditions for both grazing and hay production in the transition zone and panhandle of Oklahoma. It is incredibly cold tolerant, with yield potential similar to Midland 99. Other prominent cultivars of clonal forage bermudagrass include Greenfield, Alicia, Callie, Brazos, Grazer, Russell, Florakirk, and World Feeder (Elder, 1955; Watson, 1974; Eichhorn et al., 1984; Eichhorn et al., 1986; Ball et al., 1996; Mislevy et al., 1995; Gordon, 1989).

Seeded bermudagrass has received considerably less focus from breeding efforts until recent years. Most seeded varieties are derivatives of common bermudagrass (*C. dactylon* var. *dactylon*), with one of the earlier varieties, NK-37, being selected from Giant bermudagrass (*C. dactylon* var. *aridus*) (Hanson, 1972). Guymon is another seed-propagated cultivar, possessing exceptional cold tolerance in its adaptations to the transition zone (Taliaferro et al., 1983). Initially released as a turf grass, Cheyenne has experienced significant adoption as a result of its forage production and cold tolerance in comparison to other seeded cultivars (Samudio and Brede, 1998). Released by Johnston Seed Co. in 1999, Wrangler is an exceptional seeded cultivar with cold tolerance needed for profitable production in the transition zone. In addition to stand alone seeded cultivars, many blends are

available that feature 2-3 cultivars of commercialized germplasm, or various ecotypes of common and giant bermudagrass.

Bermudagrass Seed Production in Oklahoma

The semi-arid nature of the Oklahoma Panhandle mimics the climate of the Yuma, AZ area. The summers feature low humidity, warm temperatures and limited precipitation. Access to irrigation water from the Ogallala Aquifer allows producers to closely control irrigation inputs (Taghvaeian et al., 2017). A prior study has suggested that economical seed yields of bermudagrass can be achieved under growing conditions witnessed in Oklahoma, where altered moist and dry weather conditions produce substantially higher seed yield (Ahring et al., 1974). Ahring et al. (1982) further demonstrated the potential for bermudagrass seed production in Oklahoma under different management practices. Their results showed that despite differences in management treatments, economical seed yields were achieved. Redfearn and Wu (2013) note that the niche market of farming bermudagrass for seed has significant potential for growth in Oklahoma, as varieties produced and developed in this area have substantially improved cold hardiness and fit to colder regions when compared to lineages of bermudagrass seed from Arizona and California.

Unlike most common crops, slight water stress will contribute to greater seed yield for bermudagrass. Ahring et al. (1982) described this phenomenon in detail, beginning by highlighting the need for climatic conditions that result in a rainfall distribution of 5.5, 4.0, and 3.5 inches of precipitation throughout May, June, and July, respectively. In the Panhandle of Oklahoma, supplemental irrigation is likely needed to achieve these distributions, but can serve a substantial benefit when compared to the uncontrolled nature of

precipitation in dryland systems of central Oklahoma under day time temperatures of 86 to 100°F. The authors continued to discuss the need for alternate wet and dry cycles to induce slight water deficit stress, which contributes to and amplifies seed stalk development. Three to four cycles of stress are necessary to achieve desired seedstalk development and flowering (Ahring et al., 1982). Each cycle should consist of two weeks of irrigation, followed by two weeks of dry/stressing conditions to the plant.

Traditional Breeding Methods

Bermudagrass displays a high level of self-incompatibility (Burton, 1947; Burton and Hart, 1967; Richardson et al., 1977; Kenna et al., 1983). Recent work by Tan et al. (2015) observed an outcrossing rate of 99.86% in common bermudagrass under field conditions. Degrees of self-sterility will vary by species and biotype. A high affinity for outcrossing warrants considerable advantages in breeding protocols for clonal hybrid development and F_1 seed production. Controlled crosses are possible, yet rarely used in breeding programs due to the small floral anatomy making manual emasculation a time consuming and tedious process (Richardson, 1958; Burton, 1947; Burton, 1965).

As a result of the natural self-incompatibility crosses can be made in a field setting using isolated plots, or crossing blocks (Burton, 1965). The primary focus of many forage bermudagrass breeding programs has been the development of clonally propagated F_1 hybrids. This objective allows for simple, direct selection of elite progeny (Taliaferro et al., 2004). Heterosis of hybrid plants is an exceptional quality displayed by many progeny in hybridized populations (Burton, 1956, 1959). Typical methods center on early work by Burton (1947, 1954) in the development of Coastal bermudagrass using a form of recurrent

selection. Basic procedures center on parent genotypes planted in strips adjacent to each other and seed heads harvested at maturity throughout the growing season. Seed is germinated in the greenhouse and subsequently planted in high density selection nurseries. From here, selection is centered upon protocols and progeny performance, which determines advancement to replicated field studies in time (years) and location, and ultimate commercialization.

Selection protocols are dependent on the trait of interest. In forage breeding, biomass yield, cold tolerance, and forage quality have been the primary traits of interest in determining the commercial value of progeny plants. Forage quality can be determined through in vitro or in vivo digestibility tests (Tilley and Terry, 1963; Lowrey et al., 1968; Monson et al., 1969). In addition to modern laboratory methods for determining crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF), near infrared reflectance spectroscopy (NIRS) offers significant advantages in terms of efficiency for processing large volumes of samples (Stuth et al., 2003). For such traits as cold tolerance, laboratory procedures exist using freeze and growth chambers (Anderson et al., 2002), however these methods are not efficient for large progeny populations. Testing for cold tolerance in the field features planting in environments within or north of the transition zone. Subjection to harsh winter conditions and visual or digital measurement of winterkill are the most efficient methods of determining cold tolerance in large populations. Direct selections for biomass and seed yield are focused on manual harvest of plots, however, several indirect selections methods exist that add incredible efficiency to advancing yields.

Indirect Selection Methods

Understanding associations of observable morphological characteristics with terminal traits allows for efficient means of selection as an alternative to hand harvesting plots in single plant nurseries. Using a set of common bermudagrass turf-type collections from China, Wu et al. (2007) identified plant height, spring greenup, foliage density, and internode length to be positively associated with biomass production. Additionally, it was observed that winterkill is negatively associated with forage yield. Utilizing these traits as indirect selection methods offers substantial advantage to efficiency of single plant nursery selections. Similar work was conducted by Wu et al. (2006) for morphological traits associated with seed yield. The authors identified inflorescence prolificacy and seed set percentage to be beneficial traits for indirect selection of seed yield.

Molecular Breeding Methods

Many commercial forage bermudagrass cultivars are a result of interspecific hybridization between *C. dactylon* and *C. nlemfuensis* and the exploitation of fixed heterosis in F_1 hybrids in interspecific and intraspecific progeny (Taliaferro et al., 2004). Selecting progeny has been dependent on observable morphological characteristics, however, the onset and ease of application for molecular breeding techniques offers tremendous potential to improving efficiency and accuracy of breeding programs.

Marker assisted selection (MAS) offers immense benefits to determining true hybrids in progeny pools. Fang et al. (2015) demonstrated the ability of SSR markers to readily differentiate and identify unknown bermudagrass germplasm. Although primarily focused on turf bermudagrass, work has been conducted in developing SSR markers, linkage maps and

quantitative trait loci (QTL) (Guo et al., 2017; Tan et al., 2012; Tan et al., 2014; Harris-Shultz et al., 2010). However, the transferability of these turf SSR markers for genotyping forage bermudagrass provides a great opportunity to accelerate selection procedures of mass progeny pools.

Associations of molecular markers and traits of interest offer another method of increased precision in selecting progeny plants. Gitau et al. (2017) identified 41 SSR markers that had significant associations with traits related to forage quality in *C. dactylon*. Continued development of marker-trait-associations (MTA) will allow for enhanced efficiency of breeding programs. Identifying associations of employed markers with terminal traits for yield and QTL of cold tolerance and forage quality will have an immense impact on forage bermudagrass breeding.

Transferability of SSR Markers

Molecular markers that are transferable among species provides researchers with economical means to employ such technologies as SSR markers in species with little molecular marker development has occurred. Dayanandan et al. (1997) noted that the success of transfer rates largely depends on the genetic relatedness between the species of interest. Several studies have determined the transferability of SSR markers between species. Xie et al. (2010) investigated the transferability of SSR from several cereal crops to orchardgrass (*Dactylis glomerata*). Results of this study found 15 of the 50 cereal expressed sequence tagged (EST)-SSRs screened produced 90 total bands from 74 accessions of orchardgrass, resulting in 68 being polymorphic. Hernandez et al. (2001) witnessed a transferability rate of 74.5% when evaluating the efficacy of maize SSRs' in sugarcane. Saha et al. (2006) tested

the transferability of 511 tall fescue SSR primer pairs in several cereal, forage and turf grasses. Among these 6 species evaluated, 38% of the genomic SSR primer pairs were transferable from tall fescue.

Wang et al. (2005) evaluated the transfer rate of SSR markers from wheat, rice, maize, and sorghum to several other minor grass species, including *Cynodon*. Of the 210 SSR markers used in this study, an average transferability rate of 54% was witnessed for bermudagrass. Sorghum SSR markers had the highest transfer rate to bermudagrass at 62%, and maize the lowest at 42%. Tan et al. (2012) tested the transferability of 354 sorghum SSR markers to bermudagrass, and found transfer rates of 57%, 27% and 22% to *C. transvaalensis* T577, Tifton 10 (*C. dactylon*) and Zebra (*C. dactylon*), respectively.

Tan et al. (2012) aimed to develop SSR markers for bermudagrass by using ESTs from the National Center for Biotechnology Information and from examining the transferability of sorghum SSR primer pairs. Of the 20,237 identified *Cynodon* ESTs, 303 were selected and identified as producing reliable bands in at least one of pool of varieties from *C. dactylon* and *C. transvaalensis.* *Cynodon* genotypes used in the transferability study between bermudagrass and sorghum included T577 (*C. transvaalensis*), Tifton 10 (*C. dactylon*) and Zebra (*C. dactylon*). *Cynodon transvaalensis* (T577) achieved the highest rate of sorghum SSR transferability at 57%, while the two *C. dactylon* varieties, Tifton 10 and Zebra, witnessed rates of 27 and 22%, respectively. Results from Saha et al. (2006) are indicative of transferability rates among related species being somewhat dependent on the conservation of SSR flanking regions within related species. In other words, the variation experienced over the course of speciation can determine the success rate of transferability. Increased genetic distance of species is expected to incur lesser degrees of transferability.

Additional studies have further demonstrated the transferability of SSR markers among a range of species (Brown et al., 1996; Cordeiro et al., 2001; Harris-Shultz et al., 2012; Roder et al., 1995; Thiel et al., 2003; Varshney et al., 2005). Identification of transferable SSR markers allows researchers to economically and efficiently expand tools of selection for breeding programs and genetic studies.

Future Prospects

Continued focus should be placed on improving forage quality, forage yield potential, and seed yield of bermudagrass. Considerable work has been implemented in enhancing cold tolerance. Further advancement of seed and biomass yield, in addition to forge quality of cold tolerant germplasm will lead to expanded territorial range of forage bermudagrass. Additionally, molecular research producing novel SSR, QTL, and MTA data will lead to heightened efficiency of modern breeding programs and the continued implementation of forage bermudagrass as a marquee forage crop.

CHAPTER II

GENETIC VARIABILITY AND INTERRELATIONSHIPS OF MORPHOLOGICAL, ADAPTIVE, AND BIOMASS TRAITS IN FORAGE BERMUDAGRASS

ABSTRACT

Bermudagrass is a major source of grazed and stockpiled forage for the livestock industry. Understanding interrelationships of morphological, adaptive, and biomass traits allows breeders to make efficient decisions during the selection process when breeding new cultivars. Limited work has been done in evaluating these trait relationships with biomass yield, a key performance trait in forage germplasm. A collection of 104 cold tolerant genotypes of bermudagrass were evaluated in a randomized complete block design with 3 replications at the Oklahoma Panhandle Research and Extension Center in Goodwell, OK from 2017 – 2019 in order to characterize the relationships of 18 morphological, adaptive, and biomass traits. Biomass yield was found to be significantly ($\alpha = .05$, $P < .05$) correlated with leaf length, internode length, spring greenup, early vigor, and plant height. Path analysis indicated that selection for taller plants with early, vigorous spring growth is strong criteria for the indirect selection of biomass yield.

Furthermore, substantial variability is displayed in this experimental population, providing ample genetic resources for future varietal improvement initiatives.

INTRODUCTION

During the 2019 growing season, 52 million ha of land in the United States was used hay production (NASS-USDA, 2020). Taliaferro et al. (2004) estimated that bermudagrass (*Cynodon* spp.) is grown on 10 – 12 million hectares (ha), a number that has likely seen considerable growth over the years. A robust combination of drought and heat tolerance (Burton et al., 1957; Taliaferro et al., 2004) make bermudagrass a reliable option for grazing and hay production in environments with variable weather patterns. Bermudagrass breeding initiatives have achieved considerable genetic gains over the years with improved cold tolerance, yield, and digestibility in commercial cultivars (Burton et al., 1993; Nelson and Burns, 2006; Wu et al., 2009).

Focus on cold tolerance has allowed for the expansion of bermudagrass into the transition zone of the United States through the release of cultivars such as Goodwell, Hardie, Midland 99, and Ozark (Wu and Taliaferro, 2009; Taliaferro and Richardson, 1980; Taliaferro et al., 2002; Richardson and Taliaferro, 2005). In addition to cold tolerance, productive biomass is a basic requirement of any forage bermudagrass cultivar. Breeders evaluate thousands of progeny plants each year, routinely utilizing indirect selection in high density greenhouse and field nurseries. Direct quantification of yield in these settings is difficult and time consuming. Breeders can indirectly select for primary traits of interest by identifying easily observable morphological traits that have strong relationships with their trait of interest. Understanding interrelationships of biomass yield

with morphological and adaptive traits is critical to developing proper indirect selection protocols in forage bermudagrass breeding.

Burton (1947) documented the strong correlation (r = 0.80) between first year forage yield and four-year total yield of bermudagrass, showcasing initial biomass yield selection in the first growing season to be representative of production in following years. Wu et al. (2007) indicated the positive correlation coefficients between biomass yield and spring greenup (r = 0.37 – 0.40 across 3 ratings), plant height (r = 0.30 with seedhead; r = 0.30 without seedhead), internode length (r = 0.26), and sod density (r = 0.20), in addition to the negative relationship with winterkill rate (r = -0.29). Harlan and de Wet (1969) noted the extensive genetic variability of bermudagrass, which can provide a range of traits to evaluate for indirect selection. Aside from the aforementioned studies, limited work has been conducted in evaluating the interrelationships of biomass yield and secondary traits in forage germplasm. Additionally, many bermudagrass traits have proven to be heritable and offer real value to breeders. Of specific interest to this study, Stefaniak et al. (2009) observed spring greenup to be a trait with moderate heritability, indicating its ability to be selected for and advanced in breeding cycles.

Utilizing methods established by Dewey and Lu (1959), path coefficient analysis can be employed to more readily identify secondary traits to be used for indirect selection. Das et al. (2004) and Kang (1994) note the benefit of quantifying the direct and indirect effects of traits, as correlation coefficients fail to provide adequate insight to complex trait interactions and their impact on primary traits. Wu et al. (2007) utilized path analysis in their study with bermudagrass, identifying plant height, spring greenup, internode diameter and length, and winterkill rate to all contribute significant direct

effects upon biomass yield and presenting confidence in these secondary traits use for indirect selection. Continued investigation can confirm these selection parameters and further document the genetic variability of bermudagrass used in forage production. The objective of this study was to analyze the genetic variability of 100 experimental bermudagrass genotypes and explore the interrelationships of 18 adaptive, morphological, and biomass traits.

MATERIALS AND METHODS

Plant Materials

Experimental accessions were selected from three populations of cold tolerant bermudagrass in Stillwater, OK. The selection of 100 genotypes was based on characteristics related to seed production, focused on inflorescence prolificacy and flowering time. Material was gathered from each of the selected plots and propagated in a greenhouse, in addition to the four commercial cultivars as standards, Goodwell, Midland, Midland 99, and Wrangler. Plants were grown under ideal growing conditions for 5 weeks prior to transplant into the field. For each genotype, 14 plants were grown in separate 3.8 cm diameter cone-tainers. Water, fertilizer, and commercial pesticides were applied as needed.

Trial Design, Establishment, and Management

The trial was established in 2017 on a Gruver clay loam in Goodwell, OK at the Oklahoma Panhandle Research and Extension Center. A randomized complete block design containing 104 genotypes was employed with 3 replications. Data was collected from 2017 – 2019 growing seasons. Plot dimensions measured 2.7 by 2.7 m, separated by

1 m alleys. A 2 m alley separated each replication. Total dimensions measured 100.9 m long by 43.4 m wide. Four plants were used to establish each plot, via direct transplantation of greenhouse germplasm into a finely tilled seedbed. Potassium (K) and phosphorus (P) rates were based on soil test results, while urea was incorporated prior to planting at a rate of 112.1 kg nitrogen (N) ha^{-1}. Ronstar Flo (Bayer Crop Science, Monheim am Rhein, Germany) was applied pre-emergent to control weed competition during establishment at a rate of 6.4 L product ha^{-1}. Irrigation was applied immediately after planting. Glyphosate was applied to alleys as needed to prevent overgrowth and contamination of neighboring plots throughout the duration of the experiment at a rate of 2.3 L product ha^{-1}, in addition to other commercial pesticides used for weed control as needed. Irrigation was applied throughout 2017 as needed to encourage rapid stand establishment. During 2018 and 2019, plots received supplement irrigation at rates of 25.4 – 50.8 mm, in addition to any natural precipitation. This irrigation was applied in order to encourage sufficient regrowth to recover from any sustained winterkill. Irrigation rates were based on sprinkler capacity of the research station. Following regrowth, plots were mowed down to 7.6 cm height and allowed to grow for 2 weeks. At the conclusion of the two-week period, irrigation scheduling entered altering 2-week wet/dry cycles to encourage inflorescence production. During wet periods, irrigation was used to supplement rainfall in order to achieve weekly watering rates of approximately 50.8 mm. Dry periods included complete withholding of irrigation water. In the presence of rainfall events over 25.4 mm, the 2-week dry cycle started over. Throughout 2018 and 2019, total N applied was 224.2 kg urea ha^{-1}, applied in 112.1 kg urea ha^{-1} applications following spring greenup, and again following seed harvest in the fall. Phosphorus and potassium

were applied each spring based on soil test results from the Oklahoma State University Soil, Water, and Forage Analytical Laboratory. After seed harvest, biomass was swathed and baled off, allowing plants enough time to recover in preparation for winter dormancy.

Data Collection and Analysis

Data collection included physical measurements and visual ratings or morphological and adaptive traits (Table 1). Ratings were based on a 1 – 9 scale used in the National Turf Evaluation Program (NTEP), with 1 indicating worst and 9 representing best relative performance throughout the trial. Traits rated on the 1 – 9 scale included establishment rate, spring greenup, and early vigor. For establishment rate, a rating of 1 indicated no to very slow lateral stolon spread, while a 9 expressed rapid, vigorous growth of stolons. Greenup ratings of 1 indicated little to no shoot growth in the early spring, while 9 was representative of vigorous, upright shoot growth throughout the plot. Early vigor ratings of 1 indicated light green coloration, with little upright growth and sparse canopy density, while a 9 was indicative of lush, dense, dark green canopy cover. Winterkill was visually assessed on a percent dead plot area following spring greenup, with 0% representing no visually identifiable dead area, and 100% representative of complete winterkill and no visible bermudagrass growth. Plant heights without seedhead and with seedhead were measured in the field with a metric ruler. Height with seedhead was determined by the high point of the infloresence standing upright in the plot without manipulation, while height without seedhead was identified by recording the height at which the vegetative growth stopped. Prior to biomass harvest, 5 stem samples were randomly collected from each plot within each replication, placed on ice, and stored at -20°C prior to morphological measurements. Morphological traits were

quantified with a digital caliper and included first and second leaf length/width, 2nd and 3rd internode diameter, and 2nd internode length. First leaf was identified as the first fully emerged leaf with a visible collar region, while 2nd leaf was the leaf directly below the 1st leaf. Second and 3rd internodes were based off the identification of the first visible node. Biomass samples were collected by hand clipping a 0.3 m by 0.3 m area in the middle of each plot. These samples were oven dried for 48 hours, weighed, and converted to Mg ha^{-1} by dividing the dry weight in g by .09, and subsequently multiplying that value by .01. Plot means from the 5 stem samples were used for statistical analysis. Data was analyzed with procedures in SAS 9.4 (SAS, 2014). Analysis of variance (ANOVA) was performed with PROC MIXED, with genotype, replication, and year serving as random effects, in addition to their respective interactions of genotype x year and genotype x replication. Descriptive statistics were generated with PROC MEANS, and biomass yield was separated with Fischer's Least Significant Difference (LSD) at a probability level of 0.05 and 0.01 with PROC GLM as result of there being no missing data points. Mean separation data was utilized in establishing thresholds for genotype selection in the breeding process. Stepwise selection was performed with PROC REG, and Pearson correlation coefficients were generated with PROC CORR. Path analysis was conducted with PROC IML to properly identify traits used for indirect selection. Path analysis was conducted with rationale developed by Dewey and Lu (1959) in order to partition direct and indirect effects of trait interrelationships.

RESULTS

Trait Variability

Analysis of variance (ANOVA) identified significant ($\alpha = .05$, $P < .05$) variability among 16 of the 18 observed traits, with the exceptions being greenup and winterkill (Table 1). Year had a significant effect ($\alpha = .05$, $P < .05$) on all 17 traits containing two years of data, while replication effects were significant in 8 of the 18 traits (Table 1). With the exceptions of first and second leaf width, average leaf width, second internode length, and early vigor, traits with 2 years of data displayed significant ($\alpha = .05$, $P < .05$) genotype x year interactions (Table 1). Significance was more limited within genotype x replication, as first leaf length, average leaf length, second internode length, height without seedhead, average height, and biomass yield displayed significant ($\alpha = .05$, $P < .05$) interactions (Table 1).

As a result of significant genotype x year interactions (Table 1), means and ranges of 18 performance traits are separated by year in Table 2. Wide variability was evident in annual ranges of trait means in comparison to 4 commercial standards. Experimental entries collectively displayed quicker establishment rates following planting than commercial standards in 2017 (Table 2). Furthermore, experimental entries experienced lesser rates of collective winterkill than Goodwell and Midland 99 during 2018 and 2019, while Wrangler experienced the least among all evaluated genotypes (Table 2). Spring greenup was more vigorous in experimental entries in 2018, while Goodwell, Midland, and Wrangler exceeded the experimental mean in 2019 (Table 2). Biomass yield was highly variable in the experimental genotypes, ranging from 2.7 – 12.3 Mg ha^{-1} in 2018

and 5.3 – 18.9 Mg ha^{-1} in 2019 (Table 2). Commercial standards displayed a range in biomass yield between 7.3 – 8.0 Mg ha^{-1} in 2018 and 11.1 – 12.4 Mg ha^{-1} in 2019. Midland 99 displayed larger sizes of morphological features in both years (Table 2). Wide variability was evident in the ranges of morphological measurements for the experimental entries, but to varying degrees displayed longer leaves, comparable leaf widths, internode diameters, and internode length to most of the 4 standards (Table 2). Observed plant heights indicated a lower canopy for Wrangler in comparison to the experimental mean during both years, while all standards fell in the upper range of the plant heights for the experimental entries (Table 2).

Restricted maximum likelihood variance component estimates are presented in Table 3. With the exception of greenup and winterkill, all other observed traits displayed significant ($P < 0.01$ or 0.05) genotypic variance components (σ^2_G) and associated standard errors. No statistical difference was detected among year variance components (σ^2_Y), in addition to early vigor being the only trait with a significant ($\alpha = .05$, $P < .05$) replication variance component estimate (σ^2_R). Significant ($\alpha = .05$, $P < .05$) genotypic x year interaction variance estimates (σ^2_{GxY}) were present for 12 of the 17 traits with 2-year data, while significant genotypic x replication variance estimates (σ^2_{GxR}) were documented in biomass yield, first and average leaf length, internode length, height without seedhead, and average plant height.

Biomass Yield Component Analysis and Relationships

Table 4 displays significant ($\alpha = .05$, $P < .05$) Pearson correlation coefficients among 17 traits observed through 2018 and 2019. Biomass yield displayed significant (α

= .05, *P* <.05), positive correlations with 8 traits, first leaf length (r = 0.27), average leaf length (r = 0.32), second internode length (r = 0.55), greenup (r = 0.27), early vigor (r = 0.48), height with seedhead (r = 0.65), height without seedhead (r = 0.72), and average height (r = 0.70). Winterkill produced no correlation with biomass yield, however, significant (α = .05, *P* <.05), negative relationships were present between winterkill and several traits, most notably, early vigor (r = -0.33), spring greenup (r = -0.72), and height without seedhead (r = -0.65). Various correlations were evident among all traits, showcasing the positive and negative interactions that multiple traits can place upon the performance of one another.

Stepwise selection was performed with all traits to analyze their predictive impact on biomass yield and results displayed in Table 5. From the model, height with seedhead, second internode diameter, winterkill, and greenup were identified to be significant (α = .05, *P* <.0001). This model accounted for 43.7% of the variability witnessed in biomass yield. Height with seedhead accounted for the greatest portion of the model, representing 33.7% of biomass variability. Path analysis was performed to gauge the direct effects of average height, early vigor, greenup, second internode length, and average leaf length on biomass yield. Results of the path analysis are displayed in Table 6, showcasing the direct and indirect effects. Of the 5 descriptor traits, average plant height had the largest direct effect on biomass yield at 0.63, followed by early vigor displaying a positive direct effect of 0.20. Spring greenup and second internode length contributed direct effects of 0.04 and 0.08, respectively. Average leaf length expressed a negative effect on biomass yield of -0.15. Based on the path analysis, selection of taller plants that display earlier, vigorous spring greenup can aid in the indirect selection of biomass yield.

DISCUSSION

Variability in trait expression was present across genotypes. Environment influenced trait expression across growing seasons. Specifically, biomass yield had a significant genotype x year interaction. Multiple studies have reported similar variability of forage yield across growing seasons and/or locations (Avis et al., 1980; Mohamed et al., 1990; Wu et al., 2007). Across growing seasons, correlation coefficients between key morphological traits and biomass yield remained consistent. Although trait performance may change across environments, the positive and negative relationships among traits are relatively stable. Selecting for taller plants with early, vigorous spring growth will concurrently select for increased biomass production. Longer internodes were also the source of a moderate direct effect on biomass yield, this is to be expected as plant height and internode length are highly correlated ($r = 0.70$). Harlan (1970b) notes the large geographic distribution of bermudagrass, as Wu et al. (2007) observed similar path analysis results when evaluating a collection of Chinese turf-type bermudagrass accessions. Within their study, greenup, plant height, and internode length contributed substantial direct effects to biomass yield, similarly to what was witnessed in the evaluation of our bermudagrass population. Prior research has documented winterkill to have a negative correlation with biomass yield (Wu et al., 2007). This relationship was absent in our study, likely due to the focus placed on cold tolerance in the selection of germplasm for this nursery, thus limiting biomass yield variability in response to winterkill. The negative relationship between winterkill and spring greenup indicates the nature of more cold tolerant germplasm to express earlier spring growth. Furthermore, early vigor and spring greenup show a modest relationship, illustrating the ability of cold

tolerant germplasm to have more growth in early weeks of the growing season. Taller plants were generally associated with larger morphological features. Of further interest, taller plants were more vigorous during early spring growth.

Variability of trait expression across the evaluated population indicates a wide range of available germplasm for continued breeding efforts. This observed variability is similar to what was encountered and described throughout multiple studies (Burton 1947; Avis et al., 1980; Harlan, 1970; Harlan et al., 1969; de Silva, 1991). Breeders should be mindful of genotype x environment interactions of key performance traits, and carefully evaluate advanced lines across multiple environments and years. Despite a lack of relationship in this study, cold tolerance should continue to be an important trait in the evaluation of bermudagrass germplasm. Furthermore, confirmed trait relationships indicate the selection of tall, vigorous plants which express early spring growth will allow for the indirect advancement of biomass yield in forage bermudagrass breeding populations.

FIGURES AND TABLES

Table 1. Analyses of variance on 18 adaptive, morphological, and biomass traits for 104 *Cynodon* genotypes.

Source	Mean squares					
	Genotype (G)	**Replication (R)**	**G x R**	**Year (Y)**	**G x Y**	**Residual**
			2 year data			
df	103	2	206	1	103	208
FLL[†]	2478.0**	311.4	457.2*	4062.7*	623.3**	311.9
SLL	2298.4**	755.7	426.6	58642.0**	767.0**	383.0
ALL	2336.9**	465.9	407.2*	23377.0**	641.7**	305.4
FLW	0.8**	0.9**	0.1	22.0**	0.1	0.1
SLW	0.8**	1.1**	0.1	19.9**	0.1	0.1
ALW	0.8**	0.9**	0.1	20.8**	0.1	0.1
SID	0.1**	0.07*	0.02	2.5**	0.03**	0.02
TID	0.1**	0.05	0.02	0.8**	0.03**	0.02
AID	0.1**	0.07*	0.02	1.6**	0.03**	0.02
SIL	1023.7**	849.1*	237.8*	14413.0**	231.6	181.3
GU	1.9	1.8**	0.3	21.9**	1.8**	0.3
WK	286.5	259.8**	37.6	14327.0**	230.7**	31.8
EV	1.5*	0.8	0.8	20.5**	1.0	0.8
HWS	103.5**	2.7	14.1	3231.0**	22.9**	11.7
HNS	110.2**	21.8	13.6**	376.2**	19.2**	9.7
AH	104.2**	7.1	13.1*	1446.0**	19.4**	9.8
BMY	10.0**	6.3	2.9**	1784.9**	2.9**	1.9
			1 year data			
df	103	2	206	N/A		
AER	1.3**	0.6	0.2			

*Significant at $\alpha = 0.05$.

**Significant at $\alpha = 0.01$.

[†]FLL, first leaf length; SLL, second leaf length; ALL, average leaf length; FLW, first leaf width; SLW, second leaf width; ALW, average leaf width; SID, second internode diameter; TID, third internode diameter; AID, average internode diameter; SIL, second internode length; GU, greenup; WK, winterkill; EV, early vigor; HWS, height with seedhead; HNS, height without seedhead; AH, average plant height; BMY, biomass yield; AER, average establishment rate.

Table 2. Means, standard deviations, and ranges for 18 adaptive, morphological, and seed traits for 2017 through 2019 of 100 experimental accessions and 4 commercial standards.

Trait†	Experimental Accessions		Standards			
			Goodwell	Midland 99	Midland	Wrangler
	Mean ± SD	Range	Mean ± SD			
			2017			
AER	6.1 ± 0.7	3.8 – 8.8	5.3 ± 0.3	4.4 ± 0.1	5.3 ± 0.4	3.2 ± 0.3
			2018			
FLL, mm	168.4 ± 29.8	86.2 – 235.0	150.1 ± 10.2	183.9 ± 23.1	123.9 ± 25.1	151.8 ± 19.8
SLL, mm	166.6 ± 31.9	80.8 – 249.3	142.7 ± 16.6	175.5 ± 9.5	117.1 ± 21.2	150.3 ± 21.1
ALL, mm	167.5 ± 30.4	84.7 – 235.6	146.4 ± 12.9	179.7 ± 15.8	120.5 ± 23.1	151.0 ± 20.5
FLW, mm	4.5 ± 0.5	3.2 – 6.0	4.2 ± 0.1	4.3 ± 0.4	3.7 ± 0.2	4.9 ± 0.3
SLW, mm	4.5 ± 0.5	3.2 – 5.8	4.3 ± 0.2	4.2 ± 0.2	3.7 ± 0.1	4.9 ± 0.2
ALW, mm	4.5 ± 0.5	3.2 – 5.9	4.3 ± 0.2	4.3 ± 0.3	3.7 ± 0.1	4.9 ± 0.3
SID, mm	1.5 ± 0.2	1.0 – 2.0	1.6 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	1.5 ± 0.2
TID, mm	1.5 ± 0.2	1.0 – 2.0	1.5 ± 0.1	1.6 ± 0.2	1.3 ± 0.2	1.5 ± 0.1
AID, mm	1.5 ± 0.2	1.0 – 2.0	1.5 ± 0.1	1.6 ± 0.2	1.2 ± 0.2	1.5 ± 0.1
SIL, mm	84.7 ± 21.2	33.8 – 151.2	79.5 ± 9.7	87.0 ± 16.1	68.3 ± 8.2	88.5 ± 7.1
GU	5.3 ± 1.2	1.0 – 8.0	4.3 ± 0.6	3.0 ± 0.0	4.3 ± 0.6	4.0 ± 1.0
WK, %	17.2 ± 14.3	5.0 – 80.0	30.0 ± 8.7	36.7 ± 20.8	26.7 ± 5.8	8.3 ± 2.9
EV	5.9 ± 1.2	1.0 – 9.0	6.7 ± 2.1	6.0 ± 1.0	5.3 ± 0.6	6.0 ± 1.0
HWS, cm	44.3 ± 4.8	27.0 – 57.0	49.5 ± 5.5	49.5 ± 0.5	45.5 ± 0.5	40.0 ± 0.0
HNS, cm	37.7 ± 5.1	21.0 – 50.0	40.0 ± 7.0	44.5 ± 0.5	38.5 ± 1.5	33.0 ± 2.0
AH, cm	41.0 ± 4.9	24.5 – 53.5	44.8 ± 6.3	47.0 ± 0.5	42.0 ± 1.0	36.5 ± 1.0
BMY (Mg ha^{-1})	6.4 ±1.6	2.7 – 12.3	7.7 ± 2.1	8.0 ± 1.3	8.0 ± 1.0	7.3 ± 1.5
			2019			
FLL, mm	163.4 ± 25.0	95.9 – 246.5	122.6 ± 8.3	189.7 ± 19.8	132.1 ± 5.7	135.3 ± 18.3
SLL, mm	146.9 ± 22.9	52.0 – 201.3	114.8 ± 6.3	178.2 ± 19.6	118.5 ± 13.4	125.2 ± 2.6
ALL, mm	155.2 ± 22.7	92.4 – 217.9	118.8 ± 5.5	184.0 ± 19.7	125.3 ± 9.5	130.3 ± 9.5
FLW, mm	4.2 ± 0.4	2.9 – 5.4	3.5 ± 0.2	4.2 ± 0.1	3.0 ± 0.3	4.2 ± 0.1
SLW, mm	4.2 ± 0.5	2.9 – 5.4	3.6 ± 0.1	4.1 ± 0.1	3.0 ± 0.2	4.1 ± 0.1
ALW, mm	4.2 ± 0.4	2.9 – 5.3	3.6 ± 0.2	4.1 ± 0.1	3.0 ± 0.3	4.2 ± 0.1
SID, mm	1.3 ± 0.2	0.8 – 1.9	1.4 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	1.4 ± 0.3
TID, mm	1.4 ± 0.2	0.9 – 1.9	1.4 ± 0.2	1.6 ± 0.1	1.1 ± 0.1	1.5 ± 0.4
AID, mm	1.4 ± 0.2	0.9 – 1.9	1.4 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	1.5 ± 0.3
SIL, mm	75.3 ± 16.3	38.6 – 135.8	67.5 ± 4.8	79.4 ± 12.6	58.4 ± 8.2	62.1 ± 6.4
GU	4.9 ± 0.4	3.0 – 6.0	6.0 ± 0.0	4.7 ± 0.6	5.3 ± 0.6	5.0 ± 0.0
WK, %	7.8 ± 3.5	5.0 – 25.0	15.0 ± 0.0	15.0 ± 0.0	6.7 ± 2.9	5.0 ± 0.0
EV	5.6 ± 0.6	4.0 – 9.0	7.0 ± 0.0	5.0 ± 0.0	5.3 ± 0.6	5.7 ± 0.6
HWS, cm	48.8 ± 6.0	27.9 – 63.5	52.5 ± 3.7	59.3 ± 1.5	45.7 ± 5.1	48.3 ± 2.6
HNS, cm	39.2 ± 5.6	20.3 – 55.9	42.3 ± 2.9	50.8 ± 0.0	37.3 ± 5.3	38.1 ± 0.0
AH, cm	44.0 ± 5.7	24.1 – 59.7	47.4 ± 3.2	55.0 ± 0.8	41.5 ± 5.2	43.2 ± 1.3
BMY (Mg ha^{-1})	9.8 ± 2.2	5.3 – 18.9	12.4 ± 2.2	12.2 ± 1.6	11.1 ± 2.3	11.4 ± 3.9

†AER, average establishment rate; FLL, first leaf length; SLL, second leaf length; ALL, average leaf length; FLW, first leaf width; SLW, second leaf width; ALW, average leaf width; SID, second internode diameter; TID, third internode diameter; AID, average internode diameter; SIL, second internode length; GU, greenup; WK, winterkill; EV, early vigor; HWS, height with seedhead; HNS, height without seedhead; AH, average plant height; BMY, biomass yield.

Table 3. Variance component estimates and associated standard errors for 18 adaptive, morphological, and biomass traits in 104 *Cynodon* genotypes.

Trait	Variance components ± standard errors					
	σ^2_G	σ^2_Y	σ^2_R	σ^2_{GxY}	σ^2_{GxR}	σ^2_{Res}
BMY[†] (Mg ha^{-1})	1.02 ± 0.25**	5.74 ± 8.14	0.02 ± 0.03	0.31 ± 0.15*	0.49 ± 0.17**	1.93 ± 0.19**
FLL (mm)	285.30 ± 60.05**	11.02 ± 18.41	0.00 ± 0.00	103.79 ± 30.69**	71.94 ± 27.08**	311.92 ± 30.59**
SLL (mm)	247.97 ± 57.05**	185.50 ± 265.81	1.58 ± 3.64	128.00 ± 37.76**	21.82 ± 28.18	382.97 ± 37.55**
ALL (mm)	265.57 ± 56.90**	72.87 ± 105.96	0.28 ± 2.25	112.12 ± 31.43**	50.90 ± 25.03*	305.37 ± 29.94**
FLW (mm)	0.105 ± 0.018**	0.070 ± 0.100	0.004 ± 0.004	0.008 ± 0.007	0.004 ± 0.008	0.110 ± 0.011**
SLW (mm)	0.106 ± 0.018**	0.063 ± 0.090	0.005 ± 0.005	0.003 ± 0.006	0.002 ± 0.007	0.105 ± 0.010**
ALW (mm)	0.106 ± 0.018**	0.066 ± 0.094	0.004 ± 0.004	0.004 ± 0.006	0.002 ± 0.007	0.103 ± 0.010**
SID (mm)	0.010 ± 0.002**	0.008 ± 0.011	0.000 ± 0.000	0.004 ± 0.002**	0.000 ± 0.000	0.022 ± 0.002**
TID (mm)	0.011 ± 0.003**	0.002 ± 0.004	0.000 ± 0.000	0.003 ± 0.002*	0.000 ± 0.002	0.024 ± 0.002**
AID (mm)	0.011 ± 0.002**	0.005 ± 0.007	0.000 ± 0.000	0.003 ± 0.002*	0.000 ± 0.002	0.021 ± 0.002**
SIL (mm)	122.60 ± 24.86**	45.46 ± 65.33	2.94 ± 4.08	16.79 ± 12.28	28.25 ± 14.70*	181.27 ± 17.78**
GU	0.022 ± 0.062	0.065 ± 0.099	0.007 ± 0.009	0.490 ± 0.084**	0.006 ± 0.022	0.316 ± 0.031**
WK (%)	8.31 ± 8.58	45.18 ± 64.94	1.07 ± 1.25	66.32 ± 10.77**	2.94 ± 2.42	31.77 ± 3.12**
EV	0.083 ± 0.041*	0.063 ± 0.093	0.000 ± 0.004*	0.051 ± 0.048	0.000 ± 0.000	0.809 ± 0.056**
HWS (cm)	13.04 ± 2.48**	10.28 ± 14.65	0.00 ± 0.00	3.76 ± 1.13**	1.17 ± 0.89	11.65 ± 1.14**
HNS (cm)	14.50 ± 2.61**	1.14 ± 1.71	0.04 ± 0.11	3.20 ± 0.95**	1.99 ± 0.82**	9.65 ± 0.95**
AH (cm)	13.61 ± 2.48**	4.57 ± 6.55	0.00 ± 0.00	3.17 ± 0.96**	1.59 ± 0.80*	9.85 ± 0.97**
AER[‡]	0.369 ± 0.062**		0.004 ± 0.006		0.000 ± 0.000	0.224 ± 0.022**

*Estimate significant at $\alpha = 0.05$.

** Estimate significant at $\alpha = 0.01$.

[†]FLL, first leaf length; SLL, second leaf length; ALL, average leaf length; FLW, first leaf width; SLW, second leaf width; ALW, average leaf width; SID, second internode diameter; TID, third internode diameter; AID, average internode diameter; SIL, second internode length; GU, greenup; WK, winterkill; EV, early vigor; HWS, height with seedhead; HNS, height without seedhead; AH, average plant height; BMY, biomass yield; AER, average establishment rate.

[‡]Trait had 1 year of data.

Table 4. Significant ($p < 0.05$) correlation coefficients of 17 adaptive, morphological, and biomass traits of 104 *Cynodon* genotypes.

	BMY†	FLL	SLL	ALL	FLW	SLW	ALW	SID	TID	AID	SIL	GU	WK	EV	HWS	HNS
2	0.27	-														
3	-	0.96	-													
4	0.32	0.99	0.99	-												
5	-	0.45	0.44	0.45	-											
6	-	0.47	0.47	0.47	0.99	-										
7	-	0.46	0.46	0.46	1.00	1.00	-									
8	-	0.55	0.51	0.54	0.66	0.68	0.67	-								
9		0.53	0.47	0.51	0.64	0.66	0.65	0.94	-							
10	-	0.55	0.51	0.54	0.66	0.67	0.67	0.98	0.98	-						
11	0.55	0.43	0.50	0.47	-	-	-	0.20	0.21	0.21	-					
12	0.27	0.24	0.30	0.27	-	-	-	-	-	-	0.31	-				
13	-	-0.30	-0.35	-0.33	-	-	-	-	-	-	-0.23	-0.72	-			
14	0.48	0.22	0.30	0.26	-	-	-	-	-	-	0.39	0.38	-0.33	-		
15	0.65	0.52	0.57	0.55	-	-	-	0.28	0.21	0.26	0.69	0.27	-	0.40	-	
16	0.72	0.56	0.62	0.60	-	-	-	0.29	0.22	0.26	0.70	0.26	-0.65	0.44	0.95	-
17	0.70	0.55	0.60	0.58	-	-	-	0.29	0.22	0.26	0.70	0.27	-	0.43	0.99	0.99

†1 = biomass yield (BMY); 2 = first leaf length (FLL); 3 = second leaf length (SLL); 4 = average leaf length (ALL); 5 = first leaf width (FLW); 6 = second leaf width (SLW); 7 = average leaf width (ALW); 8 = second internode diameter (SID); 9 = third internode diameter (TID); 10 = average internode diameter (AID); 11 = second internode length (SIL); 12 = greenup (GU); 13 = winterkill (WK); 14 = early vigor (EV); 15 = height with seedhead (HWS); 16 = height without seedhead (HNS); 17 = average height (AH).

Table 5. Stepwise selection of predictive traits for biomass yield for 104 *Cynodon* genotypes.

Trait	Partial R^2	Model R^2	P value
Height with seedhead	0.3366	0.3366	< .0001
Second internode diameter	0.0519	0.3885	< .0001
Winterkill	0.0321	0.4205	< .0001
Greenup	0.0163	0.4369	< .0001

Table 6. Path analysis of direct and indirect effects of 5 traits on biomass yield for 104 *Cynodon* genotypes.

Trait	Direct effect	Indirect effect					Biomass Correlation Coefficient
		AH	EV	GU	SIL	ALL	
AH†	0.627	-	0.087	0.010	0.057	-0.086	0.696
EV	0.204	0.268	-	0.015	0.034	-0.039	0.479
GU	0.039	0.167	0.077	-	0.026	-0.041	0.268
SIL	0.082	0.441	0.080	0.012	-	-0.070	0.545
ALL	-0.149	0.364	0.054	0.011	0.038	-	0.318

†AH, average height; EV, early vigor; GU, greenup; SIL, second internode length; ALL, average leaf length.

CHAPTER III

FORAGE BERMUDAGRASS RELATIONSHIPS AMONG SEED YIELD AND ITS COMPONENTS

ABSTRACT

Seed propagated bermudagrass offers considerable advantages over clonally propagated cultivars in terms of access to planting equipment, logistics of shipping and storage, and associated labor costs. However, limited cold tolerant, seeded forage-type bermudagrass cultivars are available for the transition zone of the United States. Germplasm characterization provides valuable resources for breeders in understanding relationships and variability of seed yield and its components for use in cultivar development. Accordingly, the objective of this experiment was to characterize seed yield and its components in a forage germplasm collection. From 2017 – 2019, 104 accessions of cold tolerant bermudagrass were evaluated for interrelationships of 14 adaptive, reproductive, and seed traits with seed yield. Significant ($\alpha = .05$, $P < .05$) positive correlations were observed between seed yield and seeds infloresence^{-1}, seed set, inflorescence prolificacy, and seed weight. Additionally, seed yield was negatively correlated with early vigor, plant height, and biomass yield. These relationships suggest the difficulty in selecting for

both biomass and seed yield. Path analysis indicated that the selection for high rates of seedhead production, exceptional fertility, and heavier seeds will indirectly select for increased seed yield.

INTRODUCTION

Bermudagrass (*Cynodon* spp.) is a widely distributed, warm-season, perennial grass popular for its excellent turf, soil stabilization, and forage production capabilities (Harlan, 1970; Taliaferro, 1995). The utilization of bermudagrass in forage production has likely expanded from Taliaferro et al. (2004) original estimate of 10 – 12 million hectare (ha) in the United States. Breeding initiatives have produced clonal, cold tolerant varieties such as Goodwell, Midland 99, Hardie, and Ozark, allowing for the northern expansion of forage bermudagrass (Wu and Taliaferro, 2009; Taliaferro and Richardson, 1980; Taliaferro et al., 2002; Richardson and Taliaferro, 2005). Over the years, interest has increased in the production of seeded propagated bermudagrass. Seeded cultivars offer several advantages in terms of planting equipment, logistics of shipping and storage, and overall labor costs (Ahring et al., 1974; Tan, 2013; Guo et al., 2017). A unique aspect of bermudagrass seed production is that the positive effect of alternating wet and dry irrigation cycles can have on increasing seedhead production (Ahring et al., 1974; Ahring et al., 1982).

Many seeded cultivars are produced in the southwestern United States (Baltensperger et al., 1993; Kneebone, 1966), however, many of these cultivars lack the cold tolerance needed for productive use in the transition zone (Redfearn and Wu, 2013). Primary options for cold tolerant, seeded cultivars include Wrangler (Johnston Seed

Company, Enid, OK) and Guymon (Taliaferro et al., 1983), released in 1999 and 1983, respectively. Genetic variability of bermudagrass has been well documented (Burton, 1947; Wu et al., 2006; Wu et al., 2007; Anderson et al., 2009; Jewell et al., 2012; Guo et al., 2017). High levels of self-incompatibility are a major driver in the heterozygous and diverse nature of the present variability in bermudagrass (Burton and Hart, 1967; Richardson et al., 1978; Tan et al., 2013). The genetic diversity and reproductive mechanisms of bermudagrass provide considerable opportunity for furthered development of new seeded cultivars.

Seed yield improvement can be difficult, if not impossible to efficiently quantify in greenhouses and high density selection nurseries, and understanding the associations of seed yield and secondary traits is critical to breeders in the selection process. Early work by Cluff and Baltensperger (1991) demonstrated a correlation between seed set percentage and seed yield. Additionally, Guo et al. (2017) noted the negative relationships between raceme length and seed set percentage. Wu et al. (2006) evaluated interrelationships of reproductive traits in 114 turf-type accessions of Chinese bermudagrass, identifying seed yield to be correlated with inflorescence prolificacy, percent seed set, and seeds infloresence^{-1}. Furthermore, path analysis showed inflorescence prolificacy and seed set to have the strongest direct effects on seed yield. Path analysis allows for accurate quantification of direct and indirect effects traits play on primary traits, accounting for the complex interrelationships correlation coefficients fail to address on their own (Das et al., 2004; Kang, 1994). Aside from the previously mentioned studies, limited work has been conducted to further investigate reproductive and adaptive trait relationships with seed yield in bermudagrass. The objective of this

study is to evaluate the genetic variability of seed yield and its components in a collection of 104 forage bermudagrass genotypes, in addition to identifying proper traits for use in indirect selection via path analysis of phenotypic data.

MATERIALS AND METHODS

Germplasm Preparation

A collection of 100 genotypes were evaluated and selected from 3 seeded forage bermudagrass nurseries in Stillwater, OK. Selection criteria were based on seedhead abundance and flowering date. Germplasm was transplanted into a greenhouse and grown under ideal growing conditions for 5 weeks with irrigation, fertilizer, and pesticides being applied as needed. For each accession, 14 plants were grown in 3.8 cm diameter cone-tainers. Four commercial standards, Goodwell, Midland, Midland 99, and Wrangler were grown as well.

Experimental Design, Establishment, and Maintenance

The trial was located at the Oklahoma Panhandle Research and Extension Center (OPREC), located in Goodwell, OK. A randomized complete block design, with 3 replications was used, evaluating 104 total genotypes. Each plot measured 2.7 by 2.7 m, separated by 1 m allies, while a 2 m alley separated each replication. The entire trial measured 100.9 m long and 43.4 m wide. Plants were established in June 2017 on a finely tilled Gruver clay loam by planting 4 plugs of each genotype equally spaced in their respective plots. Prior to planting, urea was incorporated into the seedbed at a rate of 112.1 kg N ha^{-1}, in addition to phosphorus (P) and potassium (K) rates based on soil test results from the Oklahoma State University Soil, Water, and Forage Analytical

Laboratory (SWAFL). Immediately after planting, Ronstar Flo (Bayer Crop Science, Monheim am Rhein, Germany) was applied at a rate of 6.4 L product ha^{-1} pre-emergent, followed by irrigation. Plots were kept well-watered throughout establishment. To prevent contamination of neighboring plots, glyphosate was applied to alleys as needed to control overgrowth throughout the duration of the experiment at a rate of 2.3 L product ha^{-1}. During the spring of 2018 and 2019, irrigation was applied at weekly rates of 25.4 – 50.8 mm prior to induced wet/dry cycles. Weekly rates were based on sprinkler capacity at OPREC. Following plant recovery from any sustained winterkill, plots were mowed down to 7.6 cm height to encourage equal starts to summer growth. Two weeks post mowing, all plots entered alternate, 2-week wet/dry cycles to promote reproductive growth. Wet cycles featured weekly irrigation of approximately 50.8 mm, in addition to any received rainfall. Dry cycles incurred the complete withholding of irrigation, while the occurrence of rainfall greater than 25.4 mm resulted in the restart of the respective dry cycle. Urea N was applied at a total rate of 224.2 kg N ha^{-1} in split 112.1 kg N ha^{-1} during each growing season in 2018 and 2019. The first application was applied following the conclusion of spring greenup, and the second was applied after seed harvest in the fall. All biomass was swathed and baled away following seed harvest, providing plots time to recover before winter dormancy. During both 2018 and 2019, P and K were applied in the spring based on SWAFL soil test results, in addition to commercial herbicides used as needed for weed control.

Data Collection and Analysis

All observed traits are presented in Table 7. Plants were visually assessed for establishment rate in 2017, in addition to spring greenup, early vigor, inflorescence

prolificacy and winterkill in 2018 and 2019. Visual ratings for establishment, greenup, inflorescence prolificacy, and vigor were based on a 1 – 9 scale used by the National Turf Evaluation Program (NTEP), with 1 signifying relative worse performance across the trial, and 9 being best. Establishment ratings of 1 represented limited to no lateral growth of stolons, while 9 signified rapid lateral and upward growth of stolons and shoots. Greenup rating of 1 represent plots with few to none emerged shoots, while ratings of 9 featured upright shoots and dense canopy cover throughout the plot. An early vigor rating of 1 was associated with light green color and sparse canopy cover with weak early growth, while ratings of 9 featured dense, dark green growth with erect, rapid canopy growth. Inflorescence prolificacy ratings were a function of seedhead density, with 1 representing few to no seedheads, and a rating of 9 showcasing dense, prolific seedheads throughout the plot. Winterkill was quantified as a percentage of dead plot area following spring greenup, 0% was representative of no visual observance of dead plot area, while 100% featured no observable bermudagrass growth. Physical measurements of seedhead features were based on 5 mature seedheads randomly collected from each plot within each replication prior to seed harvest in both 2018 and 2019. Traits examined from each seedhead included raceme number and length, seeds infloresence^{-1}, seed set, and 1000 seed weight. Raceme number was visually counted, and length was measured with a metric ruler. Seeds infloresence^{-1} was determined by soaking each seedhead in a 20% bleach v/v solution for up to 24 hours. Following bleaching, seeds turn a pale orange and are easily identifiable. To determine seed set percentage, seeds infloresence^{-1} was divided over spikelet number infloresence^{-1}. As it was impractical to count spikelet number for all 1,560 seedheads, a model was developed through regressing the spikelet number with

raceme length of 100 seedheads. The linear regression equation used was: $y = 0.82x + 26.42$ ($r^2 = 0.79$, $P < 0.001$). Seed weight was determined by separating out 100 seeds from the harvested seed of each plot, weighing it, and multiplying by 10. Seed harvest featured the manual clipping of a 0.3 by 0.3 m area of each plot when seedheads were mature. Samples were oven dried for 48 hours at 65°C. Seed was hand rubbed and cleaned using a Model B South Dakota seed blower. Cleaned samples were weighed and converted from g 0.09 m^{-1} to a kg ha^{-1} scale.

Data analysis utilized SAS 9.4 (SAS, 2014). Plot means of each seed yield component were generated from the 5 seedhead samples and used for data analysis. Genotype, replication, and year served as random effects in analysis of variance (ANOVA), conduced with PROC MIXED. Means, standard deviations, and ranges of traits were generated with PROC MEANS and seed yield least significant differences were obtained with PROC GLM at a 5% and 1% probability level. The use of PROC GLM was based on prior variety testing experiments and lack of missing data points within separated traits. Mean separation data was utilized in establishing thresholds for selection in the breeding process. Stepwise selection and spikelet number infloresence-1 regression model generation were conducted in PROC REG. Significant ($P < 0.05$) correlation coefficients were generated in PROC CORR. Following concepts developed by Dewey and Lu (1959), path analysis was performed with PROC IML. Selection of top performing accessions was based on the relative performance indices of observed traits in comparison to the top performing observation of the respective trait throughout the trial and growing season. Performance indices were summed across traits and across growing season, where the top 10 were selected off seed and biomass yield.

RESULTS

Trait Variability

Analysis of variance (ANOVA) results are displayed in Table 7 for 14 observed traits. Significant ($\alpha = .05$, $P < .05$) genotypic differences occurred among 12 of the 14 observed morphological and adaptive traits. With the exception of inflorescence prolificacy and raceme number, significant differences ($\alpha = .05$, $P < .05$) were attributed to effects generated by year. Replication differences were more conservative, with significant differences ($\alpha = .05$, $P < .05$) occurring among 7 of the 14 traits. Genotype x replication interaction was significant ($\alpha = .05$, $P < .05$) in raceme length, seeds infloresence^{-1}, seed set, raceme number, height without seedhead, average height, and seed yield. Furthermore, genotype x year interactions were significant ($\alpha = .05$, $P < .05$) in 10 of 13 traits observed in 2018 and 2019.

Displayed in Table 8, means, associated standard deviations, and ranges of 14 traits further support wide variation among genotypes. Wrangler serves as the primary standard of comparison for seed components and yield, due to it being the only seeded standard in the trial. Due to significant genotype x year interactions, means were separated by year. Wrangler had better mean seed set, lowest average winterkill, and highest seed yield in both 2018 and 2019 in comparison to the 100 experimental plant mean and other standard cultivars. Experimental entries produced a range of 2 – 8 for inflorescence prolificacy ratings, with an average of 5.6 in 2018. These metrics were similar to Wrangler, with a mean inflorescence prolificacy of 5.6. Experimental range for 2019 was relatively similar, with a range of 1 – 9 with a mean of 5.8. With the exception

of Wrangler, experimental entries had a lower mean winterkill in both 2018 and 2019 when compared to Goodwell, Midland, and Midland 99. Average seed weight was higher in experimental entries during 2018, while Wrangler had heavier seeds in 2019. Wrangler had highest mean seed set percentages in both 2018 and 2019. Midland 99 had the tallest plant canopy both years, falling in the upper range of experimental entry plant heights. Experimental genotypes displayed more vigorous average spring greenup in 2018, while Goodwell produced the strongest greenup in 2019. Wrangler had the highest mean seed yield at 372.1 kg ha^{-1} in 2018, and 245.7 kg ha^{-1} in 2019. These yields fell in the middle of the experimental entry ranges of 2.9 – 536.0 kg ha^{-1} in 2018, and 2.9 – 536.5 kg ha^{-1} in 2019.

Variance component estimates and associated standard errors are represented in Table 9. Genotypic variance estimates (σ^2_G) were significant ($\alpha = .05$, $P < .05$) for all traits with the exception of greenup and winterkill. Variance estimates for year (σ^2_Y) were not significant ($\alpha = .05$, $P < .05$), while early vigor was the lone trait to have a significant ($\alpha = .05$, $P < .05$) replication variance estimate (σ^2_R). Genotype x year variance estimates (σ^2_{GxY}) were significant ($\alpha = .05$, $P < .05$) for all traits observed over 2018 and 2019 with the exception of raceme length, seed weight, and early vigor. Genotype x replication variance estimates (σ^2_{GxR}) was significant ($\alpha = .05$, $P < .05$) for 7 of the 14 observed traits. The observed variance estimates for seed yield ad it's components show significant variation among the evaluated genotypes, in addition to substantial variation experienced between 2018 and 2019.

Trait Relationships and Path Analyses

Significant ($\alpha = .05$, $P < .05$) correlation coefficients are given in Table 10. Seed yield was positively correlated with seeds inflorescence^{-1} (r = 0.64), seed set (r = 0.65), inflorescence prolificacy (r = 0.55), and seed weight (r = 0.32). Moderate, negative relationships were witnessed between seed yield and early vigor (r = -0.19), height with seedhead (r = -0.33), height without seedhead (r = -0.42), average height (r = -0.38), and biomass yield (r = -0.28). Biomass yield had further negative relationships with seeds inflorescence^{-1} (r = -0.22), seed set (r = -0.23), inflorescence prolificacy (r = -0.29), and seed weight (r = -0.22). Taller plants generally displayed lower levels of seedhead production (r = -0.30). Seed set percentage was negatively affected by higher rates of winterkill to a degree (r = -0.26). Furthermore, stronger seed set was accompanied by heavier seeds (r = 0.30).

Stepwise selection was performed on all traits to generate a model which illustrates the predictive impact on total seed yield, these results are displayed in Table 11. Seed set and inflorescence prolificacy were identified as strong predictors of seed yield ($\alpha = .05$, $P < .0001$). The model accounted for 63.7% of the variability witnessed in seed yield, with seed set generating 42.3% and inflorescence prolificacy contributing 21.4% of observed model predictive power.

Path analysis was performed with traits that generated significant correlation coefficients ($\alpha = .05$, $P < .05$) with seed yield. Direct effects to seed yield and associated correlation coefficients of 5 traits are presented in Table 12. Inflorescence prolificacy had the largest direct effect on seed yield at 0.41, followed by seeds inflorescnce^{-1} at 0.38.

Seed set and seed weight had smaller direct effects of 0.17 and 0.09, respectively. Average plant height produced the lone negative, direct effect with seed yield at -0.13. These results indicate the need to select fertile plants with abundant seedheads producing heavy seeds in order to indirectly select for seed yield. Additionally, care should be taken when selecting plants with taller canopies due to the negative relationship of seed yield and plant height.

Top Performing Selections

Utilizing a selection index based on relative trait performance, the top ten accessions were identified for future breeding objectives. Top accessions and their associated statistics are presented in Table 13. Various combinations of these selected genotypes will be tested for combining ability, compatible performance, and uniformity of progeny trait expression. Selected plants were G-19-1, G-19-8, G-19-17, G-19-20, G-19-30, G-19-31, G-19-37, G-19-70, G-19-78, and G-19-83. Biomass yield of all accession is presented in Table 14 and seed yield is presented in Table 15, in addition to associated LSDs. Wrangler produced the highest seed yield throughout the duration of the experiment, with G-19-70 producing the greatest seed yield amongst the experimental accessions when combining growing season performance. When considering biomass yield, G-19-86 was the top producing accession, but was moderate in terms of seed yield.

DISCUSSION

High levels of genetic variability were observed for the evaluated traits among the genotypes, which is in agreement with previous studies (Harlan and de Wet, 1969; Richardson et al., 1978; Wu et al., 2006; Guo et al., 2017). Furthermore, significant

genotype by year interactions of key traits such as inflorescence prolificacy, seed set, and seed yield, illustrate the impact environmental variation has on trait expression. Year produced a significant interaction with genotypic expression in these same traits in a study conducted by Wu et al. (2006). Guo et al. (2017) reported a significant genotype by year effect on inflorescence prolificacy, but not for seed set percentage. Fertility and seed yield means and associated ranges were similar to what was witnessed by Kenna et al. (1983), but lower than what was observed by Wu et a. (2006). Weather conditions likely played a role in our lower levels of average seed set percentage and seed yield.

Despite this, several genotypes performed well in terms of seed production and fertility when being compared to the commercial standard, Wrangler. Multiple experimental accessions have potential for synthetic varietal development. Evaluating combining ability, compatible growth habits, and uniformity of progeny performance are critical elements of furthered testing among the selected accessions. In addition to biomass and seed yield, traits that are critical for synthetic population development are establishment rate, greenup, early vigor, and maturity. Uniform establishment rates, early vigor, and greenup expression ensure that certain parents are not overtaken within the field by adjacent parents. Similar maturity is also of high importance, as plants undergoing pollination at uniform intervals throughout the growing season ensures the potential for maximum seed production. Ultimately, a synthetic cultivar, which is a scheme that relies on open pollination among several parent plants, will be the targeted objective of continued breeding evaluations among the top accessions.

Trait relationships with seed yield provided valuable insight into secondary traits to be used for indirect selection. Strong correlation coefficients were observed between

seed yield and seeds inflorescence^{-1}, seed set, and inflorescence prolificacy. Additionally, a moderate relationship was observed between seed yield and seed weight. Of particular interest, biomass yield was negatively associated with seed yield, indicating the difficult obstacle breeders can encounter when trying to select for both seed and forage yield. Seed yield also experienced negative correlations with early vigor and plant height. Early vigor and plant height are traits with known, positive associations to biomass yield (Wu et al., 2007). Interrelationships of these traits and their impact on heightened biomass production is a likely explanation for the relative contribution to seed yield drag, as the genes associated with early vigor and plant height favor vegetative growth over reproductive. When selecting for seed yield, breeders should take care to avoid selecting for traits with isolated associations to biomass yield, as they may have a negative impact on seed production. Overall, observed correlation coefficients were in agreement with previous research. Wu et al. (2006) documented similar relationships among seed yield and inflorescence prolificacy, seed set, and seeds inflorescence^{-1}. Kenna et al. (1983) and Ahring et al. (1974) also reported positive correlations between seed yield and seed set.

Path analysis results mirrored the observed correlation coefficients, as inflorescence prolificacy provided the largest direct effect to seed yield, followed by seeds inflorescence^{-1}, seed set, and seed weight. Plant height had a negative effect on overall seed yield. Das et al. (2004) and Kang (1994) both noted the advantage path analysis contributes in accounting for interactions among traits as they relate to primary traits of interest. Wu et al. (2006) observed inflorescence prolificacy to have a similar direct effect on seed yield as to what was observed in our study. Seed set contributed a larger direct effect in Wu et al. (2006), while seeds inflorescence^{-1} exerted a modest

negative direct effect on seed yield. Furthermore, Wu et al. (2006) mentioned seed weight as potential variable of interest for future work. Seed weight contributed a small, but positive direct effect to seed yield, indicating that seed size can be a determining factor in this selection process. Overall, selection of plants with high rates of seedhead production which express strong fertility and larger seeds will aid in leveraging selection for seed yield.

Table 7. Analyses of variance on 14 adaptive, morphological, and seed traits for 104 *Cynodon* genotypes.

Source	Mean squares					
	Genotype (G)	Replication (R)	G x R	Year (Y)	G x Y	Residual
2 year data						
df	103	2	206	1	103	208
RL	8434.0**	22888.0**	1763.7*	52097.0**	1564.1	1206.5
SDI	1485.9**	1883.5**	237.6**	1061.2*	243.7**	142.9
SDS	173.1**	102.5*	27.4**	298.8**	28.6**	16.2
IP	6.4**	3.8*	0.9	10.5	2.9**	0.9
RN	1.5**	4.1**	0.2*	0.4	0.2**	0.1
SWD	0.0082**	0.0058	0.0023	0.5284**	0.0024	0.0020
GU	1.9	1.8**	0.3	21.9**	1.8**	0.3
WK	286.5	259.8**	37.6	14327.0**	230.7**	31.8
EV	1.5*	0.8	0.8	20.5**	1.0	0.8
HWS	103.5**	2.7	14.1	3231.0**	22.9**	11.7
HNS	110.2**	21.8	13.6**	376.2**	19.2**	9.7
AH	104.2**	7.1	13.1*	1446.0**	19.4**	9.8
SDY	17382.0**	2612.5	3419.7**	180962.0**	3728.8**	2242.5
1 year data						
df	103	2	206	N/A		
AER	1.3**	0.6	0.2			

*Significant at $\alpha = 0.05$.

**Significant at $\alpha = 0.01$.

†RL, raceme length; SDI, seeds inflorescence^{-1}; SDS, seed set; IP, inflorescence prolificacy; RN, raceme number; SWD, seed weight; GU, greenup; WK, winterkill; EV, early vigor; HWS, height with seedhead; HNS, height without seedhead; AH, average plant height; BMY, biomass yield; AER, average establishment rate.

Table 8. Means, standard deviations, and ranges for 14 adaptive, morphological, and seed traits for 2017 through 2019 of 100 experimental accessions and 4 commercial standards.

Trait†	Experimental Accessions		Standards			
			Goodwell	Midland 99	Midland	Wrangler
	Mean ± SD	Range	Mean ± SD			
			2017			
AER	6.1 ± 0.7	3.8 – 8.8	5.3 ± 0.3	4.4 ± 0.1	5.3 ± 0.4	3.2 ± 0.3
			2018			
RL, mm	341.5 ± 52.8	173.2 – 513.4	354.9 ± 3.8	399.9 ± 69.5	265.1 ± 21.3	352.3 ± 25.0
SDI	27.5 ± 20.9	0.0 – 90.2	9.7 ± 6.4	1.9 ± 1.9	4.6 ± 1.9	55.2 ± 20.5
SDS, %	9.1 ± 7.0	0.0 – 35.1	3.1 ± 2.0	0.6 ± 0.6	1.9 ± 0.9	17.7 ± 7.0
IP	5.6 ± 1.1	2.0 – 8.0	5.3 ± 0.6	2.3 ± 0.6	6.3 ± 0.6	6.0 ± 0.0
RN	5.5 ± 0.6	4-0 – 9.0	5.5 ± 0.3	5.9 ± 0.6	4.6 ± 0.2	5.6 ± 0.4
SDW, g	0.38 ± 0.05	0.25 – 0.53	0.35 ± 0.05	0.32 ± 0.02	0.30 ± 0.58	0.37 ± 0.02
GU	5.3 ± 1.2	1.0 – 8.0	4.3 ± 0.6	3.0 ± 0.0	4.3 ± 0.6	4.0 ± 1.0
WK, %	17.2 ± 14.3	5.0 – 80.0	30.0 ± 8.7	36.7 ± 20.8	26.7 ± 5.8	8.3 ± 2.9
EV	5.9 ± 1.2	1.0 – 9.0	6.7 ± 2.1	6.0 ± 1.0	5.3 ± 0.6	6.0 ± 1.0
HWS, cm	44.3 ± 4.8	27.0 – 57.0	49.5 ± 5.5	49.5 ± 0.5	45.5 ± 0.5	40.0 ± 0.0
HNS, cm	37.7 ± 5.1	21.0 – 50.0	40.0 ± 7.0	44.5 ± 0.5	38.5 ± 1.5	33.0 ± 2.0
AH, cm	41.0 ± 4.9	24.5 – 53.5	44.8 ± 6.3	47.0 ± 0.5	42.0 ± 1.0	36.5 ± 1.0
SDY, kg ha^{-1}	98.3 ± 83.6	2.9 – 536.0	84.8 ± 47.1	4.0 ± 3.6	35.3 ± 16.0	372.1 ± 91.8
			2019			
RL, mm	324.0 ± 51.5	215.2 – 481.0	316.8 ± 39.2	330.7 ± 10.2	262.6 ± 13.1	303.5 ± 52.7
SDI	29.9 ± 19.4	0.0 – 98.0	16.1 ± 8.1	2.6 ± 1.9	11.3 ± 1.6	73.9 ± 16.8
SDS, %	10.4 ± 6.6	0.0 – 31.6	5.8 ± 3.4	0.9 ± 0.7	4.6 ± 0.4	27.5 ± 6.2
IP	5.8 ± 1.8	1.0 – 9.0	6.3 ± 1.2	6.7 ± 0.6	6.7 ± 0.6	7.7 ± 1.5
RN	5.4 ± 0.6	4.0 – 8.6	5.2 ± 0.4	5.1 ± 0.1	4.6 ± 0.3	4.8 ± 0.7
SDW, g	0.32 ± 0.06	0.13 – 0.50	0.29 ± 0.04	0.31 ± 0.08	0.23 ± 0.02	0.35 ± 0.08
GU	4.9 ± 0.4	3.0 – 6.0	6.0 ± 0.0	4.7 ± 0.6	5.3 ± 0.6	5.0 ± 0.0
WK, %	7.8 ± 3.5	5.0 – 25.0	15.0 ± 0.0	15.0 ± 0.0	6.7 ± 2.9	5.0 ± 0.0
EV	5.6 ± 0.6	4.0 – 9.0	7.0 ± 0.0	5.0 ± 0.0	5.3 ± 0.6	5.7 ± 0.6
HWS, cm	48.8 ± 6.0	27.9 – 63.5	52.5 ± 3.7	59.3 ± 1.5	45.7 ± 5.1	48.3 ± 2.6
HNS, cm	39.2 ± 5.6	20.3 – 55.9	42.3 ± 2.9	50.8 ± 0.0	37.3 ± 5.3	38.1 ± 0.0
AH, cm	44.0 ± 5.7	24.1 – 59.7	47.4 ± 3.2	55.0 ± 0.8	41.5 ± 5.2	43.2 ± 1.3
SDY, kg ha^{-1}	64.4 ± 53.8	2.9 – 536.5	62.1 ± 15.8	35.6 ± 19.6	47.7 ± 16.1	245.7 ± 173.5

†AER, average establishment rate; RL, raceme length; SDI, seed inflorescence^{-1}; SDS, seed set; IP, inflorescence prolificacy; RN, raceme number; SDW, seed weight; GU, greenup; WK, winterkill; EV, early vigor; HWS, height with seedhead; HNS, height without seedhead; AH, average plant height; BMY, biomass yield.

Table 9. Variance component estimates and associated standard errors for 14 adaptive, morphological, and seed yield traits in 104 *Cynodon* genotypes.

Trait	Variance components ± standard errors					
	σ^2_G	σ^2_Y	σ^2_R	σ^2_{GxY}	σ^2_{GxR}	σ^2_{Res}
SDY† (kg ha^{-1})	2279.48 ± 439.18**	544.32 ± 785.81	0.00 ± 0.00	397.16 ± 187.49*	486.91 ± 203.52**	2338.28 ± 234.58**
RL	1052.12 ± 202.27**	161.96 ± 236.14	101.56 ±110.04	119.19 ± 82.66	278.59 ± 105.11**	1206.50 ± 118.31**
SDI	191.26 ± 35.27**	2.62 ± 4.81	7.91 ± 9.06	33.58 ± 12.24**	47.36 ± 13.64**	142.91 ± 14.01**
SDS	22.24 ± 4.11**	0.87 ± 1.35	0.36 ± 0.49	4.11 ± 1.43**	5.59 ± 1.57**	16.23 ± 1.59**
IP	0.58 ± 0.17**	0.02 ± 0.05	0.01 ± 0.02	0.69 ± 0.14**	0.03 ± 0.06	0.86 ± 0.08**
RN	0.210 ± 0.035**	0.001 ± 0.002	0.019 ± 0.020	0.023 ± 0.010*	0.021 ± 0.011*	0.134 ± 0.013**
SDW	0.0009 ± 0.0002**	0.0017 ± 0.0024	0.00002 ± 0.00003	0.0001 ± 0.0001	0.0001 ± 0.0002	0.0020 ± 0.0002**
GU	0.022 ± 0.062	0.065 ± 0.099	0.007 ± 0.009	0.490 ± 0.084**	0.006 ± 0.022	0.316 ± 0.031**
WK (%)	8.31 ± 8.58	45.18 ± 64.94	1.07 ± 1.25	66.32 ± 10.77**	2.94 ± 2.42	31.77 ± 3.12**
EV	0.083 ± 0.041*	0.063 ± 0.093	0.000 ± 0.004*	0.051 ± 0.048	0.000 ± 0.000	0.809 ± 0.056**
HWS (cm)	13.04 ± 2.48**	10.28 ± 14.65	0.00 ± 0.00	3.76 ± 1.13**	1.17 ± 0.89	11.65 ± 1.14**
HNS (cm)	14.50 ± 2.61**	1.14 ± 1.71	0.04 ± 0.11	3.20 ± 0.95**	1.99 ± 0.82**	9.65 ± 0.95**
AH (cm)	13.61 ± 2.48**	4.57 ± 6.55	0.00 ± 0.00	3.17 ± 0.96**	1.59 ± 0.80*	9.85 ± 0.97**
AER‡	0.369 ± 0.062**		0.004 ± 0.006		0.000 ± 0.000	0.224 ± 0.022**

*Estimate significant at $\alpha = 0.05$.

** Estimate significant at $\alpha = 0.01$.

†SDY, seed yield; RL, raceme length; SDI, seed infloresence^{-1}; SDS, seed set; IP, inflorescence prolificacy; RN, raceme number; SDW, 1000 seed weight; GU, greenup; WK, winterkill; EV, early vigor; HWS, height with seedhead; HNS, height without seedhead; AH, average plant height; BMY, biomass yield; AER, average establishment rate.

‡Trait had 1 year of data.

Table 10. Significant ($p < 0.05$) correlation coefficients of 13 adaptive, morphological, and seed traits, and biomass yield of 104 *Cynodon* genotypes.

	SDY†	BMY	RL	SDI	SDS	IP	RN	SDW	GU	WK	EV	HWS	HNS
2	-0.28												
3	0.92	-											
4	0.64	-0.22	-	-									
5	0.65	-0.23	-	0.98	-								
6	0.55	-0.29	-	-	-	-							
7	-	-	0.64	-	-	-	-						
8	0.32	-0.22	-	0.28	0.30	-	-	-					
9	-	0.27	-	-	-	-	-	-	-				
10	-	-	-	-0.25	-0.26	-	-	-	-0.72	-			
11	-0.19	0.48	-	-	-	-0.19	-	-	0.38	-0.33	-		
12	-0.33	0.65	0.29	-	-	-0.30	-	-	0.27	-	0.40	-	
13	-0.42	0.72	0.24	-	-0.21	-0.43	-	-	0.26	-	0.44	0.95	-
14	-0.38	0.70	0.27	-	-0.20	-0.37	-	-	0.27	-	0.43	0.99	0.99

†1 = seed yield (SDY); 2 = biomass yield (BMY); 3 = raceme length (RL); 4 = seeds inflorescence^{-1} (SDI); 5 = seed set (SDS); 6 = inflorescence prolificacy (IP); 7 = raceme number (RN); 8 = seed weight (SDW); 9 = greenup (GU); 10 = winterkill (WK); 11 = early vigor (EV); 12 = height with seedhead (HWS); 13 = height without seedhead (HNS); 14 = average height (AH).

Table 11. Stepwise selection of predictive traits for seed yield for 104 *Cynodon* genotypes

Trait	Partial R^2	Model R^2	P value
Seed set	0.4229	0.4229	< .0001
Inflorescence prolificacy	0.2136	0.6365	< .0001

Table 12. Path analysis of direct and indirect effects of 5 traits on seed yield for 104 *Cynodon* genotypes.

Trait	Direct effect	Indirect effect					Seed Yield Correlation Coefficient
		SDI	SDS	IP	SDW	AH	
SDI†	0.378	-	0.165	0.056	0.024	0.020	0.643
SDS	0.169	0.371	-	0.061	0.025	0.025	0.650
IP	0.414	0.051	0.025	-	0.015	0.047	0.552
SDW	0.085	0.105	0.050	0.072	-	0.007	0.319
AH	-0.127	-0.060	-0.034	-0.155	-0.005	-	-0.380

†SDI, seeds inflorescence^{-1}; SDS, seed set IP, inflorescence prolificacy; SDW, 1000 seed weight; AH, average height.

Table 13. Performance statistics of top 10 selections from Goodwell nursery.

ID	BMY‡, Mg ha^{-1}	SDY, kg ha^{-1}	AER†	EV	GU	WK	MAT	IP	SS
G-19-1	10.1	123.9	6.1	6.0	5.7	8.3	1.7	5.3	19.0
G-19-8	8.8	138.1	5.9	6.2	6.0	6.7	2.3	5.5	14.5
G-19-17	6.4	216.7	5.6	6.5	5.3	8.3	1.0	6.0	21.6
G-19-20	7.3	180.5	6.6	5.3	4.3	15.0	3.0	6.8	14.4
G-19-30	7.8	178.4	6.1	5.7	5.5	6.7	1.7	6.2	20.7
G-19-31	9.3	130.8	5.6	6.0	5.8	5.8	1.7	7.3	16.7
G-19-37	6.0	220.7	6.0	4.8	4.3	20.0	3.0	6.0	24.9
G-19-70	9.7	226.9	6.3	6.2	5.0	13.3	2.3	7.0	14.2
G-19-78	6.1	225.8	2.8	5.0	4.2	16.7	1.7	7.0	15.8
G-19-83	8.2	206.2	6.3	5.2	6.2	5.0	3.0	8.3	8.7
LSD 0.05	2.7	146.7	3.8	1.1	1.2	10.7	0.9	1.2	8.8
LSD 0.01	3.5	195.5	5.1	1.4	1.5	14.2	1.3	1.7	11.7

‡BMY, biomass yield; SDY, seed yield; AER, average establishment rate; EV, early vigor; GU, greenup; WK, winterkill; MAT, maturity; IP, inflorescence prolificacy; SS, seed set.

†Establishment rate, early vigor, inflorescence prolificacy, and greenup rated on a 1 – 9 scale; Winterkill rated as percent dead plot area following greenup; Maturity rated on 1 – 3 scale; Seed set is percentage seeds spikelets^{-1}.

Table 14. Mean biomass yield (Mg ha^{-1}) of 100 experimental *Cynodon* genotypes and 4 commercial standards.

ID	2018	Rank	2019	Rank	2 year avg.	Rank
G-19-86	10.4	1	10.1	41	10.2	7
G-19-64	9.5	2	12.2	11	10.8	2
G-19-89	9.4	3	12.1	12	10.8	3
G-19-6	9.1	4	15.1	1	12.1	1
G-19-70	8.8	5	10.6	33	9.7	14
G-19-51	8.5	6	10.7	32	9.6	15
G-19-46	8.5	7	10.1	42	9.3	22
G-19-44	8.4	8	9.7	52	9.1	28
G-19-21	8.4	9	12.2	10	10.3	6
G-19-35	8.3	10	9.5	63	8.9	32
G-19-58	8.3	11	12.8	4	10.6	5
G-19-97	8.2	12	11.3	21	9.8	13
G-19-92	8.1	13	10.5	34	9.3	21
G-19-60	8.0	14	11.8	15	9.9	11
G-19-45	8.0	17	13.4	2	10.7	4
G-19-31	7.8	18	10.8	26	9.3	19
G-19-1	7.8	19	12.4	6	10.1	8
G-19-91	7.8	20	11.8	14	9.8	12
G-19-71	7.7	22	10.2	39	9.0	30
G-19-61	7.7	23	9.5	59	8.6	37
G-19-8	7.5	24	10.1	40	8.8	33
G-19-94	7.5	25	11.0	23	9.2	23
G-19-65	7.5	26	10.7	30	9.1	27
G-19-98	7.4	27	9.9	47	8.7	35
G-19-42	7.3	28	9.0	75	8.2	47
G-19-34	7.1	30	9.7	54	8.4	40
G-19-75	7.1	31	9.7	55	8.4	41
G-19-74	6.9	32	9.6	58	8.2	44
G-19-14	6.8	33	8.5	85	7.7	66
G-19-59	6.8	34	9.1	73	8.0	55
G-19-22	6.8	35	9.2	70	8.0	53
G-19-15	6.7	36	12.2	8	9.5	17
G-19-95	6.7	37	10.8	25	8.8	34
G-19-66	6.6	38	7.2	100	6.9	88
G-19-96	6.6	39	10.0	43	8.3	42
G-19-33	6.6	40	10.0	46	8.3	43
G-19-3	6.6	41	11.7	17	9.1	25
G-19-81	6.6	42	9.1	74	7.8	61
G-19-87	6.5	43	11.9	13	9.2	24
G-19-48	6.5	44	9.9	50	8.2	46
G-19-38	6.4	45	9.6	56	8.0	52
G-19-83	6.4	46	10.0	45	8.2	45

G-19-27	6.4	47	10.4	35	8.4	39
G-19-84	6.4	48	9.4	67	7.9	56
G-19-29	6.4	49	9.3	69	7.8	60
G-19-90	6.4	50	11.5	19	8.9	31
G-19-47	6.4	51	7.1	102	6.7	92
G-19-2	6.3	52	7.8	90	7.1	84
G-19-54	6.3	53	9.8	51	8.0	54
G-19-16	6.2	54	10.7	31	8.5	38
G-19-9	6.2	55	11.7	16	9.0	29
G-19-99	6.2	56	12.6	5	9.4	18
G-19-62	6.1	57	7.8	91	7.0	87
G-19-30	6.1	58	9.5	61	7.8	62
G-19-23	6.0	59	9.4	66	7.7	64
G-19-12	6.0	60	7.7	92	6.9	91
G-19-93	6.0	61	9.2	71	7.6	69
G-19-25	6.0	62	8.8	79	7.4	75
G-19-39	6.0	63	9.5	60	7.8	63
G-19-79	6.0	64	8.8	80	7.4	76
G-19-67	5.9	65	6.9	103	6.4	95
G-19-32	5.9	66	8.1	88	7.0	86
G-19-88	5.9	67	8.5	84	7.2	80
G-19-49	5.9	68	7.9	89	6.9	90
G-19-52	5.8	69	9.9	48	7.9	57
G-19-50	5.8	70	8.9	78	7.4	78
G-19-20	5.8	71	8.8	82	7.3	79
G-19-11	5.7	72	9.4	68	7.5	71
G-19-36	5.7	73	9.4	65	7.6	70
G-19-28	5.7	74	10.4	36	8.1	50
G-19-19	5.7	75	10.0	44	7.8	59
G-19-57	5.7	76	10.4	37	8.1	51
G-19-55	5.7	77	11.6	18	8.6	36
G-19-100	5.6	78	9.6	57	7.6	67
G-19-17	5.6	79	7.3	99	6.4	96
G-19-69	5.6	80	10.7	29	8.1	48
G-19-77	5.5	81	9.2	72	7.4	77
G-19-43	5.5	82	8.8	81	7.1	83
G-19-41	5.5	83	9.5	62	7.5	72
G-19-80	5.4	84	9.5	64	7.4	73
G-19-73	5.4	85	12.9	3	9.1	26
G-19-53	5.4	86	9.0	77	7.2	81
G-19-5	5.3	87	8.5	86	6.9	89
G-19-18	5.3	88	9.0	76	7.2	82
G-19-72	5.2	89	11.0	24	8.1	49
G-19-63	5.2	90	7.3	97	6.3	97
G-19-4	5.0	91	10.2	38	7.6	68
G-19-76	5.0	92	10.7	28	7.9	58

G-19-56	5.0	93	9.9	49	7.4	74
G-19-7	4.9	94	8.3	87	6.6	94
G-19-78	4.9	95	7.3	98	6.1	99
G-19-24	4.8	96	7.6	94	6.2	98
G-19-10	4.7	97	7.3	96	6.0	101
G-19-82	4.7	98	10.7	27	7.7	65
G-19-85	4.7	99	8.6	83	6.6	93
G-19-26	4.5	100	7.6	93	6.1	100
G-19-37	4.4	101	7.5	95	6.0	102
G-19-13	4.4	102	9.7	53	7.1	85
G-19-40	4.3	103	6.6	104	5.5	103
G-19-68	3.7	104	7.2	101	5.4	104
Mean	6.4		9.8		8.1	
Goodwell	7.7	21	12.4	7	10.1	10
Midland 99	8.0	16	12.2	9	10.1	9
Midland	8.0	15	11.1	22	9.6	16
Wrangler	7.2	29	11.4	20	9.3	20
LSD 0.05	1.99		2.96		2.78	
LSD 0.01	2.63		3.90		3.65	

Table 15. Mean seed yield (kg ha^{-1}) of 100 experimental *Cynodon* genotypes and 4 commercial standards.

ID	2018	Rank	2019	Rank	2 year avg.	Rank
G-19-37	325.7	2	115.6	12	220.7	4
G-19-78	278.9	3	172.6	5	225.8	3
G-19-20	267.1	4	93.9	18	180.5	8
G-19-30	257.0	5	99.7	15	178.4	9
G-19-66	236.2	6	47.3	68	141.7	14
G-19-19	228.3	7	66.4	35	147.3	12
G-19-83	225.8	8	186.5	4	206.2	6
G-19-17	224.3	9	209.0	3	216.7	5
G-19-70	221.0	10	232.8	2	226.9	2
G-19-63	208.3	11	154.7	6	181.5	7
G-19-22	202.2	12	97.9	17	150.1	10
G-19-8	183.1	13	93.0	19	138.1	15
G-19-31	180.0	14	81.5	24	130.8	17
G-19-14	171.7	15	64.8	37	118.3	20
G-19-26	162.0	16	64.0	39	113.0	22
G-19-88	161.2	17	110.0	13	135.6	16
G-19-33	153.4	18	64.4	38	108.9	26
G-19-18	153.3	19	59.3	51	106.3	27
G-19-67	152.6	20	143.5	7	148.0	11
G-19-72	152.5	21	133.2	9	142.9	13
G-19-1	144.6	22	103.2	14	123.9	18
G-19-62	141.1	23	79.5	26	110.3	24
G-19-49	137.2	24	91.7	21	114.4	21
G-19-81	132.5	25	92.2	20	112.4	23
G-19-50	132.0	26	79.5	25	105.8	28
G-19-7	131.7	27	62.4	44	97.0	31
G-19-35	131.4	28	60.5	48	96.0	32
G-19-16	123.1	29	66.3	36	94.7	35
G-19-48	123.1	30	53.9	60	88.5	39
G-19-68	120.8	31	77.9	27	99.3	30
G-19-24	120.2	32	68.9	33	94.6	36
G-19-34	119.6	33	38.5	77	79.1	45
G-19-44	113.3	34	43.7	71	78.5	46
G-19-3	112.1	35	63.5	42	87.8	40
G-19-11	110.7	36	73.7	30	92.2	37
G-19-40	109.0	37	74.3	29	91.6	38
G-19-36	104.4	38	49.9	66	77.1	47
G-19-47	104.3	39	69.0	32	86.6	41
G-19-90	102.8	40	87.0	23	94.9	34
G-19-52	101.7	41	60.1	49	80.9	43
G-19-87	101.7	42	88.7	22	95.2	33
G-19-84	98.3	43	140.7	8	119.5	19

G-19-80	91.9	44	126.4	10	109.1	25
G-19-73	90.0	45	121.5	11	105.7	29
G-19-13	89.1	46	53.8	61	71.5	50
G-19-27	84.4	48	75.3	28	79.8	44
G-19-71	80.7	49	61.9	46	71.3	51
G-19-75	79.4	50	54.0	59	66.7	54
G-19-57	79.4	51	58.8	54	69.1	53
G-19-100	79.3	52	29.7	85	54.5	65
G-19-25	76.9	53	34.6	81	55.7	62
G-19-97	76.7	54	70.1	31	73.4	49
G-19-74	76.3	55	62.6	43	69.4	52
G-19-28	73.3	56	30.0	84	51.6	69
G-19-23	73.0	57	59.2	52	66.1	55
G-19-29	72.8	58	46.3	69	59.5	59
G-19-89	71.3	59	25.2	88	48.2	74
G-19-86	71.0	60	53.6	62	62.3	56
G-19-51	70.9	61	52.3	64	61.6	57
G-19-32	70.7	62	98.8	16	84.7	42
G-19-46	70.7	63	20.9	94	45.8	76
G-19-55	69.3	64	15.2	100	42.3	81
G-19-61	67.2	65	44.4	70	55.8	61
G-19-43	64.9	66	22.5	93	43.7	78
G-19-82	63.6	67	18.1	99	40.8	83
G-19-94	63.5	68	57.5	56	60.5	58
G-19-12	62.4	69	37.6	78	50.0	72
G-19-45	60.7	70	11.9	102	36.3	87
G-19-21	59.7	71	50.9	65	55.3	63
G-19-53	58.4	72	35.0	80	46.7	75
G-19-54	57.8	73	20.2	95	39.0	84
G-19-95	55.1	74	55.1	57	55.1	64
G-19-96	52.9	75	54.5	58	53.7	66
G-19-15	50.1	76	18.1	98	34.1	89
G-19-79	49.6	77	67.3	34	58.4	60
G-19-64	45.7	78	23.6	91	34.7	88
G-19-98	45.4	79	22.8	92	34.1	90
G-19-69	44.6	80	58.9	53	51.8	68
G-19-2	42.5	81	32.8	82	37.7	85
G-19-65	42.2	82	42.5	72	42.4	80
G-19-77	42.1	83	63.5	41	52.8	67
G-19-59	42.0	84	60.8	47	51.4	70
G-19-56	39.0	85	24.8	89	31.9	91
G-19-60	38.3	86	59.8	50	49.1	73
G-19-99	36.9	87	53.5	63	45.2	77
G-19-5	36.4	88	63.7	40	50.0	71
G-19-9	34.7	90	38.9	76	36.8	86
G-19-41	32.8	91	27.0	86	29.9	94

G-19-93	32.7	92	23.7	90	28.2	96
G-19-38	30.4	93	25.6	87	28.0	97
G-19-58	27.4	94	32.3	83	29.9	95
G-19-10	26.1	95	58.6	55	42.4	79
G-19-76	21.0	96	40.7	74	30.9	92
G-19-91	19.9	97	19.3	96	19.6	100
G-19-4	19.7	98	41.5	73	30.6	93
G-19-85	18.4	99	18.2	97	18.3	101
G-19-92	15.7	100	39.9	75	27.8	98
G-19-39	7.8	101	8.1	104	7.9	104
G-19-6	7.7	102	8.5	103	8.1	103
G-19-42	7.7	103	12.3	101	10.0	102
Mean	98.3		64.6		81.5	
Goodwell	84.8	47	62.1	45	73.4	48
Midland 99	4.0	104	35.6	79	19.8	99
Midland	35.3	89	47.7	67	41.5	82
Wrangler	372.1	1	245.7	1	308.9	1
LSD 0.05	98.16		70.85		65.00	
LSD 0.01	129.44		93.43		85.54	

CHAPTER IV

BROAD-SENSE HERITABILITY ESTIMATES OF COLD TOLERANCE AND BIOMASS YIELD FOR INTERSPECIFIC HYBRIDS OF FORAGE BERMUDAGRASS

ABSTRACT

Bermudagrass is a reliable forage option for livestock producers and has readily expanded into the transition zone of the United States with the introduction of cold tolerant hybrids. However, a substantial gap exists among adapted cultivars in the transition zone and hybrids grown in southern climates in terms of yield potential and forage quality. Interspecific hybridization provides promise in producing a cold tolerant hybrid with excellent yield potential and nutritive value. The objective of this study was to evaluate the genetic variation of interspecific hybrids (*Cynodon dactylon* x *C. nlemfuensis*) for cold tolerance, biomass yield, and forage quality, in addition to generating broad-sense heritability estimates for multiple adaptive and performance traits. A collection of 98 interspecific hybrids were evaluated in a randomized complete block design with three replications in Perkins and Stillwater, OK during the 2019 growing season. Trait evaluations included winterkill rate, biomass yield, and several other plant

performance metrics. Broad-sense heritability estimates ranged from 0.53 – 0.91 with biomass yield and winterkill expressing broad-sense heritability estimates of 0.53 and 0.90, respectively. Several traits were correlated with biomass production, indicating the potential value for indirect selection. Observed genetic diversity and hybrid performance suggests commercialization potential among top performing hybrids.

INTRODUCTION

Bermudagrass (*Cynodon* spp.) is a robust warm season grass that sees employments in landscapes, soil stabilization, and agriculture (Taliaferro et al., 2004). Tolerance to a wide range of biotic and abiotic stressors, in addition to the ability to persist under intense grazing and defoliation events make bermudagrass an excellent option as a stockpiled or grazed forage (Burton et al., 1957; Quisenberry, 1990; Rouquette et al., 1998; Smiley et al., 1992; Taliaferro et al., 2004; Xiang et al., 2017). Focus on incorporating cold tolerance into the genetic makeup of new cultivars has allowed for the northern expansion of bermudagrass into the transition zone of the United States with such cultivars as Goodwell, Hardie, Midland 99, and Ozark (Wu and Taliaferro, 2009; Taliaferro and Richardson, 1980; Taliaferro et al., 2002; Richardson and Taliaferro, 2005). Hybrid bermudagrass has provided a tremendous benefit to livestock production over the years (Nelson and Burns, 2006). Grown extensively throughout the southern United States and on over 1 million hectares (ha) in Brazil, Tifton 85 is often regarded as one of the premier forage hybrids, due to its exceptional digestibility and yield potential (Burton et al., 1993; Hill et al., 2001). However, Tifton 85 lacks the necessary cold tolerance to persist within the transition zone of the United States (Anderson and Wu, 2011). A substantial gap in yield and forage quality exists

between cultivars adapted for use in the transition zone and hybrids grown in more southern climates.

Desired expression of cold tolerance, yield potential, and dry matter digestibility does not exist in any ecotypes of bermudagrass. However, breeders can utilize interspecific hybridization to combine desired characteristics of two closely related species, with much attention given to crosses involving *C. dactylon* and *C. nlemfuensis* (Wu, 2011). Simple sequence repeat (SSR) marker assisted selection can allow for accurate identification of true hybrids. Tan et al. (2014) demonstrated the ability of SSR markers to readily identify parental lineage of bermudagrass progeny.

Mechanisms of cold tolerance were attributed to dominant genes early on by Burton (1951). Although no research is available for bermudagrass, Stefaniak et al. (2009) notes cold tolerance to be an assumed quantitative trait. A family of proteins known as dehydrins have been identified to play a role in stabilizing macromolecules and cellular structures in response to dehydrating events and low temperatures (Beck et al., 2007; Close, 1997). Dehydrin expression is well documented within cold hardy germplasm, indicating its likely role in providing heightened levels of low temperature tolerance to bermudagrass (Zhang et al., 2008; Zhang et al., 2011). Additionally, structural and non-structural carbohydrate content, along with proline concentration and antioxidant activity have been shown to influence bermudagrass cold tolerance (Zhang et al., 2006). Stefaniak et al. (2009) identified heritability estimates of cold tolerance and several other traits in a population of turf bermudagrass. Their work showed broad- and narrow-sense heritability estimates of 0.89 and 0.38, respectively, for cold tolerance. Earlier work by Wofford and Baltensperger (1985) estimated the heritability of multiple

turf traits in bermudagrass, noting broad-sense heritability of 0.94 and 0.98 for vigor and clipping weight, respectively. Understanding the heritability and trait interrelationships can aid breeders in their selection process. Limited work has been conducted producing heritability estimates of cold tolerance and other adaptive traits for bermudagrass, specifically forage types. The objective of this study was to evaluate the genetic variation of interspecific hybrids for cold tolerance, biomass yield, and forage quality, in addition to generating broad-sense heritability estimates for multiple adaptive and performance traits.

MATERIALS AND METHODS

Germplasm Materials

Seed was gathered from a crossing block between experimental genotype P3 1x7 (*C. dactylon*) and Tifton 68 (*C. nlemfuensis*) in 2016. Accession P3 1x7 is a cold hardy line from the Oklahoma State University bermudagrass collection which survived 2 winters (2009 – 2011) in Champaign, IL. Tifton 68 was developed by Burton and Monson (1984) and displays superior digestibility and exceptional yield potential, but lacks cold tolerance (Anderson and Wu, 2011). Seed samples were kept separate based on seed parents. Seeds were germinated and 1,467 plants were grown in a greenhouse in the spring of 2017 in 3.8 cm cone-tainers. Plants were grown under ideal growing conditions, with irrigation, fertilizer, and pesticides applied as needed.

Hybrid Screening

Tissue samples were collected from new growth of each of the 1,467 plants in the summer of 2017 and stored at -80°C. Samples were then ground with a tissue

homogenizer (Geno/Grinder; SPEX SamplePrep, Metuchen, NJ). The phenol-chloroform DNA extraction method by Nalini and Jawali (2004) was used with slight modifications. Following extraction, DNA samples were diluted to 10 ng μL^{-1} following concentration quantification with a spectrometer (NanoDrop ND-1000; Thermo Fisher Scientific, Waltham, MA). Progeny plant DNA was then screened with four SSR markers (Figure 1) from a linkage map developed by Guo et al. (2017) that were identified to be transferable to *C. nlemfuensis*. Markers used were CDCA5-463/464, CDGA4-1343/1344, CDGA7-1667/1668, and CDCA6-529/530. Polymerase chain reaction (PCR) solution components for each reaction well included 6.54 µL nuclease free water, 1 µl 10x PCR buffer (New England BioLab, Ipswich, MA), 1 µl 1 pmol μl^{-1} forward and reverse primer, 0.2 µl 10 mM dNTPs, 0.2 µl 1 uM M13 with either 700 or 800 nm fluorescent dye (LI-COR, Lincoln, NE), 0.05 µl Taq DNA polymerase (New England BioLab, Ipswich, MA), and 1.5 µl of 10 ng μl^{-1} diluted DNA template. Reaction plates were loaded into a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). Polymerase chain reaction amplification followed a program described and used by Fang et al. (2015), where samples underwent denaturation for 5 minutes (min) at 95°C, 14 cycles of 20 seconds (s) at 94°C, 60 s at 58°C, and 30 s at 72°C, followed by 28 cycles of 20 seconds (s) at 94°C, 60 s at 55°C, and 30 s at 72°C. The final step was 10 min at 72°C, proceeded by reducing the temperature to a constant 4°C until samples were removed from the thermal cycler. Blue stop solution was added to each reaction well and then subjected to a final denaturation of 3 min at 94°C. Samples were loaded into a LI-COR 4300 DNA analyzer with 6.5% KB Plus gel (LI-COR) for a run time of 120 min. Gel images were viewed

with Saga Generation 2 Lite software. Hybrids were identified by visually determining bands shared with paternal DNA samples with consideration of maternal bands.

Field Trial Design, Establishment, and Maintenance

A total of 298 interspecific hybrids were identified. Of these hybrids, 98 were selected for replicated field-testing based on traits associated with biomass yield and forage quality. Key traits for greenhouse selection were plant height, leaf softness, and overall vigor. In addition to the 98 hybrids, 5 advanced stage experiments, EXP1, EXP2, EXP3, EXP4, and EXP5, two parent plants, Tifton 68 and P3 1x7, and 3 commercial standards, Tifton 85, Goodwell, and Midland 99, were included for evaluation and comparison. Plants were amplified in the greenhouse to provide enough material for the planting of two field nurseries. Trials were located at the Cimarron Valley Research and Extension Center in Perkins, OK and the Oklahoma Agricultural Experiment Station in Stillwater, OK. Soil types were a Teller fine sandy loam and a Kirkland silt loam in Perkins and Stillwater, respectively. Each trial was a randomized complete block design with 3 replications, each containing 108 total genotypes. Plot size measured 2 m by 2 m, separated by 1 m alleys, while 2 m alleys separated replications. Urea nitrogen (N) was applied pre plant and incorporated at a rate of 112.1 kg N ha^{-1}, in addition to phosphorus (P) and potassium (K) incorporated at rates based on soil test results. Field trials were established in May 2018 by planting 4 plugs of each genotype in their respective plots. Following planting, Ronstar Flo (Bayer Crop Science, Monheim am Rhein, Germany) was applied pre-emergent at a rate of 6.4 L product ha^{-1} and trials were irrigated as needed to promote successful establishment. Following establishment, irrigation was not used and plants were grown in a dryland system. Glyphosate was used throughout the

duration of the experiment to control alley overgrowth and prevent contamination of neighboring plots at a rate of 2.3 L product ha^{-1}. Other commercial pesticides were used as need to control insects and weeds. Urea N was applied following each harvest in 2019 at a rate of 112.1 kg N ha^{-1}, while P and K were applied in the spring of 2019 at rates determined by soil tests results from the Oklahoma State University Soil, Water, and Forage Analytical Laboratory. Following each harvest, remaining biomass was swathed and baled off.

Data Collection and Analysis

Following planting, establishment rate was visually assessed in 2018 on a 1 – 9 scale, with 1 signifying worst, and 9 being best relative performance across the location. Spring greenup and early vigor were both visually measured in 2019 using the same scale, based on National Turf Evaluation Program (NTEP) protocols. A score of 1 for establishment rate represented slow to no lateral growth of stolons, while 9 was representative of rapid, lateral and upright growth of stolons and shoots, respectively. Greenup was given a rating of 1 with a few to no emerged shoots in the plot, with 9 recognizing plots that featured dense shoot emergence throughout the plot. Early vigor ratings were based on visual appearance and health of the plot, with 1 signifying light green color with sparse canopy density, while 9 was representative of dense, upright, green foliage throughout the plot. Winterkill rate was determined by visually quantifying the percentage of dead plot area following spring greenup. A winterkill rating of 0% was representative of no visually observable dead plot area, while 100% was associated with no observable bermudagrass growth. Plant height was measured in the field prior to each harvest with a metric ruler. Biomass yield was determined by hand clipping 0.3 by 0.3 m

areas from each plot for all 3 harvests in Perkins, and 2 of the 3 harvests in Stillwater. The first harvest in Stillwater utilized a Carter Forage Harvester, that cut the middle 1 m of each plot, however, excessive accumulation of soil in sample bags from plot scalping prevented the use of this data and lead to the determination to hand clip the remaining harvests. Samples were subsequently oven dried at 65°C for 72 hours, weighed, and converted from g .09 m^{-1} to Mg ha^{-1} scale. Subsamples were collected from each harvested plot and stored for forage quality analysis at a later date.

Data was analyzed with SAS 9.4 (SAS, 2014). Analysis of variance (ANOVA) was conducted with PROC MIXED using genotype, location, replication with location, and genotype x replication with location as random effects. Means and descriptive statistics were generated with PROC MEANS, with least significant differences at probability levels of 5% and 1% generated with PROC GLM. Mean separation data was used in establishing thresholds for the selection process of top performing hybrids. Correlation coefficients of traits were computed with PROC CORR. Restricted maximum likelihood (REML) estimates for use in broad sense heritability calculations were also generated with PROC MIXED, using genotype and genotype x location as random effects. Broad sense heritability (H^2) was calculated with a formula used by Dong et al. (2015) due to the identical experimental design and two locations over a single growing season. The formula was: $H^2 = \sigma^2_g \ / \ [\sigma^2_g + \sigma^2_{gxl} / l + \sigma^2_{error} / lr]$, where σ^2_g, σ^2_{gxl}, and σ^2_{error}, were genotypic variance, genotype x location variance, and error variance, respectively, and l and r were the respective numbers of locations and replications. Top performing hybrids were selected for based on biomass yield and winterkill rates. Selection indices were used that quantified relative performance of forage yield and

winterkill in relation to the highest observation of each of these traits within each location. Indices were added across traits and locations with restrictions enforced for minimum allowable forage yield and maximum allowable winterkill to prevent overcompensation of each index for the underperformance of the other. Top hybrids, commercial standards, and advanced experimental plant samples were submitted to the OSU Soil, Water, and Forage Analytical Lab (SWAFL) for crude protein (CP), acid-detergent fiber (ADF), and neutral-detergent fiber (NDF) quantification. Total digestible nutrients (TDN) and relative forage quality (RFQ) were calculated from ADF and NDF values with the following formulas:

$$TDN, \% = 88.9 - (0.779 \times ADF) \qquad RFQ = [(120 / NDF) \times TDN] / 1.23$$

RESULTS

Trait Variability

Analyses of variance for 7 performance traits are presented in Table 16. Genotypic and replication within location variation were significant ($\alpha = .05$, $P < .01$) for all traits. Location variation was only significant ($\alpha = .05$, $P < .01$) for biomass yield, in addition the location variance of biomass yield being higher than the genotypic variance. This is suggestive of strong environmental effects on biomass yield between the two locations, however genotype x location interaction was not significant for biomass yield. A large proportion of the variation experienced at each location can be attributed to genetic effects. Furthermore, greenup, winterkill, early vigor, and height with seedhead all had experienced significant ($\alpha = .05$, $P < .01$) genotype x location interactions. None of

the 7 traits experienced significant ($\alpha = .05$, $P < .05$) genotype x replication interactions within location interactions.

Traits variability is further illustrated in observed means, associated standard deviations, and ranges of 7 performance traits displayed in Table 17. Due to significant genotype x location interactions for 4 of the 7 traits, values are separated by location. Biomass yield was variable among the 98 hybrids, experiencing cumulative production ranges of 0.0 – 34.1 and 0.0 – 24.1 Mg ha^{-1} in Perkins and Stillwater, respectively. Each location was harvested 3 times, however, only 2 harvests from Stillwater were viable due to equipment malfunction during the first harvest. Rates of winterkill were variable, encompassing a range of 5 – 100% at both locations. Among the 5 standards, Tifton 68 experienced the highest rate of winterkill at both locations. Tifton 85 expressed the quickest establishment rate among the standards, achieving mean ratings of 6.3 and 6.8 at Perkins and Stillwater, respectively. These ratings fell in the upper end of the ranges displayed for experimental hybrids at both locations. With the exception of early vigor, greenup, and winterkill, mean performance of experimental hybrids is similar to what was observed from Tifton 68. Early vigor, greenup, and winterkill experienced by hybrid plants is more closely associated with the performance of the cold tolerant parent, P3 1x7.

Broad Sense Heritability

Restricted maximum likelihood (REML) estimates of variance components and broad sense heritability estimates of 7 performance traits are presented in Table 18. Genotypic variance (σ^2_G) and genotype x location (σ^2_{GxE}) are used to calculate broad sense heritability on a clonal basis as was previously conducted by Dong et al. (2015) due

to the near identical experimental design of the two studies. Genotypic variance estimates were significant ($\alpha = .05$, $P < .01$) for all traits. Significant ($\alpha = .05$, $P < .05$) genotype x environment interactions were observed for all traits, with the exception of establishment rate. A large proportion of variation was accounted for by genotypic variance with a limited GxE interaction, with the exception of biomass yield. A large degree of genotype x environment variation was witnessed for biomass yield, indicating the role certain environmental conditions played on observed levels of forage production. Broad sense heritability estimates were moderate – high for all traits. Establishment rate, greenup, winterkill, early vigor, height without seedhead, and height with seedhead produced heritability estimates of 0.89, 0.84, 0.90, 0.90, 0.88, and 0.91, respectively. Broad sense heritability for biomass yield was more moderate, at 0.53.

Trait Relationships

Significant ($\alpha = .05$, $P < .05$) correlation coefficients are given in Table 19. Biomass yield exhibited a moderate, positive correlation with greenup ($r = 0.44$), early vigor ($r = 0.52$), height without seedhead ($r = 0.42$), and height without seedhead ($r = 0.43$). Although a weaker correlation, establishment rate was positively correlated with biomass yield ($r = 0.19$). A moderate, negative relationship was expected and observed between winterkill and biomass yield ($r = -0.45$). Various relationships were witnessed among traits. Winterkill had a strong, negative association with early vigor ($r = -0.89$) and greenup ($r = -0.83$), while greenup and early vigor displayed a strong, positive correlation ($r = 0.87$). Taller plants displayed earlier, more vigorous spring growth, in addition to faster rates of establishment. These relationships show the early, vigorous spring growth of taller plants can be a potential candidate for indirect selection for

biomass yield. Furthermore, the avoidance of plants that experience high rates of winterkill will further advance yield in the selection process.

Plant Selection and Forage Quality

In total, 25 hybrids were selected based on selection indices that quantified relative forage yield and winterkill rates across locations. Selected hybrids were IH-19-80, IH-19-132, IH-19-605, IH-19-834, IH-19-841, IH-19-852, IH-19-855, IH-19-906, IH-19-908, IH-19-925, IH-19-1024, IH-19-1027, IH-19-1031, IH-19-1043, IH-19-1049, IH-19-1067, IH-19-1088, IH-19-1110, IH-19-1120, IH-19-1129, IH-19-1131, IH-19-1143, IH-19-1156, IH-19-1199, and IH-19-1329. Wet chemistry for CP, ADF, and NDF can become costly with the large number of samples collected in this experiment, due to this, forage quality was only assessed for the top performing hybrids. Forage quality data and adaptive trait performance of aforementioned hybrids are reported in Tables 20 and 21 for Perkins and Stillwater, respectively. Perkins hybrid CP, ADF, and TDN produced ranges of 10.2 – 12.3%, 32.5 – 36.2%, and 60.7 – 63.6%, respectively, while the respective means for CP, ADF, and TDN were 34.4%, 11.1%, and 62.1%. Range for Stillwater hybrid CP was 10.9 – 14.4%, with a mean of 12.2%. Accession IH-19-1067 had the highest CP of 12.3% at Perkins, while IH-19-1088 was the highest at Stillwater with 14.4%. Furthermore, Stillwater ADF recorded a range of 31.0 – 35.2%, with a mean of 32.7%, while Stillwater TDN was 61.5 – 64.8% and a mean of 63.4%. Parental mean CP for P3 1x7 and Tifton 68 at Perkins was 11.9% and 12.9%, respectively. Stillwater mean parent CP was 12.2% and 13.7% for P3 1x7 and Tifton 68, respectively. The ADF for both P3 1x7 and Tifton 68 was 33.0% at Stillwater. Alternatively, P3 1x7 had a lower ADF than Tifton 68 at Perkins, where P3 1x7 ADF was 11.9% and Tifton 68 was 12.9%.

Among experimental hybrids, IH-19-852 had the highest ADF at both Perkins and Stillwater with respective values of 36.2% and 35.2%. Experimental hybrid IH-19-834 had the highest TDN at Stillwater, recording a value of 64.8%, while IH-19-605 had the highest TDN in Perkins at 63.6%. Tifton 68 and P3 1x7 had similar TDN in Perkins, however, P3 1x7 was over 1% higher in Stillwater. Relative forage quality (RFQ) was low across all genotypes, however, mean RFQ of experimental hybrids was higher than the RFQ observed from commercial standards. Furthermore, the observed NDF within the hybrids was considerably lower than NDF of commercial cultivars. Additional data for winterkill and biomass yield of all tested accessions and commercial cultivars is presented in Table 22.

DISCUSSION

High variability was evident throughout all traits of the evaluated hybrids. Accordingly, bermudagrass germplasm has demonstrated substantial variability throughout many studies (Harlan and de Wet, 1969; Guo et al., 2017; Stefaniak et al., 2009; Wu et al., 2006; Wu et al., 2007). Biomass yield was highly variable, with large ranges topping 43.5 and 29.3 Mg ha^{-1} in Perkins and Stillwater, respectively. These high yields would be uncommon in large scale production settings. The values are largely attributed to the 0.09 m^2 sampling area, in addition to harvest intervals that approached 8 weeks, leading to high levels of mature biomass accumulation. Spring greenup, winterkill rate, and early vigor all experienced significant variability between the two locations. As Perkins was a sandy loan, compared to the silt loam of Stillwater, the insulating factors attributed with the contrasting soil types and their associated properties likely played a role in this observed variability. Early vigor, greenup, and winterkill all displayed

significant relationships among one another, which was further witnessed by Wu et al. (2007). Additionally, significant correlations between biomass yield and greenup, winterkill, vigor, and plant height provide valuable insight into exploitable relationships for the selection process. Wu et al. (2007) also identified significant correlations between the aforementioned traits and biomass yield. It can be concluded that early, vigorous spring growth provides genotypes with a longer window to accumulate biomass, ultimately resulting in higher yields.

Comparative performance of hybrid trait means to at least one of the parents indicates no occurrence of extreme transgressive segregates, although some hybrids did perform better than the parent plants. A similar observation was made by Stefankiak et al. (2009) in their evaluation of 54 bermudagrass progeny derived from a polycross of 54 plants. Furthermore, no evidence of this phenomena was apparent in work by Wofford and Baltensperger (1985). Overall, significant genotypic variance was evident across all traits. Phenotypic variation can be attributed to hybrid genetics. Genotypic variance was higher than genotype x location variance for all traits, indicating a large genetic contribution to phenotypic expression. Biomass yield experienced a greater environmental influence on observed phenotype, but nonetheless, genetic variance was still nearly 6 times higher than genotype x location variance. As noted by Stefankiak et al. (2009), recurrent selection would be a valuable tool with populations displaying this type of dynamic. Taliaferro et al. (2004) emphasized the need for F_1 hybrids with strong combining ability if this breeding method is to be employed.

Selected plants show potential for development of F_1 commercial hybrids. Winterkill rates of selected plants were highly comparable to cold tolerant standards

Goodwell and Midland 99, while the biomass yield was in line with what was observed with Tifton 85. Forage quality results were variable across selected accessions, however, potential exists with the various expressions of biomass yield and winterkill for selection of superior quality within plants. Forage quality was low across all genotypes, likely a result of the 8-9 week harvest intervals. Hancock et al. (2017) notes the sacrifice of decreased forage quality over time as biomass accumulation increases. Despite the low RFQ values, comparisons among the experimental hybrid means and Tifton 85 show a higher degree of relative quality amongst the experimental hybrids. Further evaluations of top hybrids will allow for a more concentrated effort of evaluating yield and quality within shorter harvest intervals.

Broad-sense heritability of the measured traits was moderate to high, ranging from 0.53 – 0.91. These values indicate a high potential for breeders to improve these traits through selection. Our winterkill heritability of 0.90 was highly comparable to the broad-sense heritability of cold tolerance observed by Stefankiak et al. (2009), where the authors documented a broad-sense heritability of 0.89. Wofford and Baltensperger (1985) noted a broad-sense heritability of 0.94 for vigor, comparable to our value of 0.90. Although, Wofford and Baltensperger (1985) evaluated clipping weight of turfgrass, it still provides comparison to our value of biomass yield. Their calculated heritability of 0.98 is higher than our observation of 0.53, resulting from limited environmental interactions in comparison to their observed genetic variance. Environment plays a role in the expression of yield potential, however, biomass yield will still respond to selection. Due to resource limitations, forage quality evaluations were limited to the top 25 selections and heritability was not calculated from this smaller sample size. However,

prior research has demonstrated the broad-sense heritability of dry matter digestibility to range from 0.27 – 0.69, indicating its potential for improvement through selection (Burton and Monson, 1972). Although all three traits are not present at desired levels in any current cultivar, the ability to breed and select for cold tolerance, yield, and quality is evident in the release of such hybrids as Goodwell and Tifton 85 (Burton et al., 1993; Wu et al., 2009). Understanding heritability and trait relationships will allow for the continued improvement of forage bermudagrass, further expanding the agronomic range through the introduction of cold tolerant hybrids with high yield potential and improved forage quality.

TABLES AND FIGURES

Table 16. Analysis of variance on 7 performance traits for 98 *Cynodon* interspecific hybrids.

Source	Mean squares					
	Genotype (G)	**Location (L)**	**G x L**	**Replication (L)**	**G x Replication(L)**	**Residual**
df	*97*	*1*	*97*	*4*	*387*	*1*
BMY (Mg ha^{-1})	132.45**	3825.17**	22.93	162.31**	24.93	1.62
ER†	3.71**	1.13	0.42	3.05**	0.34	0.50
GU†	10.17**	19.23	1.39**	14.81**	0.73	0.00
WK (%)	0.30**	0.04	0.03**	0.18**	0.19	0.00
EV†	9.50**	2.73	0.90**	6.46**	0.62	0.50
df	*94*	*1*	*92*	*4*	*363*	*1*
HNS (cm)	471.46 **	552.28	34.74	213.86**	27.16	64.98
df	*88*	*1*	*84*	*4*	*309*	*1*
HWS (cm)	480.66**	29.32	50.84**	164.57**	31.43	115.52

*Significant at α = 0.05.

**Significant at α = 0.01.

†Rated on 1-9 visual scale, 1 being worst, 9 being best.

Table 17. Means, standard deviations, and ranges for 7 adaptive, morphological, and biomass traits for 98 interspecific hybrids, 2 parent plants, and 3 other commercial hybrids in Perkins and Stillwater, OK.

Trait†	Experimental Hybrids		Standards				
			P3 1x7	Tifton 68	Tifton 85	Goodwell	Midland 99
	Mean ± SD	Range	Mean ± SD				
Perkins							
BMY† (Mg ha^{-1})	19.6 ± 7.6	0.0 – 34.1	11.5 ± 8.1	22.5 ± 10.9	23.5 ± 4.2	22.6 ± 4.1	19.2 ± 6.5
ER	5.7 ± 1.0	2.5 – 8.5	2.2 ± 0.8	5.2 ± 0.3	6.3 ± 0.6	4.7 ± 0.6	3.8 ± 0.8
GU	4.8 ± 1.5	1.0 – 7.5	3.5 ± 2.8	2.0 ± 1.3	4.3 ± 0.8	6.3 ± 0.3	5.7 ± 0.6
WK (%)	18.3 ± 24.6	5.0 – 100.0	16.7 ± 20.2	76.3 ± 20.3	18.3 ± 2.9	10.0 ± 0.0	15.0 ± 5.0
EV	5.3 ± 1.4	1.0 – 8.0	4.3 ± 2.1	3.7 ± 1.5	5.0 ± 0.0	7.0 ± 1.0	6.7 ± 0.6
HNS (cm)	34.7 ± 10.2	7.6 – 63.5	24.8 ± 4.5	36.8 ± 11.4	53.3 ± 5.1	35.1 ± 2.6	44.0 ± 3.7
HWS (cm)	48.1 ± 11.1	15.2 – 76.2	29.9 ± 4.5	38.1 ± 0.0	61.8 ± 2.9	37.7 ± 1.9	54.2 ± 3.9
Stillwater							
BMY† (Mg ha^{-1})	14.6 ± 5.3	0.0 – 24.1	8.8 ± 2.5	16.4 ± 4.3	22.3 ± 0.9	13.7 ± 4.3	15.1 ± 1.9
ER	5.8 ± 0.9	2.0 – 8.0	4.2 ± 0.8	6.2 ± 0.6	6.8 ± 0.3	5.0 ± 0.9	5.0 ± 0.5
GU	4.5 – 1.7	1.0 – 7.5	4.0 ± 1.3	2.2 ± 1.0	4.0 ± 0.5	7.3 ± 0.6	6.3 ± 0.8
WK (%)	20.0 ± 27.4	5.0 – 100.0	6.7 ± 2.9	58.3 ± 31.8	13.3 ± 2.9	8.3 ± 2.9	8.3 ± 2.9
EV	5.2 ± 1.5	1.0 – 8.0	5.0 ± 1.0	3.3 ± 1.2	5.0 ± 0.0	7.3 ± 0.6	6.7 ± 0.6
HNS (cm)	36.4 ± 10.3	10.2 – 66.0	18.6 ± 3.9	39.0 ± 2.6	51.2 ± 1.9	36.4 ± 4.5	47.4 ± 5.3
HWS (cm)	48.1 ± 10.6	20.3 – 69.9	27.9 ± 4.6	54.2 ± 8.2	61.8 ± 2.6	38.5 ± 7.4	58.4 ± 4.6

†BMY, biomass yield; ER, establishment rate; GU, greenup; WK, winterkill; EV, early vigor; HNS, height without seedhead; HWS, height with seedhead.

Table 18. Variance component estimate, standard error, and broad sense heritability for performance traits of interspecific hybrids grown in Perkins and Stillwater, OK.

Trait	Variance component estimates ± SE			Broad sense heritability
	σ^2_G	σ^2_{GxE}	σ^2_{Error}	H^2
Establishment rate†	0.549 ± 0.090**	0.018 ± 0.022	0.371 ± 0.027**	0.89
Greenup†	1.432 ± 0.246**	0.236 ± 0.078**	0.872 ± 0.062**	0.84
Winterkill (%)	0.044 ± 0.007**	0.003 ± 0.002*	0.021 ± 0.002**	0.90
Early vigor†	1.430 ± 0.229**	0.082 ± 0.047*	0.680 ± 0.049**	0.90
Height without seedhead (cm)	73.809 ± 11.840**	3.496 ± 2.093*	29.348 ± 2.166**	0.88
Height with seedhead (cm)	87.303 ± 15.103**	5.402 ± 3.008*	34.189 ± 2.770**	0.91
Biomass yield (Mg ha^{-1})	11.751 ± 3.456**	11.892 ± 3.011**	26.264 ± 1.875**	0.53

*Significant at $\alpha = 0.05$

**Significant at $\alpha = 0.01$

†Rated on 1-9 visual scale, 1 being worst, 9 being best.

Table 19. Significant ($p < 0.05$) correlation coefficients of 7 performance traits among *Cynodon* interspecific hybrids.

	BMY	ER	GU	WK	EV	HNS	HWS
Biomass yield (BMY)	-						
Establishment Rate (ER)	0.19	-					
Greenup (GU)	0.44	0.27	-				
Winterkill (WK)	-0.45	-0.20	-0.83	-			
Early vigor (EV)	0.52	0.28	0.87	-0.89	-		
Height without seedhead (HNS)	0.42	0.36	0.44	-0.33	0.55	-	
Height with seedhead (HWS)	0.43	0.36	0.45	-0.30	0.54	0.77	-

Table 20. Adaptive trait, forage quality, and biomass yield performance of 25 selected hybrids, 2 parents, and 3 commercial cultivars at Perkins, OK in 2019.

ID	BMY†, Mg ha^{-1}	WK, %	CP, %	ADF, %	NDF, %	TDN, %	RFQ
IH-19-80	26.9	15.0	11.6	34.9	67.4	61.7	89.5
IH-19-132	20.8	6.7	11.4	33.8	67.5	62.6	90.4
IH-19-605	20.7	6.7	11.8	32.5	66.1	63.6	94.0
IH-19-834	16.7	15.0	11.2	32.6	68.1	63.5	91.1
IH-19-841	22.0	11.7	10.9	34.8	67.1	61.8	89.9
IH-19-852	34.1	11.7	10.8	36.2	67.3	60.7	88.4
IH-19-855	25.6	10.0	11.7	35.4	67.8	61.4	88.6
IH-19-906	27.4	6.7	10.7	33.5	67.1	62.8	91.4
IH-19-908	23.4	6.7	11.1	33.8	68.7	62.6	88.9
IH-19-925	23.7	6.7	11.0	34.4	66.1	62.1	91.8
IH-19-1024	25.0	5.0	10.7	34.8	67.3	61.8	89.6
IH-19-1027	20.0	6.7	11.8	33.9	66.4	62.5	91.9
IH-19-1031	25.0	13.3	10.2	34.3	68.5	62.2	88.6
IH-19-1043	26.1	8.3	11.8	33.2	66.7	63.1	92.3
IH-19-1049	25.7	5.0	10.3	35.2	66.1	61.5	90.9
IH-19-1067	28.0	20.0	12.3	33.1	66.4	63.1	92.8
IH-19-1088	25.5	8.3	11.3	35.3	67.5	61.4	88.9
IH-19-1110	24.2	8.3	12.0	32.8	66.2	63.3	93.4
IH-19-1120	25.7	10.0	10.7	35.0	67.4	61.6	89.2
IH-19-1129	24.2	10.0	11.2	35.4	67.6	61.3	88.6
IH-19-1131	22.9	6.7	10.8	34.7	65.1	61.9	93.6
IH-19-1143	23.7	10.0	11.0	34.7	68.1	61.9	88.7
IH-19-1156	26.4	10.0	10.7	36.1	67.5	60.8	88.0
IH-19-1199	19.3	8.3	10.3	35.8	67.5	61.0	88.2
IH-19-1329	25.0	11.7	11.2	33.0	65.4	63.2	94.5
			Parents				
Tifton 68	22.5	76.3	12.9	33.0	65.8	63.2	93.7
P3 1x7	11.5	16.7	11.9	31.9	67.4	64.0	92.7
			Commercial Cultivars				
Goodwell	22.6	10.0	10.2	35.2	69.2	61.5	86.9
Midland 99	19.2	15.0	11.3	34.0	65.7	62.4	92.8
Tifton 85	23.4	18.3	11.2	35.6	67.8	61.2	88.3
LSD 0.05	10.2	18.6	1.4	1.5	2.3	1.2	4.0
LSD 0.01	13.5	24.5	1.9	2.0	3.0	1.6	5.3

†BMY, biomass yield; WK, winterkill; CP, crude protein; ADF, acid-detergent fiber; NDF, neutral-detergent fiber; TDN, total digestible nutrient; RFQ, relative forage quality.

Table 21. Adaptive trait, forage quality, and biomass yield performance of 25 selected hybrids, 2 parents, and 3 commercial cultivars at Stillwater, OK in 2019.

ID	BMY†, Mg ha^{-1}	WK, %	CP, %	ADF, %	NDF, %	TDN, %	RFQ
IH-19-80	19.7	10.0	13.9	33.5	66.0	62.8	93.3
IH-19-132	16.3	5.0	12.7	31.5	64.0	64.4	98.3
IH-19-605	15.3	10.0	13.0	31.6	62.5	64.3	100.6
IH-19-834	19.5	6.7	11.6	31.0	64.7	64.8	97.8
IH-19-841	17.9	10.0	12.1	31.9	63.4	64.1	98.8
IH-19-852	21.9	13.3	11.8	35.2	65.4	61.5	92.2
IH-19-855	18.8	10.0	13.5	33.0	64.2	63.2	96.1
IH-19-906	21.6	11.7	11.3	32.1	64.6	63.9	96.5
IH-19-908	20.0	8.3	12.2	32.4	65.9	63.7	95.1
IH-19-925	17.9	11.7	11.1	35.1	66.6	61.5	90.3
IH-19-1024	14.3	6.7	11.0	32.5	64.9	63.6	95.7
IH-19-1027	17.9	6.7	13.4	31.0	62.6	64.8	101.1
IH-19-1031	24.1	10.0	11.6	32.4	65.3	63.7	95.4
IH-19-1043	18.2	8.3	12.0	33.6	63.2	64.7	96.9
IH-19-1049	18.4	6.7	11.6	32.3	64.8	63.8	96.2
IH-19-1067	22.5	10.0	12.5	32.5	66.3	63.6	94.8
IH-19-1088	20.1	11.7	14.4	32.3	63.6	63.8	98.0
IH-19-1110	14.8	11.7	11.9	31.5	64.9	64.4	97.1
IH-19-1120	18.2	8.3	13.0	32.5	65.0	63.6	95.6
IH-19-1129	19.6	5.0	10.9	35.0	65.8	61.7	91.6
IH-19-1131	16.4	11.7	11.2	32.3	66.5	63.7	93.7
IH-19-1143	17.0	8.3	12.3	33.1	66.6	63.1	93.1
IH-19-1156	22.5	10.0	11.4	33.7	64.9	62.6	94.3
IH-19-1199	20.9	10.0	11.2	33.5	64.7	62.8	95.1
IH-19-1329	21.0	10.0	12.2	31.8	63.9	64.1	98.3
			Parents				
Tifton 68	16.4	58.3	13.7	33.0	63.1	63.2	97.7
P3 1x7	8.8	6.7	12.2	33.0	64.1	63.2	96.2
			Commercial Cultivars				
Goodwell	13.7	8.3	10.0	32.5	66.5	63.6	93.7
Midland 99	15.2	8.3	11.4	31.8	64.4	64.2	97.2
Tifton 85	22.3	13.3	13.8	35.0	67.5	61.6	89.4
LSD 0.05	5.4	25.9	1.7	2.2	3.4	1.7	5.7
LSD 0.01	7.2	34.2	2.2	2.9	4.5	2.3	7.5

†BMY, biomass yield; WK, winterkill; CP, crude protein; ADF, acid-detergent fiber; NDF, neutral-detergent fiber; TDN, total digestible nutrient; RFQ, relative forage quality.

Table 22. Mean separations of biomass yield and winterkill for 98 interspecific hybrids, 5 late stage experimental varieties, 3 commercial standards, and 2 parental genotypes of *Cynodon* spp. at Perkins and Stillwater, OK.

ID	Perkins, OK			Stillwater, OK		
	BMY† (Mg ha^{-1})	WK (%)	Rank	BMY (Mg ha^{-1})	WK (%)	Rank
IH-19-20	20.8	36.7	94	14.4	6.7	18
IH-19-80	26.9	15.0	83	19.7	10.0	43
IH-19-84	21.0	10.0	49	11.3	46.7	96
IH-19-94	22.8	13.3	71	11.3	46.7	97
IH-19-96	16.9	6.7	21	14.9	5.0	5
IH-19-116	18.5	11.7	65	12.2	13.3	79
IH-19-118	21.7	8.3	31	15.4	8.3	33
IH-19-132	20.8	6.7	14	16.3	5.0	2
IH-19-199	16.3	15.0	82	12.4	13.3	72
IH-19-230	13.4	13.3	74	13.4	15.0	81
IH-19-232	2.5	99.7	107	1.1	100.0	107
IH-19-237	0.0	99.3	105	0.0	100.0	108
IH-19-269	6.1	99.7	106	0.0	99.7	105
IH-19-270	14.2	8.3	40	14.3	8.3	36
IH-19-273	8.7	100.0	108	8.9	99.7	104
IH-19-348	20.2	6.7	16	15.9	5.0	3
IH-19-377	21.8	8.3	41	18.3	6.7	11
IH-19-392	18.8	10.0	55	15.5	10.0	45
IH-19-413	20.7	13.3	75	13.4	10.0	58
IH-19-476	19.5	20.0	90	15.6	6.7	15
IH-19-581	13.2	16.7	85	15.2	18.3	84
IH-19-584	8.9	89.7	104	0.0	99.7	106
IH-19-586	15.8	6.7	23	10.3	38.0	91
IH-19-598	20.6	40.0	95	12.5	41.3	94
IH-19-605	20.7	6.7	15	15.3	10.0	46
IH-19-646	17.5	10.0	51	14.2	10.0	50
IH-19-648	16.8	5.0	5	16.1	6.7	13
IH-19-663	10.2	16.7	87	11.2	10.0	59
IH-19-683	14.4	71.7	100	8.3	96.0	103
IH-19-690	18.6	10.0	57	16.3	49.7	98
IH-19-693	9.4	13.3	79	7.6	10.0	60
IH-19-732	18.1	11.7	66	18.0	53.0	99
IH-19-759	18.3	8.3	37	14.3	11.7	67
IH-19-792	18.5	10.0	50	12.2	15.0	82
IH-19-802	11.0	41.7	96	15.5	43.3	95
IH-19-826	19.2	6.7	19	13.1	6.7	23
IH-19-834	25.6	15.0	80	19.5	6.7	9
IH-19-841	22.0	11.7	61	17.9	10.0	44
IH-19-843	16.7	6.7	22	14.3	6.7	19
IH-19-846	18.5	8.3	36	12.3	13.3	73

IH-19-849	23.8	10.0	46	11.7	16.7	83
IH-19-852	34.1	11.7	58	21.9	13.3	75
IH-19-855	25.6	10.0	52	18.8	10.0	56
IH-19-862	20.0	11.7	63	14.3	71.0	101
IH-19-875	20.0	6.7	18	15.6	13.3	77
IH-19-887	11.8	6.7	25	12.1	5.0	8
IH-19-902	17.7	11.7	68	13.3	6.7	22
IH-19-906	27.4	6.7	6	21.6	11.7	61
IH-19-908	23.4	6.7	11	20.0	8.3	29
IH-19-925	23.7	6.7	9	17.9	11.7	62
IH-19-936	19.3	8.3	42	18.1	21.7	85
IH-19-954	21.9	43.3	97	22.5	35.0	89
IH-19-1002	15.7	8.3	39	8.6	39.7	92
IH-19-1012	19.2	33.3	93	22.6	40.0	93
IH-19-1015	9.6	53.3	99	8.8	35.0	90
IH-19-1016	23.2	10.0	53	13.8	11.7	71
IH-19-1024	25.0	5.0	2	14.3	6.7	20
IH-19-1027	20.0	6.7	17	17.9	6.7	12
IH-19-1030	20.0	25.0	92	15.4	15.0	80
IH-19-1031	25.0	13.3	70	24.1	10.0	52
IH-19-1042	25.2	6.7	8	14.2	10.0	48
IH-19-1043	26.1	8.3	27	18.2	8.3	31
IH-19-1049	25.7	5.0	1	18.4	6.7	10
IH-19-1052	25.8	6.7	7	12.1	8.3	39
IH-19-1067	28.0	20.0	89	22.5	10.0	53
IH-19-1071	22.6	11.7	69	17.6	28.3	87
IH-19-1076	23.0	5.0	3	11.5	6.7	26
IH-19-1084	23.1	10.0	54	13.2	8.3	38
IH-19-1088	25.5	8.3	28	20.1	11.7	70
IH-19-1094	27.5	8.3	26	15.2	10.0	47
IH-19-1100	23.0	8.3	30	13.7	6.7	21
IH-19-1110	24.2	8.3	29	14.8	11.7	66
IH-19-1114	19.6	8.3	34	12.0	6.7	24
IH-19-1118	26.5	11.7	59	11.6	6.7	25
IH-19-1120	25.7	10.0	44	18.2	8.3	30
IH-19-1129	24.2	10.0	45	19.6	5.0	1
IH-19-1131	22.9	6.7	12	16.4	11.7	63
IH-19-1143	23.7	10.0	47	17.0	8.3	32
IH-19-1144	17.7	50.0	98	15.8	11.7	64
IH-19-1148	19.0	6.7	20	12.3	5.0	7
IH-19-1150	20.1	13.3	77	14.4	8.3	35
IH-19-1156	26.4	10.0	43	22.5	10.0	54
IH-19-1173	22.0	6.7	13	15.8	11.7	65
IH-19-1178	13.2	13.3	78	16.0	6.7	14
IH-19-1198	17.9	86.3	103	13.1	30.0	88
IH-19-1199	19.3	8.3	35	20.9	10.0	55

IH-19-1223	17.9	11.7	67	12.2	8.3	41
IH-19-1294	19.6	13.3	72	14.2	10.0	49
IH-19-1306	16.1	13.3	73	15.2	21.7	86
IH-19-1307	20.3	13.3	76	16.0	13.3	76
IH-19-1308	16.8	8.3	38	15.1	6.7	16
IH-19-1312	20.6	25.0	91	13.6	11.7	69
IH-19-1329	25.0	11.7	60	21.0	10.0	42
IH-19-1352	21.0	8.3	32	9.5	6.7	27
IH-19-1364	21.5	11.7	62	14.9	13.3	78
IH-19-1415	20.5	8.3	33	13.7	11.7	68
IH-19-1432	23.6	6.7	10	12.1	8.3	40
IH-19-1435	22.3	5.0	4	16.7	10.0	57
Mean	19.7	18.3		14.6	20.0	
			Commercial Standards			
Goodwell	22.6	10.0	48	13.7	8.3	37
Midland 99	19.2	15.0	81	15.2	8.3	34
Tifton 85	23.4	18.3	88	22.3	13.3	74
			Parents			
P1 3x7	11.5	16.7	86	8.8	6.7	28
Tifton 68	22.5	76.3	101	16.4	58.3	100
			Late Stage Experimental Selections			
EXP1	14.2	6.7	24	13.4	5.0	6
EXP2	18.7	10.0	56	15.0	5.0	4
EXP3	19.9	11.7	64	14.6	6.7	17
EXP4	14.9	81.7	102	17.7	88.3	102
EXP5	15.3	15.0	84	13.2	10.0	51
LSD 0.05	10.2	18.6		5.4	25.9	
LSD 0.01	13.5	24.5		7.2	34.2	

[†]BMY, biomass yield; WK, winterkill.

CHAPTER V

IDENTIFICATION OF TRANSFERABLE SSR MARKERS FOR USE IN *CYNODON NLEMFUENSIS*

ABSTRACT

Development of F_1 interspecific hybrid cultivars between *Cynodon dactylon* and *C. nlemfuensis* is a major focus of forage bermudagrass breeding programs. Molecular markers can be used to accurately identify targeted hybrids. However, no simple sequence repeat (SSR) markers exist for use with *C. nlemfuensis*. Accordingly, the objective of this study was to develop SSR markers for *C. nlemfuensis* and to assess the genetic diversity 6 *Cynodon* genotypes to gauge the effectiveness of transferable markers. A collection of 246 SSR primer pairs (PPs) developed and mapped in *C. dactylon* were tested on 5 genotypes of *C. nlemfuensis*. Effectiveness of transferable markers was confirmed through their use in assessing the genetic relatedness of the tested germplasm. In total, 215 SSR markers were transferable to *C. nlemfuensis*, with a transferability rate that ranged from 75.8 – 87.9%. Amplified alleles were relatively conservative with a majority of markers amplifying 1 – 2 alleles SSR PP^{-1}. Two accessions of Tifton 85 were the most closely related with a similarity coefficient of 0.99, indicating the efficacy and

accuracy of the markers. Additionally, Tifton 68, a parent of Tifton 85, expressed a relatedness of 0.70 to Tifton 85. Transferable markers will facilitate efficient identification of hybrid progenies in interspecific crosses of forage bermudagrass.

INTRODUCTION

Bermudagrass (*Cynodon* spp.) is a robust warm season grass, often employed in landscape, soil stabilization, and agricultural settings (Taliaferro et al., 2004). With a base chromosome count being 9, bermudagrass is most commonly found in diploid (2n=2x=18) and tetraploid (2n=4x=36) cytotypes (Forbes and Burton, 1963; Harlan et al., 1970a; de Silva and Snaydon, 1995). Wide geographic distribution has curated 9 species of within the *Cynodon* genus (Harlan et al., 1970b; Taliaferro, 1995). Taliaferro et al. (2004) notes how breeding efforts have demonstrated considerable focus on the improvement of *C. dactylon* and *C. nlemfuensis* for forage use. Additionally, these efforts have focused on yield, forage quality, and cold tolerance. Improved cold tolerance has led to the northern expansion of bermudagrass past 36°N latitude with such cultivars as Goodwell, Hardie, Midland 99, and Ozark (Wu and Taliaferro, 2009; Taliaferro and Richardson, 1980; Taliaferro et al., 2002; Richardson and Taliaferro, 2005). Exploitation of fixed heterosis within interspecific F_1 hybrids has become a common theme, specifically with crosses between *C. dactylon* and *C. nlemfuensis* (Taliaferro, et al., 2004; Wu, 2011).

Molecular breeding methods have become an important tool in hybrid development of many crops. Considerable work has been implemented with *C. dactylon*, where a primary focus has been placed on turf types. Multiple studies have reported the

identification of useful simple sequence repeat (SSR) markers in bermudagrass (Guo et al., 2017; Harris-Shultz et al., 2010; Tan et al., 2012; Tan et al., 2014a). These markers improve breeder's ability to accurately genotype and understand germplasm through marker assisted selection, linkage mapping, and identification of quantitative trait loci. Furthermore, molecular markers are an important component of understanding the diversity of genotypes. Assessments of germplasm collections with SSR markers have proven to be efficient and accurate (Anderson et al., 2009; Harris-Shultz et al., 2010; Jewell et al., 2012). Both Fang et al. (2015) and Tan et al. (2014b) further demonstrated the discriminatory power of SSR markers to readily identify parental relationships of bermudagrass plants.

A majority of the molecular marker work of bermudagrass has been centralized on *C. dactylon*, with no attention given to *C. nlemfuensis*. Interspecific hybridization between the two species is a popular method for breeders. But it is difficult to separate targeted hybrids between two selected parents from selfed progeny of the seed parent or non-targeted hybrids between the seed parent and contamination pollen from bermudagrass plants in surrounding areas. Breeding programs witness increased accuracy in hybrid identification with the use of marker assisted selection. Developing novel sets of SSR markers for a species can be time consuming and resource intensive. However, the option to utilize transferable markers from another species, especially *C. dactylon*, is a method that provides considerable promise. Success of transferability is dependent on the genetic relatedness of targeted species (Dayanandan et al., 1997). Successful SSR transferability studies are well documented throughout a variety of crops (Brown et al., 1996; Cordeiro et al., 2001; Varshney et al., 2005; Xie et al., 2010). Wheat,

rice, maize, and sorghum SSR markers were tested on several grasses, including *Cynodon* by Wang et al. (2005). Tan et al. (2012) identified a collection of sorghum SSRs that effectively amplified bands in *C. dactylon* and *C. transvaalensis*. Identifying transferable SSR markers for use in *C. nlemfuensis* would enhance breeding efforts and facilitate efficient genotyping of interspecific crosses of bermudagrass. The objectives of this study were to test the transferability of *C. dactylon* SSR markers on a collection of *C. nlemfuensis* genotypes, and to investigate the genetic relatedness of tested germplasm with transferable markers.

MATERIALS AND METHODS

Plant Materials

Germplasm was collected from the Oklahoma State University (OSU) forage bermudagrass collection and National Plant Germplasm System (NPGS). Five genotypes of *C. nlemfuensis* and 1 cultivar of *C. dactylon* were utilized. Genotypes from NPGS included Florico and Tifton 85 (Tifton 85-2). Germplasm from OSU included Tifton 68, Tifton 85 (Tifton 85-1), a non-commercialized accession of *C. nlemfuensis* (CNL-1), and Zebra (*C. dactylon*). Zebra DNA was used to develop SSR markers (Guo et al., 2017). Plants of the accessions were grown in a greenhouse under ideal growing conditions. Fertilizer, irrigation, and pesticides were applied as needed.

DNA Extraction, Amplification, and Gel Electrophoresis

Plant tissue samples were collected from new growth on the 6 genotypes separately and placed in storage at -80°C for 72 hours. Samples were then ground with a tissue homogenizer (Geno/Grinder; SPEX SamplePrep, Metuchen, NJ) for 2 minutes.

Extraction of DNA from ground tissue followed phenol-chloroform methods described by Nalini and Jawali (2004) with minimal modification. Concentrated DNA was quantified with a spectrometer (NanoDrop ND-1000; Thermo Fischer, Waltham, MA) and diluted to 10 ng uL-1. Primer pairs for 246 SSR markers developed with a *C. dactylon* linkage map by Guo et al. (2017) were tested on the 6 genotypes (Figure 1). These SSR markers developed with Zebra, which served as the control in our testing process. Components of the polymerase chain reaction (PCR) solution included 6.54 µL nuclease free water, 1 µl 10x PCR buffer (New England BioLab, Ipswich, MA), 1 µl 1 pmol μl^{-1} forward and reverse primer, 0.2 µl 10 mM dNTPs, 0.2 µl 1 uM M13 with either 700 or 800 nm fluorescent dye (LI-COR, Lincoln, NE), 0.05 µl Taq DNA polymerase (New England BioLab, Ipswich, MA), and 1.5 µl of 10 ng μl^{-1} of diluted DNA template. Each sample was replicated twice and a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA) was used for PCR reactions. A program by Fang et al. (2015) was employed. The reaction cycle was 5 minutes (min) at 95°C, 14 cycles of 20 seconds (s) at 94°C, 60 s at 58°C, and 30 s at 72°C, followed by 28 cycles of 20 seconds (s) at 94°C, 60 s at 55°C, and 30 s at 72°C. Completed reactions were kept at 4°C once removed from thermal cycler. Blue stop solution was added to each reaction well prior to a final 3 min denaturation at 94°C. A LI-COR 4300 DNA analyzer (LI-COR Biosciences, Lincoln, NE) was used for gel electrophoresis with a 6.5% KB Plus gel (LI-COR). Each gel experienced a runtime of 120 min. Images were visualized with Saga Generation 2 Lite software (LI-COR).

Gel Scoring, Data Analysis, and Genetic Diversity

Binary matrix scoring of gels was utilized when evaluating each primer pair. Scores of 0 (no band) and 1 (clear band) were used, 9 signified a failed reaction. A maximum of 4 scored bands was allowed for each genotype. To assess transferability, only primer pairs that produced bands in Tifton 68, CNL-1, or Florico were identified as transferable. As Tifton 85 is an interspecific hybrid between *C. nlemfuensis* and *C. dactylon*, bands produced only in Tifton 85-1 and Tifton 85-2 could not defined as transferable due to the likelihood that these primer pairs are amplifying the *C. dactylon* portion of the Tifton 85 genome. Excel (Microsoft Corporation, Redmond, WA) was used for data management and quantification of transferability and allele amplification rate. Assessment of genetic diversity was performed with methods described by Tan et al. (2012). Scored SSR data was analyzed using cluster analysis and unweighted pair-group method of arithmetic averages (UPGMA) were generated with NTSYS-pc software (Exeter Software, Setauket, NY).

RESULTS

Transfer rate of SSR markers

Transfer rates of *C. dactylon* SSR primer pairs were relatively high across the 5 evaluated genotypes. Zebra bermudagrass served as the *C. dactylon* control. Of the 246 SSR primer pairs tested, 215 primer pair provided clear data and amplified bands in Zebra, for an effective control rate of 87.4%. Primer pairs that did not produce bands in Zebra are a result of failed PCR reactions or issues with clarity of gel images. Data from the 215 useable bands displayed a transfer rate that ranged from 75.8 – 87.9% among the

5 genotypes, depicted in Table 23. Tifton 85-1 and Tifton 85-2 provided the highest number of transferable primer pairs at 187 and 189, respectively. Tifton 68 achieved a transfer rate of 81.9%, followed by CNL-1 at 76.7%, and Florico at 75.8%. The complete list of transferable SSR primer pairs and number of amplified bands across the 5 genotypes is presented in Table 24. Of the 215 primer pairs, 182 produced clear, reproducible bands in at least one of the evaluated genotypes, resulting in an overall transfer rate of 84.7%. Primer pairs that only produced bands in Tifton 85 were not counted as transferable. Tifton 85 is an interspecific hybrid from *C. dactylon* x *C. nlemfuensis*, therefore, bands produced only in Tifton 85 are likely to be amplifying the *C. dactylon* genome.

Distribution of amplified alleles in the tested genotypes is presented in Figure 2. All primer pairs amplified 0 – 4 alleles across the 6 genotypes. Serving as the control, Zebra encountered successful amplification with 215 primer pairs. Of these primer pairs, Zebra expressed band amplification rates of 59.5% and 31.6% for one and two band amplifications, respectively. The 5 *C. nlemfuensis* genotypes were likewise relatively conservative for 1 and 2 allele amplifications of the successful primer pairs. Outside of Zebra, Florico accounted for the largest percentage of single band amplification at a rate of 49.7%, accompanied by a rate of 35.6% for two bands from the 162 effective primer pairs. Band numbers in CNL-1 were highest for 1 (40.6%) and 2 bands (37.6%). Tifton 85-1 amplified 1 band at a rate of 32.6% and 2 bands at 40.1%. Tifton 85-2 expressed similar amplification patterns, at 33.3% for 1 band, and 39.7% for 2 bands. Tifton 68 witnessed a more uniform distribution of 1, 2, 3, and 4 bands, with 28.4%, 37.5%, 22.7%, and 11.4%, respectively.

Genetic diversity of tested genotypes

Utilizing 226 SSR markers, genetic relatedness among the 6 genotypes was assessed with 1106 amplified bands. Genetic similarity coefficients are displayed in Table 25. Further depiction of the unweighted pair group method with arithmetic average (UPGMA) tree is represented in Figure 3. Tifton 85-1 and Tifton 85-2 were the most similar, as expected, with a similarity coefficient of 0.99. Although Tifton 85-1 and Tifton 85-2 are the same variety, the subjective nature of SSR scoring could be the result of the slight difference of observed banding pattern between the two biotypes. As a parent of Tifton 85, Tifton 68 was highly similar to both biotypes (0.70). Additionally, Tifton 68 and CNL-1 expressed a relatedness of 0.58, while CNL-1 achieved a similarity coefficient of 0.55 with both Tifton 85 biotypes. Florico displayed modest relationships with CNL-1 (0.63), Tifton 85-1 and Tifton 85-2 (0.70), and Tifton 68 (0.58). Zebra was the most genetically distant from the *C. nlemfuensis* genotypes, expressing coefficients of 0.43, 0.44, 0.48, 0.44, and 0.49 with Tifton 68, Tifton 85-1, CNL-1, Tifton 85-2, and Florico, respectively. The *C. dactylon* SSR markers provided effective and efficient discriminatory ability to distinguish genetic relatedness of the tested *C. nlemfuensis* genotypes.

DISCUSSION

High transferability rates were witnessed across all 5 genotypes, ranging from 75.8 – 87.9%. As *C. dactylon* and *C. nlemfuensis* are relatively close in terms of species relation, this high transfer rate is not unexpected. Dayanandan et al. (1997) notes transfer success is likely to be strongly associated with the genetic relatedness of target species.

As stargrass and common bermudagrass belong to the same genus, their genetic relatedness is close as characterized by Assefa et al. (1999). Tan et al. (2012) reported sorghum SSR markers to display a transferable range of 22.0 – 56.8% among 3 bermudagrass genotypes. Distant relatedness of sorghum and bermudagrass is evident of the lower transfer rates. In our study, higher transfer rates encountered with Tifton 85 germplasm (T85-1, T85-2) are a representation of the *C. dactylon* genome that makes up a portion of this interspecific hybrids DNA. Tan et al. (2012) witnessed relatively conservative band amplification in their transferable markers, incurring high frequencies of only 1 – 2 bands marker-1. Similar results were documented in our study, where frequencies of 1 – 2 band amplifications were predominant across transferable markers. These findings continue to represent the conservative nature of the evaluated primer pairs in terms of allele amplification.

Genetic diversity of tested germplasm was used to assess the effectiveness and discriminatory power of the transferable markers. Data from 226 SSR markers, amplifying 1106 alleles provided sufficient information for analysis. Tifton 85 (T85-1, T-85-2) germplasm was the most closely related at 0.99. Lack of a perfect relationship is likely the result of subjective scoring of the observed alleles within the two accessions. Tifton 68 (T68) was highly similar to Tifton 85 germplasm, with a similarity coefficient of 0.70. Anderson et al. (2009) documented a similar relationship between Tifton 68 and Tifton 85. As a parent of Tifton 85, it is to be expected that Tifton 68 has such strong relation (Burton et al., 1993). Morphology of Tifton 85 is also suggestive of the substantial influence and portion of the genome Tifton 68 accounts for. The accession from the OSU greenhouse, CNL-1, displayed a moderate relationship to the other *C.*

nlemfuensis genotypes, suggesting it's lack of use in commercial breeding purposes to this date. Florico was most closely related to CNL-1 with a similarity coefficient of 0.63. Originally native to Kenya, Florico was introduced by the Florida Agricultural Experiment Station as an ecotype selection from Puerto Rico (Mislevy et al., 1993). This variety lacks the ability to persist outside of tropical climates, leading to its relative isolation from the genetic pool used in the Tifton breeding program. Zebra was the most genetic distant from the *C. nlemfuensis* genotypes, as was expected, expressing similarity coefficients that ranged from 0.43 – 0.49. Evaluated SSR markers showed ample ability for use in assessing genetic variability and distinguishing progeny lineages. This collection of markers will facilitate heightened efficiency in expanded breeding efforts of forage bermudagrass. For genetic diversity analysis of larger genotype collections, the use of highly polymorphic bands should be a priority.

Identification of effective, transferable SSR markers saves time and resources in breeding programs and should be considered for species with limited molecular tool development. Incorporation of molecular breeding procedures will allow for increased efficiency in selections procedures, and further aid in interspecific hybridization between established species and species with limited molecular tool development. Furthermore, the validation of discriminatory power amongst transferable SSR markers is paramount, as this confirmation provides valuable evidence for their inclusion and use in modern breeding programs. The collection of transferable markers will readily serve future breeding initiatives involving *C. nlemfuensis*.

Table 23. Transferability rate of 215 *C. dactylon* SSR markers in 5 genotypes of *C. nlemfuensis*.

Genotype	Transferable SSR primer pairs	Transferability rate, %
Tifton 68	176	81.9
Tifton 85-1	187	87.0
CNL-1	165	76.7
Tifton 85-2	189	87.9
Florico	163	75.8

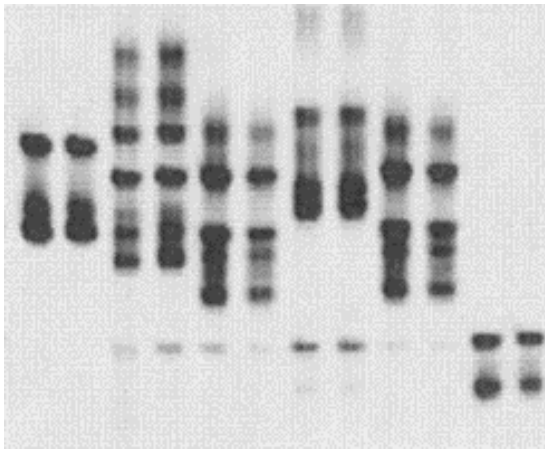

Figure 1. Transferability testing of SSR markers, replicated twice in order from L-R: Zebra, T68, T85-1, CNL-1, T85-2, Florico.

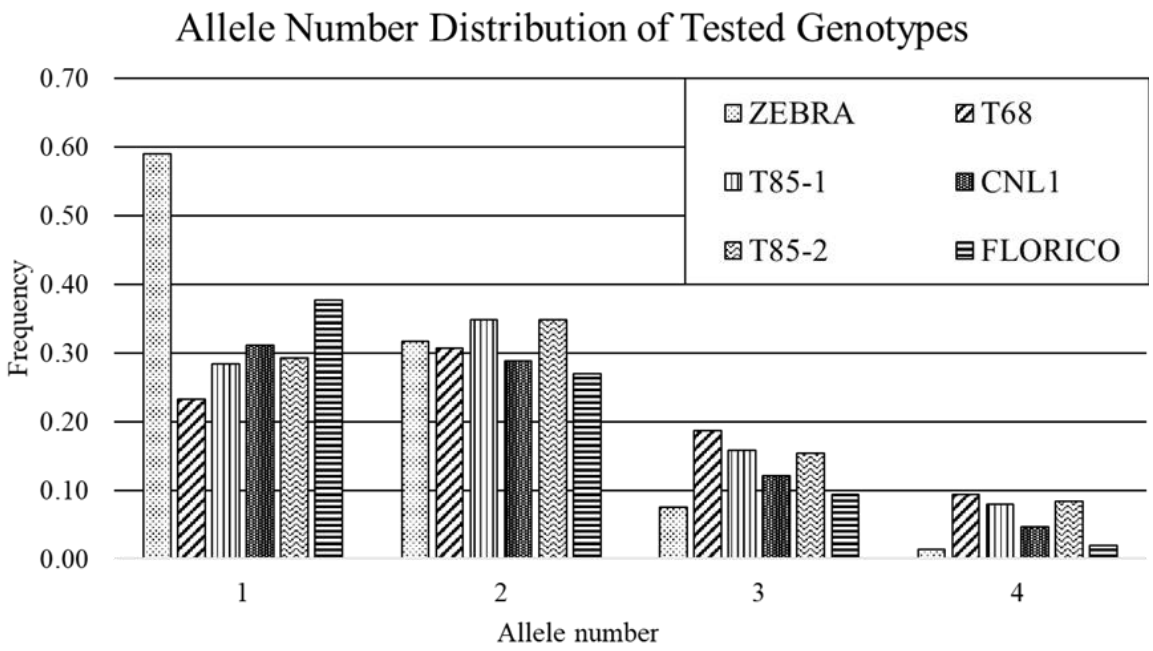

Figure 2. Amplified allele frequencies across tested genotypes.

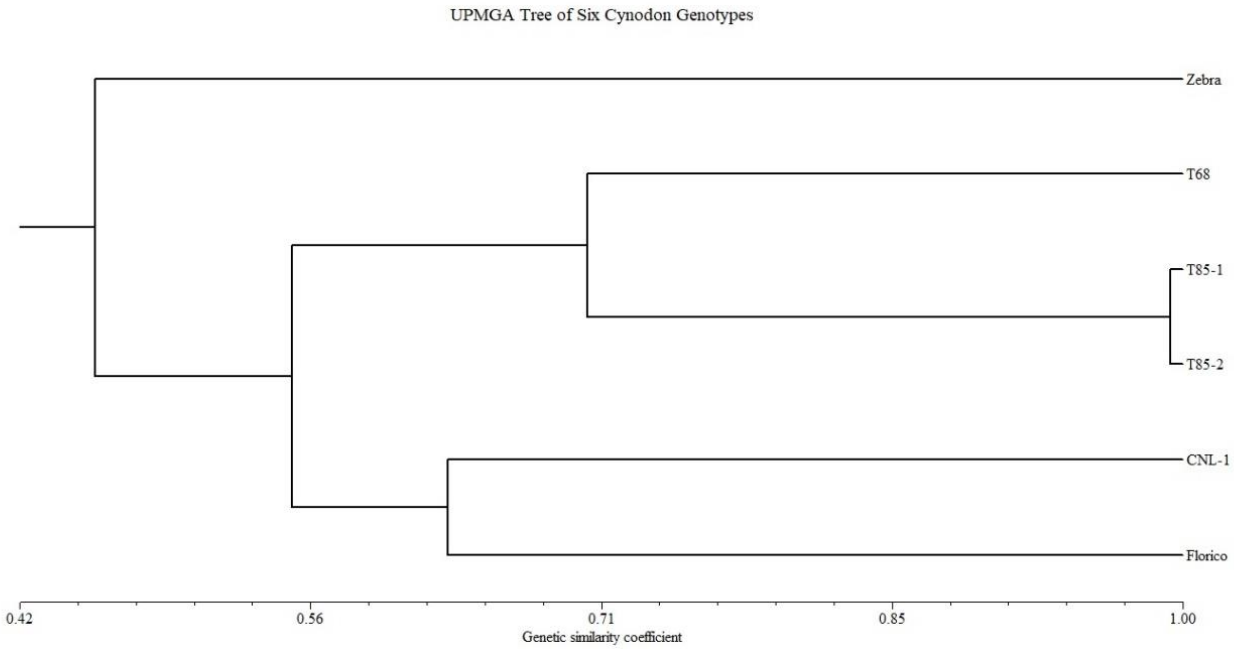

Figure 3. Dendogram of genetic similarity coefficients among tested genotypes.

Table 24. Transferable *C. dactylon* SSR primer pairs and associated number of amplified bands across 5 genotypes of *C. nlemfuensis*.

SSR primer pair ID	Linkage group†	Number of amplified bands genotype^{-1}				
		T68	T85-1	CNL-1	T85-2	Florico
CDCA5-475/476	1	1	0	1	0	1
CDGA1-783/784	1	2	1	2	1	1
CDGA2-1089/1090	1	2	2	1	2	0
CDCGA5-1465/1466	1	1	1	2	1	1
CDCA1-39/40	1	2	2	1	2	1
CDCA6-529/530	1	3	1	1	1	1
CDCA6-543/544	1	2	0	3	1	2
CDGA4-1301/1302_120	1	2	2	1	2	1
CDCA2-197/198	1	2	2	1	2	2
CDAAC5-2527/2528	1	2	1	2	1	2
CDCA3-313/314	1	2	2	2	2	2
CDGA4-1355/1356	1	3	2	3	2	2
CDGA8-1807/1808	1	3	3	0	3	2
CDGA5-1425/1426	1	3	3	2	3	1
CDGA7-1601/1602	2	1	2	1	2	1
CDGA1-877/878_250	2	1	2	2	2	1
CDCA1- 11/12	2	2	2	1	2	1
CDCA2-449/450	2	1	2	2	2	2
CDGA4-1313/1314	3	1	1	1	1	0
CDGA4-1323/1324	3	2	1	1	1	1
CDCA7-675/676	3	1	2	1	2	1
CDCA5-491/492	3	2	2	2	2	0
CDCA3-245/246	3	2	3	0	3	1
CDAAC5-2563/2564	3	2	3	2	3	2
CDCA7-667/668	3	2	3	2	3	2
CDCA1- 7/8	3	4	3	3	3	1
CDGA3-1135/1136	3	4	3	3	3	2
CDGA4-1249/1250	3	3	4	4	4	1
CDCA4-319/320	4	2	2	1	2	2
CDCA6-589/590	4	3	2	2	2	2
CDGA1-829/830	4	3	3	2	3	2
CDCA8-709/710	4	4	4	2	4	2
CDCA7-703/704	4	4	4	4	4	3
CDGA3-1189/1190	5	1	1	0	1	1
CDCA1-59/60	5	2	1	1	1	1
CDCA1-21/22	5	1	2	0	2	2
CDGA3-1103/1104	5	2	1	1	1	1
CDATG1-1905/1906	5	1	1	2	1	1
CDCA6-583/584	5	2	2	1	2	1
CDGA4-1331/1332	5	1	1	3	1	2
CDCA6-549/550	5	2	2	2	2	2
CDCA8-773/774	5	3	3	0	3	3

CDCA2-213/214	5	3	3	3	3	3
CDCA3-263/264	6	2	3	3	3	3
CDCA5-501/502	6	4	4	3	4	3
CDCA7-623/624	7	1	0	0	0	0
CDCA5-409/410	7	1	0	1	1	0
CDCA6-565/566	7	1	1	0	1	0
CDGA8-1795/1796	7	1	1	0	1	0
CDGA2-965/966	7	0	1	1	1	1
CDGA3-1119/1120	7	1	2	1	2	0
CDCA1-115/116	7	1	2	1	2	1
CDCA7-695/696	7	1	2	1	2	1
CDATG3-1999/2000	7	2	1	2	1	1
CDCA5-469/470_230	7	2	2	2	2	2
CDGA1-787/788	7	1	2	2	2	2
CDGA5-1455/1456_160	7	2	2	2	2	2
CDGA2-991/992	7	3	2	2	2	2
CDGA1-791/792	7	2	3	3	3	1
CDCA5-465/466	7	3	2	3	2	3
CDCA5-505/506	7	2	3	3	4	2
CDGA2-1027/1028	7	2	4	2	4	1
CDGA4-1307/1308	7	2	3	2	3	3
CDCA3-237/238	7	4	4	2	4	1
CDCA7-671/672	7	3	4	3	4	1
CDGA4-1295/1296	7	3	4	4	4	2
CDCA5-503/504	7	3	4	4	4	4
CDGA1-865/866	8	1	1	1	1	1
CDGA4-1335/1336	8	1	2	0	2	0
CDGA1-921/922	8	2	2	2	2	1
CDGA2-1037/1038	8	3	2	2	2	2
CDAAC3-2439/2440	8	3	2	2	2	1
CDCA6-571/572	8	4	2	2	2	1
CDCA8-717/718	8	4	2	3	2	1
CDGA2-1011/1012	8	3	2	2	2	2
CDGA5-1439/1440	8	4	2	1	2	2
CDAAC7-2675/2676	8	2	3	4	3	3
CDGA1-805/806	9	2	1	1	1	2
CDGA1-815/816	9	2	2	2	2	1
CDCA6-577/578	9	1	2	3	2	2
CDGA4-1269/1270	9	2	3	4	3	2
CDGA5-1467/1468_120	9	4	2	4	2	3
CDCA6-559/560	9	4	4	4	4	3
CDGA3-1147/1148	10	1	1	0	1	0
CDAAC8-2725/2726	10	1	1	0	1	0
CDCA1-45/46	10	1	1	2	1	1
CDCA1-89/90	10	1	2	1	2	1
CDGA5-1369/1370	10	2	2	2	2	3

CDGA3-1211/1212	10	3	3	2	3	3
CDCA1-63/64	11	2	1	2	1	1
CDGA5-1461/1462	11	1	1	1	1	1
CDGA3-1177/1178	11	2	1	2	1	1
CDCA2-227/228	11	1	2	1	2	1
CDGA4-1347/1348	11	3	4	4	4	2
CDGA5-1381/1382	12	0	1	0	1	1
CDGA3-1157/1158	12	2	1	0	1	0
CDCA4-401/402	12	1	1	1	1	1
CDCA1-19/20	12	1	2	1	2	1
CDCA7-645/646	12	1	2	1	2	2
CDCA7-693/694	12	1	2	2	2	1
CDGA4-1333/1334	12	2	2	1	2	1
CDGA4-1343/1344	12	2	2	2	2	2
CDGA1-939/940	12	3	3	2	3	1
CDCA2-141/142	12	4	3	1	3	2
CDCA3-295/296	12	4	2	3	2	2
CDGA2-1015/1016	12	2	3	3	3	2
CDCA1-57/58	13	1	1	1	1	1
CDGA7-1665/1666	13	2	1	0	1	1
CDCA1-95/96	13	1	1	1	1	2
CDCA8-729/730	13	2	1	1	1	2
CDGA3-1161/1162	13	1	2	1	2	2
CDGA4-1281/1282	13	1	2	1	2	2
CDGA2-1021/1022	13	2	2	1	2	2
CDGA4-1253/1254	13	2	2	2	2	1
CDGA5-1391/1392	13	2	2	2	2	2
CDGA3-1167/1168	13	2	3	3	3	0
CDATG3-2001/2002	14	1	1	1	1	1
CDGA3-1219/1220	14	1	1	2	1	1
CDGA1-827/828	14	2	2	1	2	1
CDGA4-1261/1262	14	2	1	3	1	1
CDCA2-179/180	14	1	3	1	3	1
CDGA1-915/916	14	1	2	1	2	1
CDGA6-1475/1476	14	3	4	2	4	1
CDCA7-635/636	14	4	3	2	3	3
CDGA2-1003/1004	15	2	1	1	1	1
CDGA3-1215/1216	15	1	1	1	1	1
CDAAC5-2537/2538	15	2	1	1	1	1
CDCA5-411/412	15	1	2	1	2	2
CDCA2-207/208	15	3	2	1	2	1
CDGA1-933/934	15	2	2	2	2	1
CDATG1-1875/1876	15	1	3	1	3	1
CDCA7-615/616	15	3	3	1	3	0
CDGA1-847/848	15	2	2	2	2	2
CDGA2-957/958	15	2	3	2	3	1

CDAAC2-2361/2362	15	3	2	1	2	2
CDCA6-587/588	15	3	2	2	2	4
CDCA7-611/612	15	3	3	1	3	2
CDAAC7-2693/2694_285	15	3	3	1	3	1
CDCA4-399/400	15	4	3	2	3	3
CDGA4-1345/1346	15	4	4	3	4	4
CDCA7-639/640	16	0	1	1	1	0
CDGA2-1073/1074	16	0	1	1	1	0
CDCA7-691/692	16	1	1	1	1	2
CDGA2-999/1000	16	2	1	1	1	1
CDGA3-1225/1226	16	2	1	2	1	1
CDGA5-1459/1460	16	2	2	1	2	0
CDCA4-335/336	16	1	2	0	2	2
CDCA2-125/125	16	3	2	2	2	2
CDCA7-641/642	16	3	2	3	2	2
CDGA5-1471/1472	16	4	2	2	2	3
CDGA1-935/936	17	0	0	1	0	0
CDCA5-461/462	17	2	1	2	1	1
CDGA5-1359/1360	17	1	2	2	2	1
CDGA7-1697/1698	17	2	1	2	1	3
CDATG1-1857/1858	17	3	2	1	2	1
CDGA3-1197/1198	17	3	4	1	4	2
CDCA2-177/178	17	3	4	2	4	2
CDGA4-1239/1240	17	2	3	2	3	2
CDCA4-325/326	17	4	4	2	4	3
CDCA1-81/82	18	0	0	0	0	1
CDCA8-731/732	18	1	0	1	0	0
CDCA4-389/390	18	1	1	0	1	1
CDCA1-27/28	18	1	1	1	1	1
CDCA6-569/570	18	1	1	1	1	1
CDCA6-521/522	18	2	1	1	1	1
CDCA6-605/606	18	2	1	1	1	1
CDCA8-711/712	18	2	1	1	1	1
CDGA5-1443/1444	18	2	1	2	1	1
CDCA3-243/244	18	2	2	2	2	1
CDCA5-437/438	18	3	1	2	1	2
CDGA3-1159/1160	18	2	2	4	2	1
CDGA5-1441/1442	18	2	3	1	3	1
CDCA1-53/54	18	3	2	3	2	2
CDGA8-1783/1784	18	3	2	1	2	2
CDCA1-111/112	18	3	2	3	2	3
CDGA5-1395/1396	18	3	2	2	2	3
CDGA5-1417/1418	18	3	3	3	3	1
CDCA5-473/474	18	3	3	3	3	2
CDGA5-1363/1364	18	3	3	3	3	2
CDGA7-1667/1668	18	4	2	2	2	3

CDCA3-247/248	18	2	4	2	4	2
CDAAC1-2245/2246	18	4	2	3	2	4

[†]Associated marker linkage group from Guo et al. (2017).

Table 25. Similarity coefficients of 6 *Cynodon spp.* genotypes.

	Zebra	Tifton 68	Tifton 85-1	CNL-1	Tifton 85-2
Tifton 68	0.43	-			
Tifton 85-1	0.44	0.70	-		
CNL-1	0.48	0.58	0.55	-	
Tifton 85-2	0.44	0.70	0.99	0.55	-
Florico	0.49	0.58	0.54	0.63	0.54

CHAPTER VI

MOLECULAR GENETIC DIVERSITY OF FORAGE BERMUDAGRASS CULTIVARS AS DETERMINED BY SSR MARKERS

ABSTRACT

Bermudagrass breeding efforts have expanded the geographic range of productive forage stands. Understanding the genetic diversity of current commercial cultivars enables informed decisions regarding future breeding objectives. However, limited analysis has been conducted in investigating the genetic diversity of major forage bermudagrass cultivars. The objective of this study was to characterize the genetic diversity of major forage bermudagrass commercial cultivars and experimental accessions with SSR markers. A collection of 31 accessions of forage bermudagrass experimental accession and commercial cultivars were analyzed with 52 SSR markers. Genetic similarity coefficients ranged from 0.772 – 0.939, indicating a relatively narrow genetic base among major hybrids. Allele amplification was highly conservative for 1 – 2 bands genotype^{-1} across markers. Understanding the genetic relatedness of cultivars provides valuable insight into future breeding goals, where breeders should work to incorporate diverse collections of novel germplasm into established breeding pipelines.

INTRODUCTION

Forage bermudagrass (*Cynodon* spp.) occupies a major role in the agricultural value chain, as it is grown on an estimated 10 – 12 million ha (Taliaferro et al, 2004). Breeding efforts have contributed to the expansion of bermudagrass use in agronomic settings. A cross between Tift and a South African accession, Coastal was one of the earliest hybrids of forage bermudagrass (Myers, 1951, Burton 1948). Coastal served a parent for the release of other prominent forage hybrids, including Coastcross-1, Tifton 44, Midland, and Ozark (Burton, 1972; Burton and Monson, 1978; Hein, 1953; Richardson et al., 2005). Ozark and Coastcross-1 ultimately became half-sibs of later hybrids as the parents used in those original crosses served as a parent for Guymon, Grazer, Wrangler (Johnston Seed Co., Enid, OK), and Tifton 68 (Taliaferro et al., 1983; Eichhorn et al., 1986; Burton and Monson, 1984). Goodwell and Tifton 85 are also relatives of Ozark and Coastcross-1, respectively (Wu et al., 2009; Burton et al., 1993). Early focus was placed on improving forage yield and quality, which was well displayed in such hybrids as Tifton 68 and Tifton 85 (Burton and Monson, 1984; Burton et al., 1993). Breeders have made another focus on improving cold hardiness. With such hybrids as Goodwell, Hardie, Midland, Midland 99, Ozark, and Tifton 44, bermudagrass is now capable of profitable production within the transition zone of the United States (Wu et al., 2009; Taliaferro et al., 2002; Richardson et al. 2005; Taliaferro and Richardson, 1980; Burton and Monson, 1978; Hein, 1953). Understanding the relationships of commercial cultivars allows for informed decisions in future breeding initiatives.

Multiple studies have reported the large diversity that exists within bermudagrass germplasm (Harlan and de Wet, 1969; Guo et al., 2017; Stefaniak et al., 2009; Wu et al., 2006; Wu et al., 2007). However, few studies have quantified this diversity on a molecular level. One major advantage of molecular genetic diversity characterization is not affected by environment. Anderson et al. (2009) utilized amplified fragment length polymorphisms (AFLP) to assess the diversity of a core collection of forage bermudagrass accessions. Furthermore, Jewell et al. (2012) analyzed the genetic diversity of a core collection of Australian bermudagrass with expressed sequence-tag simple sequence repeat (EST-SSR) markers. Both of these studies demonstrated large variability among the core accessions of forage bermudagrass. Despite this, Karaca et al. (2002) noted a lack of diversity, and a relatively narrow genetic base within commercial cultivars of bermudagrass. Their study reported a genetic similarity coefficient range of 0.608 – 0.977 utilizing a variety of molecular marker technologies. Further studies into the genetic relationships of commercial cultivars will allow more definitive decisions on breeding objectives and directions. Simple sequence repeat (SSR) markers are a robust option for diversity analyses, Fang et al. (2015) and Tan et al. (2014b) demonstrated the discriminatory power of SSR markers to identify relationships of turf bermudagrass plants. The ability of SSR markers to produce distinct allelic amplification, in addition to their codominant and high polymorphic nature make them an excellent tool for genetic studies. Bermudagrass is known to be highly heterozygous due to its outcrossing nature (Tan et al., 2014), allowing for even more information to be gained from the use of SSR markers. The objective of this study was to characterize the genetic diversity of major

forage bermudagrass commercial cultivars and experimental accessions with SSR markers.

MATERIALS AND METHODS

Plant Materials

Germplasm resources were collected from the Oklahoma State University forage bermudagrass collection. In total, 31 genotypes were evaluated – 30 forage type bermudagrass cultivars and experimental selections, and Zebra, which served as DNA templates in the development of SSR markers, therefore the control for SSR amplification in this study. The 30 forage genotypes included Alicia (Private commercialization, Edna, TX), Coastal, Coastcross-1, Goodwell, Grazer, Guymon, Midland, Midland 99, Ozark, Tifton 44, Tifton 68, Tifton 85, World Feeder (Burton, 1948; Myers, 1951; Hein, 1953; Richardson et al., 2005; Burton, 1972; Burton and Monson, 1978; Gordon, 1989; Taliaferro et al., 1983; Wu et al., 2009; Eichhorn et al., 1986; Burton and Monson, 1984; Burton et al., 1993; Taliaferro et al., 2002), Wrangler (Johnston Seed Co., Enid, OK), 7 ecotypes of Greenfield (Elder, 1955), and 9 late stage experimental accessions from the OSU grass breeding program, 3200W-R14-C8, 3200W-R9-C8, 5200E-1, 56-10, G-19-6, G-19-64, G-19-86, G-19-89, and T44-1. Plant material was collected and grown in a greenhouse under ideal growing conditions, receiving irrigation, fertilizer, and pesticide applications as needed.

DNA Extraction, Amplification, and Gel Electrophoresis

New tissue growth was sampled from each genotype and placed in a freezer at -80°C for 72 hours. Samples were ground with a Geno/Grinder in preparation for DNA

extraction (Geno/Grinder; SPEX SamplePrep, Metuchen, NJ). Phenol-chloroform DNA extraction was used in accordance with methods described by Nalini and Jawali (2004) with minimal modification. Concentrated DNA samples were then diluted to ng uL-1 based on spectrometer quantitation (NanoDrop ND-1000; Thermo Fischer, Waltham, MA). A total of 54 primer pairs were selected from a linkage map developed by Guo et al. (2017) (Table 27), of which 52 provided useable data. Care was taken to select 3 primer pairs uniformly spaced across each linkage group. Reaction well components for the polymerase chain reaction (PCR) solution included 6.54 µL nuclease free water, 1 µl 10x PCR buffer (New England BioLab, Ipswich, MA), 1 µl 1 pmol µl–1 forward and reverse primer, 0.2 µl 10 mM dNTPs, 0.2 µl 1 uM M13 with either 700 or 800 nm fluorescent dye (LI-COR, Lincoln, NE), 0.05 µl Taq DNA polymerase (New England BioLab, Ipswich, MA), and 1.5 µl of 10 ng μl^{-1} diluted DNA template. Samples were replicated twice and PCR reactions were performed with a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). Fang et al. (2015) provided a PCR program for our experiment, which featured a reaction cycle of 5 minutes (min) at 95°C, 14 cycles of 20 seconds (s) at 94°C, 60 s at 58°C, and 30 s at 72°C, followed by 28 cycles of 20 seconds (s) at 94°C, 60 s at 55°C, and 30 s at 72°C. Reaction products were stored at 4°C once removed from the thermal cycler. To terminate the reaction, blue stop solution was added prior to a final 3 min denaturation at 94°C. Gel-electrophoresis was conducted with a LI-COR 4300 DNA analyzer (LI-COR Biosciences, Lincoln, NE) with a 6.5% KB Plus gel (LI-COR) ran for 120 min. Sage Generation 2 Lite software was used to view gel images (LI-COR).

Gel Scoring, Data Analysis, and Genetic Diversity

Banding patterns were scored as one (1) for presence, zero (0) for absence, and nine (9) for missing data (Figure 4). Four scored bands from each primer pair were allowed for each genotype as reported by Guo et al. (2017). Excel (Microsoft Corporation, Redmond, WA) was the primary database for calculation of allele amplification rates. Methods described by Tan et al. (2012) were employed to assess genetic diversity. Cluster analysis and unweighted pair-group method of arithmetic averages (UPGMA) were generated through NTSYS-pc software (Exeter Software, Setauket, NY) to produce the dendrogram and similarity coefficients. Similarity coefficients range from 0 – 1, with values approaching 1 indicating a higher degree of relatedness among the analyzed alleles.

RESULTS

Amplified Allele Distribution

Allele amplification was relatively conservative for 1 and 2 bands across all genotypes. On average, 1 band was amplified 43% of the time, and 2 bands amplified 32% of the time (Figure 5). Limited allelic richness in the tested SSR markers could be a contributing factor the narrow nature of the observed genetic base.

Genetic Diversity Analysis

Genetic similarity coefficients indicate a relatively narrow base within the evaluated germplasm. From the 52 SSR markers, 710 alleles were amplified, producing similarity coefficients that ranged from 0.772 – 0.939, and an overall mean of 0.833.

Genetic similarity coefficients are displayed in Table 26, in addition to visualized relationship with a UPGMA in Figure 6. Seven ecotypes of Greenfield were collected throughout Oklahoma and Arkansas. For these 7 ecotypes, the Greenfield from the OSU forage collection served as the reference cultivar. Results indicate a moderate level of contamination within these fields, as similarity coefficients ranged from 0.811 – 0.873. Greenfield-Shrimplin was the most closely related to Greenfield-OSU, while Greenfield-Caudell was the most distant. Aside from Greenfield ecotypes, Greenfield-OSU was most closely related to an experimental Chinese accession of bermudagrass, G-19-89. Four experimental accessions of Chinese bermudagrass from OSU were evaluated, G-19-6, G-19-64, G-19-86, and G-19-89. Relation among these genotypes was high, ranging from 0.896 – 0.924. Closest relation was between G-19-86 and G-19-89 at 0.924, while G-19-6 and G-19-64 were the most distant at 0.896. These minor distances are suggestive of the geographic origin shared among the 4 ecotypes. Four experimental varieties from the OSU forage collection, 3200W-R9-C8, 3200W-R14-C-4, 5200E-1, and 56-10, were modest in their genetic similarity, ranging from 0.820 – 0.869 with a mean of 0.834. Zebra, a common type bermudagrass used as reference standard for SSR amplification, was expected to be the most genetically distant from the forage types. This was confirmed with the relatively modest similarity coefficient average of 0.814 and range of 0.780 – 0.844. Zebra was most closely related to the Chinese accessions. World Feeder and Alicia displayed a relationship of 0.841.

Midland, Ozark, Coatcross-1, and Tifton 44 exhibited genetic similarities of 0.873, 0.852, 0.834, and 0.877 with Coastal, respectively. Ozark displayed relationships of 0.849, 0.851, and 0.859 to Guymon, Goodwell, and Wrangler, respectively. Wrangler

and Midland 99 exhibited a relatedness of 0.854, in addition to a relationship of 0.856 between Wrangler and Goodwell. Midland 99 and Goodwell displayed a coefficient of 0.839. Additionally, Guymon displayed relationships to Goodwell and Wrangler of 0.841 and 0.852, respectively. Coastcross-1 contains established relationships with Grazer, Tifton 68, and Tifton 85 of 0.824, 0.835, and 0.815, respectively. Grazer was modestly similar to Tifton 68 and Tifton 85, with similarity coefficients of 0.828 and 0.834, respectively. Tifton 68 and Tifton 85 displayed a high relationship of 0.901. Tifton 44 and a mutant of itself, T44-1, had a relationship of 0.897. Coastal and T44-1 expressed a relatedness of 0.882.

DISCUSSION

A narrow genetic base was apparent in the evaluated germplasm. Karaca et al. (2002) documented a similar observation, with a genetic similarity coefficient range of 0.608 – 0.977. Our range of 0.772 – 0.939 is likely biased upward to a certain degree. Although 52 SSR primer pairs were used, several lacked significant polymorphism within the tested genotypes. Additionally, the allele frequency is descriptive of the conservative nature of these markers. Tan et al. (2012) witnessed a similar situation of allele amplification being conservative for 1 to 2 bands. However, the narrow genetic base was still evident. This narrow base is a result of the interrelationships that exist among many of the prominent forage hybrids.

Coastal is one of the earliest hybrids, resulting from a cross between Tif bermudagrass and an accession from South Africa (Burton, 1948; Myers, 1951). Coastal has ultimately served as a parent for 5 of the evaluated genotypes in this study, Midland,

Ozark, Coastcross-1, Tifton 44, and T44-1 (Hein, 1953; Richardson et al., 2005; Burton, 1972; Burton and Monson, 1978). Coastal displayed a relatedness of 0.834, 0.873, 0.852, 0.877, and 0.882 to Coastcross-1, Midland, Ozark, Tifton 44, T44-1, respectively. An expressed relation of 0.897 between Tifton 44 and T44-1 is to be expected, as T44-1 was collected from a field of Tifton 44 infected with *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *graminis* (Dr. Jerry Legg, personal communication). World Feeder is a mutant of Alicia, where a relation of 0.841 was observed (Gordon, 1989). This relationship can be suggestive of a mutation event leading to a distinguishable variation between the two cultivars.

The second parent involved in the Ozark hybridization, A9959 (PI 253302), is an accession from Yugoslavia. This accession was hybridized with A12156 to create Guymon, in addition to being incorporated into early hybridization events in the development of Goodwell (Taliaferro et al., 1983; Wu et al., 2009). Ozark expressed relationships to Guymon and Goodwell of 0.849 and 0.851, respectively. Additionally, A9959 is one of the parent plants used for the production of Wrangler seed (Johnston Seed Co., Enid, OK). Wrangler had a relatedness of 0.856, 0.852, and 0.859 with Goodwell, Guymon, and Ozark, respectively. Goodwell and Midland 99 are derivatives of an early hybridization event, ultimately displaying a relatedness of 0.939 (Taliaferro et al., 2002; Wu et al., 2011).

Along with Coastal, an accession from Kenya (PI 255445) was used in the Coastcross-1 hybridization (Burton, 1972). This Kenyan accession was further used to develop Grazer in conjunction with PI 320876 (Eichhorn et al., 1986). The relationship between Coastcross-1 and Grazer was 0.824. Tifton 68 is a half-sib with Coastcross-1

and Grazer, as PI255445 was also used in the Tifton 68 hybridization (Burton and Monson, 1984). Tifton 68 expressed respective relations of 0.835 and 0.828 with Coastcross-1 and Grazer. In the hybridization of Tifton 85, Tifton 68 was crossed with PI 290884 (Burton et al., 1993). Genetic similarity between Tifton 68 and Tifton 85 was 0.901. Relationships among the aforementioned commercial cultivars are well established, as there is substantial pedigree information linking many together along their respective family trees. The narrow genetic base observed in this study further illustrates these close relationships.

Greenfield is an early cultivar released by Oklahoma State University as an ecotype selection from a global germplasm collection, which survived multiple winters in Stillwater, OK (Elder, 1955). Seven genotypes of Greenfield were evaluated from fields in Oklahoma and Arkansas. These accessions lacked the genetic similarity you would have expected from a pure cultivar, exhibiting a coefficient range of 0.811 – 0.873. Over the years, contamination from neighboring fields and ditches, in addition to outside pollen and subsequent seedling establishment in following years likely played a significant role in diluting the genetic purity of the original Greenfield plantings. This is an illustration of the need for proper management of bermudagrass stands to ensure longevity of investment.

Four accessions of Chinese decent, G-19-6, G-19-64, G-19-86, and G-19-89 displayed a range of 0.896 – 0.924. These plants have been selected for seed yield characteristics and biomass yield. The narrow genetic base among the 4 accessions could be attributed to underlying associations with traits of interest; however, that assertion would need more robust testing for confirmation. Ranges in the genetic relationships of 4

experimental varieties from the OSU forage collection, 3200W-R9-C8, 3200W-R14-C-4, 5200E-1, and 56-10, were 0.820 – 0.869. The potential for strong genetic relationships is likely in these accessions, as their close proximity and selection pressure are likely to encourage similar allele frequency for certain traits. Overall, the genetic similarity of commercial cultivars is high, with a relatively narrow genetic base serving a wide array of germplasm. Over the years, selection has focused on biomass yield, quality, and cold tolerance. By using similar parents across a variety of hybridizations, in addition to selecting for a small range of traits, the likelihood of selecting certain allele frequencies escalates across generations. It can be assumed that the traits of interest offer some form of linkage to other traits, which further compounds the genetic similarity of many cultivars with similar pedigrees. A compounding effect over generations can eventually lead to a genetic bottleneck within breeding pipelines. Breeders should seek opportunities to incorporate novels source of germplasm into breeding lines in order to capitalize on the rich diversity present within the bermudagrass genome. This will allow the continued advancement of an industry scientists have revolutionized through innovative hybridizations and breeding strategies.

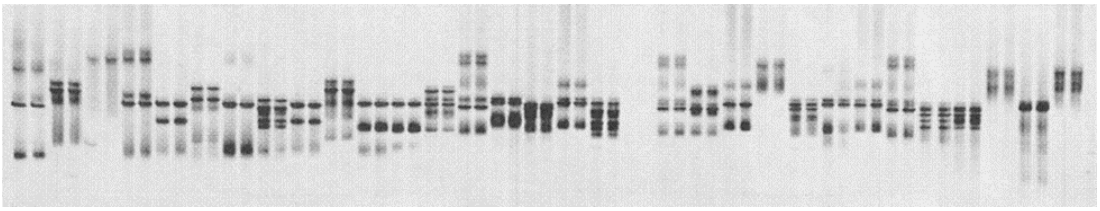

Figure 4. Gel image from genetic diversity analysis with SSR markers.

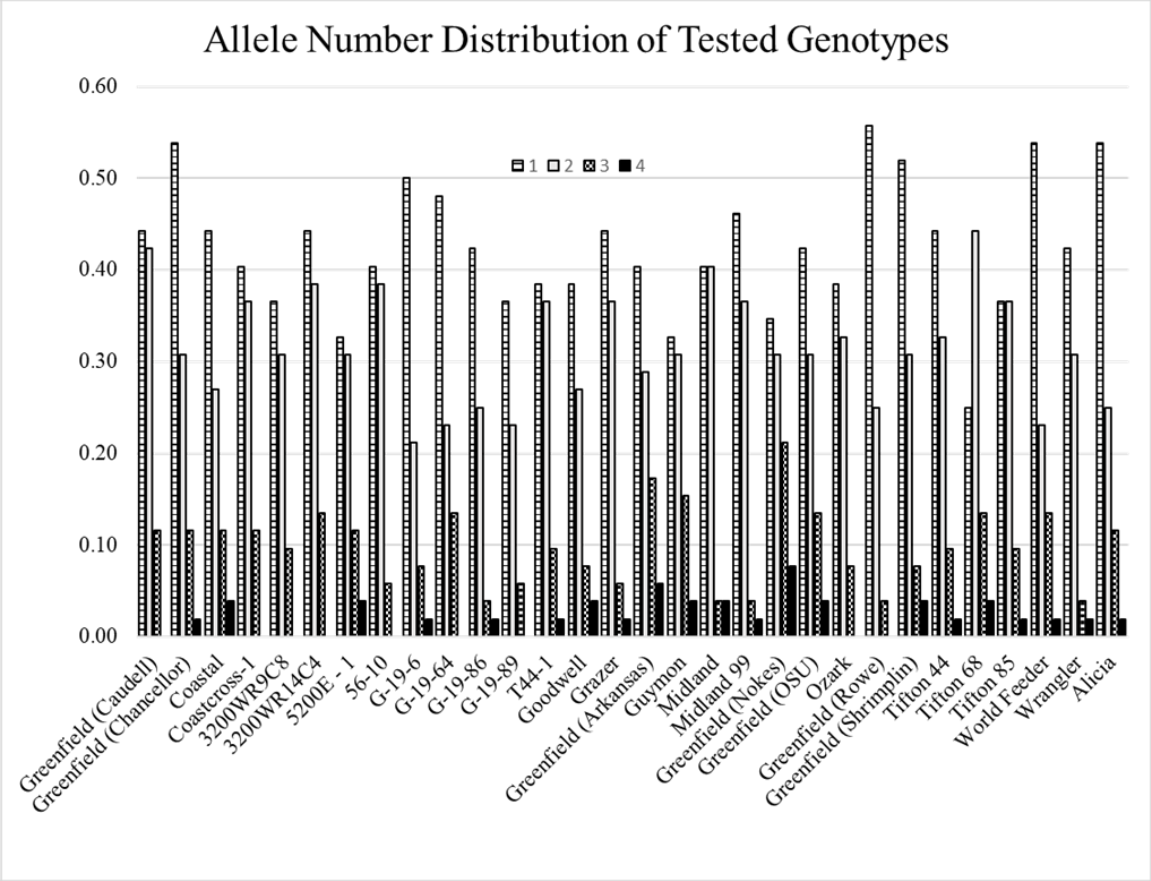

Figure 5. Allele amplification distribution across tested genotypes.

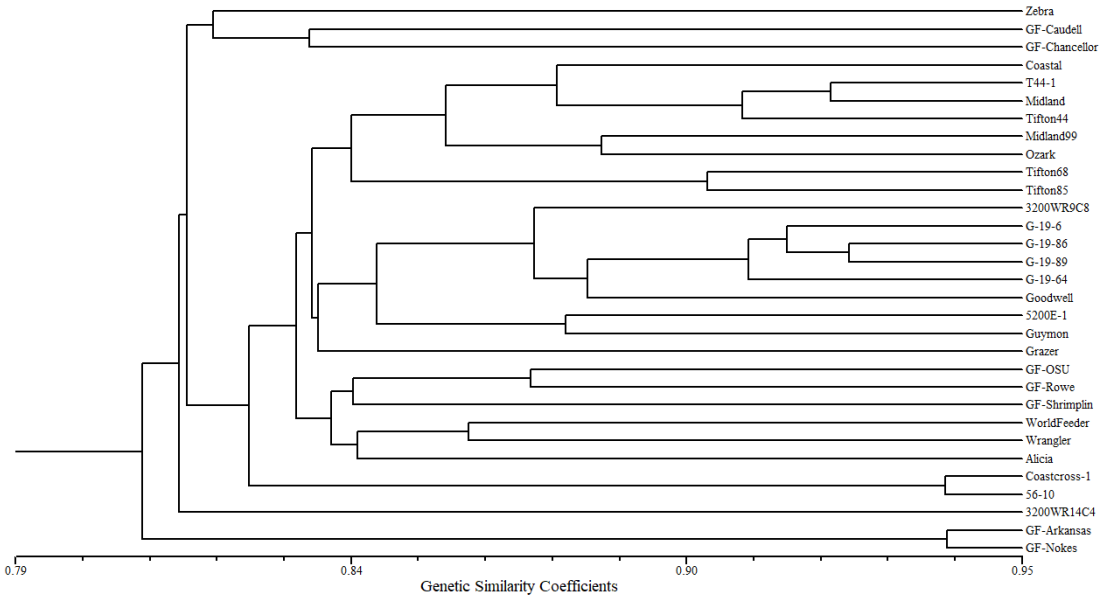

Figure 6. Tree plot of genetic relationships among 31 forage bermudagrass genotypes.

Table 26. Genetic similarity coefficients of 31 forage bermudagrass accessions.

	1†	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2	0.82																													
3	0.82	0.84																												
4	0.81	0.82	0.84																											
5	0.82	0.80	0.81	0.83																										
6	0.83	0.83	0.84	0.85	0.82																									
7	0.80	0.82	0.82	0.82	0.81	0.84																								
8	0.82	0.81	0.82	0.83	0.82	0.87	0.83																							
9	0.82	0.81	0.83	0.84	0.94	0.82	0.83	0.83																						
10	0.84	0.82	0.82	0.84	0.83	0.88	0.82	0.86	0.83																					
11	0.82	0.81	0.82	0.84	0.81	0.86	0.81	0.84	0.82	0.90																				
12	0.84	0.82	0.84	0.85	0.84	0.87	0.82	0.85	0.85	0.92	0.92																			
13	0.84	0.84	0.85	0.86	0.84	0.89	0.84	0.86	0.85	0.91	0.91	0.92																		
14	0.80	0.81	0.82	0.88	0.82	0.83	0.80	0.83	0.84	0.84	0.83	0.86	0.86																	
15	0.82	0.82	0.82	0.83	0.82	0.87	0.81	0.84	0.82	0.87	0.88	0.89	0.89	0.84																
16	0.81	0.81	0.81	0.83	0.82	0.84	0.81	0.82	0.83	0.84	0.83	0.85	0.87	0.82	0.84															
17	0.79	0.83	0.81	0.82	0.78	0.83	0.81	0.80	0.79	0.83	0.82	0.83	0.84	0.80	0.82	0.80														
18	0.80	0.80	0.81	0.83	0.82	0.85	0.80	0.88	0.82	0.85	0.82	0.85	0.86	0.83	0.84	0.83	0.82													
19	0.80	0.81	0.82	0.87	0.82	0.82	0.81	0.83	0.84	0.84	0.82	0.85	0.85	0.92	0.84	0.84	0.82	0.83												
20	0.81	0.83	0.84	0.86	0.85	0.85	0.83	0.84	0.86	0.85	0.83	0.85	0.88	0.87	0.84	0.85	0.81	0.83	0.87											
21	0.78	0.82	0.81	0.81	0.77	0.81	0.80	0.79	0.79	0.82	0.81	0.81	0.82	0.80	0.81	0.80	0.94	0.81	0.80	0.80										
22	0.80	0.81	0.85	0.82	0.80	0.84	0.82	0.83	0.81	0.83	0.82	0.84	0.85	0.82	0.82	0.81	0.82	0.82	0.82	0.84	0.83									
23	0.83	0.82	0.84	0.85	0.84	0.86	0.83	0.85	0.85	0.86	0.84	0.86	0.87	0.85	0.85	0.83	0.83	0.85	0.85	0.89	0.81	0.84								
24	0.83	0.83	0.84	0.84	0.83	0.86	0.84	0.85	0.83	0.86	0.84	0.87	0.87	0.85	0.86	0.84	0.83	0.85	0.86	0.87	0.83	0.87	0.87							
25	0.81	0.81	0.82	0.82	0.80	0.82	0.80	0.82	0.81	0.83	0.83	0.84	0.85	0.83	0.83	0.81	0.84	0.80	0.83	0.84	0.84	0.83	0.83	0.86						
26	0.81	0.81	0.82	0.88	0.83	0.83	0.81	0.83	0.83	0.85	0.83	0.85	0.85	0.90	0.84	0.83	0.82	0.83	0.92	0.87	0.81	0.83	0.86	0.86	0.84					
27	0.79	0.80	0.81	0.84	0.84	0.82	0.81	0.82	0.84	0.83	0.82	0.84	0.85	0.83	0.83	0.83	0.80	0.84	0.84	0.84	0.79	0.82	0.84	0.84	0.81	0.87				
28	0.80	0.80	0.81	0.84	0.82	0.82	0.82	0.81	0.83	0.83	0.83	0.84	0.86	0.85	0.83	0.83	0.81	0.83	0.86	0.85	0.80	0.83	0.83	0.84	0.81	0.86	0.90			
29	0.81	0.81	0.83	0.82	0.80	0.84	0.81	0.81	0.82	0.83	0.83	0.84	0.86	0.81	0.83	0.82	0.83	0.83	0.83	0.84	0.82	0.84	0.85	0.85	0.85	0.84	0.82	0.83		
30	0.83	0.82	0.83	0.85	0.83	0.87	0.82	0.85	0.84	0.85	0.85	0.87	0.87	0.85	0.86	0.85	0.83	0.85	0.86	0.85	0.82	0.83	0.86	0.86	0.84	0.86	0.86	0.84	0.86	
31	0.81	0.81	0.82	0.83	0.80	0.84	0.81	0.82	0.81	0.83	0.83	0.85	0.85	0.82	0.83	0.81	0.81	0.81	0.82	0.84	0.81	0.82	0.84	0.86	0.84	0.83	0.82	0.82	0.84	0.85

†1, Zebra; 2, Greenfield (Caudell); 3, Greenfield (Chancellor); 4, Coastal; 5, Coastcross-1; 6, 3200W-R9-C8; 7, 3200W-R14-C4; 8, 5200E-1, 9, 56-10; 10, G-19-6; 11, G-19-64; 12, G-19-86; 13, G-19-89; 14, T44-1; 15, Goodwell; 16, Grazer; 17, Greenfield (Arkansas); 18, Guymon; 19, Midland; 20, Midland 99; 21, Greenfield (Nokes); 22, Greenfield (OSU); 23, Ozark; 24, Greenfield (Rowe); 25, Greenfield (Shrimplin); 26, Tifton 44; 27, Tifton 68; 28, Tifton 85; 29, World Feeder; 30, Wrangler; 31, Alicia.

Table 27. Guo et al. (2017) SSR primer pairs used for molecular genetic diversity assessment.

Primer Pair ID	Linkage Group
CDAAC6-2611/2612	1
CDGA1-783/784	1
CDCA1-39/40	1
CDCA3-263/264	6
CDATGA-2059/2060	6
CDCA5-501/502	6
CDCA5-449/450	2
CDAAC4-2463/2464	2
CDGA7-1601/1602	2
CDCA3-245/246	3
CDCA7-681/682	3
CDCA7-675/676	3
CDCA5-471/472	4
CDCA2-229/230	4
CDCA7-703/704	4
CDGA4-1331/1332	5
CDCA1-59/60	5
CDGA3-1103/1104	5
CDCA8-725/726	7
CDCA5-409/410	7
CDGA5-1399/1400	7
CDGA4-1335/1336	8
CDGA1-921/922	8
CDGA1-865/866	8
CDGA1-815/816	9
CDCA6-559/560	9
CDGA1-805/806	9
CDCA1-25/26	10
CDGA5-1369/1370	10
CDGA3-1143/1144	10
CDGA3-1177/1178	11
CDCA2-227/228	11
CDGA4-1347/1348	11
CDCA5-431/432	12
CDGA1-939/940	12
CDGA4-1343/1344	12
CDCA1-57/58	13
CDGA1-807/808	13
CDGA2-1021/1022	13
CDGA3-1219/1220	14
CDGA1-827/828	14
CDCA7-635/636	14

CDGA3-1215/1216	15
CDCA5-411/412	15
CDCA7-615/616	15
CDGA3-1225/1226	16
CDCA7-641/642	16
CDCA4-373/374	16
CDCA1-9/10	17
CDGA1-935/936	17
CDGA7-1697/1698	17
CDCA1-81/82	18
CDCA4-389/390	18
CDCA7-651/652	18
CDAAC6-2611/2612	1
CDGA1-783/784	1

REFERENCES

Chapter 1

Ahring, R.M., C.M. Taliaferro, and R.D. Morrison. 1974. Seed production of several strains and hybrids of bermudagrass, Cynodon dactylon (L.) Pers. 1. Crop Sci. 14:93-95. doi:10.2135/c-ropsci1974.0011183X001400010028x

Ahring, R.M., C.M. Taliaferro, and W.L. Richardson. 1982b. Management of bermudagrass for seed production in Oklahoma. Oklahoma Agricultural Experiment Station. T-158.

Anderson, J., C.M. Taliaferro, and D. Martin. 2002. Freeze tolerance of bermudagrass. Crop Sci. 42:975-977. doi:10.2135/cropsci2002.9750

Anderson, W.F., A. Maas, and P. Ozias-Akins. 2009. Genetic variability of a forage bermudagrass core collection. Crop Sci. 49:1347-1358. doi: 10.2135/cropsci200-8.06.0330

Ball, D.M., M.M. Eichhorn, Jr., R.A. Burdett, Jr., and D.M. Bice. 1996. Registration of 'Russell' bermudagrass. Crop Sci. 36:467. doi:10.2135/cropsci1996.001118-3X003600020046x

Brown, S.M., M.S. Hopkins, S.E. Mitchell, M.L. Senior, T.Y. Wang, R.R. Duncan, F. Gonzalez-Candelas, and S. Kresovich. 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. Theor. Appl. Genet. 93:190-198. doi:10.1007/BF00225745

Burton, G.W. 1943. ‘Coastal’ bermudagrass. Circular 10. Georgia Coastal Plain Exp.

Burton, G.W. 1947. Breeding bermudagrass for the southweastern United States. J. Am. Soc. Agron. 39:551-569. doi:10.2134/agronj1947.00021962003900070001x

Burton, G.W. 1954. Coastal bermduagrass. Cir. 10. Ga Agric. Exp. Stn., Athens.

Burton, G.W. 1956. Utilization of heterosis in pasture plant breeding. p. 3-12. *In* G.J. Neale (ed.) Proc. Int. Grassl. Congr., 7th, Palmerston North, NZ. 6-12 Nov. 1956. Wright and Carman, Wellington, NZ.

Burton, G.W. 1959. Principles of breeding veteatively propagated plants. p. 661-670. *In* Proc. Congr. Int. Soc. Sugarcane Technologists, 10th. Elsevier Publ. Co., Amsterdam, The Netherlands.

Burton, G.W. 1962. Registration of Varieties of Bermudagrass. Crop Sci. 2:352-253.

Burton, G.W. 1965. Breeding better bermudagrass. p. 93-96. *In.* L.P. Jordao et al. (ed. comm.) Proc. Int. Grassl. Congr., 9th, Sao Paulo, Brazil. 7-20 Jan. 1965. Dep. Nacl. de Producao Anim., Sao Paulo, Brazil.

Burton, G.W. 1972. Registration of ‘Coastcross-1’ bermudagrass. Crop Sci. 12:125. doi: 10.2135/cropsci1972.0011183X001200010049x

Burton, G.W., R.N. Gates, and G.M. Hill. 1993. Registration of ‘Tifton 85’ bermudagrass. Crop Sci. 33:644-645. doi: 10.2135/cropsci1993.0011183X003300030045x

Burton, G.W., and W.G. Monson. 1978. Registration of 'Tifton 44' bermudagrass. Crop Sci. 18:911. doi:10.2135/cropsci1978.0011183X001800050065x

Burton, G.W., and W.G. Monson. 1984. Registration of 'Tifton 68' bermudagrass. Crop Sci. 24:1211. doi:10.2135/cropsci1984.0011183X002400060051x

Burton, G.W., and W.G. Monson. 1988. Registration of 'Tifton 78' bermudagrass. Crop Sci. 28:187-188. doi:10.2135/cropsci1988.0011183X002800010047x

Burton, G.W., G.M. Prine, and J.E. Jackson. 1957. Studies of drought tolerance and water use of several southern grasses. Agron. J. 49:498-503. doi: 10.2134/agronj1957.000-21962004900090010x

Burton, G.W., R.N. Gates, and G.M. Hill. 1993. Registration of 'Tifton 85' bermudagrass. Crop Sci. 33:644-645. doi: 10.2135/cropsci1993.0011183X003300030045x

Chengcheng, T. Y.Q. Wu, C.M. Taliaferro, M.P. Anderson, C. Tauer, T. Samuels. 2012. Development of simple sequence repeat markers for bermudagrass from its expressed sequence tag sequences and preexisting sorghum SSR markers. Mol Breeding. 29:23-30. doi:10.1007/s11032-010-9521-2

Cordeiro, G.M., R. Casu, C.L. McIntyre, J.M. Manners, and R.J. Henry. 2001. Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci. 160:1115-1123. doi:10.1016/S0168-9452(01)00365-X

Dayanandan, S. K.S. Bawa, and R.V. Kesseli. 1997. Conservation of microsatellites among tropical trees (*Leguminosae*). Am. J. Bot. 84:1658-1663. doi:10.2307/2446463

de Silva, P.H.A.U, and R.W. Snaydon. 1995. Chromosome number in Cynodon dactylon in relation to ecological conditions. Ann. Bot. (London) 76:535-537. doi: 10.100-6/anbo.1995.1129

de Wet, J.M.J., and J.R. Harlan. 1971. South African species of Cyndon (Gramineae). J.S. Afric. Bot. 37:53-56.

Eichhorn, Jr., M.M., B.D. Nelson, C.R. Montgomery, A.V. Davis, and W.B. Hallmark. 1984. Brazos – A new bermudagrass for pastures and hay in Louisiana. P. 18-19. Vol. 27. *In* D.E.T. Didier (ed.) Louisiana agriculture. La. Agric. Exp. Stn., Baton Rouge.

Eichhorn, Jr., M.M., W.M. Oliver, W.B. Hallmark, W.A. Young, A.V. Davis, and B.D. Nelson. 1986. Registration of 'Grazer' bermudagrass. Crop Sci. 26:835. doi: 10.2135/crop-sci1986.0011183X002600040046x

Elder, W.C. 1955. Greenfield bermudagrass. Bull. B-455. Okla. Agric. Exp. Stn., Stillwater.

Fang, T., Y.Q. Wu, S. Makaju, T. Tribble, D.L. Martin, and J.Q. Moss. 2015. Identification of turf bermudagrass on the Oklahoma State University baseball field and three experimental clones as revealed with simple sequence repeat markers. HortTechnol. 25:714-724. doi: 10.21273/HORTTECH.25.6.714

Felder, M.R. 1967. Chromosome associations in triploid Cynodon hybrids. M.S. Thesis. Oklahoma State Univ., Stillwater.

Forbes, I., and G.W. Burton. 1963. Chromosome numbers and meiosis in some Cynodon species and hybrids. Crop Sci. 3:75-79. doi:10.2135/cropsci1963.00-11183X00030001-0023x

Gitau, M.M., J. Fan, Y. Xie, and J. Fu. 2017. Genetic diversity and association mapping of forage quality in diverse bermudagrass accessions. Euphytica (2017) 213-234. doi: 10.100-7/s10681-017-2024-z

Gordon, L.R. 1989. World Feeder bermudagrass. U.S. Plant Patent 7081. Date issued: 19 Dec.

Gulsen, O., S. Sever-Mutlu, N. Mutlu, M. Tuna, O. Karaguzel, R.C. Shearman, T.P. Riordan, and T.M. Heng-Moss. 2009. Polyploidy creates higher diversity among Cynodon accessions as assessed by molecular markers. Theor. Appl. Genet. 118:1309-1319. doi:10.1007/s00122-009-0982-9

Guo, Y., Y.Q. Wu, J.A. Anderson, J.Q. Moss. L. Zhu, and J. Fu. 2016. SSR marker development, linkage mapping, and QTL analysis for establishment rate in common bermudagrass. Plant Genome 10. doi: 10.3835/plantgenome2016.07.0074

Hanson, A.A. 1972. Grass varieties in the United States. USDA-ARS Agric. Handb. 170. U.S. Gov. Print. Office. Washington, D.C.

Harlan, J.R., J.M.J. de Wet, K.M. Rawal, M.R. Felder, and W.L. Richardson. 1970a. Cytogenetic studies in *Cynodon* L.C. Rich. (Gramineae). Crop Sci. 10:288-291. doi: 10.2135/crops-ci1970.0011183X001000030023x

Harlan, J.R., J.M.J. De Wet, W.W. Huffine, and J.R. Deakin. 1970b. A guide to the species of *Cynodon* (Gramineae). Oklahoma Agricultural Experiment Station Bulletin B-673.

Harris-Shultz, K.R., S.R. Milla-Lewis, and J.A. Brady. 2012. Transferability of SSR and RGA markers developed in *Cynodon* spp. To *Zoysia* spp. Plant Mol. Biol. Rep. 30:1264-1269. doi: 10.1007/s11105-012-0417-7

Harris-Shultz, K.R., B.M. Schwartz, W.W. Hanna, and J.A. Brady. 2010. Development, linkage mapping, and use of microsatellites in bermudagrass. J. Am. Soc. Hort. Sci. 135:511-520. doi: 10.21273/JASHS.135.6.511

Hernandez, P., G. Oorado, D. Laurie, A. Martin, J. Snape. 2001. Microsatellites and RFLP probes from maize are efficient sources of molecular markers for the biomass energy crop *Miscanthus*. Theor. Appl. Genet. 102:616-622. doi: 10.1007/s001220051688

Hurcombe, R. 1947. A cytological and morphological study of cultivated Cynodon species. J. South. Afric. Bot. 13:107-116.

Jewell, M.C., Y. Zhou, D.S. Loch, I.D. Godwin, and C.J. Lambrides. 2012. Maximizing genetic, morphological, and geographic diversity in a core collection of Australian bermudagrass. Crop Sci. 52:879-889. doi: 10.2135/cropsci2011.09.0497

Johnston, R.A. 1975. Cytogenetics of some hexaploid 3 tetraploid hybrids in Cynodon. M.S. thesis. Oklahoma State Univ., Stillwater.

Kang, S.Y., G.J. Lee, K.B. Lim, H.J. Lee, I.S. Park, S.J. Chung, J.B. Kim, D.S. Kim, and H.K. Rhee. 2008. Genetic diversity among Korean bermudagrass (Cynodon spp.) ecotypes characterized by morphological, cytological and molecular approaches. Mol. Cells. 25(2):163-171.

Karaca, M., S. Saha, A. Zipg, J.N. Jenkins, and D.J. Lang. 2002. Genetic diversity among forage bermudagrass (Cynodon spp.): Evidence from chloroplast and nuclear DNA fingerprinting. Crop Sci. 42:2118-2127. doi: 10.2135/cropsci2002.2118

Kenna, M.P., C.M. Taliaferro, and W.L. Richardson. 1983. Comparative fertility and seed yields of parental bermudagrass clones and their singlecross F1 and F2 populations. Crop Sci. 23:1133-1135. doi: 10.2135/cropsci1983.0011183X002300060026x

Lowrey, R.S., G.W. Burton, J.C. Johnson, Jr., W.H. Marchant, and W.C. McCormick. 1968. *In vivo* studies with Coastcross-1 and other Bermudas. Bull. 55. Ga. Agric. Exp. Stn., Athens.

Malik, C.P., and R.C. Tripathi. 1968. Cytological evolution within the Cynodon dactylon complex. Biol. Zentralbl. 87:625-627.

Mislevy, P., W.F. Brown, R.S. Kalmbacher, L.S. Dunavin, W.S. Judd, T.A. Kucharek, O.C. Ruelke, J.W. Noling, R.M. Sonoda, and R.L. Stanley, Jr. 1995. Florakirk bermudagrass. Circ. S-395. Fla. Agric. Exp. Stn., Gainseville.

Moffett, A.A., and R. Hurcombe. 1949. Chromosome numbers of South African grasses. Heredity. 3:369-373. doi:10.1038/hdy.1949.27

Monson, W.G., R.S. Lowrey, and I. Forbes, Jr. 1969. *In vitro* nylon bag vs. two-stage *in vitro* digestion: Comparison of two techniques for estimating dry-matter digestibility of forages. Agron. J. 61:587-589. doi:10.2134/agronj1969.00021962006100040032x

Powell, J.B., G.W. Burton, and C.M. Taliaferro. 1968. A hexaploid clone from a tetraploid 3 diploid cross in Cynodon. Crop Sci. 8:184-185. doi:10.2135/cropsci1968.001118-3X000800020014x

Quisenberry, S.S. 1990. Plant resistance to insects and mites in forage and turf grasses. Fla. Entomol. 73:411-421.

Redfearn, D. and Y.Q. Wu. 2013. Choosing, establishing, and managing bermudagrass varieties in Oklahoma. Oklahoma Cooperative Extension Service. PSS-2583.

Richardson, W.L. 1958. A technique of emasculating small grass florets. Indian J. Genet. Plant Breed. 18:69-73.

Richardson, W.L., and C.M. Taliaferro. 2005. Bermudagrass hybrid named 'Ozark.' US Plant Patent 15, 576 P3. Date issued: Feb. 22.

Ricahrdson, W.L., C.M. Taliaferro, and R.M. Abring. 1978. Fertility of eight bermudagrass clones and open-pollinated progeny from them. Crop Sci. 18:332-334. doi: 10.2135/cropsci-1978.0011183X001800020035x

Roder, M.S., J. Plaschke, S.U. Konig, A. Borner, M.E. Sorrells, S.D. Tanksley, and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246:327-333. doi:10.1007/BF00288605

Rouquette, F.M., Jr., G.R. Smith, and V.A. Haby. 1997. Bermudagrass pastures under long-term stocking rates and fertility regimens. p. 73-74. *In* J.G. Buchanan-Smith et al. (ed.) Proc. Int. Grassl. Congr. 18th, Winnipeg, MB, and Saskatoon, SK, Canada, 8-17 June 1997. Grasslands 2000, Toronto, Dep. Of Plant Sci., Univ. or Manitoba, Winnipeg.

Rouquette, F.M., Jr., J.L. Kerby, and G.H. Nimr. 1998. Pasture and animal performance from bermudagrass pastures at three stocking rates with or without nitrogen fertilizer. p. 65-66. *In* Texas A&M Univ. Res. Ctr. Tech. Rep. 98-1. Tex. Agric. Exp. Stn., College Station.

Saha, M.C., J.D. Cooper. M.A.R. Mian, K. Chekhozskiy, and G.D. May. 2006. Tall fescue genomic SSR markers: development and transferability across multiple grass species. Theor. Appl. Genet. 113:1449-1458. doi:10.1007/s00122-006-0391-2

Samudio, S.H., and A.D. Brede. 1998. Registration of 'Cheyenne' bermudagrass. Crop Sci. 38:279. doi:10.2135/cropsci1998.0011183X003800010053x

Smiley, R.W., R.H. Dernoeden, and B.B. Clark. 1992. Compendium of turfgrass diseases. Am. Phytopathol. Soc. Press, St. Paul, MN.

Stuth, J., A. Jama, D. Tolleson. 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crops Research. 84:45-56. doi:10.1-016/S0378-4290(03)00140-0

Taghvaeian, S., R.S. Frazier, D. Livingston, G. Fox. 2017. The Ogallala Aquifer. Oklahoma Cooperative Extension Service. BAE-1531.

Taliaferro, C.M. 1995. Diversity and vulnerability of Bermuda turfgrass species. Crop Sci. 35:327-332. doi:10.2135/cropsci1995.0011183X003500020006x

Taliaferro, C.M., and W.L. Richardson. 1980. Registration of Hardie bermudagrass. Crop Sci. 20:413. doi:10.2135/cropsci1980.0011183X002000030039x

Taliaferro, C.M., R.M. Ahring, and W.L. Richardson. 1983. Registration of 'Guymon' bermudagrass. Crop Sci. 23:1219. doi:10.2135/cropsci1983.0011183X002300060054x

Taliaferro, C.M., J.A. Anderson, W.L. Richardson, J.L. Baker, et al. 2002. Registration of Midland 99 forage bermudagrass. Crop Sci. 42:2212. doi:10.2135/cropsci2002.2212

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In: L.E. Moser, B.L. Burson, and L.E. Sollenberger, editors, Warm-season (C_4) grasses. ASA, CSSA, and SSSA, Madison, Wisconsin. p. 417-464. doi:10.2134/agronmonogr45.c12

Tan, C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, M.W. Smith, and J.Q. Moss. 2015. Selfing and outcrossing fertility in common bermudagrass under open pollinating conditions examined by SSR markers. Crop Sci. 54:1832-1837. doi:10.2135/cropsci2013.12.0816

Tan, C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, and M.W. Smith. 2014. Development and characterization of genomic SSR markers in *Cynodon transvaalensis* Burtt-Davy. Mol. Gen. and Genom. 289:523-531. doi:10.1007/s00438-014-0829-1

Tan, C., Y.Q. Wu, C.M. Taliaferro, M.P. Anderson, C. Tauer, and T. Samuels. 2012. Development of simple sequence repeat markers for bermudagrass from its expressed sequence tag seuqnces and preexisintg sorghum SSR markers. Mol. Breed. 29:23-30. doi:10.1007/s11032-010-9521-2

Thiel, T., W. Michalek, R. Varshney, A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 106:411-422. doi:10.1007/s00122-002-1031-0:

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18:104-111.

Varshney, R.K., R. Sigmund, A. Borner, V. Korzun, N. Stein, M.E. Sorrells, P. Langridge, and A. Graner. 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci. 168:195-202. doi:10.1016/j.plantsci.2004.08.001

Wang, M.L., N.A. Barkley, J.-K. Yu, R.E. Dean, M.L. Newman, M.E. Sorrells, and G.A. Pederson. 2005. Transfer of simple sequence repeat (SSR) markers from major cereal crops to minor grass species for germplasm characterization and evaluation. Plant Genetic Res. 3:45-47. doi:10.1079/PGR200461

Watson, V.H. 1974. A chronological review of the selection, research, and distribution of a bermudagrass called Callie. Rep. (Unnumbered) Miss. Agric. For. Exp. Stn., Starkville.

Wu, Y.Q., C.M. Taliaferro, G.H. Bai, D.L. Martin, J.A. Anderson, M.P. Anderson, and R.M. Edwards. 2006. Genetic analyses of Chinese Cynodon accessions by flow cytometry and AFLP markers. Crop Sci. 46:917-926. doi:10.2135/cropsci2005.08.0256

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson. 2007. Genetic variability and relationshps for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. Crop Sci. 47:1985-1994. doi:10.2135/cropsci2007.01.0047

Wu, Y.Q., C.M. Taliaferro, and R. Kochenower. 2009. Forage bermudagrass plant named 'Goodwell.' Plant patent PP22,101. Available online: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP22101.PN. [Accessed Mar 9 2020].

Xie, W.G., X.Q. Zhang, H.W. Cai, W. Liu, and Y. Peng. 2010. Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.). Biochem. Syst. Ecol. 38:740-749. doi:10.1016/j.bse.2010.06.009

Chapter 2

Avis, J.D., C.M. Taliaferro, and D. Holbert. 1980. Genotype x interaction study of bermudagrass yields in Oklahoma. Proc. Okla. Acad. Sci. 60:69-74.

Burton, G.W. 1947. Breeding bermudagrass for the southeastern United States. Agron. J. 39:551-569. doi:10.2134/agronj1947.00021962002900070001x

Burton, G.W., R.N. Gates, and G.M. Hill. 1993. Registration of 'Tifton 85' bermudagrass. Crop Sci. 33:644-645. doi: 10.2135/cropsci1993.0011183X00-3300030045x

Das, M.K., R.G. Fuentes, C.M. Taliaferro. 2004. Genetic variability and trait relationships in switchgrass. Crop Sci. 44:443-448. doi:10.2135/cropsci2004.4430

de Silva, P.H.A.U. 1991. The ecological genetics of *Cynodon dactylon* (L.) Pers. in Sri Lanka. Ph.D. dissertation. University of Reading. UK.

Dewey, D.R., and K.H. Lu. 1959. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agron. J. 51:515-518. doi:10.2134/agronj1959.00-021962005100090002x

Harlan, J.R. 1970. *Cynodon* species and their value for grazing or hay. Herbage Abs. 40:233-238.

Harlan, J.R., J.M.J. de Wet, W.W. Huffine, and J.R. Deakin. 1970. A guide to the species of *Cynodon* (Gramineae). Stillwater, OK, USA: Oklahoma Agricultural Experiment Station Bulletin B-673.

Harlan, J.R., and J.M.J. de Wet. 1969. Sources of variation in *Cynodon dactylon* (L.) Pers. Crop Sci. 9:774-778. doi:10.2135/cropsci1969.0011183X000900060031x

Kang, M.S. 1994. Applied quantitative genetics. M.S. Kang Publisher, Baton Rouge, LA.

Mohamed, C., C.M. Taliaferro, and R.W. McNew. 1990. Genotype-environment interactions of bermudagrass forage yields. Crop Sci. 30:49-53. doi:10.2135/cropsci1990.0011183X003-000010011x

NASS-USDA. 2020. National statistics for hay. National Agriculture Statistics Service – United States Department of Agriculture. Washington, D.C. [Accessed Mar 9 2020]. Available online: https://www.nass.usda.gov/Statistics_by_Subject/result.php?3DB-8E0DC-49F4-3535-B02D00136C9791A3&sector=CROPS&group=FIELD%20CR-OPS&comm=HAY

Nelson, C.J., and J.C. Burns. 2006. Fifty years of grassland science leads to change. Crop Sci. 46:2204-2217. doi:10.2135/cropsci2006.04.0278gas

Richardson, W.L. and C.M. Taliaferro. 2005. Bermudagrass hybrid named 'Ozark.' Plant patent PP15,576. Available online: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP1557- 6.PN [Accessed Mar 9 2020].

SAS Institute Inc. 2014. SAS User's Guide. Cary, NC: SAS Institute Inc.

Stefaniank, T.R., C.A. Rodgers, R. VanDyke, D.W. Williams, and T.D. Phillips. 2009. The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. Crop Sci. 49:1489-1495. doi:10.2135/cropsci2008.09.0564

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In: L.E. Moser, B.L. Burson, and L.E. Sollenberger, editors, Warm-season (C_4) grasses. ASA, CSSA, and SSSA, Madison, Wisconsin. p. 417-464. doi:10.2134/agronmonogr45.c12

Taliaferro, C.M., and W.L. Richardson. 1980. Registration of Hardie bermudagrass. Crop Sci. 20:413. doi:10.2135/cropsci198-.0011183X002000030039x

Taliaferro, C.M., J.A. Anderson, W.L. Richardson, J.L. Baker, J.L. Moyer, T.L. Hansen, R.L. Kallenbach, and R.J. Crawford. 2002. Registration of 'Midland 99' forage bermudagrass. Crop Sci. 42:2212-2213. doi:10.2135/cropsci2002.2212

Wu, Y.Q., C.M. Taliaferro, and R. Kochenower. 2009. Forage bermudagrass plant named 'Goodwell.' Plant patent PP22,101. Available online: http://patft.uspto.gov/neta-cgi/nphParser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP22101.PN. [Accessed Mar 9 2020].

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson. 2007. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. Crop Sci. 47:1985-1994. doi:10.2135/cropsci2007.01.0047

Chapter 3

Ahring, R.M., C.M. Taliaferro, and R.D. Morrison. 1974. Seed production of several strains and hybrids of bermudagrass, *Cynodon dactylon* (L.) Pers. Crop Sci. 14:93-95. doi:10.2135/crop-sci1974.0011183X001400010028x

Ahring, R.M., C.M. Taliaferro, and W.L. Richardson. 1982. Single-cross bermudagrass seed production under different management. Agron. J. 74:445-446. doi:10.2134/agronj1982.00219320074000300l1x

Anderson, W.F., A. Maas, and P. Ozias-Akins. 2009. Genetic variability of a forage bermudagrass core collection. Crop Sci. 49:1347-1358. doi:10.2135/cropsci2-008.06.0330

Baltensperger, A.A., B. Dossey, L. Taylor, and J. Klingenberg. 1993. Bermudagrass, *Cynodon dactylon* (L.) Pers., seed production and variety development. Int. Turfgrass Soc. Res. J. 7:829-838.

Burton, G.W. 1947. Breeding bermudagrass for the southeastern United States. J. Am. Soc. Agron. 39:551-569. doi:10.2134/agronj1947.00021962003900070001x

Burton, G.W., and R.H. Hart. 1967. Use of self-incompatibility to produce commercial seed-propagated F_1 hybrids. Crop Sci. 7:524-527. doi:10.2135/cropsci1967.00-11183X000700050035x

Cluff, G.J., and A.A. Baltensperger. 1991. Heritability estimates of seed yield and seed yield components in bermudagrass. Bull. 759. College of Agriculture and Home Economics, New Mexico State Univ. Las Cruces, NM.

Das, M.K., R.G. Fuentes, C.M. Taliaferro. 2004. Genetic variability and trait relationships in switchgrass. Crop Sci. 44:443-448. doi:10.2135/cropsci2004.4430

Dewey, D.R., and K.H. Lu. 1959. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agron. J. 51:515-518. doi:10.2134/agronj1959.00-021962005100090002x

Guo, Y., Y.Q. Wu, J.Q. Moss, J.A. Anderson, and I. Zhu. 2017. Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. Crop Sci. 57:82-88. doi:10.2135/cropsci2016.05.0369

Harlan, J.R., and J.M.J. de Wet. 1969. Source of variation in *Cynodon dactylon* (L.) Pers. Crop Sci. 9:774-778. doi:10.2135/cropsci1969.0011183X000900060031x

Harlan, J.R., J.M.J. de Wet, W.W. Huffine, and J.R. Deakin. 1970. A guide to the species of *Cynodon* (Gramineae). Stillwater, OK, USA: Oklahoma Agricultural Experiment Station Bulletin B-673.

Jewell, M.C., Y. Zhou, D.S. Loch, I.D. Godwin, and C.J. Lambrides. 2012. Maximizing genetic, morphological, and geographic diversity in a core collection of Australian bermudagrass. Crop Sci. 52:879-889. doi:10.2135/cropsci2011.09.0497

Kang, M.S. 1994. Applied quantitative genetics. M.S. Kang Publisher, Baton Rouge, LA.

Redfearn, D. and Y.Q. Wu. 2013. Choosing, establishing, and managing bermudagrass varieties in Oklahoma. Oklahoma Cooperative Extension Service. PSS-2583.

Richardson, W.L. and C.M. Taliaferro. 2005. Bermudagrass hybrid named 'Ozark.' Plant patent PP15,576. Available online: http://patft.uspto.gov/netacgi/nphParser-?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP1557- 6.PN [Accessed Mar 9 2020].

Richardson, W.L., C.M. Taliaferro, and R.M. Ahring. 1978. Fertility of eight bermudagrass clones and open-pollinated progeny from them. Crop Sci. 18:332-334. doi:10.2135/cropsci1-978.0011183X001800020035x

SAS Institute Inc. 2014. SAS User's Guide. Cary, NC: SAS Institute Inc.

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In: L.E. Moser, B.L. Burson, and L.E. Sollenberger, editors, Warm-season (C_4) grasses. ASA, CSSA, and SSSA, Madison, Wisconsin. p. 417-464. 1995. doi:10.2134/agronmonogr45.c12

Taliaferro, C.M., and W.L. Richardson. 1980. Registration of Hardie bermudagrass. Crop Sci. 20:413. doi:10.2135/cropsci198-.0011183X002000030039x

Taliaferro, C.M., R.M. Ahring, and W.L. Richardson. 1983. Registration of 'Guymon' bermudagrass. Crop Sci. 23:1219. doi:10.2135/cropsci1983.0011183X-002300060054x

Taliaferro, C.M., J.A. Anderson, W.L. Richardson, J.L. Baker, J.L. Moyer, T.L. Hansen, R.L. Kallenbach, and R.J. Crawford. 2002. Registration of 'Midland 99' forage bermudagrass. Crop Sci. 42:2212-2213. doi:10.2135/cropsci2002.2212

Tan, C.C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, M.W. Smith, and J.Q. Moss. Selfing and outcrossing fertility in common bermudagrass under open-pollinating conditions examined by SSR markers. Crop Sci. 54:1832-1837. doi:10.2135/cropsci2013.12.0816

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson. 2007. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. Crop Sci. 47:1985-1994. doi:10.2135/cropsci2007.01.0047

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, C.L. Goad, and J.A. Anderson. 2006. Genetic variability and relationships for seed yield and its components in Chinese *Cynodon* accessions. Field Crops Res. 98:245-252. doi:10.1016/j.fcr.2006.02.003

Wu, Y.Q., C.M. Taliaferro, and R. Kochenower. 2009. Forage bermudagrass plant named 'Goodwell.' Plant patent PP22,101. Available online: http://patft.uspto.gov/neta-cgi/nphParser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP22101.PN. [Accessed Mar 9 2020].

Chapter 4

Anderson, J.A., and Y.Q. Wu. 2011. Freeze tolerance of forage bermudagrass. Grass For. Sci. 66:449-452. doi:10.1111/j.1365-2494.2011.00802.x

Beck, E.H., S. Fettig, C. Knake, K. Hartig, and T. Bhattarai. 2007. Specific and unspecific responses of plants to cold and drought stress. J. Biosci. 32:501-510. doi:10.1007/s12038-007-0049-5

Burton, G.W. 1951. Intra- and inter-specific hybrids in bermudagrass. J. Hereb. 42:153-156.

Burton, G.W., R.N. Gates, and G.M. Hill. 1993. Registration of 'Tifton 85' bermudagrass. Crop Sci. 33:644-645. doi:10.2135/cropsci1993.00-11183X003300030045x

Burton, G.W., and W.G. Monson. 1972. Inheritance of dry matter digestibility in bermudagrrass, *Cynodon dactylon* (L.) Pers. Crop Sci. 12:375-378. doi:10.2135/cropsci1972.0011183X00-1200030036x

Burton, G.W., and W.G. Monson. 1984. Registration of 'Tifton 68' bermudagrass. Crop Sci. 24:1211. doi:10.2135/cropsci1984.0011183X002400060051x

Burton, G.W., G.M. Prine, and J.E. Jackson. 1957. Studies of drought tolerance and water use of several southern grasses. Agron. J. 49:498-503. doi:10.2134/agronj1957.0-00219620049-00090010x

Close, T.J. 1997. Dehydrins: A commonality in the response of plants to dehydration and low temperature. Physiol. Plant. 100:291-296. doi:10.1111/j.1399-3054.1997.tb04785.x

Dong, H., S. Thame, L. Liu, M.W. Smith, L. Yan, and Y.Q. Wu. 2015. QTL mapping for reproductive maturity in lowland switchgrass populations. Bioenerg. Res. 8:1925-1937. doi: 10.1007/s/12155-015-9651-9

Fang, T., Y.Q. Wu, S. Makaju, T. Tribble, D.L. Martin, and J.Q. Moss. 2015. Identification of turf bermudagrass on the Oklahoma State University baseball field and three experimental clones as revealed with simple sequence repeat markers. HortTechnol. 25:714-724.

Guo, Y., Y.Q. Wu, J.A. Anderson, J.Q. Moss. L. Zhu, and J. Fu. 2016. SSR marker development, linkage mapping, and QTL analysis for establishment rate in common bermudagrass. Plant Genome 10. doi:10.3835/plantgenome2016.07.0074

Guo, Y., Y.Q. Wu, J.Q. Moss, J.A. Anderson, and I. Zhu. 2017. Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. Crop Sci. 57:82-88. doi:10.2135/cropsci2016.05.0369

Harlan, J.R., and J.M.J. de Wet. 1969. Source of variation in *Cynodon dactylon* (L.) Pers. Crop Sci. 9:774-778. doi:10.2135/cropsci1969.0011183X000900060031x

Hancock, D., L. Stewart, U.K. Saha, J.K. Bernard, and B. Smith. 2017. Understanding and improving forage quality. University of Georgia Extension. Bulletin 1425.

Hill, G.M., R.N. Gates, and J.W. West. 2001. Advances in bermudagrass research involving new cultivars for beef and dairy production. J. Anim. Sci. 79:48-58. doi:10.2527/jas2001.79E-SupplE48x

Nalini, E., S. Bhagwat, and N. Jawali. 2004. A simple method for isolation of DNA from plants suitable for long-term storage and DNA marker analysis. BARC Newsletter. 249:208-214.

Nelson, C., and J.C. Burns. 2006. Fifty years of grassland science leading to change. Crop Sci. 46:2204-2217. doi:10.2135/cropsci2006.04.0278gas

Quisenberry, S.S. 1990. Plant resistance to insects and mites in forage and turf grasses. Fla. Entomol. 73:411-421. doi:10.2307/495459

Richardson, W.L. and C.M. Taliaferro. 2005. Bermudagrass hybrid named 'Ozark.' Plant patent PP15,576. Available online: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP1557- 6.PN [Accessed Mar 9 2020].

Rouquette, F.M., Jr., J.L. Kerby, and G.H. Nimr. 1998. Pasture and animal performance from bermudagrass pastures at three stocking rates with or without nitrogen fertilizer. p. 65-66. *In* Texas A&M Univ. Res. Ctr. Tech. Rep. 98-1. Tex. Agric. Exp. Stn., College Station.

SAS Institute Inc. 2014. SAS User's Guide. Cary, NC: SAS Institute Inc.

Smiley, R.W., R.H. Dernoeden, and B.B. Clark. 2005. Compendium of turfgrass diseases, third edition. Am. Phytopathol. Soc. Press, St. Paul, MN. doi:10.1094/9780890546154

Stefaniak, T.R., C.A. Rodgers, R. VanDyke, D. Williams, and T.D. Phillips. 2009. The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. Crop Sci. 49:1489-1495. doi:10.2135/cropsci2008.09.0564

Taliaferro, C.M., J.A. Anderson, W.L. Richardson, J.L. Baker, J.L. Moyer, T.L. Hansen, R.L. Kallenbach, and R.J. Crawford. 2002. Registration of 'Midland 99' forage bermudagrass. Crop Sci. 42:2212-2213. doi:10.2135/cropsci2002.2212

Taliaferro, C.M., and W.L. Richardson. 1980. Registration of Hardie bermudagrass. Crop Sci. 20:413. doi:10.2135/cropsci198-.0011183X002000030039x

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In: L.E. Moser, B.L. Busron, and L.E. Sollenberger, editors, Warm-season (C_4) grasses. ASA, CSSA, and SSSA, Madison, Wisconsin. p. 417-464. 1995. doi:10.2134/agronmonogr45.c12

Tan, C.C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, M.W. Smith, and J.Q. Moss. 2014. Selfing and outcrossing fertility in common bermudagrass under open-pollinating conditions examined by SSR markers. Crop Sci. 54:1832-1837. doi:10.2135/cropsci2013.12.0816

Wu, Y.Q. 2011. Cynodon. In: Kole, C. (ed). Wild crop relatives: Genomic breeding resources. Springer, Berlin, Heidelberg. doi:10.1007/978-3-642-14255-0_4

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson. 2007. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. Crop Sci. 47:1985-1994. doi:10.2135/cropsci2007.01.0047

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, C.L. Goad, and J.A. Anderson. 2006. Genetic variability and relationships for seed yield and its components in Chinese *Cynodon* accessions. Field Crops Res. 98:245-252. doi:10.1016/j.fcr.2006.02.003

Wu, Y.Q., C.M. Taliaferro, and R. Kochenower. 2009. Forage bermudagrass plant named 'Goodwell.' Plant patent PP22,101. Available online: http://patft.uspto.gov/netacgi/nphParser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP22101.PN. [Accessed Mar 9 2020].

Xiang, M., J.Q. Moss, D.L. Martin, K.Su, B.L. Dunn, and Y.Q. Wu. 2017. Evaluating the salinity tolerance of clonal-type bermudagrass cultivars and an experimental selection. Hort. Sci. 52(1): 185-191. doi:10.21273/HORTSCI10773-16

Zhang, X., E.H. Ervin, and A.J. LaBranche. 2006. Metabolic defense responses of seeded bermudagrass during acclimation to freezing stress. Crop Sci. 46:2598-2605. doi:10.2135/cropsci2006.02.0108

Zhang, X., K. Wang, and E.H. Ervin. 2008. Bermudagrass freezing tolerance associated with abscisic acid metabolism and dehydrin expression during cold acclimation. J. Amer. Soc. Hort. Sci. 133:542-550. doi:10.21273/JASHS.133.4.542

Zhang, X., K. Wang, E.H. Ervin, C. Waltz, and T. Murphy. 2011. Metabolic changes during cold acclimation and deacclimation in five bermudagrass varieties. I. Proline, total amino acid, protein, and dehydrin expression. Crop Sci. 51:838-846. doi:10.2135/cropsci2010.06.0345

Chapter 5

Anderson, W.F., A. Maas, and P. Ozian-Akins. 2009. Genetic variability of a forage bermudagrass core collection. Crop Sci. 49:1347-1358. doi:10.2135/cropsci2-008.06.0330

Assefa, S., C.M. Taliaferro, M.P. Anderson, B.G. de los Reyes, and R.M. Edwards. 1999. Diversity among Cynodon accessions and taxa based on DNA amplification fingerprinting. Genome. 42:465-474. doi:10.1139/g98-152

Brown, S.M., M.S. Hopkins, S.E. Mitchell, M.L. Senior, T.Y. Wang, R.R. Duncan, F. Gonzalez-Candelas, and S. Kresovich. 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. Theor. Appl. Genet. 93:190-198. doi:10.1007/BF00225745

Burton, G.W., R.N. Gates, and G.M. Hill. 1993. Registration of 'Tifton 85' bermudagrass. Crop Sci. 33:644-645. doi:10.2135/cropsci1993.0011-183X003300030045x

Cordeiro, G.M., R. Casu, C.L. McIntyre, J.M. Manners, and R.J. Henry. 2001. Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci. 160:1115-1123. doi:10.1016/S0168-9452(01)00365-X

Dayanandan, S. K.S. Bawa, and R.V. Kesseli. 1997. Conservation of microsatellites among tropical trees (*Leguminosae*). Am. J. Bot. 84:1658-1663. doi:10.2307/2446463

de Silva, P.H.A.U., and R.W. Snaydon. 1995. Chromosome number in Cynodon dactylon in relation to ecological conditions. Ann. Bot. 76:498-503. doi:10.1006/anbo.1995.1129

Fang, T., Y.Q. Wu, S. Makaju, T. Tribble, D.L. Martin, and J.Q. Moss. 2015. Identification of turf bermudagrass on the Oklahoma State University baseball field

and three experimental clones as revealed with simple sequence repeat markers. Hort. Technol. 25:714-724. doi:10.21273/HORTTECH.25.6.714

Forbes, I., and G.W. Burton. 1963. Chromosome numbers and meiosis in some Cynodon species and hybrids. Crop Sci. 3:75-79. doi:10.2135/cropsci1963.001118-3X000300010023x

Guo, Y., Y.Q. Wu, J.A. Anderson, J.Q. Moss. L. Zhu, and J. Fu. 2017. SSR marker development, linkage mapping, and QTL analysis for establishment rate in common bermudagrass. Plant Genome 10. doi:10.38535/plantgenome2016.07.0074

Harlan, J.R., J.M.J. de Wet, K.M. Rawal, M.R. Felder, and W.L. Richardson. 1970a. Cytogenic studies in Cynodon L.C. Rich. (Gramineae). Crop Sci. 10:288-291. doi:10.2135/cropsci19-70.0011183X001000030023x

Harris-Shultz, K.R., B.M. Schwarts, W.W. Hanna, and J.A. Brady. 2010. Development, linkage mapping, and use of microsatellites in bermudagrass. J. Am. Soc. Hort. Sci. 135:511-520. doi:10.21273/JASHS.135.6.511

Harlan, J.R. J.M.J. de Wet, W.W. Huffine, and J.R. Deakin. 1970b. A guide to the species of *Cynodon* (Gramineae). Oklahoma Agricultural Experiment Station Bulletin B-673.

Jewell, M.C., Yi. Zhou, D.S. Loch, I.D. Godwin, and C.J. Lambrides. 2012. Maximizing genetic, morphological, and geographic diversity in a core collection of Australian bermudagrass. Crop Sci. 52:879-889. doi:10.2135/cropsci2011.09.0497

Mislevy, P., W.F. Brown, R. Caro-Costas, J. Vicente-Chandler, L.S. Dunavin, D.W. Hall, R.S. Kalmbacher, A.J. Overman, O.C. Ruelke, R.M. Sonoda, A. Sotomayor-Rios, and M.J. Williams. 1993. Registration of 'Florico' stargrass. Crop Sci. 33:358-359. doi:10.2135/cropsci1993.0011183X003300020044x

Nalini, E., S. Bhagwat, and N. Jawali. 2004. A simple method for isolation of DNA from plants suitable for long-term storage and DNA marker analysis. BARC Newsletter. 249:208-214.

Richardson, W.L. and C.M. Taliaferro. 2005. Bermudagrass hybrid named 'Ozark.' Plant patent PP15,576. Available online: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP1557- 6.PN [Accessed Mar 9 2020].

Taliaferro, C.M. 1995. Diversity and vulnerability of Bermuda turfgrass species. Crop Sci. 35:327-332. doi:10.2135/cropsci1995.0011183X003500020006x

Taliaferro, C.M., J.A. Anderson, W.L. Richardson, J.L. Baker, J.L. Moyer, T.L. Hansen, R.L. Kallenbach, and R.J. Crawford. 2002. Registration of 'Midland 99' forage bermudagrass. Crop Sci. 42:2212-2213. doi:10.2135/cropsci2002.2212

Taliaferro, C.M., and W.L. Richardson. 1980. Registration of Hardie bermudagrass. Crop Sci. 20:413. doi:10.2135/cropsci198-.0011183X002000030039x

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In: L.E. Moser, B.L. Busron, and L.E. Sollenberger, editors, Warm-season (C_4) grasses. ASA, CSSA, and SSSA, Madison, Wisconsin. p. 417-464. 1995. doi:10.2134/agronmonogr45.c12

Tan, C., Y.Q. Wu, C.M. Taliaferro, M.P. Anderson, C. Tauer, and T. Samules. 2012. Development of simple sequence repeat markers for bermudagrass from its expressed sequence tag sequences and preexisting sorghum SSR markers. Mol. Breeding. 29:23-30. doi:10.1007/s11032-010-9521-2

Tan, C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, and M.W. Smith. 2014a. Development and characterization of genomic SSR markers in *Cynodon transvaalensis* Burtt-Davy. Mol. Gen. Genom. 289:523-531. doi:10.1007/s00438-014-0829-1

Tan, C.C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, M.W. Smith, and J.Q. Moss. 2014b. Selfing and outcrossing fertility in common bermudagrass under open-pollinating conditions examined by SSR markers. Crop Sci. 54:1832-1837. doi:10.2135/cropsci2013.12.0816

Varshney, R.K., R. Sigmund, A. Borner, V. Korzun, N. Stein, M.E. Sorrells, P. Langridge, and A. Graner. 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci. 168:195-202. doi:10.1016/j.plantsci.2004.08.001

Wang, M.L., N.A. Barkley, J.K. Yu, R.E. Dean, M.L. Newman, M.E. Sorrells, and G.A. Pederson. 2005. Transfer of simple sequence repeat (SSR) markers from major cereal crops to minor grass species for germplasm characterization and evaluation. Plant Genetic Res. 3:45-47. doi:10.1079/PGR200461

Wu, Y.Q. 2011. Cynodon. In: Kole, C. (ed). Wild crop relatives: Genomic breeding resources. Springer, Berlin, Heidelberg. doi:10.1007/978-3-642-14255-0_4

Wu, Y.Q., C.M. Taliaferro, and R. Kochenower. 2009. Forage bermudagrass plant named 'Goodwell.' Plant patent PP22,101. Available online: http://patft.uspto.gov/netacgi/nphParser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP22101.PN. [Accessed Mar 9 2020].

Xie, W.G., X.Q. Zhang, H.W. Cai, W. Liu, and Y. Peng. 2010. Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.). Biochem. Syst. Ecol. 38:740-749. doi:10.1016/j.bse.2010.06.009

Chapter 6

Anderson, W.F., A. Maas, and P. Ozian-Akins. 2009. Genetic variability of a forage bermudagrass core collection. Crop Sci. 49:1347-1358. doi:10.2135/crops-ci2008.06.0330

Burton, G.W. 1948. Coastal bermudagrass. Cir. 10. Ga. Agric. Exp. Stn., Athens.

Burton, G.W. 1972. Registration of 'Coastcross-1' bermudagrass. Crop Sci. 12:125. doi:10.2135/cropsci1972.0011183X001200010049x

Burton, G.W., R.N. Gates, and G.M. Hill. 1993. Registration of 'Tifton 85' bermudagrass. Crop Sci. 33:644-645. doi:10.2135/cropsci1993.0011183X003300030045x

Burton, G.W., and W.G. Monson. 1984. Registration of Tifton 68 bermudagrass. Crop Sci. 24:1211. doi:10.2135/cropsci1984.0011183X002400060051x

Burton, G.W., and W.G. Monson. 1978. Registration of 'Tifton 44' bermudagrass. Crop Sci. 18:911. doi: 10.2135/cropsci1978.0011183X001800050065x

Eichhorn, Jr., M.M., W.M. Oliver, W.B. Hallmark, W.A. Young, A.V. Davis, and B.D. Nelson. 1986. Registration of 'Grazer' bermudagrass. Crop Sci. 26:835. doi:10.2135/cropsci1986.00-11183X002600040046x

Fang, T., Y.Q. Wu, S. Makaju, T. Tribble, D.L. Martin, and J.Q. Moss. 2015. Identification of turf bermudagrass on the Oklahoma State University baseball field and three experimental clones as revealed with simple sequence repeat markers. HortTechnol. 25:714-724.

Gordon, L.R. 1989. World Feeder bermuda grass. U.S. Plant Patent 7081. Date issued: 19 Dec. 1989.

Guo, Y., Y.Q. Wu, J.A. Anderson, J.Q. Moss. L. Zhu, and J. Fu. 2016. SSR marker development, linkage mapping, and QTL analysis for establishment rate in common bermudagrass. Plant Genome 10. doi:10.3835/plantgenome2016.07.0074

Guo, Y., Y.Q. Wu, J.Q. Moss, J.A. Anderson, and I. Zhu. 2017. Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. Crop Sci. 57:82-88. doi:10.2135/cropsci2016.05.0369

Harlan, J.R., and J.M.J. de Wet. 1969. Source of variation in *Cynodon dactylon* (L.) Pers. Crop Sci. 9:774-778. doi:10.2135/cropsci1969.0011183X000900060031x

Hein, M.A. 1953. Registration of varieties and strains of bermudagrass, II [*Cynodon dactylon* (L.) Pers.]. Agron. J. 45:572-573. doi:10.2134/agronj1953.00219-62994500110016x

Jewell, M.C., Yi. Zhou, D.S. Loch, I.D. Godwin, and C.J. Lambrides. 2012. Maximizing genetic, morphological, and geographic diversity in a core collection of Australian bermudagrass. Crop Sci. 52:879-889. doi:10.2135/cropsci2011.09.0497

Karaca, M., S. Saha, A. Zlpf, J.N. Jenkins, and D.J. Lang. 2002. Genetic diversity among forage germudagrass (*Cynodon* spp.). Crop Sci. 42:2118-2127. doi:10.2135/cropsci2002.2118

Myers, W.M. 1951. Registration of varieties and strains of bermudagrass [*Cynodon dactylon* (L.) Pers.]. Agron. J. 43:240. doi:10.2134/agronj1951.000-21962004300050011x

Nalini, E., S. Bhagwat, and N. Jawali. 2004. A simple method for isolation of DNA from plants suitable for long-term storage and DNA marker analysis. BARC Newsletter. 249:208-214.

Richardson, W.L. and C.M. Taliaferro. 2005. Bermudagrass hybrid named 'Ozark.' Plant patent PP15,576. Available online: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP1557- 6.PN [Accessed Mar 9 2020].

Stefaniak, T.R., C.A. Rodgers, R. VanDyke, D. Williams, and T.D. Phillips. 2009. The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. Crop Sci. 49:1489-1495. doi:10.2135/cropsci2008.09.0564

Taliaferro, C.M., R.M. Ahring, and W.L. Richardson. 1983. Registration of 'Guymon' bermudagrass. Crop Sci. 23:219. doi:10.2135/cropsci1983.0011183X002300060054x

Taliaferro, C.M., J.A. Anderson, W.L. Richardson, J.L. Baker, J.L. Moyer, T.L. Hansen, R.L. Kallenbach, and R.J. Crawford. 2002. Registration of 'Midland 99' forage bermudagrass. Crop Sci. 42:2212-2213. doi:10.2135/cropsci2002.2212

Taliaferro, C.M., and W.L. Richardson. 1980. Registration of Hardie bermudagrass. Crop Sci. 20:413. doi:10.2135/cropsci1980.0011183X002000030039x

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In: L.E. Moser, B.L. Busron, and L.E. Sollenberger, editors, Warm-season (C_4) grasses. ASA, CSSA, and SSSA, Madison, Wisconsin. p. 417-464. 1995. doi:10.2134/agronmonogr45.c12

Tan, C., Y.Q. Wu, C.M. Taliaferro, M.P. Anderson, C. Tauer, and T. Samules. 2012. Development of simple sequence repeat markers for bermudagrass from its expressed sequence tag sequences and preexisting sorghum SSR markers. Mol. Breeding. 29:23-30. doi:10.1007/s11032-010-9521-2

Tan, C.C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, M.W. Smith, and J.Q. Moss. 2014. Selfing and outcrossing fertility in common bermudagrass under open-pollinating conditions examined by SSR markers. Crop Sci. 54:1832-1837. doi:10.2135/cropsci2013.12.0816

Wu, Y.Q., C.M. Taliaferro, and R. Kochenower. 2009. Forage bermudagrass plant named 'Goodwell.' Plant patent PP22,101. Available online: http://patft.uspto.gov/netacgi/nphParser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=PP22101.PN. [Accessed Mar 9 2020].

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson. 2007. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. Crop Sci. 47:1985-1994. doi:10.2135/cropsci2007.01.0047

Wu, Y.Q., C.M. Taliaferro, D.L. Martin, C.L. Goad, and J.A. Anderson. 2006. Genetic variability and relationships for seed yield and its components in Chinese *Cynodon* accessions. Field Crops Res. 98:245-252. doi:10.1016/j.fcr.2006.02.003

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