GENETIC AND QUANTITATIVE TRAIT LOCI MAPPING IN AFRICAN BERMUDAGRASS AND CHARACTERIZATION OF WINTER SURVIVABILITY AND DROUGHT RESPONSE IN TURF-TYPE BERMUDAGRASS

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Abstract: African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) is frequently used to cross with common bermudagrass (*C. dactylon* Pers.) in the creation of F_1 hybrid cultivars that most widely used in the worldwide turf industry. The species has some unique morphological, adaptive, and reproductive characteristics that contribute to turfgrass quality improvement. However, molecular resources in this species are limited. Winterkill is a major concern for turf-type bermudagrasses (*C. dactylon* and *C. dactylon* × *C. transvaalensis*) when cultivated in the U.S. transition zone. Water scarcity is a widespread issue in the turf industry. It would be valuable for new cultivars to combine the two traits. Accordingly, the objectives of this study were to construct a high-density genetic map; to quantify genetic variability and to identify quantitative trait loci (QTL) associated with adaptive, morphological, and reproductive traits; to estimate reliability for spring greenup and drought response in turf-type bermudagrass selections. A high-density linkage map for African bermudagrass was constructed using a genotyping-by-sequencing approach based on 109 S_1 progeny of the African bermudagrass genotype 'OKC1163'. A total of 1,278 markers were integrated in the map with nine linkage groups, spanning 882.3 cM. Establishment rate, winter survivability traits, drought response, plant height, leaf blade width and length, stem internode diameter and length, and inflorescence prolificacy in the S_1 population with the parent were evaluated in a replicated field trial over three seasons (2018 to 2020) in Stillwater, OK. Thirty-six QTL were identified to be associated with the respective traits. In another experiment, 77 selections and seven cultivars were evaluated for spring greenup and drought response at Goodwell, OK. The reliability estimates for drought response ranged from low to moderate ($0.20 \leq i^2 \leq 0.63$) and the estimates for spring greenup were low ($0.08 \leq i^2 \leq 0.27$), indicating that winter survivability and drought resistance may be evaluated in separate trials. The research developed important genetic resources for understanding the genetic structure of the traits and towards marker-assisted selection, and added useful information to the knowledge pool for improving the important traits in breeding turf bermudagrass cultivars.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

Chapter II

Chapter III

Table Page

Chapter IV

Chapter V

Table Page

LIST OF FIGURES

Figure Page

Chapter II

Figure Page

Chapter III

Chapter IV

Figure Page

CHAPTER I

GENERAL INTRODUCTION

RESEARCH OUTLINE

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy), named by its place of origin, is indigenous to the southwestern Transvaal, Orange Free State and the northern part of the central Cape Province of South Africa (Harlan et al., 1970a). Usually, African bermudagrass can be found in wet areas such as river banks (Harlan et al., 1970b). The cold hardiness of African bermudagrass allows it to adapt to much colder regions beyond its natural distribution (Harlan et al., 1970a). It has been confirmed that African bermudagrass can survive the climate of 39 °N latitude in the United States (Taliaferro, 1992).

African bermudagrass has unique morphological features such as fine-textured, erect leaves, and a yellowish-green color as distinguished from other bermudagrass species (Harlan et al., 1970b). The stolons and stems are slender with shortened internodes length (Kenworthy, 2006). When reproducing vegetatively, its rhizomatous and stoloniferous growth habit allows African bermudagrass to form a dense sod. African bermudagrass can also reproduce sexually through seed production. It has two to four racemes per inflorescence with the spikelets loosely arranged (Harlan et al., 1970b). Each African bermudagrass flower has one pistil and three anthers (Taliaferro, 2004). It is

highly self-incompatible. Therefore, different genotypes must be planted together in order to set seed (Harlan et al., 1970b). African bermudagrass usually flowers during the spring and in summer, seedheads start to hide inside the canopy. Seed shattering at maturity is quite common for African bermudagrass (Taliaferro, 2004).

Together with eight bermudagrass species, African bermudagrass belongs to the genus *Cynodon*, subtribe Eleusininae, tribe Cynodonteae, subfamily Chloridoideae, and family Poaceae (Harlan et al., 1970a; Soreng et al., 2015). The base chromosome number per genome of the *Cynodon* species is nine, with an estimated genome size of 540Mbp per copy (Forbes and Burton, 1963; Harlan et al., 1970b; Taliaferro et al., 1997). African bermudagrass is diploid with 18 chromosomes, and generally crosses readily with tetraploid (2*n*=4*x*=36) *C. dactylon* var. *dactylon*, despite distinctive morphological features between these two species. African bermudagrass can also cross with diploid *C. nlemfuensis* var. *nlemfuensis* (de Wet and Harlan, 1970).

African bermudagrass can be used as a turfgrass. Cultivars such as 'Florida' and 'Uganda' have been used on sporting surfaces worldwide (Juska and Hanson, 1964; Roux, 1969). However, its high water and nutrient demand, thatch buildup tendency, and turfgrass quality decline in summer limits use as turf (Taliaferro, 1992). The primary use of African bermudagrass is to serve as a parent crossing with *C. dactylon* var. *dactylon* to produce interspecific hybrids, which combines the desirable turf characteristics of the two species (Burton, 1991; Taliaferro, 1992, Taliaferro, 1995). Dr. Glenn Burton, University of Georgia Coastal Plain Experiment Station, Tifton, Georgia, began breeding bermudagrass for turf in 1946. He was the first to make crosses between *C. dactylon* var. *dactylon* and *C. transvaalensis* and to evaluate the hybrids for improved turfgrass

characteristics. Besides the controlled crossing, interspecific hybrid bermudagrass cultivar development also utilized naturally occurring crosses and natural or human-induced mutations (Juska and Hanson, 1964; Burton, 1991).

Genotypic and environmental factors and their interactions influence phenotypic expression. Understanding the genetic variance in phenotypic variation is useful for making decisions in plant breeding in order to obtain predictable selection response (Dudley and Moll, 1969). Heritability is the proportion of the progeny's observed phenotypic variation due to genetic differences (Poehlman and Sleper, 1995). Broad-sense heritability is the ratio of total genetic (additive, dominance, and epistatic) variance to phenotypic variance (Dudley and Moll, 1969). Selecting for traits with high broad-sense heritability will lead to faster process and more genetic gains in the offspring than selecting for traits with low heritability (Browning et al., 1994).

Estimating genetic variance components requires the use of an appropriate mating design (Kenworthy et al., 2006). A one-factor design is appropriate to determine the existence of genetic variability and estimate the broad-sense heritability for a single factor (trait). The random progeny of a single random-mating population is the key assumption in one-factor design (Bernardo, 2002). If the progeny is developed through some form of selection, the result is biased. However, breeders can still access the genetic effects versus nongenetic effects in phenotypic expression. In this case, using reliability can be considered the measurement of genetic versus nongenetic effects in a fixed population (Bernardo, 2002).

The heritabilities of many traits have been estimated in *C. dactylon* var. *dactylon* populations (Cluff and Baltensperger, 1991, Coffey and Baltensperger, 1989; Guo et al.,

2017; Wofford and Baltensperger, 1985). The heritability of traits for African bermudagrass is more limited than in common bermudagrass. Only Kenworthy et al. (2006) estimated the broad-sense heritability for performance traits (color, density turf quality, spring greenup, fall dormancy), reproductive traits (raceme number, raceme length, floret number, seed number, and percent seed set), and morphological traits (plant height, stolon length, internode length, and leaf length) in African bermudagrass. Kenworthy et al. (2006) reported the broad-sense heritability of African bermudagrass traits ranged from 0.42 to 0.96, indicating through recurrent selection, certain traits can be improved in the population.

Several studies have been conducted in African bermudagrass genetic diversity with amplified fragment length polymorphism (AFLP) markers (Caetano-Anolles et al., 1995; Wu et al., 2005; Zhang et al., 1999). However, African bermudagrass genetic studies, such as the construction of genetic linkage maps, are still lacking. Genetic linkage map is one of the most important tools in genetic and genome studies. As of the writing of this paper, only one African bermudagrass genetic linkage map was available. Bethel et al. (2006) constructed a framework linkage map of African bermudagrass using an interspecific hybrid population between *C. dactylon* 'T89' x *C. transvaalensis* 'T574'. The construction of the genetic map used single-dose restriction fragments (SDRFs) with approximately 62% estimated genome coverage of a total 973.4 cM with 77 markers (Bethel et al., 2006). Harris-Shultz et al. (2010) added expressed sequence tag-derived simple sequence repeat (EST-SSR) to the framework published by Bethel et al. (2006). Harris-Shultz et al. (2010) reported 8 linkage groups (LGs) were identified on T574 African bermudagrass instead of the 18 LGs identified by Bethel et al. (2006). Khanal et

al. (2017) enriched SSR markers to the SDRF framework published by Bethel et al. (2006) resulting in eleven LGs spanning 1184.7 cM based on 52 SSR and 73 RFLP markers. However, all current maps constructed with gel-based markers were in relatively low density. Single nucleotide polymorphisms (SNPs) are common mutations between related genomes and can be used as molecular markers to construct genetic linkage maps (Mammadov et al., 2012). Next-generation sequencing (NGS) technologies provide new tools for discovering SNPs markers in a fast and more affordable way for both model and non-model species (Wang et al., 2015). Genotyping-by-sequencing (GBS), a protocol under NGS, has been developed using appropriate methylation-sensitive restriction enzymes to digest genome while avoiding the repetitive genome regions to reduce the complexity of targeted genomes (Elshire et al., 2011). The GBS approach has been applied in linkage map construction for many crop species such as switchgrass (*Panicum virgatum* L.) (Fielder et al., 2015), zoysiagrass (*Zoysia japonica* Steud.) (Holloway et al., 2018; Wang et al., 2015), St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) (Yu et al., 2018), and common bermudagrass (Fang et al., 2020). However, a high-density linkage map is not available for African bermudagrass.

Linkage maps can be used for quantitative trait loci (QTL) mapping, which is an important tool to identify the genomic regions responsible for phenotypic variation. Using a high-density linkage map will increase the chance of having markers closely linked to the genomic regions contributing to the traits of interest. QTL mapping can also help the detection of inter and intra-locus interactions and genotype x environment interactions (Paterson et al. 1988; Stuber et al. 1992). Compared to the major crops, the QTL mapping in African bermudagrass is limited. Dunne et al. (2016) analyzed the QTL

for flowering and seedhead characteristics in African bermudagrass. Khanal et al. (2019) reported the QTL for morphological traits such as leaf length, leaf width, plant height, stolon internode length, and length of the longest stolon. Many morphological and reproductive traits in African bermudagrass are complex quantitative traits and influenced by environments. Different from using phenotypic recurrent selection, molecular markers are not influenced by the environment. Therefore, using molecular markers to assist selection will increase the selection reliability with fewer field evaluations.

Bermudagrass has good drought resistance, heat tolerance, and wear tolerance compared to many other turfgrass species. However, its ability to survive freezing temperatures limits adaptation in the northern portion of the United States transition zone (Beard, 1973). When bermudagrass is grown into the transition zone, winter injury periodically occurs resulting in substantial cost for re-establishment and loss of use for extended periods. Efforts have been made to develop turf bermudagrass cultivars with improved winter survivability. In the 1960s, J. R. Harlan and his colleagues collected bermudagrass germplasm around the world to conduct biosystematics investigation and some of the germplasms collection have been used in improving bermudagrass winter survivability (Harlan and de Wet, 1969; Taliaferro et al., 2004b). 'Patriot' (2n=4x=36) released by Oklahoma State University (OSU) in 2002 was an interspecific hybrid with high turfgrass quality and improved winter survivability (Taliaferro, 2004b). Subsequently, three additional interspecific F_1 bermudagrass (2n=3x=27) cultivars (Latitude 36®, Northbridge®, and Tahoma 31®) were released (Wu et al., 2013; Wu et al., 2014; Wu et al., 2019). As urban and suburban areas expand, large areas of previously non-irrigated lands are being converted to irrigated lawns and landscapes. The increasing

irrigation demand has exacerbated water scarcity issues, especially in summer. Turfgrass consumers in the transition zone need new bermudagrass cultivars that combine high visual quality, sufficient winter hardiness, and improved drought resistance. Although bermudagrass is relatively drought resistant, studies have reported phenotypic variations for drought resistance in bermudagrass (Chalmers et al., 2008; Steinke et al., 2011; Yu, 2017). However, most breeding programs do not focus on enhancing winter hardiness and drought resistance together due to their climate limitations. The climate of Oklahoma provides an ideal opportunity to screen warm-season turfgrasses for winter hardiness and drought resistance simultaneously.

REFERENCES

Beard, J.B., 1973. Turfgrass: Science and culture. Prentice-Hall, Englewood Cliffs, NJ.

Bernardo, R., 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Bethel, C.M., E.B. Sciara, J.C. Estill, J.E. Bowers, W.W. Hanna, and A.H. Paterson. 2006. A framework linkage map of bermudagrass (*Cynodon dactylon* × *transvaalensis*) based on single-dose restriction fragments. Theor. Appl. Genet. 112:727–737.

Browning, S., T.P. Riordan, R.K. Johnson and J. Johnson-Cicalese. 1994. Heritability estimates of turf-type characteristics in buffalograss. HortScience 29:204–205.

Burton, G.W. 1991. A history of turf research at Tifton. U.S. Golf Assoc. Green Section Record. 29:12–14.

Caetano-Anolles, G., L.M. Callahan, P.E. Williams, K.R. Weaver, and P.M. Gresshoff. 1995. DNA amplification fingerprinting analysis of bermudagrass (*Cynodon*): genetic relationships between species and interspecific crosses. Theor. Appl. Genet. 91:228–235.

Chalmers, D.R., Steinke, K.S., White, R., Thomas, J.C., Fipps, G., 2008. Evaluation of sixty-day drought survival in San Antonio of established turfgrass species and cultivars. Final report to the San Antonio Water System & the Turfgrass Producers of Texas. pp. 1–60.

Cluff, G.J., and A.A. Baltensperger. 1991. Heritability estimates for seed yield and seed yield components in bermudagrass. New Mexico Agric. Exp. Stn. Bull. 759.

Coffey, B.N., and A.A. Baltensperger. 1989. Heritability estimates for selected turfgrass characteristics of bermudagrass evaluated under shade, p. 117- 119. In H. Takatoh (ed.). Proc. Int. Turfgrass Res. Conf., 6th, 31 July- 5 Aug. 1989, Tokyo, Jpn. Intl. Turfgrass Soc. and Jpn Soc. Turfgrass Sci., Tokyo.

de Wet, J.M.J., and J.R. Harlan. 1970. Biosystematics of *Cynodon* L.C. Rich. (Gramineae). Taxon 19:565–569.

Dudley, J. and R. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9:257–262.

Dunne, J.C. 2016. Breeding for combined shade and cold tolerance in bermudagrass (*Cynodon* spp.) and the identification of QTL associated with seed head traits. Ph.D. dissertation. North Carolina State Univ., Raleigh, NC.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One 6:e19379.

Fiedler, J.D., C. Lanzatella, M. Okada, J. Jenkins, J. Schmutz, and C.M. Tobias. 2015. High-density single nucleotide polymorphism linkage maps of lowland switchgrass using genotyping-by-sequencing. Plant Genome doi:10.3835/plantgenome2014.10.0065

Forbes, I., and G.W. Burton. 1963. Chromosome numbers and meiosis in some Cynodon species and hybrids. Crop Sci. 3:75–79.

Guo, Y., Wu, Y.Q., Moss, J.Q., Anderson, J.A., Zhu, L., 2017. Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. Crop Sci. 57, 1–7.

Harlan, J.R. and J. De Wet. 1969. Sources of variation in *Cynodon dactylon* (L). Pers. Crop Science 9: 774–778.

Harlan, J.R., J.M.J. de Wet, and K.M. Rawal. 1970a. Geographic distribution of the species of Cynodon L.C. Rich (Gramineae). East Afr. Agric. Forest. J. 36:220–226.

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

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. Amer. Soc. Hort. Sci. 135:511–520.

Holloway, H.M.P., X.W. Yu, J.C. Dunne, B.M. Schwartz, A.J. Patton, C. Arellano, et al. 2018. A SNP-based high-density linkage map of zoysiagrass (*Zoysia japonica* Steud.) and its use for the identification of QTL associated with winter hardiness. Mol. Breed. 38:10–24.

Juska, F.V., and A.A. Hanson. 1964. Evaluation of bermudagrass varieties for general-purpose turf. Dep. Agric., Agric. Res. Ser., Agric. Handbook no. 270. U.S. Gov. Print. Office, Washington, DC.

Kenworthy, K.E., C.M. Taliaferro, B.F. Carver, D.L. Martin, J.A. Anderson and G.E. Bell. 2006. Genetic variation in *Cynodon transvaalensis* Burtt-Davy. Crop Sci. 46:2376–2381.

Khanal, S., C. Kim, S.A. Auckland, L.K. Rainville, J. Adhikari, B.M. Schwartz, et al. 2017. SSR-enriched genetic linkage maps of bermudagrass (*Cynodon dactylon* × *transvaalensis*), and their comparison with allied plant genomes. Theor. Appl. Genet. 130:819–839.

Khanal, S., J.C. Dunne, B.M. Schwartz, C. Kim, S. Milla-Lewis, P.L. Raymer, et al. 2019. Molecular dissection of quantitative variation in bermudagrass hybrids (*Cynodon dactylon* x *transvaalensis*): morphological traits. G3: Genes, Genomes, Genetics. 400061.402019.

Mammadov, J., R. Aggarwal, R. Buyyarapu, and S. Kumpatla. 2012. SNP markers and their impact on plant breeding. Int. J. Plant Genomics doi:10.1155/2012/728398

Paterson A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln, and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721–726.

Poehlman, J.M., and D.A. Sleper. 1995. Breeding field crops. 4th ed. Iowa State Univ. Press, Ames, IA.

Roux, W.M. 1969. Grass, a history of Frankenwald. Oxford University Press, Cape Town, South Africa.

Steinke, K., D. Chalmers, J. Thomas, and R. White, 2011. Bermudagrass and buffalograss drought response and recovery at two soil depths. Crop Sci. 51:1215–1223.

Stuber C.W., S.E. Lincoln, D.W. Wolff, T. Helentjaris, and E.S. Lander. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839.

Taliaferro, C.M. 1992. Out of Africa - A new look at “African” bermudagrass. U.S. Golf Assoc. Green Section Record. 30:10–12.

Taliaferro, C.M. 1995. Diversity and vulnerability of bermuda turfgrass species. Crop Sci. 35:327–332.

Taliaferro, C.M., A.A. Hopkins, J.C. Henthom, C.D. Murphy, and R.M. Edwards. 1997. Use of flow cytometry to estimate ploidy level in Cynodon species. Int. Turfgrass Soc. Res. J. 8:385–392.

Taliaferro, C.M., F.M. Rouquette, Jr., and P. Mislevy. 2004. Bermudagrass and stargrass. In L.E. Moser et al. (ed.) Warm-season (C4) grasses. ASA Monogr. 45. ASA, CSSA, and SSSA, Madison, WI.

Wofford, D.S., and A.A. Baltensperger. 1985. Heritability estimates for turfgrass characteristics in bermudagrass. Crop Sci. 25:133–136.

Wu, Y.Q., C.M. Taliaferro, G. Bai and M.A. Anderson. 2005. Genetic diversity of *Cynodon transvaalensis* Burtt-Davy and its relatedness to hexaploid *C. dactylon* (L.) Pers. as indicated by AFLP markers. Crop Sci. 45:848–853.

Wu, Y.Q., D.L. Martin, C.M. Taliaferro, J.A. Anderson, and J.Q. Moss. 2013. Northbridge turf bermudagrass. USPP 24,116. U.S. Patent and Trademark Office.

Wu, Y.Q., D.L. Martin, C.M. Taliaferro, J.A. Anderson, and J.Q. Moss. 2014. Latitude 36 turf bermudagrass. USPP 24,271. U.S. Patent and Trademark Office.

Wu, Y.Q., D.L. Martin, J.Q. Moss, N.R. Walker, and C.H. Fontanier, 2019. Bermudagrass plant named 'OKC 1131'. USPP 2019/0364715 P1. U.S. Patent and Trademark Office.

Yu, S., 2017. Advanced turf-type bermudagrass and zoysiagrass experimental genotypes show marked variation in responses to drought stress under field conditions. M.S. thesis, Oklahoma State Univ., Stillwater, OK.

Yu, X., J.A. Kimball, and S.R. Milla-Lewis. 2018. High density genetic maps of St. Augustinegrass and applications to comparative genomic analysis and QTL mapping for turf quality traits. BMC Plant Biol. 18:346–357.

Zhang, L., P. Ozias-Akins, G. Kochert, S. Kresovich, R. Dean, and W. Hanna. 1999. Differentiation of bermudagrass (*Cynodon* spp.) genotypes by AFLP analyses. Theor. Appl. Genet. 98:895–902.

CHAPTER II

GENETIC AND QTL MAPPING IN AFRICAN BERMUDAGRASS

ABSTRACT

Cynodon transvaalensis Burtt-Davy is frequently used to cross with *C. dactylon* Pers. in the creation of F_1 hybrid cultivars that are some of the most widely used in the worldwide turf industry. However, molecular resource development in this species is limited. Accordingly, the objectives of this study were to construct a high-density genetic map, and to identify genomic regions associated with the establishment rate. In this study, we constructed the first high-density linkage map for African bermudagrass using a genotyping by sequencing approach based on 109 S_1 progeny. A total of 1,246 single nucleotide polymorphisms and 32 simple sequence repeat markers were integrated in the linkage map. The total length of nine linkage groups was 882.3 cM, with an average distance of 0.69 cM per interval. Four genomic regions were identified to be associated with sod establishment rate. The results provide important genetic resources towards understanding the genome as well as marker-assisted selection for improving the establishment rate in bermudagrass breeding.

INTRODUCTION

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) originated in southwestern Transvaal and the northern part of the central Cape Province of South Africa (Harlan et al., 1970a). The species has distinct morphological features, including unique yellowish-green color, slender stolons, short internodes, fine-textured leaves, and small stature as compared with other species in *Cynodon* L.C. Rich. (Harlan et al., 1970b). A small number of African bermudagrass cultivars such as Florida and Uganda were used on putting greens and tennis courts (Juska and Hanson, 1964). The usage of African bermudagrass as turfgrass is limited due to the high demand for water and fertilizers, reduced turf quality in summer, and purple color under cool temperatures (Taliaferro, 1992). However, the primary use of African bermudagrass is to serve as a parent to cross with tetraploid or hexaploid common bermudagrass to create interspecific F_1 hybrid cultivars (Burton, 1991; Taliaferro et al., 2006). Numerous hybrid cultivars have been widely used in the turf industry across the world since the 1960s. African bermudagrass contributes genes for several important traits related to turfgrass performance, including fine leaf blades, high sod density, tolerance to low mowing heights, and cold hardiness (Harlan et al., 1970a).

The base chromosome number of *Cynodon* species is nine (Forbes and Burton, 1963) and an estimated genome size of *C. transvaalensis* (2*n*=18) is ~540 Mbp in the haploid genome (Taliaferro and Lamle, 1997). The species is a largely self-incompatible, cross-pollinated taxon (Burton and Hart, 1967; Richardson et al., 1978). Kenworthy et al. (2006) estimated the broad-sense heritability of 21 African bermudagrasses traits related to turf performance, inflorescence, and morphology as having a range from 0.42 to 0.96,

indicating certain traits can be improved through recurrent selection. Studies have been conducted to quantify African bermudagrass genetic diversity with DNA markers (Wu et al., 2005; Zhang et al., 1999). However, African bermudagrass molecular resource development, such as the construction of linkage maps, lags behind some other turfgrass species, like zoysiagrass (*Zoysia* spp.) (Huang et al., 2016; Wang et al., 2015) and St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] (Yu et al., 2018). Linkage maps are one of the most important molecular tools in genetic and genomic research. To date, three African bermudagrass genetic linkage maps have been published based on one population (Bethel et al., 2006; Harris-Shultz et al., 2010; Khanal et al., 2017). The most recent map was composed of 125 single-dose restriction fragments (SDRF) and simple sequence repeat (SSR) markers (Khanal et al., 2017), and no high-resolution linkage map is available for African bermudagrass. Therefore, in order to advance the efficiency for mapping and discovery of quantitative trait loci (QTLs)/genes in African bermudagrass, a high-resolution linkage map is required (Mammadov et al., 2012).

Single nucleotide polymorphisms (SNPs) are the most abundant DNA polymorphisms that can be utilized as molecular markers to construct linkage maps (Mammadov et al., 2012). Next-generation sequencing (NGS) technologies provide efficient methods for discovering SNP markers in a fast and affordable way for both model and non-model species (Wang et al., 2015). Genotyping-by-sequencing (GBS), an approach based on NGS, has been developed to generate a large volume of SNPs using appropriate methylation-sensitive restriction enzymes to digest genomic DNA, while avoiding repetitive genomic regions to reduce the complexity of targeted genomes (Elshire et al., 2011). The GBS approach has been applied in SNP development and

linkage map construction in many grass species used for turf, biomass, and forage, such as switchgrass (*Panicum virgatum* L.) (Fielder et al., 2015), zoysiagrass (Holloway et al., 2018), St. Augustinegrass (Yu et al., 2018), and Chinese silvergrass (*Miscanthus sinensis* Andersson) (Dong et al., 2018; Liu et al., 2016). However, to date, no African bermudagrass linkage map has been constructed with SNP markers.

As popular interspecific hybrid bermudagrass (*C. dactylon* × *C. transvaalensis)* cultivars such as Tifway, Latitude 36®, Northbridge®, Tahoma 31® and TifTuf® are sterile, they can only be vegetatively propagated (Schwartz et al., 2018; Wu et al., 2013; Wu et al., 2014; Wu et al., 2019). Therefore, a fast establishment rate is critical to sod and sprig production. However, information on the genetic basis for controlling the establishment rate is not available. Accordingly, the objectives of this study were to construct a high-density genetic linkage map, and to identify QTLs associated with the establishment rate.

MATERIALS AND METHODS

Plant Materials

A mapping population was created by selfing a *C. transvaalensis* genotype OKC1163 (NTEP, 2017). The parent plant was planted into a 25 x 50 cm tray with greenhouse soil (Berger BM2, Berger, QC, Canada) in a greenhouse on Agronomy Farm, Oklahoma State University (OSU) in the spring of 2014. The temperature regime was maximum 35/21°C (day/night). Sixteen hours of photoperiod to meet the minimal 25 $mol \cdot m^{-2} \cdot d^{-1}$ daily light integral. The plant was placed on a bench that was relatively isolated from other bermudagrass plants, allowing it to produce selfed seed. Mature inflorescences were hand collected from the parent plant in June 2014 and stored after drying at ambient temperature for two weeks. Germination of 600 seeds was carried out in the greenhouse, and seedlings were transplanted into 3.8 cm diameter cone-tainers in March 2017. Initial visual screening to exclude presumed interspecific crossed progeny was conducted two months after germination based on typical *C. transvaalensis* morphological characteristics (*i.e.*, fine leaf blades, yellowish-green color, and short internodes). One-hundred twenty-four plants were selected from more than 300 putative selfed progeny.

Accurate selfed progeny identification was performed using SSR markers to remove unintended crossed progeny. Approximately 1g of fresh leaf tissue from each of the selected progeny plants in the greenhouse was collected and immediately placed in an icebox and then stored in a -80 °C freezer. A tissue homogenizer (Geno/Grinder; SPEX SamplePrep, Metuchen, NJ) was used to grind leaf samples for 90 seconds at 1,500 RPM. DNA was extracted following a modified phenol-chloroform extraction method (Nalini et

al., 2004). A NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to quantify DNA concentration, and each DNA sample was then diluted to 10 ng μL^{-1} in preparation for polymerase chain reaction (PCR). Eleven *C. transvaalensis* SSR primer pairs (PPs) (CTGA7-1983/1984, CTGA7-1987/1988, CTGA7-1993/1994, CTGA7-1995/1996, CTAAG8-2499/2500, CTAAG8-2501/2502, CTAAG8-2503/2504, CTAAG8-2505/2506, CTAAG8-2507/2508, CTAAT1-2509/2510, CTAAT1-2513/2514) developed by Tan et al. (2012) were employed to genotype the progeny. The PCR and gel electrophoresis were conducted following the procedures described by Fang et al. (2015). Progeny with different bands from the parent were identified as cross-pollinated, which were excluded from the study. In total, 109 self-pollinated progenies were retained for the following experiments.

Experimental Design, Establishment, and Management in A Replicated Field Trial

The 109 first-generation selfed (S_1) progeny and the parent were grown in 3.8 cm diameter cone-taniers with daily irrigation and bi-weekly fertilization with 12-4-8 (N-P-K) (Miracle Gro Lawn Products Inc., Marysville, OH) in the greenhouse since March 2017. To collect phenotypic data for turf establishment rate, greenhouse grown plants were transplanted to a nursery on the OSU agronomy farm (36°12'N lat; 97°08'W long), Stillwater, OK on 20 June 2017. The experiment was arranged as a randomized complete block design with three replications. Plot size was 0.9 by 0.9 m with 0.3 m wide alleys between neighboring plots. Each plot was established with one plant in the center. The soil type was a Kirkland silt loam (fine, mixed, superactive, thermic Udertic Paleustoll). Ronstar® 2G herbicide (oxidiazon; Bayer Environmental Science, Montvale, NJ) was applied at 2.2 kg ha^{-1} of active ingredient one day after transplanting to prevent weeds.

Based on the results of a soil chemical analysis, 289 kg ha^{-1} of 17-17-17 (N-P-K) (Chouteau Lime Co., Pryor, OK) was applied pre-plant and another 107 kg ha^{-1} of 46-0-0 (N-P-K) (Chouteau Lime Co., Pryor, OK) was applied on 10 August 2017 to promote growth. During the establishment phase, Permit® (halosulfuron-methyl; Gowan, Yuma, AZ) was spot sprayed to control yellow nutsedge (*Cyperus esculentus* L.) and Scimitar® (lambda-cyhalothrin; Syngenta, Greensboro, NC) to control fall armyworm [*Spodoptera frugiperda* (J.E. Smith)]. Alleys were sprayed with a 2% solution (v/v) of Roundup® [glyphosate; Monsanto, St. Louis, MO) and 0.25% nonionic surfactant to prevent contamination of adjacent plots. Irrigation was applied to prevent drought stress as predicted by weather data from a nearby weather station (Oklahoma Mesonet, http://www.mesonet.org/index.php/agriculture/monitor). Hand weeding was implemented as needed.

Genotyping-by-sequencing

Leaf samples of the 109 S_1 progenies and their parent were submitted to the University of Wisconsin-Madison Biotechnology Center for sequencing. DNA was extracted from the leaves of each plant using a standard CTAB protocol (Doyle, 1987). Each of the 109 progeny DNA sample was replicated twice and the parent sample was replicated 35 times in 48-plex reaction libraries. Libraries were prepared following the protocol described by Elshire et al. (2011) with minor modifications. Fifty µg of genomic DNA was digested with restriction enzyme 5-bp cutter *ApeK*I (New England Biolabs, Ipswich, MA). Then the digested DNA was ligated with barcode adapters (Elshire et al., 2011) amenable to Illumina sequencing with T4 ligase (New England Biolabs, Ipswich, MA). Adapter-ligated samples were pooled and amplified to provide library quantities

amenable for sequencing. The quality and quantity of the finished libraries were assessed using the Agilent Bioanalyzer High Sensitivity Chip (Agilent Technologies, Inc., Santa Clara, CA) and Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY), respectively. A cluster was generated using HiSeq SR Cluster Kit v3 cBot kits (Illumina, San Diego, CA, USA). The whole population was sequenced in three lanes on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) with 100 bp single-end reads. SNP discovery was conducted using the UNEAK pipeline in TASSEL V4.3.5 with following settings: c=5, e=0.03, mnMAF=0.05, mxMAF=0.5, mnC=0.1, mxC=1. Raw SNPs were filtered to improve quality with the following steps: 1. SNPs with missing data were removed; 2. all redundant SNPs were removed; and 3. SNPs were removed if the parent showed 'hh' or 'kk' genotypes. For diploid species, a minimal sequencing depth of 6x is needed to ensure 98% accuracy of calling heterozygotes by assuming a binomial distribution at two alleles per locus. The remaining SNPs were filtered again to improve the confidence of correct calling with the following steps: 1. SNPs were removed if more than 10% of individual genotypes had less than 6x total reading depth; 2. the genotypes with the reading depth of only 1x and 2x were converted into missing data; and 3. SNPs with more than 5% of missing data were removed.

Ssr Markers and Gel Scoring

To investigate the relevance of genetic maps between *C. dactylon* and *C. transvaalensis*, SSR markers that were mapped in a *C. dactylon* genetic map (Guo et al., 2017) were genotyped in the *C. transvaalensis* S_1 population. Two hundred and fifty-four *C. dactylon* SSR PPs were genotyped in this population based on Guo et al. (2017). Segregation classes, hh, hk, kk were used for scoring gels (Guo et al., 2017). Briefly, for

a heterozygous locus of the parent with two alleles, h and k, when only an upper band appeared in a progeny sample, it was scored as homozygous ‘hh’; when only one lower band was present, it was scored as homozygous ‘kk’; when two bands were present, the genotype was scored as heterozygous ‘hk; and the missing genotype (no band showing up) was scored as ‘-’.

Linkage Analysis

Linkage analysis was carried out using JoinMap 5.0 (Van Ooijen, 2006a). In JoinMap, it was reported that cross-pollination (CP) was not appropriate for the selfed population, and the markers segregate types <hkxhk> in coupling {00} and repulsion {11} phases in both parents would cause singularity errors in QTL analysis (Dong et al., 2015). Therefore, population type F_2 was used. The genotypes (hh, hk, kk) were converted to (a, h, b) based on the coupling (00) or repulsion (11) phase that was calculated by JoinMap. According to Dong et al. (2015), for (00) phase markers, hh>a, hk>h, kk>b; and for (11) phase markers, hh>b, hk>h, kk>a. Markers segregation ratios were calculated using Chi-square analysis to test the goodness of fit to the expected 1:2:1 ratio. The distorted segregation markers were kept for linkage analysis as it was expected that selfing-depression would be common in the selfed population. Van Ooijen (2006a) recommended using a starting logarithm of odds (LOD) value of 10 for a stringent test. Then we gradually decreased LOD value to regroup markers. A minimum LOD value of eight was selected because of using a smaller LOD value, markers from different linkage groups were grouped together. The regression mapping algorithm was employed with default settings except for using Kosambi as the mapping function to correct the linkage distance (Wang et al., 2015). Due to JoinMap regression algorithm not being able to

handle a large number of markers in a single group, marker order was initially determined based on the maximum likelihood algorithm with default settings. According to the result, redundant markers located at the same positions were manually removed. Subsequently, the regression algorithm was used to reestimate intermarker spacing. Another dataset excluding segregation distorted markers (chi-squared test, $P < 0.05$) was utilized to construct the linkage map with the same settings. Comparisons between the two linkage maps were drawn with MapChart (Voorrips, 2002).

Phenotypic Evaluation of Establishment Rate and QTL Analysis

The first set of visual establishment ratings on percent green cover (Cover1) was collected on 21 August 2017. A 0.9 by 0.9 m frame was used to mark the plot size to assist the percent green cover assessment. To obtain objective data, an image was taken in each plot (Cover2) on 10 October 2017 with a digital camera (Canon Powershot G1X, Canon U.S.A., Inc., Melville, NY) mounted on a custom-built lightbox having four compact fluorescent light bulbs as light sources (Richardson et al., 2001). Images were analyzed by Turf Analyzer (Karcher et al., 2017). After *C. transvaalensis* plants went dormant, the third set of visual percent green cover ratings was collected on 10 November 2017 (Cover3) to quantify the first growing season total growth. All the percent green cover data were analyzed by SAS 9.4 (SAS Institute Inc., Cary, NC). A General Linear Models Procedure (PROC GLM) with split plot in time where genotype as main plot and date as subplot was utilized to conduct the analysis of variance (ANOVA) test.

Mean percent green cover data for each genotype (N=3) were analyzed by date for QTL mapping with MapQTL 6.0 (Van Ooijen, 2006b). LOD threshold was calculated

by a permutation test of 1,000 iterations at a 95% confidence level. The interval mapping (IM) method with a regression algorithm was initially selected to detect QTL. Settings for calculation options were 'yes' for QTL analysis; fit dominance for F2 (IM); '1' for mapping step size; '5' for maximum number of neighboring markers; '200' for maximum number of iterations; '$1.0e^{-8}$' functional tolerance value; '$P = 0.02$' for automatic cofactor selection. Then the QTLs found in IM were further analyzed using multiple QTL model (MQM). Markers close to the peak LOD values in each LG were selected as cofactors in MQM analysis. The MQM approach was repeated until the selected cofactors consistently had the highest LOD values in each LG. A QTL graph was drawn using MapChart (Voorrips, 2002).

Linkage Map Comparison

The African bermudagrass linkage map based on OKC1163 was compared with the common bermudagrass linkage map reported by Guo et al. (2017). The *C. dactylon* SSR markers mapped on the *C. transvaalensis* linkages were used to reveal the relationship between the maps of the two species. Related linkage groups between *C. transvaalensis* and *C. dactylon* were drawn using MapChart (Voorrips, 2002).

RESULTS

Genotyping by Sequencing and SNP Calling

With the 109 progeny and the parent sequenced, a total of more than 712 million raw reads (71.2 Gbp) were obtained. The number of reads of each selfed progeny ranged from 2.1 to 7.5 million, with an average of 4.7 million. The parent was sequenced 35 times, a greater depth than progeny to accurately detect the segregation of SNPs. A total of 169 million raw reads were generated from the parent, and the number of reads for each replicate of the parent ranged from 2.8 to 6.8 million, with an average of 4.8 million reads. After the SNP calling and the sequenced reads were trimmed to 64 bp in length, 94,909 raw SNPs were obtained. After stringent filtering, 2,026 high-quality SNP markers were utilized for the subsequent linkage analysis.

SSR Marker Testing

Two hundred and fifty-four *C. dactylon* SSR PPs were screened on the parent and three progenies with two replications. One hundred and twenty-three PPs (50.4%) amplified bands representing alleles, in which 36 (29.3%) PPs were polymorphic. Those polymorphic PPs were genotyped in the whole population. Primer pair CDCA2-177 (5'-CACGACGTTGTAAAACGACTTGATGCACTTCCAACCAGT-3') and CDCA2-178 (5'-CGCACAGATTTGCTGATTCT-3') amplified each of two alleles at two loci. Totally 37 SSR markers were used for linkage map construction.

Linkage Mapping

Among the 2,063 SNP and SSR markers, 1,985 (96.2%) were mapped into 9 LGs, while the remaining 78 isolated markers were not able to group into any LG. Since no reference genome was available for *C. transvaalensis*, the designation of 1-9 LGs was

based on the grouping numbers of JoinMap. After removing redundant markers located at the same positions in each LG calculated by maximum likelihood algorithm, 1,278 markers (1,246 SNP and 32 SSR) (Supplementary file 1) were analyzed again with the regression mapping algorithm. A summary of the LGs is given in Table 1, and graphically displayed in Fig. 1. Nine LGs were generated with a total length of 882.28 cM with an average distance of 0.69 cM between neighboring markers. The number of markers in each LG ranged from 61 (LG9) to 267 (LG3), with an average of 142. The length of each LG ranged from 47.91 (LG7) to 143.60 cM (LG8). LG8 had the lowest marker density at a spacing of 2.05 cM per interval, while LG7 had the highest marker density at 0.37 cM per interval. Thirty-two of 37 (86.5%) SSR markers were mapped into eight LGs, and no SSR marker was mapped into LG2 (Table 2). The number of SSR markers in LGs1, 3, 4, 5, 6, 7, 8, and 9 were 9, 7, 3, 2, 3, 1, 2, and 5, respectively. Two loci amplified by PP CDCA2-177/178 were mapped, one on LG3 and another on LG5. One gap > 15 cM was found on LG8.

Segregation distortion ($P < 0.05$) was observed on 569 of 1,278 (44.5%) mapped loci. Segregation distortion loci were found in all nine LGs. The percentage of segregation distortion loci in each LG ranged from 3.4% (LG3) to 100% (LG7). The distorted loci were found unevenly distributed across LGs except LG7. The majority of the distorted loci were clustered together and formed segregation distortion regions at ends of LGs (Fig. 1). The distorted loci clustered in one end of LGs 1, 4, and 6 spanning more than 60, 40, and 80 cM, respectively. Among the 32 mapped SSR loci, seven (21.9%) were distorted ($P < 0.05$). The distorted SSR loci were mapped on LGs 1, 4, 6, 7, and 9.

To investigate how distortion segregation markers affected grouping, loci orders, and map length, a second linkage map was constructed without segregation distortion markers (Fig. 1). In the above mapping analysis, all markers on LG7 were distorted in segregation. Therefore, eight LGs were created with a LOD value of eight. The number of markers in each undistorted LG ranged from 12 (LG6a) to 256 (LG3a). Compared with each LG in the map that consisted of distorted and undistorted loci, the removal of distorted loci did not change the grouping of undistorted markers (Fig. 1). The total length of the undistorted linkage map was 499.77 cM, with an average distance of 0.71 cM per marker interval (Table 1). This linkage map with undistorted markers was 43.4% shorter than the map that included both distorted and undistorted loci. No gap larger than 15 cM was observed on the LGs1a-9a.

Establishment Rate (ER) and QTL Analysis

Three sets of percent green cover data were taken to quantify the establishment rate for each of the progeny and the parent. The ANOVA results indicated a significant evaluation date by genotype effect (Supplementary file 2 Table S1). The means and associated standard deviations for Cover1, Cover2, and Cover3 in the progeny population were 18.14 ± 11.93. 44.77 ± 32.74, and 58.15 ± 27.06, respectively (Supplementary file 3). The results of the percent green cover of the parent in Cover1, Cover2, and Cover3 were 22.67 ± 13.65, 66.87 ± 36.84, and 85.00 ± 15.00, respectively. Genotype means were highly and positively correlated (0.83 to 0.93) across evaluation dates.

The LOD threshold calculated for detecting ER QTL was four in the QTL analysis. Four QTLs for ER, designated *QCTER1-4*, were identified in the progeny population. They were located on LGs1, 3, and 6, which explained 8.9 to 32.7% of the

total phenotypic variation (Table 2). Among these QTLs, *QCTER2*, with the peak LOD values at positions 103.26 and 103.07 cM on LG3 was detected in all three datasets, explaining 17.8-32.7 % of the total phenotypic variation (Fig. 2). Other three QTLs were identified in only one dataset each (Table 2). The major QTL, *QCTER2* was closely linked to SNP markers, TP64377/TP12397 on LG3. The intra-locus effects of the QTLs linked markers are given in Table 2. The means and associated standard deviations for the percent green cover of the QTLs linked markers are given in Table 3.

Linkage Map Comparison

The incorporation of *C. dactylon* SSR markers in this *C. transvaalensis* linkage map allowed for an investigation of the genomic relationship between the two species. Among *C. transvaalensis* LGs, except LG2, each had at least one SSR marker. Each of *C. transvaalensis* LGs5, 6, 7, and 8 was connected to one *C. dactylon* LG, and each of *C. transvaalensis* LGs 1 and 4 corresponded to two *C. dactylon* LGs. The remaining two *C. transvaalensis* LGs (LGs3 and 9) were each bridged to three *C. dactylon* LGs ((Supplementary file 2 Fig. S1 to S8).

DISCUSSION

Selfed Mapping Population

Although *Cynodon* spp. can be easily propagated vegetatively by rhizomes or stolons, most *Cynodon* spp. plants still have the ability to sexually reproduce by seed (Richardson et al., 1978). Kneebone (1967) reported a low percent seed set produced by self-pollination with bagging, suggesting the mode of sexual reproduction in the bermudagrass taxon is through a high degree of cross-pollination with relatively high self-incompatibility (Richardson et al., 1978). However, *C. transvaalensis* genotype OKC1163 was able to produce a large amount of selfed seed, from which an S_1 population was developed for the linkage map construction in this study. Since the male and female gametes were produced on the same parent, only one genetic linkage map was constructed. Several other studies used S_1 populations to construct genetic linkage maps for outcrossing species such as *C. dactylon* (Guo et al., 2017), sugarcane (*Saccharum* spp.) (Andru et al., 2011), and switchgrass (Dong et al., 2015; Liu et al., 2012). Population size is an important factor in linkage map construction, which affects the linkage map accuracy. *C. transvaalensis* linkage maps reported by Bethel et al. (2006) and Harris-Shultz et al. (2010) included 113 and 118 gametes in linkage analysis. In this study, 218 gametes of 109 S_1 progeny were used to calculate genetic distances to form the *C. transvaalensis* linkage map. More gametes in this linkage analysis provided a higher level of accuracy in calculating the recombination frequency.

Linkage Map

Using the GBS approach, 109 selfed progeny and the parent OKC1163 were sequenced, and more than 94,000 SNP markers were generated. Eventually, 1,246 SNP

and 32 SSR markers were used to construct this map in *C. transvaalensis*. To our knowledge, this map was the first high-density genetic linkage map in the species. Bethel et al. (2006) constructed a framework linkage map of *C. transvaalensis* using an interspecific hybrid population between *C. dactylon* T89 x *C. transvaalensis* T574 based on single-dose restriction fragments (SDRFs). The T574 *C. transvaalensis* map was constructed with 77 markers on 18 LGs, with an average marker spacing of 16.5 cM (Bethel et al., 2006). Harris-Shultz et al. (2010) reported a T574 map based on 36 single-dose amplified fragments (SDAFs) spanning 311.1 cM. Khanal et al. (2017) enriched SSR markers to the SDRF framework of Bethel et al. (2006), producing eleven LGs spanning 1,184.7 cM with 52 SSR and 73 SDRF markers. Compared to the total markers used in the maps by Bethel et al. (2006), Harris-Shultz et al. (2010), and Khanal et al. (2017), 1,278 markers, more than 10 times of previously mapped, were included in the OKC1163 map.

C. transvaalensis can easily cross with *C. dactylon*, producing interspecific hybrids. To investigate the relationship between *C. transvaalensis* and *C. dactylon* LGs, we tested 244 *C. dactylon* SSR PPs, in which 36 PPs were polymorphic and one PP was multiallelic. The multiallelic PP CDCA2-177/178 mapped to LG3 and LG5 may indicate paralogous loci in *C. transvaalensis* genome. None of the published *C. transvaalensis* maps has reported primer pairs that were able to amplify two loci. However, in tetraploid species, Guo et al. (2017) reported four multiallelic PPs, which identified three homologous LGs in *C. dactylon*.

Each *C. transvaalensis* LG corresponded to at least one *C. dactylon* LG as compared to the linkage map by Guo et al. (2017) (Fig. S1-S8). Khanal et al. (2017) also

reported that most *C. transvaalensis* LGs corresponded to two *C. dactylon* LGs and some *C. transvaalensis* LGs corresponded to either one or three *C. dactylon* LGs. *C. dactylon* was considered an allopolyploid tetraploid species (Guo et al., 2015; Gong et al., 2013; Fang et al., 2020). More research is needed to definitively characterize the genomic relationship between the two closely related species. Once the whole genome sequence is available in one or both species, it is feasible to fully establish the relationship.

Segregation Distortion

Segregation distortion is a common phenomenon in cross-pollinated species like *C. dactylon* (Guo et al., 2017; Khanal et al., 2017), switchgrass (Fiedler et al., 2015; Liu et al., 2012), and zoysiagrass (Huang et al., 2016; Wang et al., 2015). Linkage maps constructed with segregation distorted markers may lead to incorrect marker orders due to spurious linkages (Cloutier et al., 1997). In this self-pollinated *C. transvaalensis* population, 44.5% of mapped markers were segregation distorted. The segregation distortion rate in this population was slightly higher compared to the segregation distortion rate of the self-pollinated *C. dactylon* population reported by Guo et al. (2017). Khanal et al. (2017) reported 12.1% of distorted loci in the *C. transvaalensis* parental map that derived from a *C. dactylon* × *C. transvaalensis* population. Our results are consistent with the higher tendency for segregation distortion due to selfing that theoretically generates homozygous loci of lethal alleles in the self-pollinated population as compared with the cross-pollinated population (Guo et al., 2017; Liu et al., 2012).

Most distorted markers were clustered near the central part or ends of the LGs. In LGs1, 2, and 4 of this map, most distorted markers were clustered at one half of LGs. But the distorted markers in LGs5 and 9 were scattered at both ends. All the markers in LG7

were distorted. Guo et al. (2017) also reported that the markers in two *C. dactylon* LGs were all distorted. We found that the total length of the linkage map with distorted markers was over 90% longer than the map that only included undistorted markers. Fielder et al. (2015) and Huang et al. (2016) reported 23% and around 10% increased length when adding distorted markers into switchgrass and zoysiagrass linkage maps based on cross-pollinated populations, respectively.

When comparing the shared markers and their orders between the distorted- and undistorted-marker linkage maps, we observed rearrangements on LGs1, 2, 3, 4, and 5. In LGs2 and 5, reversed marker orders were observed between neighboring markers (Fig. 1). LG1 experienced more extensive marker rearrangements, some markers were relocated to a new position a few markers away. On LGs3 and 4, marker orders were inverted at one end of each. If distorted markers were clustered on one or two ends of an LG, the marker orders appeared not to be affected by adding distorted markers. However, if distorted markers were located in the middle of an LG, marker orders were likely to be disrupted. This suggested that segregation distortion markers affected the linkage analysis, yet differently upon their locations in an LG. However, it is still recommended to keep distorted markers in linkage analysis to better understand LGs (Van Ooijen, 2006a). Segregation distortion marker clustering regions have not been analyzed in the *C. transvaalensis* genome before. The clustering regions of distorted markers usually contain lethal genes or incompatibility genes (Lefebvre et al., 1995). Therefore, having the knowledge of distorted loci are important in conventional breeding since distorted loci are important factors in sexual reproduction (*i.e.*, seed set) (Shinozuka et al., 2010).

QTLs of Establishment Rate in C. transvaalensis

This was the first QTL study of the establishment rate in *C. transvaalensis* based on a high-density linkage map. The results clearly showed that the *C. transvaalensis* ER was controlled by multiple loci. Totally, four QTLs were identified in this study. The QTL on LG3, *QCTER2*, with peak LOD value near TP64377/TP12397 were detected consistently across all three datasets. The consistency of QTLs at the same location in a LG is a good indication of the reliability of the results. One QTL, *QCTER1* with peak LOD value at 60.22 cM on LG1 was only detected in Cover1, another QTL, *QCTER3* at 37.48 cM on LG1 only detected in Cover3, and the QTL, *QCTER4* at 36.84 cM on LG6 was only detected in Cover3, suggest theses QTLs may play roles in different growth and developmental stages. Besides differences in growth and development stages, unrepeatable QTLs were likely the result of the gene by environment interaction, which may be affected by environmental conditions (i.e., soil type, water, light, and temperature) (Johnson et al., 2012). Guo et al. (2017) identified five QTLs associated with *C. dactylon* ER. They reported one QTL on *C. dactylon* LG18 to be reliable. In this study, we identified *QCTER4* in *C. transvaalensis* LG6, orthologous to *C. dactylon* LG18. In addition, the orthologous *C. transvaalensis* LG3 and *C. dactylon* LG16, both carried QTLs associated with ER. The QTL analysis in this study was based on a high-density genetic linkage map, which showed a greater detection resolution and the QTLs were mapped to smaller regions compared to the QTL mapping by Guo et al. (2017). Markers closely linked to QTLs provide a valuable tool in marker-assisted selection for ER. Once the whole genome sequence of *C. transvaalensis* is available, the sequences of these tightly linked SNP markers can anchor the physical locations of QTLs on *C. transvaalensis* chromosomes and be converted to breeder friendly PCR-based markers

that are specific for those respective QTLs. Those markers will be useful in marker-assisted selection for developing fast ER bermudagrass hybrid cultivars.

REFERENCES

Andru, S., Y.B. Pan, S. Thongthawee, D.M. Burner, and C.A. Kimbeng. 2011. Genetic analysis of the sugarcane (*Saccharum* spp.) cultivar 'LCP 85–384'. I. Linkage mapping using AFLP, SSR, and TRAP markers. Theor. Appl. Genet. 123:77–93.

Bethel, C.M., E.B. Sciara, J.C. Estill, J.E. Bowers, W.W. Hanna, and A.H. Paterson. 2006. A framework linkage map of bermudagrass (*Cynodon dactylon* × *transvaalensis*) based on single-dose restriction fragments. Theor. Appl. Genet. 112:727–737.

Burton, G.W. 1991. A history of turf research at Tifton. U.S. Golf Association Green Section Record 29:12–14.

Burton, G.W., and R.H. Hart. 1967. Use of self-incompatibility to produce commercial seed-propagated F_1 bermudagrass hybrids. Crop Sci.7:524–527.

Cloutier, S., M. Cappadocia, and B.S. Landry. 1997. Analysis of RFLP mapping inaccuracy in *Brassica napus* L. Theor. Appl. Genet. 95:83–91.

Dong, H., S, Thames, L. Liu, M.W. Smith, L. Yan, and Y.Q. Wu. 2015. QTL mapping for reproductive maturity in lowland switchgrass populations. BioEnergy Res. 8:1925–1937.

Dong, H., S. Liu, L.V. Clark, S, Sharma, J.M. Gifford, J.A. Juvik, et al. 2018. Genetic mapping of biomass yield in three interconnected Miscanthus populations. GCB Bioenergy 10:165–185.

Doyle, J.J. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One 6:e19379.

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

Fang, T., H. Dong, S. Yu, J.Q. Moss, C.H. Fontanier, D.L. Martin, J. Fu, and Y.Q. Wu. 2020. Sequence-based genetic mapping of *Cynodon dactylon* Pers. reveals new insights into genome evolution in Poaceae. Commun. Biol. doi:10.1038/s42003-020-1086-y

Fiedler, J.D., C. Lanzatella, M. Okada, J. Jenkins, J. Schmutz, and C.M. Tobias. 2015. High-density single nucleotide polymorphism linkage maps of lowland switchgrass using genotyping-by-sequencing. Plant Genome doi:10.3835/plantgenome2014.10.0065

Forbes, I., and G.W. Burton. 1963. Chromosome numbers and meiosis in some *Cynodon* species and hybrids. Crop Sci. 3:75–79.

Gong, Z., C. Xue, M. Zhang, and M. Wang. 2013. Distribution of rDNA loci and genome differentiation in tetraploid *Cynodon*. India J. Genet. Plant Breed. 73:459–461.

Guo, Y., Y.Q. Wu, J.A. Anderson, J.Q. Moss, and L. Zhu. 2015. Disomic inheritance and segregation distortion of SSR markers in two populations of *Cynodon dactylon* (L.) Pers. var. *dactylon*. PLoS One 10:e0136332.

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 doi:10.3835/plantgenome2016.07.0074

Harlan, J.R., J.M.J. de Wet, and K.M. Rawal. 1970a. Geographic distribution of the species of *Cynodon* L.C. Rich (*Gramineae*). East Afr. Agric. Fores. J. 36:220–226.

Harlan, J.R., J.M.J. de Wet, W.W. Huffine, and J.R. Deakin. 1970b. A guide to the species of *Cynodon* (Gramineae). Bull. Okla. Agric. Exp. Stn. B-673:37–37.

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. Amer. Soc. Hort. Sci. 135:511–520.

Holloway, H.M.P., X. Yu, J.C. Dunne, B.M. Schwartz, A.J. Patton, C. Arellano, et al. 2018. A SNP-based high-density linkage map of zoysiagrass (*Zoysia japonica* Steud.) and its use for the identification of QTL associated with winter hardiness. Mol. Breed. 38:10–24.

Huang, X., F. Wang, R. Singh, J.A. Reinert, M.C. Engelke, A.D. Genovesi, et al. 2016. Construction of high-resolution genetic maps of *Zoysia matrella* (L.) Merrill and applications to comparative genomic analysis and QTL mapping of resistance to fall armyworm. BMC Genomics 17:562–578.

Juska, F.V., and A.A. Hanson. 1964. Evaluation of bermudagrass varieties for general purpose turf. USDA-ARS, Agric. Handbook No. 270.

Johnson, E.B., J.E. Haggard, and D.A. St. Clair. 2012. Fractionation, stability, and isolate-specificity of QTL for resistance to *Phytophthora infestans* in cultivated tomato (*Solanum lycopersicum*). G3: Genes, Genomes, Genet. 2:1145–1159.

Karcher, D.E., C.J. Purcell, M.D. Richardson, L.C. Purcell, and W.H. Kenneth. 2017. New Java program to rapidly quantify several turfgrass parameters from digital images. CSSA Oral presentation. Retrieved from https://scisoc.confex.com/crops/2017am/webprogram/Paper109313.html

Kenworthy, K.E., C.M. Taliaferro, B.F. Carver, D.L. Martin, J.A. Anderson, and G.E. Bell. 2006. Genetic variation in *Cynodon transvaalensis* Burtt-Davy. Crop Sci. 46:2376–2381.

Khanal, S., C. Kim, S.A. Auckland, L.K. Rainville, J. Adhikari, B.M. Schwartz, et al. 2017. SSR-enriched genetic linkage maps of bermudagrass (*Cynodon dactylon* × *transvaalensis*), and their comparison with allied plant genomes. Theor. Appl. Genet. 130:819–839.

Kneebone, W.R. 1967. Breeding seeded bermudagrass; unique Arizona research problem. Prog. Agric. Ariz. 19:4–5.

Lefebvre, V., A. Palloix, C. Caranta, and E. Pochard. 1995. Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome 38:112–121.

Liu, L., Y.Q. Wu, Y. Wang, and T Samuels. 2012. A high-density simple sequence repeat-based genetic linkage map of switchgrass. G3: Genes, Genomes, Genet. 2:357–370.

Liu, S., L.V. Clark, K. Swaminathan, J.M. Gifford, J.A. Juvik, and E.J. Sacks. 2016. High-density genetic map of *Miscanthus sinensis* reveals inheritance of zebra stripe. GCB Bioenergy 8:616–630.

Mammadov, J., R. Aggarwal, R. Buyyarapu, and S. Kumpatla. 2012. SNP markers and their impact on plant breeding. Int. J. Plant Genomics doi:10.1155/2012/728398

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

National Turfgrass Evaluation Program (NTEP). 2017. Final Report NTEP No. 18-14. 2013 National Bermudagrass Test. Retrieved from http://www.ntep.org/reports/bg13/bg13_18-14f/bg13_18-14f.htm

Richardson, M.D., D.E. Karcher, and L.C. Purcell. 2001. Quantifying turfgrass cover using digital image analysis. Crop Sci. 41:1884–1881.

Richardson, 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.

Schwartz, B.M., W.W. Hanna, L.L. Baxter, P.L. Raymer, P.C. Waltz, A.R. Kowalewski, et al. 2018. ‘DT-1’, a drought-tolerant triploid turf bermudagrass. HortScience 53:1711–1714.

Shinozuka, H., N.O.I. Cogan, K.F. Smith, G.C. Spangenberg, and J.W. Forster. 2010. Fine-scale comparative genetic and physical mapping supports map-based cloning strategies for the self-incompatibility loci of perennial ryegrass (*Lolium perenne* L.). Plant Mol. Biol. 72:343–355.

Taliaferro, C.M. 1992. Out of Africa—A new look at "African" bermudagrass. U.S. Golf Association Green Section Record 30:10–12.

Taliaferro, C.M., and J.T. Lamle. 1997. Use of flow cytometry to estimate ploidy level in *Cynodon* species. Int. Turfgrass Soc. Res. J. 8:385–392.

Taliaferro, C.M., D.L. Martin, J.A. Anderson, and M.P. Anderson. 2006. Patriot turf bermudagrass. USPP 16,801. U.S. Patent and Trademark Office.

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 sequences and preexisting sorghum SSR markers. Mol. Breed. 29:23–30.

Van Ooijen, J.W. 2006a. JoinMap 5: Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, the Netherlands.

Van Ooijen, J.W. 2006b. MapQTL 6: Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma B.V., Wageningen, the Netherlands.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93:77–78.

Wang, F., R. Singh, A.D. Genovesi, C. Wai, X. Huang, A. Chandra, et al. 2015. Sequence-tagged high-density genetic maps of *Zoysia japonica* provide insights into genome evolution in Chloridoideae. Plant J. 82:744–757.

Wu, Y.Q., C.M. Taliaferro, G. Bai, and M.P. Anderson. 2005. Genetic diversity of *Cynodon transvaalensis* Burtt-Davy and its relatedness to hexaploid *C. dactylon* (L.) Pers. as indicated by AFLP markers. Crop Sci. 45:848–853.

Wu, Y.Q., D.L. Martin, C.M. Taliaferro, J.A. Anderson, and J.Q. Moss. 2013. Northbridge turf bermudagrass. USPP 24,116. U.S. Patent and Trademark Office.

Wu, Y.Q., D.L. Martin, C.M. Taliaferro, J.A. Anderson, and J.Q. Moss. 2014. Latitude 36 turf bermudagrass. USPP 24,271. U.S. Patent and Trademark Office.

Wu, Y.Q., D.L. Martin, J.Q. Moss, N.R. Walker, and C.H. Fontanier. 2019. Bermudagrass plant named 'OKC 1131'. USPP 2019/0364715 P1. U.S. Patent and Trademark Office.

Yu, X., J.A. Kimball, and S.R. Milla-Lewis. 2018. High density genetic maps of St. Augustinegrass and applications to comparative genomic analysis and QTL mapping for turf quality traits. BMC Plant Biol. 18:346–357.

Zhang, L., P. Ozias-Akins, G. Kochert, S. Kresovich, R. Dean, and W.W. Hanna. 1999. Differentiation of bermudagrass (*Cynodon* spp.) genotypes by AFLP analyses. Theor. Appl. Genet. 98:895–902.

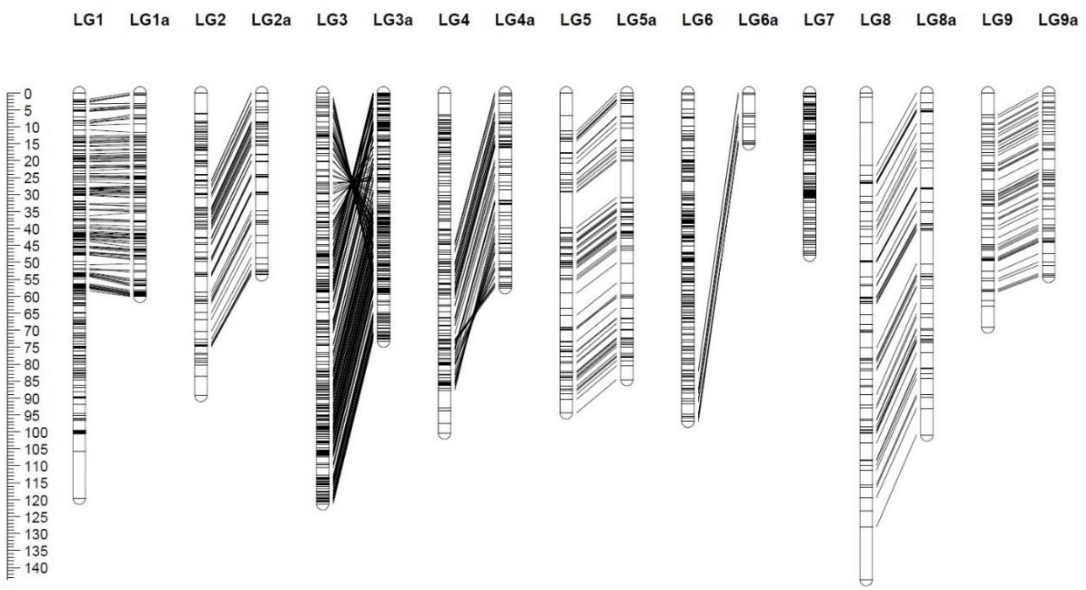

Fig. 1. Two *Cynodon transvaalensis* linkage maps, one (LG1-9) constructed with both undistorted and distorted markers and another (LGa1-9) only with undistorted loci (with suffix 'a' after each LG). Black lines between two LGs connect shared markers on the two maps showing how distorted loci affected the loci order. Length scale in cM on the left sides.

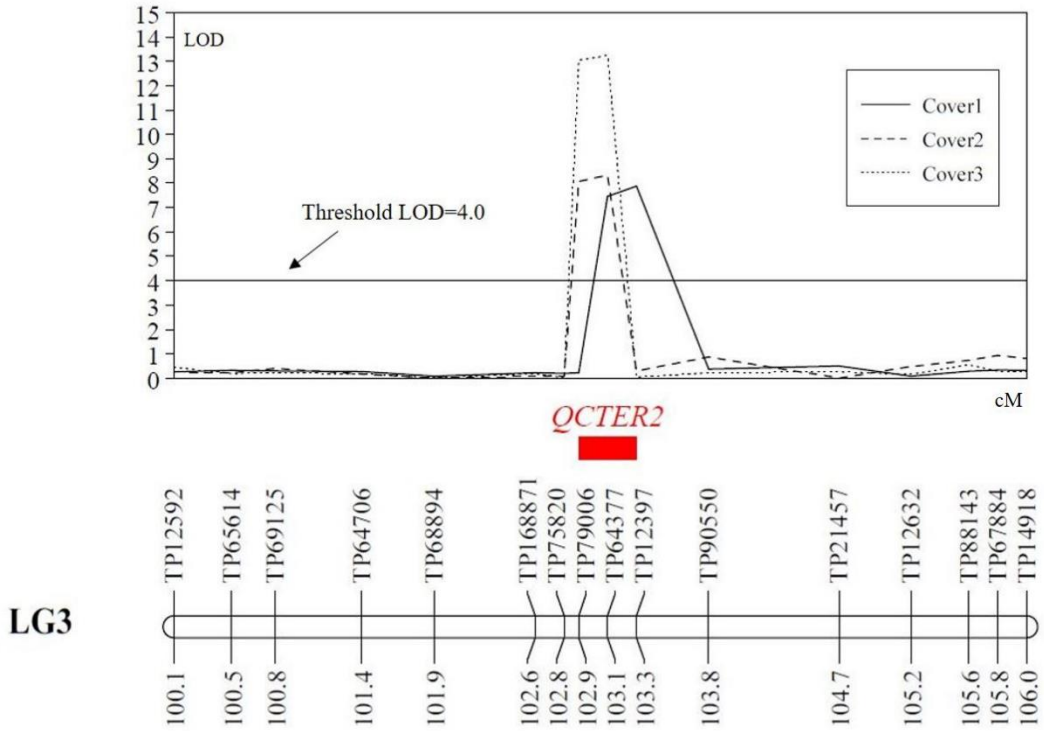

Fig. 2. A major quantitative trait locus, *QCTER2* associated with African bermudagrass establishment rate identified at three different time points located at the region on linkage group 3, where genetic distances in centiMorgan (cM) are given on the lower side of the map while SNP marker designations are shown on the upper side of the map bar. The horizontal line with a LOD value of 4.0 indicates the 95% significant threshold for QTL detection.

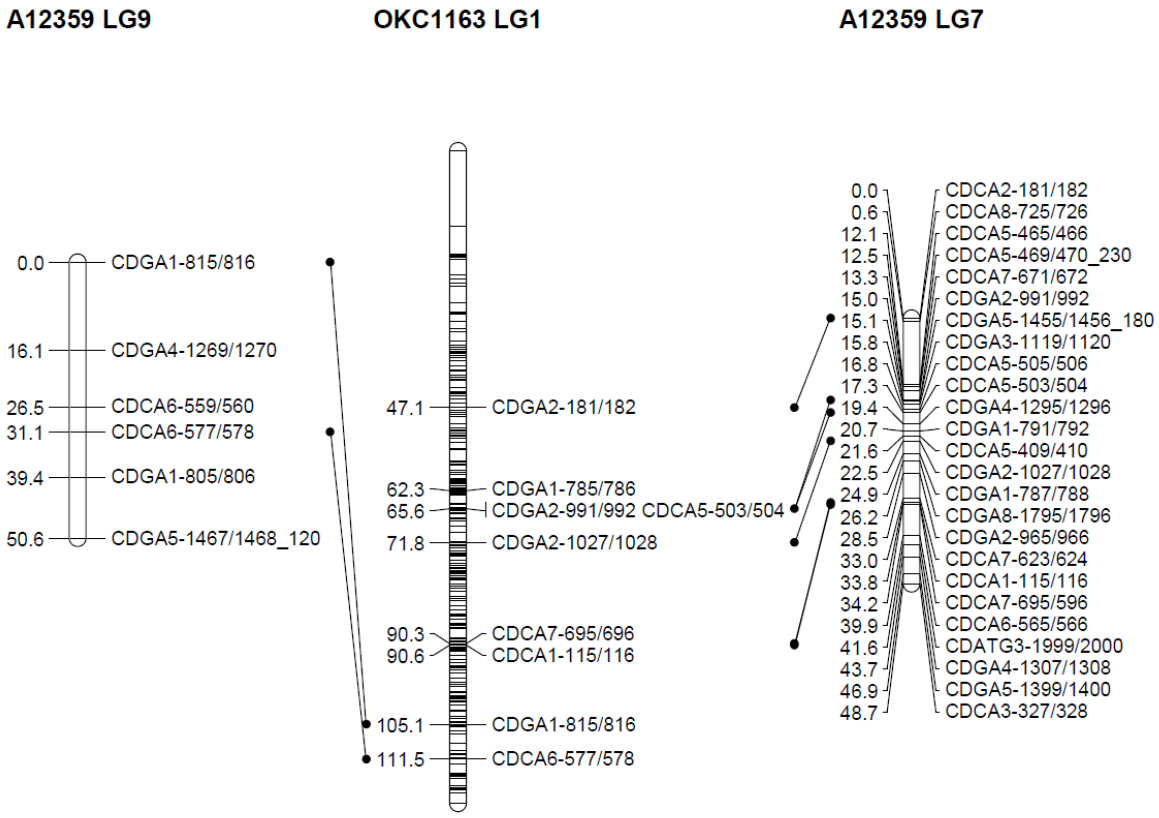

Fig. S1 African bermudagrass LG1 corresponding to common bermudagrass LGs7 and 9 (Guo et al. 2017).

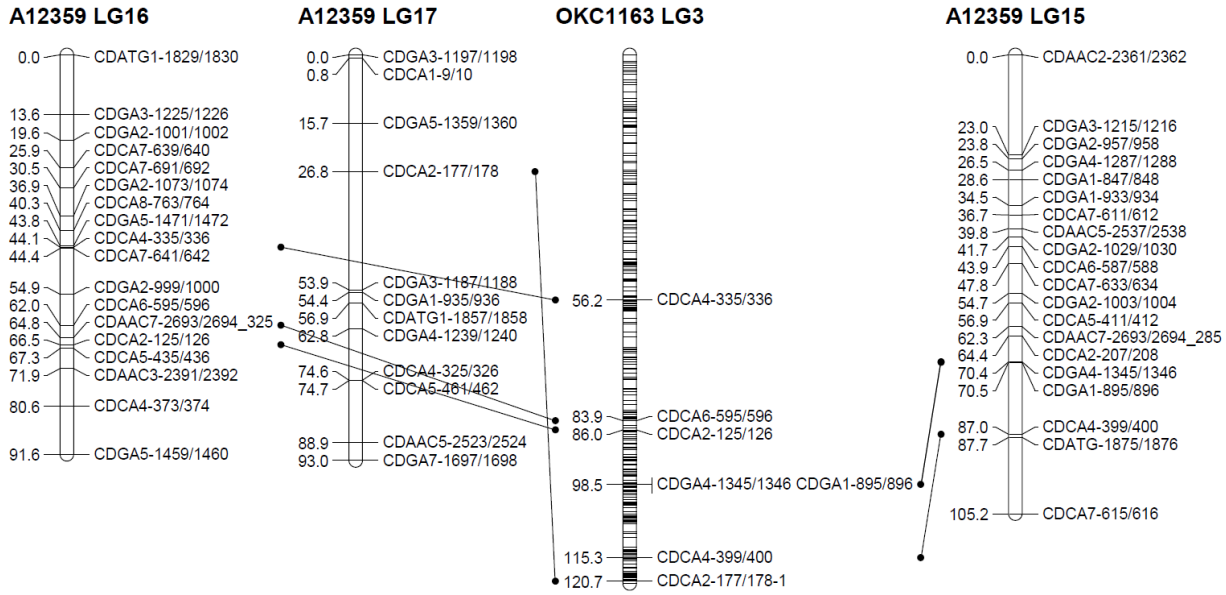

Fig. S2 African bermudagrass LG3 corresponding to common bermudagrass LGs15, 16, and 17 (Guo et al. 2017).

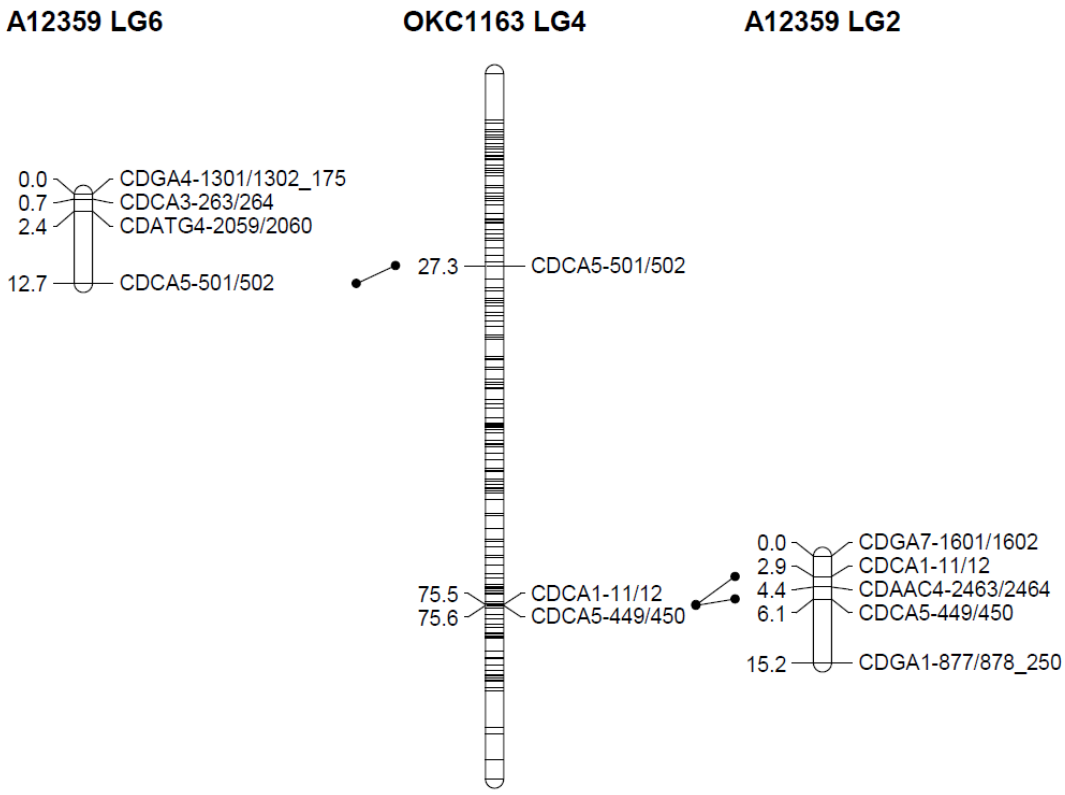

Fig. S3 African bermudagrass LG4 corresponding to common bermudagrass LGs2 and 6 (Guo et al. 2017).

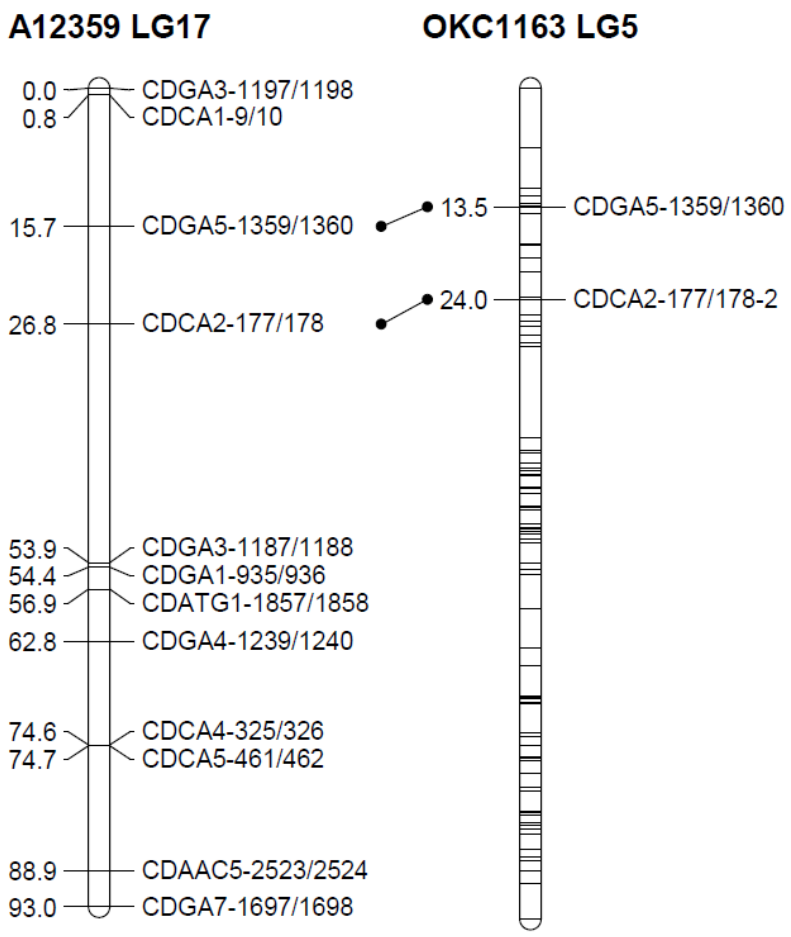

Fig. S4 African bermudagrass LG5 corresponding to common bermudagrass LG17 (Guo et al. 2017).

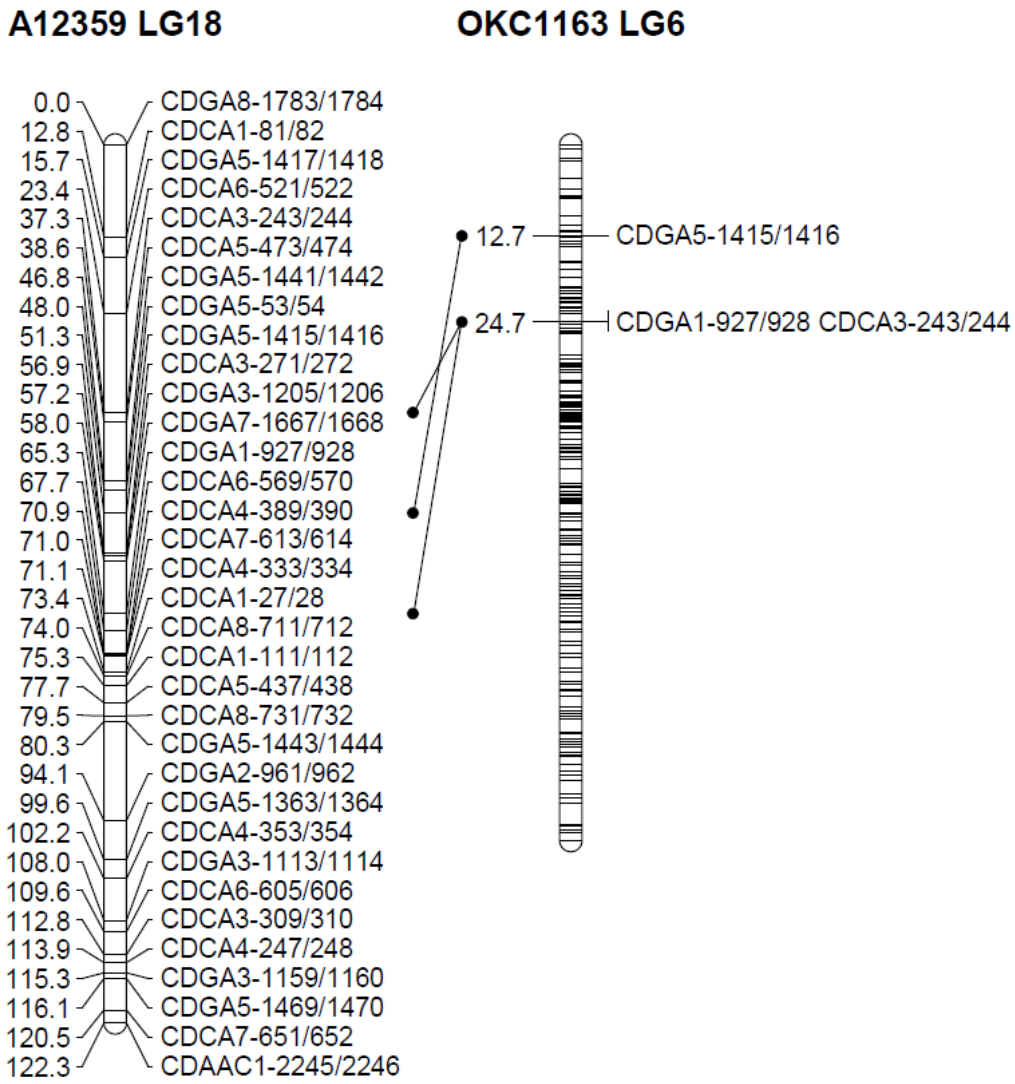

Fig. S5 African bermudagrass LG6 corresponding to common bermudagrass LG18 (Guo et al. 2017).

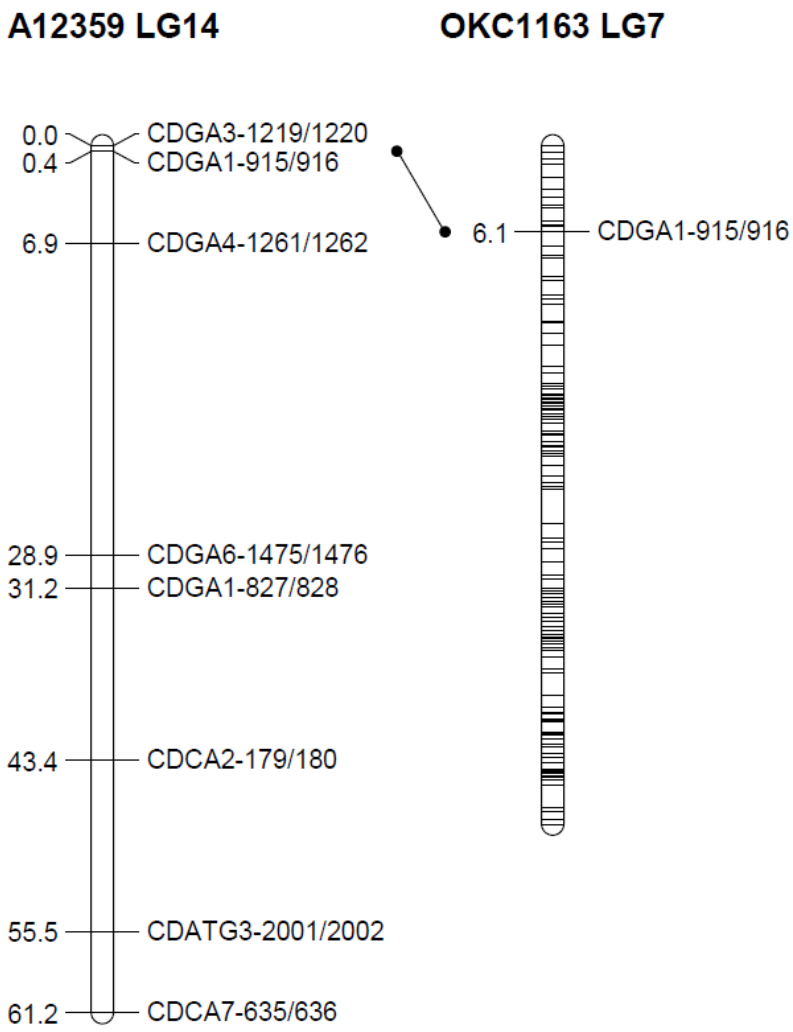

Fig. S6 African bermudagrass LG7 corresponding to common bermudagrass LG14 (Guo et al. 2017).

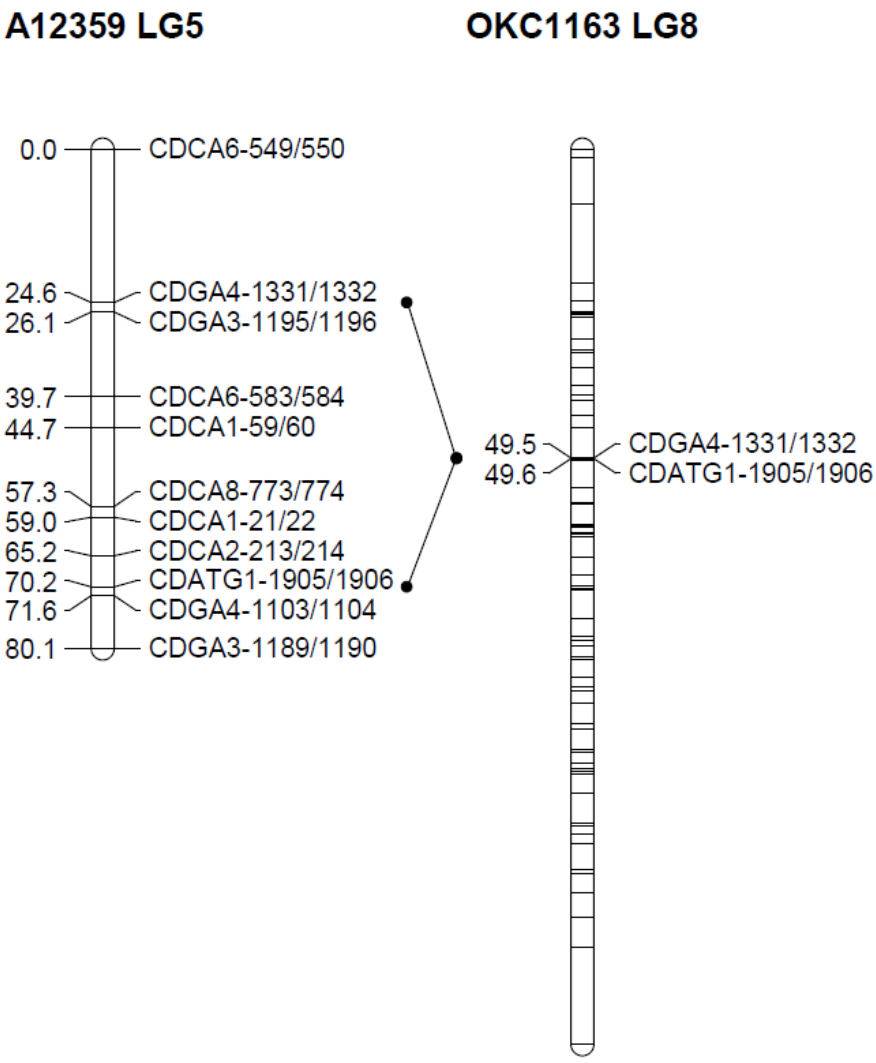

Fig. S7 African bermudagrass LG8 corresponding to common bermudagrass LG5 (Guo et al. 2017).

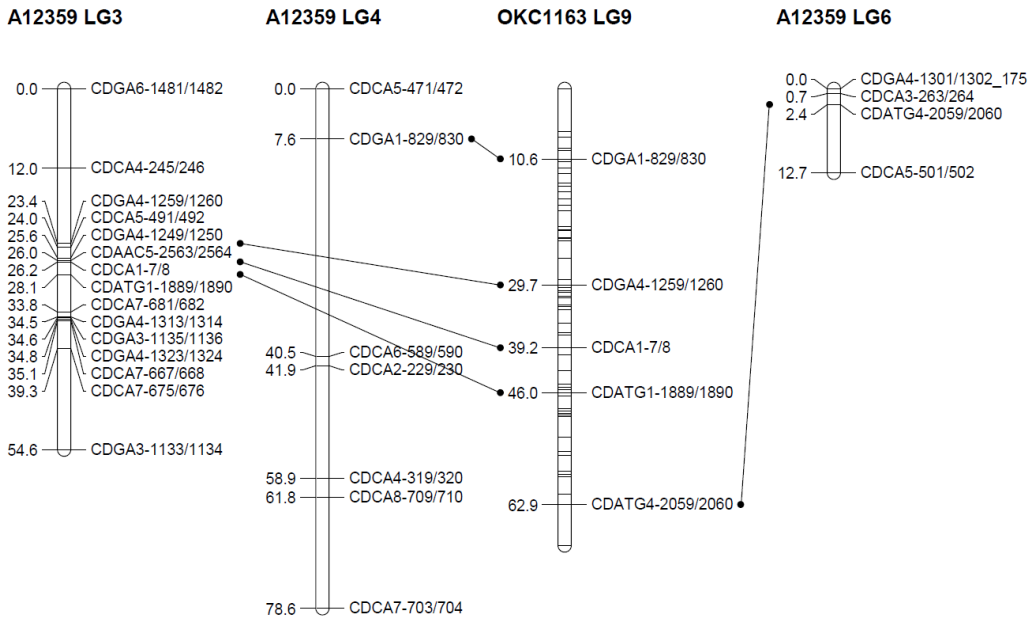

Fig. S8 African bermudagrass LG9 corresponding to common bermudagrass LGs3, 4, and 6 (Guo et al. 2017).

Table 1. Linkage group (LG) assignment, loci numbers, LG length, marker interval, and the number of gaps in *Cynodon transvaalensis* genetic maps constructed with all markers and with only undistorted markers based on a self-pollinated population of OKC1163.

Linkage map with all markers					Linkage map with undistorted markers				
LG	Total loci	Total length (cM)	cM/ Marker interval	Gap (>15 cM)	LG	Total loci	Total Length (cM)	cM/ Marker interval	Gap (>15 cM)
LG1	230	119.57	0.52	0	LG1a	131	60.60	0.46	0
LG2	98	89.27	0.91	0	LG2a	53	53.70	1.01	0
LG3	267	121.24	0.45	0	LG3a	256	73.18	0.29	0
LG4	157	100.33	0.64	0	LG4a	77	57.47	0.75	0
LG5	77	94.43	1.23	0	LG5a	68	84.60	1.24	0
LG6	188	96.88	0.52	0	LG6a	12	15.04	1.25	0
LG7	130	47.91	0.37	0					
LG8	70	143.60	2.05	1	LG8a	55	100.97	1.84	0
LG9	61	69.11	1.13	0	LG9a	56	54.21	0.97	0
Total	1278	882.28		1	Total	708	499.77		0
Average	142	98.03	0.69	0.11	Average	88.5	62.47	0.71	0

Table 2. Quantitative trait loci identified using interval mapping for establishment rate in a self-pollinated population from *Cynodon transvaalensis* OKC1163.

Dataset	LG	QTL	LOD peak	Position of LOD peak (cM)	Locus	Phenotypic variance explained	Additive	Dominance
Cover1	LG1	*QCTER1*	5.51	60.22	TP101403	12.10%	-12.38	13.61
	LG3	*QCTER2*	7.88	103.26	TP12397	17.80%	-3.62	5.98
Cover2	LG3	*QCTER2*	8.33	103.07	TP64377	26.60%	-14.09	17.04
Cover3	LG1	*QCTER3*	6.37	37.48	TP81783	13.40%	12.31	-0.84
	LG3	*QCTER2*	12.23	103.07	TP64377	32.70%	-13.84	18.40
	LG6	*QCTER4*	4.39	36.84	TP70570	8.90%	24.07	28.53

[†]LG, linkage group; QTL, quantitative trait loci; LOD, logarithm of the odds.

Table 3. Means and standard deviations of three percent green cover data collections in an S_1 population of two SNP markers that are closely linked to a major QTL, *QCTER2*.

Marker	Genotype	Cover1%	Cover2%	Cover3%
			Mean ± SD	
TP64377	A (kk)	9.94 ± 5.38b[†]	21.24 ± 16.97b	35.22 ± 18.83b
	H (hk)	20.16 ± 6.89a	52.75 ± 20.60a	65.77 ± 16.33a
	B (hh)	19.25 ± 9.46a	48.29 ± 25.34a	59.76 ± 22.08a
TP12397	A (hh)	9.94 ± 5.38b	21.24 ± 16.97b	35.22 ± 18.83b
	H (hk)	20.46 ± 7.23a	53.32 ± 21.75a	66.04 ± 17.43a
	B (kk)	20.32 ± 10.88a	50.19 ± 25.40a	61.90 ± 22.02a

[†]Same letter within a column in each marker is not significant at $P < .05$.

Table S1. Analysis of variance for the effects of entry, date, block and their interaction, on establishment rate during the growing season of 2017.

Source	df	SS	MS	F
Entry	108	325871	3017	27.76***[a]
Block	2	16553	8276	76.14***
Entry*Block	216	165222	765	7.04***
Date	2	267691	133845	1231***
Entry*Date	216	59620	276	2.54***
Date*Block*Entry	432	45760	109	
Total	980			

[a]***, significant at $P < 0.1\%$.

CHAPTER III

GENETIC VARIABILITY AND QTL MAPPING OF WINTER SURVIVABILITY AND DROUGHT RESPONSE IN AFRICAN BERMUDAGRASS

ABSTRACT

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) has been extensively used to cross with common bermudagrass (*C. dactylon* Pers. var. *dactylon*) in the creation of F_1 hybrid cultivars that are widely used in the worldwide turf industry. Turf bermudagrass is susceptible to winterkill when grown in the transition zone and water scarcity in urban areas is an increasing concern of broad societal significance. Improvement in the winter hardiness and drought resistance of bermudagrass will benefit the turfgrass industry. However, little information on molecular basis for winter survivability and drought resistance is available in African bermudagrass. Accordingly, the objectives of this study were to quantify genetic variability and to identify quantitative trait loci (QTL) associated with winter survivability traits and drought response. A total of 109 first-generation self-pollinated (S_1) progeny of *C. transvaalensis* 'OKC1163' were evaluated in a field trial with three replications in a randomized complete block design for three seasons. Significant genetic variance existed for all traits, and the broad-sense heritability estimates ranged from 0.36 to 0.81. Seven QTLs identified for winter survivability and two for drought response, and five of them were

recurrent QTL in mapping the phenotypic data on to a preexisting dense linkage map. Co-localizations were found between winter survivability and drought response QTL, which explained the significant correlation between the two traits. The results provide important genetic resources towards understanding the genetic basis associated with winter survivability and drought resistance as well as marker-assisted selection for turf bermudagrass improvement.

INTRODUCTION

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) is a diploid ($2n = 2x = 18$) species with origins in the southwestern Transvaal and the northern part of the central Cape Province of South Africa (Harlan et al. 1970a; Harlan et al. 1970b). The species has unique morphological characteristics such as fine leaf texture and high sod density. It can adapt to much colder climates beyond its natural distribution due to its cold hardiness (Harlan et al. 1970a). African bermudagrasses have been crossed with common bermudagrasses (*C. dactylon* Pers. var. *dactylon*) to produce fine-textured, high quality, interspecific F_1 hybrid cultivars, such as 'Tifgreen' and 'Tifway' (Taliaferro, 1992). Interspecific hybrid bermudagrasses (*C. dactylon* × *C. transvaalensis*) have been widely used on sports fields, golf courses and home lawns in the southern and transition zone of the United States. Therefore, African bermudagrass is essential to the turfgrass industry (Burton 1991; Taliaferro et al. 2006).

When grown in the northern areas of the transition zone, bermudagrass can be vulnerable to winterkill (Taliaferro et al. 2004). Breeding efforts are needed to develop turf bermudagrass cultivars with improved freeze tolerance (Anderson and Taliaferro 2002). Freezing tolerance can be defined as the ability of nodes within rhizomes and stolons of bermudagrass to survive low winter temperatures and resume normal growth in the spring (Levitt, 1980). A series of physiological changes occurs at 8/2 ^{o}C (day/night), including shifts in protein synthesis, osmotic protectants, and fatty acid saturation (Fontanier et al. 2020; Munshaw et al. 2006; Zhang et al. 2006) that can gradually increase the plant hardiness under freezing temperatures (Levitt 1980). Field winter survivability evaluation has been widely used in identifying superior freeze tolerant

genotypes in bermudagrass (Stefaniak et al. 2009; Wu and Anderson 2011). Due to the highly negative correlation between winterkill and earliness of spring regrowth (Wu et al. 2007), early spring greenup has been used as a proxy for selecting winter hardiness. Substantial efforts have been devoted to understanding bermudagrass spring greenup (Dunne et al. 2019; Guo et al. 2017; Kenworthy et al. 2006; Stefaniak et al. 2009; Wu et al. 2007). Previous studies showed that the heritability of African bermudagrass spring greenup were high (Kenworthy et al. 2006), indicating genetic factors play a major role in African bermudagrass winter survivability. However, the genetic mechanisms in association with African bermudagrass winter survivability are elusive.

Another important challenge for the turfgrass industry is the limited water resource for irrigation due to the large areas of previously non-irrigated lands are being converted to irrigated lawns along with urbanization. Using drought resistant turfgrass species and cultivars has been identified as a key management strategy to address this concern (Carrow 1995; Marcum et al. 1995). Therefore, developing bermudagrass cultivars with improved drought resistance could have broad impacts on reducing municipal water consumption. To identify superior drought resistant genotypes, turfgrass breeders often evaluate turf canopy responses under diminishing soil moisture using visual and sensor-based measurements. Substantial variations have been reported in bermudagrass canopy drought response (Richardson et al. 2010; Steinke et al. 2011; Yu 2017). However, the genetic mechanisms associated with African bermudagrass canopy response to drought stress are unknown.

Quantitative trait loci (QTL) identification has been used as a primary method to identify genomic regions on a linkage map or chromosomes that are associated with trait

phenotypes. Previous studies have shown that cold tolerance is a complex quantitative trait and QTL associated with the trait have been identified in meadow fescue (*Festuca pratensis* Huds.) (Alm et al. 2011), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Wang et al. 2014), St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] (Kimball et al. 2018), perennial ryegrass (*Lolium perenne* L.) (Paina et al. 2016), and zoysiagrass (*Zoysia japonica* Steud.) (Holloway et al. 2018). Similarly, drought resistance is also a quantitative trait and QTL of drought resistance have been detected in St. Augustinegrass (Yu et al. 2019), meadow fescue (Alm et al. 2011), and creeping bentgrass (*Agrostis* spp.) (Merewitz et al. 2012). So far, no QTL for winter survivability and drought response has been reported in African bermudagrass. Recently, a high-density African bermudagrass linkage map with 1,278 markers was reported (Yu et al. 2020). This map provides a solid foundation in mapping QTL for winter survivability and drought response. Accordingly, the objectives in the present study were to quantify the genetic variability and detect the genomic regions associated with winter survivability and drought response in African bermudagrass.

MATERIALS AND METHODS

Plant Materials and Field Experiment

A total of 109 first-generation self-pollinated progenies (S_1) constituted the mapping population as described by Yu et al. (2020). The parent 'OKC1163' and its S_1 progeny were transplanted to a field nursery on the Oklahoma State University Agronomy Farm (36°12' N. lat.; -97°08' W. long.), Stillwater, OK on June 20, 2017. The experimental design was a randomized complete block with three replications. The soil type was Kirkland silt loam (fine, mixed, superactive, thermic Udertic Paleustoll). The plot size was 0.9 x 0.9 m and alleys were 0.3 m wide between neighboring plots.

Based on the results of soil analysis, 289 kg ha^{-1} of 17-17-17 (N-P-K) (Chouteau Lime Co., Pryor, OK) was applied as base fertilizer before transplanting. Ronstar 2G herbicide (oxidiazon; Bayer Environmental Science, Montvale, NJ) was applied at 2.3 kg ha^{-1} of active ingredient one day after transplanting to prevent weeds. Another 107 kg ha^{-1} of urea (46-0-0, N-P-K) (Chouteau Lime Co., Pryor, OK) was applied on August 10, 2017 to promote growth. In 2018, 107 kg ha^{-1} of urea (46-0-0, N-P-K) was applied in the second week of May and another 107 kg ha^{-1} of urea (46-0-0, N-P-K) was applied in the second week of August to promote growth. In 2019, based on the soil test, 289 kg ha^{-1} of 17-5-5 (N-P-K) (Chouteau Lime Co., Pryor, OK) was applied in the second week of May and 107 kg ha^{-1} of urea (46-0-0, N-P-K) was applied in the second week of August. Plots were mowed at 2.5 cm in late March in 2018, 2019, and 2020 to remove the dormant canopy for spring regrowth. After most plots greened up, the trial was only mowed at 3.8 cm to achieve consistent growth in early May from 2018 to 2020. In the middle of August in 2019, plots were mowed at 3.8 cm to remove the wilted canopy for recovering

from drought stress. Permit (Halosulfuron-methylmethyl; Gowan Company, Yuma, AZ) was spot sprayed to control yellow nutsedge (*Cyperus esculentus* L.) in 2017 and 2018. Scimitar (Lambda-cyhalothrin; Syngenta, Greensboro, NC) was used to control fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] in 2017. Alleys were sprayed with 2% Roundup (glyphosate; Monsanto, St. Louis, MO) and 0.25% nonionic surfactant to prevent excessive stolon growth to avoid contamination. Based on the weather data from the Oklahoma Mesonet, adequate irrigation was applied to prevent any drought stress in 2017 during the establishment phase, and no supplemental irrigation was applied from 2018 to 2020. Hand weeding was frequently implemented throughout the study.

Data Collection

Spring greenup (SG) data were collected in the spring from 2018 to 2020. One image was taken per plot on March 26, April 3, and April 13 in 2018, April 17 and April 26 in 2019 and April 17 and April 28 in 2020 with a digital camera (Canon Powershot G1X, Canon U.S.A., Inc., Melville, NY) mounted on a custom-built lightbox with four compact fluorescent light bulbs as light sources (Richardson et al. 2001). Images were analyzed by Turf Analyzer 1.01 (Karcher et al. 2017) for percent green cover (SGPGC). Visual SG ratings were taken on March 25, April 2, and April 12 in 2018, April 16, April 22, and April 29 in 2019, and April 7, April 13 and April 23 in 2020. The scale for spring greenup was 1-9, where 1 represented no greenup and 9 represented completely greenup (Morris and Shearman, 2000). Winterkill (WK) visual ratings were taken on May 10, 2018, May 17, 2019, and May 12, 2020 to estimate the percentage of ground coverage killed by the winter low temperatures. Leaf firing (LF) visual ratings were taken on

August 6, 2019 and August 26, 2020 on a 1-9 scale, where 1 represented completely straw-colored leaves and 9 represented completely green leaves.

Statistical Analysis

All effects were considered as random effects because year and rating date within the year were not chosen based on expected environmental conditions (Gordon et al. 1972), and the information on traits performance for this S_1 population was unknown. Analysis of variance (ANOVA) for SG, SGPGC, WK, and LF was conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) MIXED Procedure (PROC MIXED) with repeated measurements and variance components were estimated using TYPE3 method of moments estimation (Chang et al., 2016). Pearson correlation coefficients were calculated using the PROC CORR.

The broad-sense heritability (*H*) for WK and LF calculated using the following formula: $H= \sigma_G^2/ (\sigma_G^2+ \sigma_{GY}^2/Y+ \sigma_E^2/RY)$. The broad-sense heritability (*H*) for SG and SGPGC calculated using the following formula: $H= \sigma_G^2/ (\sigma_G^2+ \sigma_{GY}^2/Y+ \sigma_{GR}^2/R+\sigma_{GDY}^2/DY+\sigma_E^2/RDY)$, where σ_G^2 represents the variance of genotype, σ_{GY}^2 represents the variance of genotype by year interaction, σ_{GR}^2 represents the variance of genotype by replication interaction, σ_{GD}^2 represents the variance of genotype by date, σ_{GDY}^2 represents the variance of genotype by date within the year interaction, σ_E^2 represents the error variance, R represents the number of replication, Y represents the number of year, and D represents the number of rating date (Hallauer 1970).

Linkage Map and QTL Analysis

The detailed information about sequencing and linkage analyses were reported by Yu et al. (2020). QTL analysis was performed using MapQTL 6.0 (Van Ooijen 2006).

The mean values of traits from each rating date were analyzed separately. The genome-wide logarithm of the odds (LOD) threshold value was calculated by a 1,000 permutation test and determined at a significant *p*-value of 0.05. The Interval mapping (IM) method with default settings were initially selected to detect QTL. Then the QTL found in IM were analyzed again using multiple QTL model (MQM). Markers close to the peak LOD values in each linkage group (LG) were selected as cofactors in MQM analysis. The MQM approach was repeated until the selected cofactors consistently have the highest LOD values in each LG. Graphical representation of QTL was generated by using MapChart 2.2 (Voorrips 2002).

RESULTS

The means, ranges, distributions and other statistics for nine sets of SG, seven sets of SGPGC, three sets of WK, and two sets of drought response trait, LF are given in Table 1 and Fig. 1 to 4. Compared to the parent, the mean values of this S_1 population showed more winterkill, later spring greenup, and more leaf firing. Although not statistical different, the genotype '104' consistently showed less leaf firing compared to OKC1163 in both years. Similarly, the genotypes '7', '23', and '89' showed less winterkill compared to OKC1163 in three years even they were not statistically different.

The ANOVA results of SG, SGPGC, WK, and LF are presented in Table 2. Significant ($P < 0.01$) genotype effects were found for each of the four traits. The effects of replication were significant ($P < 0.05$) for all the traits except for SGPGC. The effects of genotype by replication interactions were highly significant ($P < 0.0001$) for all the traits except LF. Year effects were not significant for SG and SGPGC but highly significant ($P < 0.0001$) for WK and LF. Genotype by year interactions were significant ($P < 0.05$) for all winter survivability and drought response traits. The genotype by date within year interaction in SGPGC was highly significant while the SG genotype by date within year was not significant.

The variance components estimate and broad-sense heritability for winter survivability and drought response traits are given in Table 2. The variance for SG genotype by date within the year was negative as estimated by TYPE3 method of moments estimation. In this case, the variance component was considered zero since the negative variance value was logically impossible. The largest variance component was error variances for all the winter survivability related traits while the largest variance for

drought response was genotype effect. The broad-sense heritability estimates for winter survivability related traits ranged from 0.36 to 0.54 (Table 2). Digital image analysis had a heritability estimate of 0.41, which was better than 0.36 of visual ratings, indicating the technology improvement evaluation of this trait. The largest variance component was genotype variance for LF, and the broad-sense heritability was high at 0.81.

Correlation Analysis

Due to the significant genotype by year interactions, data were analyzed by year. Significant correlation coefficients among winter survivability traits are presented in Table 3. Highly positive relationships ($r > 0.75$) were found between SG and SGPGC in each year. A highly negative relationship was found for SG and WK in 2018 and 2020, while relationships between SG and WK were only moderately negative in 2019. Leaf firing was found to be significantly correlated with winter survivability in 2020. However, in 2019, LF only correlated with SGPGC. Correlation coefficients between phenotypes of the same traits were calculated between years (Table 4). All the traits had significant correlations between years except for the SGPGC correlation between 2018 and 2020. The highest correlation between years was LF between 2019 and 2020 (0.7, $P < 0.0001$).

QTL Analyses

Spring Greenup

In total, six QTL were identified in association with SG (Table 5, Fig. 5). *QCTSG1*, between 25.55 to 36.84 cM on LG6, was consistently detected in each of the three years. It explained 10.4 to 24.3% phenotypic variation of spring green up. *QCTSG2*, detected in each of the three years, was located in the region from 77.50 to 81.99 cM on

LG4 and explained 11 to 20.9% of phenotypic variation. *QCTSG3* was detected in all the visual ratings in 2019. This QTL was located at 82.5 cM on LG1, explaining 11.3 to 14.6% of phenotypic variation. *QCTSG4* was only detected once in the visual ratings in 2019. This QTL was located at 70.93 cM on LG6 near marker TP102272, accounting for 10.2% of phenotypic variation. *QCTSG5*, located on LG1, explained 20.3% phenotypic variation, and was only detected in early visual rating in 2020. *QCTSG6*, between 105.24 and 105.78 cM on LG3, was only detected in 2020.

QCTSG1, *QCTSG2*, and *QCTSG6* were identified to varying degrees for SGPGC. *QCTSG1* and *QCTSG2* were detected every year and accounted for 10.4 to 17.3% to phenotypic variation. *QCTSG6* was only detected in 2020 and accounted for 16.1% phenotypic variation. The SG and SGPGC means and associated standard deviations for three respective genotypes of the two *QCTSG1* and *QCTSG2* linked markers are given in Table 6. The homozygous genotype B of both *QCTSG1* flanking markers and one of the *QCTSG2* flanking marker TP48420 consistently had later greenup than the genotypes A and H in three years. The homozygous genotype A of another *QCTSG2* flanking marker TP4427 had earlier greenup than the genotypes B and H in three years (Table 6).

Winterkill

QCTWK1 was detected in all three years, located on LG6 between 34.27 to 36.84 cM, and accounted for 23.1 to 35.6% of phenotypic variation (Table 5, Fig. 5). *QCTWK1* showed negatively dominant and additive intra-locus genetic effects in three years, indicating heterozygous (H) and homozygous (A) genotypes of the resistance allele reduced winterkill occurrence by 25.2-33.3% and 20.8-35.4%, respectively (Table 5). The similar magnitude of effects between A and H suggested the full dominance between

the resistance allele and non-resistance allele. The WK means and associated standard deviations for three respective genotypes of the two *QCTWK1* linked markers are given in Table 6. The homozygous genotypes B of both markers consistently had more winterkill than the genotypes A and H in three years (Table 6).

Leaf Firing

Two QTL were identified for LF. Both *QCTLF1* and *QCTLF2* were detected in 2019 and 2020. *QCTLF1* was located on LG3 between position 115.45 and 119.06 cM, accounting for 20.2 and 32.5% phenotypic variation in 2019 and 2020, respectively (Table 5, Fig. 5). *QCTLF2* was located on LG8 at position 13.71 cM in 2019 and 14.71 cM in 2020, accounting for 15.7 and 17.6% of phenotypic variation, respectively. The LF means and associated standard deviations for three respective genotypes of the two *QCTLF1* linked markers are given in Table 6. The homozygous genotypes A of both markers consistently have lower LF phenotype expression (i.e., drought susceptibility) than the genotypes B and H in the two years (Table 6).

DISCUSSION

Heritability estimates help plant breeders understand the portion of a phenotype influenced by genetic factors as opposed to environmental factors and determine what selection procedure should be implemented to make improvements (Bokmeyer et al. 2008). In this African bermudagrass S_1 population, the moderate SG heritability was due to the large genotype by year and residual variance, suggesting both genetic and environmental factors influence phenotypic expression. Bermudagrass winter survivability is affected by cold acclimation (Fontanier et al. 2020), freeze tolerance (Anderson et al. 1993; Anderson and Taliaferro 2002), and deacclimation (Chalmers and Schmidt 1979). Therefore, frequent freeze events during cold acclimation and deacclimation stages fluctuate winter survival year by year. In addition, tissue desiccation caused by drought stress in summer and local dry spots have been found altered the bermudagrass winter survivability (Deboer et al. 2017; Yu et al. 2020). This African bermudagrass population was under drought stress in the summer of 2019. The drought conditions likely caused injuries to plants in the population, which may have further decreased the SG heritability estimates. Compared to SG, the heritability estimate of SGPGC was slightly increased, which was consistent with the finding that using an objective and quantitative method, instead of visual ratings, will improve the heritability estimates due to the reduced error variance (Karcher and Richardson 2003; Kenworthy et al. 2006). The moderate heritability estimate of WK was similar to what has been reported in common bermudagrass by Wu et al. (2007). Winterkill can be used as a more reliable indicator to evaluate winter survival performance of bermudagrass genotypes.

Leaf firing is the most widely used indicator of drought response of turfgrasses (Morris and Shearman 2000). The heritability of leaf firing was 0.81, indicating genetic factors largely controlled the drought response in this African bermudagrass population. A previous study showed that the heritability of drought response was low to moderate based on a hybrid bermudagrass population (Yu et al. 2020). Stress-related traits are susceptible to environmental conditions and the heritability estimation decreased under stressed environments (Rose et al. 2007; Schwartz et al. 2009). In this study, the low genotype by year variance suggested that the environmental conditions that caused drought stress in both years were similar, which may have explained the high LF heritability. Substantial winter survivability and drought response genetic variability in this population are valuable in breeding interspecific cultivars by selecting superior plants as parents in conventional breeding. The significant correlation between winter survivability and drought response (Table 3) suggests that the possibility of improved turf bermudagrass cultivars with combined winter survivability and drought response.

QTL analysis has been used to identify molecular markers linked to cold tolerance related traits in warm-season turfgrasses such as zoysiagrass, St. Augustinegrass, and centipedegrass, and markers closely linked to major QTL showed potential to be used in marker-assisted selection for improving freeze tolerance (Holloway et al. 2018; Kimball et al. 2018; Wang et al. 2014). However, molecular markers linked to winter survivability in African bermudagrass remained unexplored due to the lack of high-density linkage maps. Recently, a high-density linkage map having 1,278 SNP and SSR markers was developed from the OKC1163 S_1 population providing a robust foundation for association study for important agronomic traits in African bermudagrass. This is the first

report of QTL identified for spring greenup, winterkill, and drought response in African bermudagrass. A total of seven QTL associated with winter survivability traits were identified and five of them were at least detected in two environments. Spring greenup QTL *QCTSG1* and *QCTSG2* were identified in all the three years, suggesting these genomic regions were consistently contributing to spring greenup in African bermudagrass. The rest of SG QTL were only detected once in a single year, suggesting these QTL were interacted with the specific environment in each year. On the contrary, the only identified winterkill QTL, *QCTWK1* was consistently detected from 2018 to 2020, indicating this is the reliable genomic region for African bermudagrass winter survivability.

Greater values of LF indicate the turfgrass ability to prevent desiccation and maintain photosynthesis that can be achieved by various mechanisms, including greater water uptake, less evapotranspiration, and osmotic adjustments (Huang 2008). Drought resistance is a complex quantitative trait in turfgrass species (Merewitz et al. 2012; Yu et al. 2019). Yu et al. (2019) reported that 18 QTL were associated with LF in St. Augustinegrass, and explained phenotypic variation between 5.1 to 20.4%. Similar findings also reported by Merewitz et al. (2012), suggested that the different environments such as the rate of dry down and microclimate conditions may affect drought response. In the present study, two QTL were consistently detected for drought response in 2019 and 2020. The repeated QTL are consistent with the high drought response heritability, suggesting these are important genomic regions associated with drought response. The major QTL *QCTLF1* associated with resistance to drought stress was located at the distal of LG3. African bermudagrass LG3 is largely syntenic to rice

chromosome one (data not shown). Rice chromosome one carries QTL that are involved in several traits like rooting depth, deep root weight, total root dry weight, and osmotic adjustment (Kamoshita et al. 2008). To understand whether the function of *QCTLF1* is associated with drought avoid mechanisms such as rooting characteristics or drought tolerant mechanisms such as osmotic adjustment, further studies are needed.

There were three genomic regions on LGs 3 and 6 in which QTL were identified for two or more traits. One genomic region on LG6 around position 36 cM harbored QTL for both SG and WK. The major drought response QTL *QCTLF1* are co-located with spring greenup *QCTSG6*, in a region of 10-14 cM on LG3.

The co-localization between *QCTSG1* and *QCTWK1* on LG6 can explain the significantly negative correlation between SG and WK in both common bermudagrass (Wu et al. 2007) and African bermudagrass. The co-location between SG and WK QTL has also been observed in St. Augustinegrass (Kimball et al. 2018). The co-location between WK and SG QTL identified in our study reinforces that this is the true genic region associated with winter survivability in African bermudagrass. Therefore, this overlapped region on LG6 could be a promising candidate region for fine mapping and used in the future to develop DNA markers in accelerating the process of breeding winter hardy bermudagrass cultivars. Co-localized QTL can also be found between different adaptive traits in African bermudagrass. Spring greenup *QCTSG1* and winterkill *QCTWK1* were co-located with establishment rate (ER) *QCTER3*; another co-localized QTL was found between *QCTSG6* and *QCTER2* (Yu et al. 2020). Holloway et al. (2018) also reported the QTL co-location between spring greenup and establishment in zoysiagrass. The overlapping between ER and SG QTL suggests these can be a

pleiotropic effect QTL or several QTL tightly linked in this genomic region. Co-locating with the major ER QTL, *QCTSG6* was only detected in 2020, might suggest that the spring greenup in 2020 was the combination of both spring greenup and reestablishment. *QCTSG6* was only detected in late spring data. It is possible that this QTL interacted with specific environment that associated with rapid growth after initial spring greenup. However, we cannot completely rule out the role that *QCTSG6* played in African bermudagrass winter survival. The primary drought response QTL *QCTLF1* can also be considered overlapped with *QCTSG6* even the distances between these QTL are greater than 10 cM because in initial QTL analyses using IM, the LOD values for both SG and LF in this genomic region were greater than their LOD threshold values, and this may explain the significant correlation was found between winter survivability and drought response, especially in 2020.The co-location of low-temperature and drought tolerance was found in meadow fescue (Alm et al. 2011) and soybean [*Glycine max* (L.) Merr.] (Zhang et al. 2012). The transcriptional factors involved in stress responses, including drought and cold responses, are well documented (Nakashima et al. 2014). But we cannot conclude that transcriptional factors play major roles in drought and low-temperature responses in African bermudagrass since *QCTSG6* was only detected in 2020. On the contrary, the connection between drought response and establishment rate was consistent. The major LF and ER QTL overlapping suggest that the fast-growing genotypes will have less drought response. Bermudagrass root length and mass affect drought response by taking up more moisture from soil profile to avoid drought stress (Yurisic, 2016). Therefore, it is possible that the below-ground growth and above-ground growth are correlated and both are control by genes in this genomic region on LG3.

Future Application for Turf Bermudagrass Improvement

Turf bermudagrass improvement relies on convention breeding. A large amount of winter survivability and drought response genetic variability within this population provides an opportunity to select elite genotypes as parents to create interspecific hybrids. Phenotypic recurrent selection cannot be used in interspecific hybrid bermudagrass ($2n = 3x = 37$) due to their reproductive sterility. The QTL identified in this study shed light on bermudagrass MAS. One major WK QTL, *QCTWK1*, has been identified in the current study can be used to select for winter survivability. Using two *QCTWK1* flanking markers, TP70570 and TP16800, genotypes A and H show low winterkill compared to genotype B. Similarly, using TP65354 and TP30607, two *QCTIF1* flanking markers, can help select genotypes with improved drought response. Genotypes B and H consistently show high LF values (i.e., drought resistance) compared to genotype A. The SNP markers close to the QTL should also provide a valuable tool for breeding resistant parents for generating F1 interspecific hybrids in turf bermudagrass. However, breeders cannot use SNP markers in MAS as no functional markers are available for these important bermudagrass genes because the whole genome sequence of African bermudagrass is not available. Kompetitive Allele Specific PCR (KASP) markers have been developed and validated in wheat based on SNP markers (Fang et al. 2020; Rasheed et al. 2016). Using the information of QTL associated SNP markers from this African bermudagrass population, KASP markers can be converted and become breeder-friendly markers in MAS for developing winter hardy and drought resistant bermudagrass hybrid cultivars.

CONCLUSION

Cold hardiness and drought resistance are two key traits in developing new turf bermudagrass cultivars. In this study, significant phenotypic variances were observed and the broad-sense heritabilities were estimated for winter survivability and drought response based on the 109 S_1 progeny of OKC 1163 African bermudagrass. Seven genomic regions associated with winter survivability and two genomic regions associated with drought response were identified in this study. Four of these QTL were repeatedly detected across environments to indicate their reliability and some genomic regions were co-located with multiple traits. These stable QTL can be used in fine-mapping to identify the genes associated with winter survivability and drought response. Also, the SNP markers closely linked to the important QTL have potential in MAS to accelerate the improvement of cold hardiness and drought resistance in bermudagrass breeding efforts.

REFERENCES

Alm V, Busso CS, Ergon Å, Rudi H, Larsen A, Humphreys MW, Rognli OA (2011) QTL analyses and comparative genetic mapping of frost tolerance, winter survival and drought tolerance in meadow fescue (*Festuca pratensis* Huds.). Theor Appl Genet 123:369–382

Anderson JA, Taliaferro CM (2002) Freeze tolerance of seed-producing turf bermudagrasses. Crop Sci 42:190–192

Anderson, JA, Taliaferro CM, Martin DL (1993) Evaluating freeze tolerance of bermudagrass in a controlled environment. HortScience 28:955

Anderson JA, Taliaferro CM, Martin DL (2003) Longer exposure durations increase freeze damage to turf bermudagrasses. Crop Sci 43:973–977

Bokmeyer JM, Bonos SA, Meyer WA (2009) Broad-sense heritability and stability analysis of brown patch resistance in tall fescue. HortScience 44:289–292

Burton GW (1991) A history of turf research at Tifton. US Golf Association Green Section Record 29:12–14

Carrow RN (1995) Drought resistance aspects of turfgrasses in the southeast: evapotranspiration and crop coefficients. Crop Sci 35:1685–1690

Chalmers D, Schmidt R (1979) Bermudagrass survival as influenced by deacclimation, low temperatures, and dormancy. Agron J 71:947–949

Chang, D., Y. Wu, L. Liu, S. Lu-Thames, H. Dong, C. Goad, et al. 2016. Quantitative trait loci mapping for tillering-related traits in two switchgrass populations. The plant genome 9. doi: 10.3835/plantgenome2016.01.0010

DeBoer EJ, Karcher DE, McCalla JH, Richardson MD (2017) Effect of late-fall wetting agent application on winter survival of ultradwarf bermudagrass putting greens. Crop Forage Turfgrass Management: e20035, doi.org/10.1002/cft2.20035

Dong H, Thames S, Liu L, Smith MW, Yan L, Wu YQ (2015) QTL mapping for reproductive maturity in lowland switchgrass populations. BioEnergy Res 8:1925–1937

Dunne JC, Tuong TD, Livingston DP, Reynolds WC, Milla-Lewis SR (2019) Field and laboratory evaluation of bermudagrass germplasm for cold hardiness and freezing tolerance. Crop Sci 59:392–399

Fang T, Lei L, Li G, Powers C, Hunger RM, Carver BF, Yan L (2020) Development and deployment of KASP markers for multiple alleles of Lr34 in wheat. Theor Appl Genet 133:2183–2195

Fontanier CH, Moss JQ, Gopinath L, Goad CL, Su K, Wu YQ (2020) Lipid composition of three bermudagrasses in response to chilling stress. J Amer Soc Hort Sci 145:95–103

Gordon IL, Byth DE, Balaam LN (1972) Variance of heritability ratios estimated from phenotypic variance components. Biometrics 28:401–415

Guo Y, Wu YQ, Moss JQ, Anderson JA, Zhu L (2017) Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. Crop Sci 57:1–7

Hallauer AR (1970) Genetic variability for yield after four cycles of reciprocal recurrent selections in maize. Crop Sci 10:482–485

Harlan JR, de Wet JMJ, Rawal KM (1970a) Geographic distribution of the species of *Cynodon* L.C. Rich (Gramineae). East Afr Agric Forest J 36:220–226

Harlan JR, de Wet JMJ, Huffine WW, Deakin JR (1970b) A guide to the species of *Cynodon* (Gramineae). Bull Okla Agric Exp Stn B 673:37

Holloway HMP, Yu X, Dunne JC, Schwartz BM, Patton AJ, Arellano C, Milla-Lewis SR (2018) A SNP-based high-density linkage map of zoysiagrass (*Zoysia japonica* Steud.) and its use for the identification of QTL associated with winter hardiness. Mol Breed 38:10–24

Huang B (2008) Mechanisms and strategies for improving drought resistance in turfgrass. Acta Horticulturae 783:221–227

Huang X, Wang F, Singh R, Reinert JA, Engelke MC, Genovesi AD, Chandra A, Yu Q (2016) Construction of high-resolution genetic maps of *Zoysia matrella* (L.) Merrill and applications to comparative genomic analysis and QTL mapping of resistance to fall armyworm. BMC Genomics 17:562–578

Kamoshita A, Babu RC, Boopathi NM, Fukai S (2008) Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crop Res 109:1–23

Karcher DE, Richardson MD (2003) Quantifying turfgrass color using digital image analysis. Crop Sci. 43:943–951

Karcher DE, Purcell CJ, Richardson MD, Purcell LC, Kenneth WH (2017) New Java program to rapidly quantify several turfgrass parameters from digital images.

CSSA Oral presentation. https://scisoc.confex.com/crops/2017am/webprogram/Paper109313.html

Kenworthy KE, Taliaferro CM, Carver BF, Martin DL, Anderson JA, Bell GE (2006) Genetic variation in *Cynodon transsvaalensis* Burtt-Davy. Crop Sci 46:2376–2381

Khanal S, Kim C, Auckland SA, Rainville LK, Adhikari J, Schwartz BM, Paterson AH (2017) SSR-enriched genetic linkage maps of bermudagrass (*Cynodon dactylon* × *transvaalensis*), and their comparison with allied plant genomes. Theor Appl Genet 130:819–839

Kimball JA, Tuong TD, Arellano C, Livingston DP Milla-Lewis SR (2018) Linkage analysis and identification of quantitative trait loci associated with freeze tolerance and turf quality traits in St. Augustinegrass. Mol Breeding 38:67

Levitt J (1980) Responses of Plants to Environmental Stress, 2nd ed. Academic Press, New York

Marcum KB, Engelke M, Morton SJ, White RH (1995) Rooting characteristics and associated drought resistance of zoysiagrasses. Agron J 87:534–538

Merewitz EB, Belanger FC, Warnke SE, Huang B (2012) Identification of quantitative trait loci linked to drought tolerance in a colonial × creeping bentgrass hybrid population. Crop Sci 52:1891–1901

Morris KN, Shearman RC (2000) NTEP Turfgrass Evaluation Guidelines. [Online]. Available at http://www.ntep.org/pdf/ratings.pdf. National Turfgrass Evaluation Program. Beltsville, MD

Munshaw GC, Ervin EH, Shang C, Askew SD, Zhang X, Lemus RW (2006) Influence of late-season iron, nitrogen, and seaweed extract on fall color retention and cold tolerance of four bermudagrass cultivars. Crop Sci 46:273–283

Paina C, Byrne SL, Studer B, Rognli OA, Asp T (2016) Using a candidate gene-based genetic linkage map to identify QTL for winter survival in perennial ryegrass. PLoS One 11:e0152004

Rasheed A, Wen W, Gao F et al (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor Appl Genet 129:1843–1860

Richardson MD, Karcher DE, McCalla J (2010) Drought tolerance of 15 bermudagrass cultivars. Arkansas Turfgrass Report 2009, Ark Ag Exp Stn Res Ser 579:112–115

Richardson MD, Karcher DE, Purcell LC (2001) Quantifying turfgrass cover using digital image analysis. Crop Sci 41:1884–1881

Rose IV LW, Das MK, Fuentes RG, Taliaferro CM (2007) Effects of high- vs. low-yield environments on selection for increased biomass yield in switchgrass. Euphytica 156:407–415

Schwartz BM, Kenworthy KE, Engelke M, Genovesi AD, Quesenberry KH (2009) Heritability estimates for turfgrass performance and stress response in *Zoysia* spp. Crop Sci 49:2113–2118

Stefaniak TR, Rodgers C, VanDyke R, Williams D, Phillips T (2009) The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. Crop Sci 49:1489–1495

Steinke K, Chalmers D, Thomas J, White R (2011) Bermudagrass and buffalograss drought response and recovery at two soil depths. Crop Sci 51:1215–1223

Taliaferro CM (1992) Out of Africa—a new look at "African" bermudagrass. USGA Green Section Record July/August:10-12

Taliaferro CM, Martin DL, Anderson JA, Anderson MP (2006) Patriot turf bermudagrass. USPP 16,801. United States Patent and Trademark Office

Taliaferro CM, Rouquette Jr FM, Mislevy P (2004) Bermudagrass and stargrass. pp 417-475 *In* Moser LE et al (eds) Warm-season (C_4) grasses. ASA Monogr 45 ASA, CSSA, and SSSA, Madison, WI

Van Ooijen JW (2006) MapQTL 6: Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma B.V., Wageningen, the Netherlands

Voorrips RE (2002) MAPCHART: software for the graphical presentation of linkage maps and QTLs. J Heredity 93:77–78

Wang P, Wang H, Zheng Y, LV Z, Liu J, Wang X (2014) Genetic mapping and QTL analysis for seed yield, vegetative characters and cold tolerance in centipedegrass (*Eremochloa ophiuroides*). Sci Hortic 176:97-104

Wang F, Singh R, Genovesi AD, Wai CM., Huang XE, Chandra A, Yu Q (2015) Sequence-tagged high-density genetic maps of *Zoysia japonica* provide insights into genome evolution in Chloridoideae. Plant J 82:744–757

Wu YQ, Taliaferro CM, Martin DL, Anderson JA, Anderson MP (2007) Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. Crop Sci 47:1985–1994

Wu YQ, Anderson JA (2011) Genetic improvement of cold hardiness in bermudagrass. In: Pessarakli M (Eds), Handbook of Plant and Crop Stress (3rd edition). CRC Press of Taylor & Francis Group, Boca Raton, FL. pp 851-865

Yu S (2017) Advanced turf-type bermudagrass and zoysiagrass experimental genotypes show marked variation in responses to drought stress under field conditions. MS thesis, Oklahoma State Univ, Stillwater, OK

Yu S, Fang T, Dong H, Yan L, Martin DL, Moss JQ, Fontanier CH, Wu YQ. 2020. Genetic and QTL mapping in African bermudagrass. Plant Genome doi.org/10.1002/tpg2.20073

Yu X, Kimball JA, Milla-Lewis SR (2018) High density genetic maps of St. Augustinegrass and applications to comparative genomic analysis and QTL mapping for turf quality traits. BMC Plant Biol 18:346–357

Yu X, Brown JM, Graham SE, Carbajal EM, Zuleta MC, Milla-Lewis SR (2019) Detection of quantitative trait loci associated with drought tolerance in St. Augustinegrass. PLoS One 14:e0224620. doi:10.1371/journal.pone.0224620

Yurisic CA (2016) Rooting characteristics and antioxidant pigment responses of three hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy] cultivars exposed to drought. MS thesis. Univ of Tennessee, Knoxville, TN

Zhang X, Ervin EH, LaBranche AJ (2006) Metabolic defense of seeded bermudagrass during acclimation to freezing stress. Crop Sci 46:2598–2605

Zhang W, Qiu P, Jiang H, Liu C, Li C, Hu G, Chen Q (2012) Dissection of genetic overlap of drought and low-temperature tolerance QTLs at the germination stage using backcross introgression lines in soybean. Mol Biol Rep 39:6087–6094

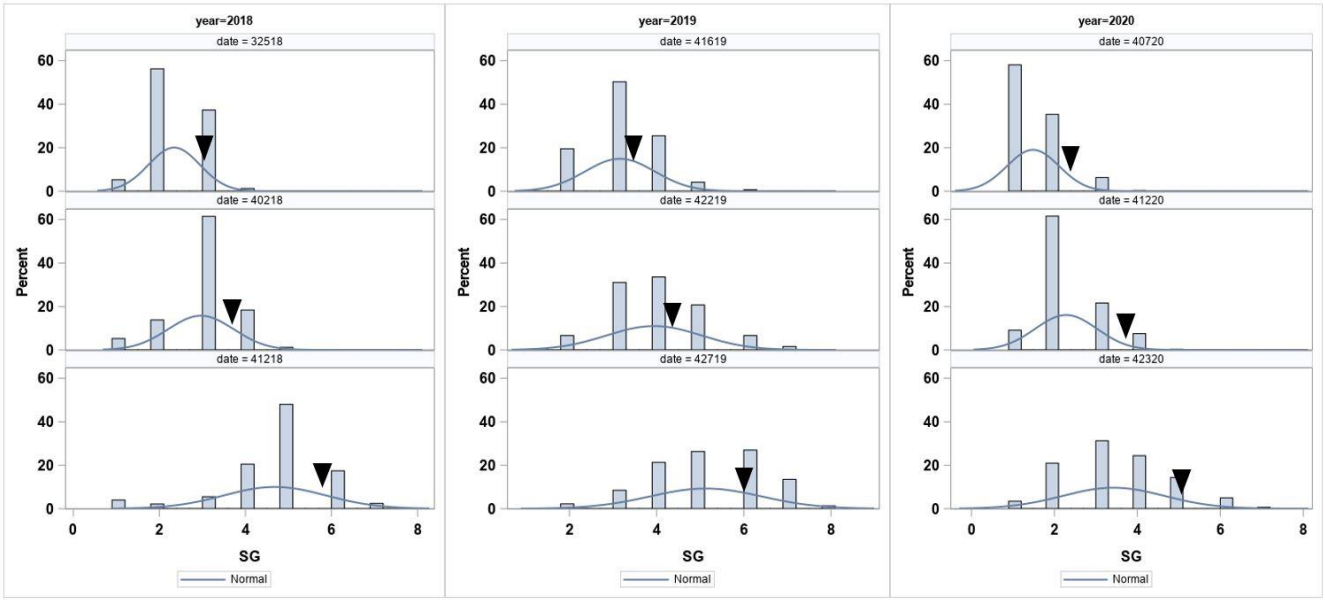

Fig. 1 Distribution of spring greenup (SG) in the OKC1163 S_1 population evaluated from 2018 to 2020. Solid triangles indicate OKC1163 means.

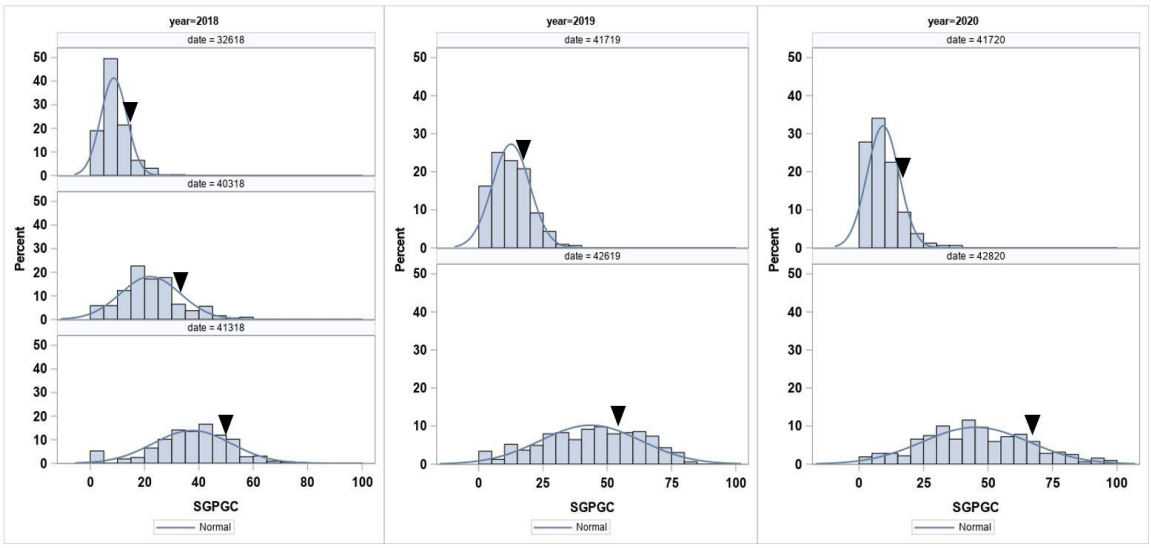

Fig. 2 Distribution of spring greenup percent green cover (SGPGC) in the OKC1163 S_1 population evaluated from 2018 to 2020. Solid triangles indicate OKC1163 means.

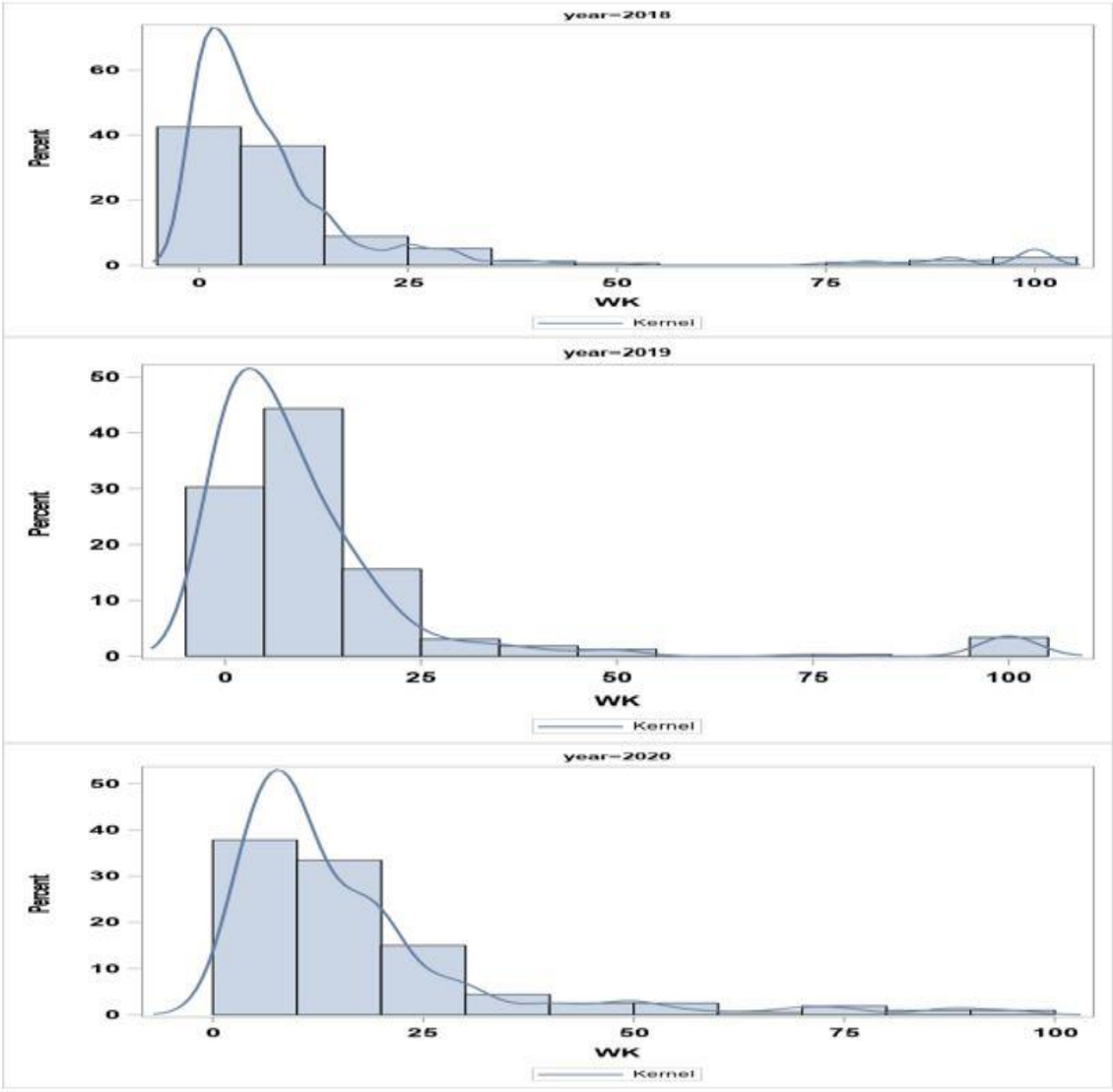

Fig. 3 Distribution of winterkill (WK) in the OKC1163 S_1 population evaluated from 2018 to 2020. Solid triangles indicate OKC1163 means.

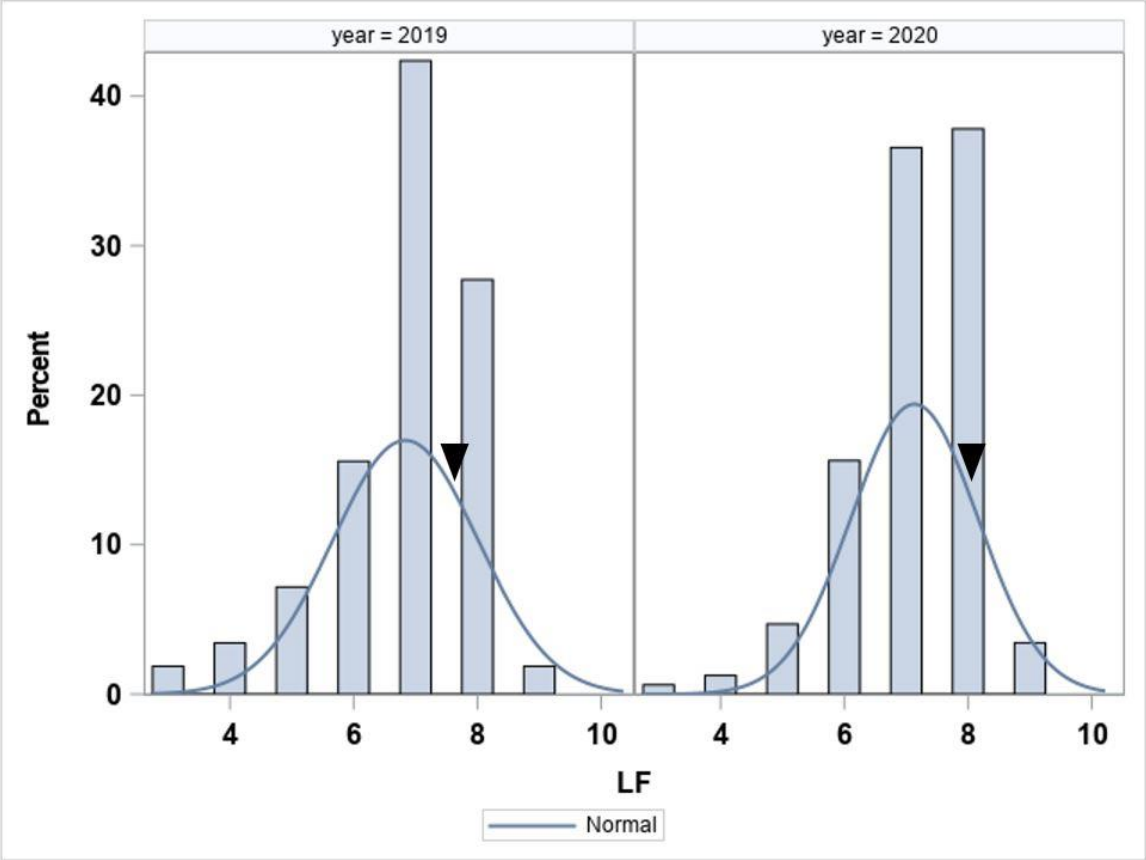

Fig. 4 Distribution of leaf firing (LF) in the OKC1163 S_1 population evaluated from 2019 to 2020. Solid triangles indicate OKC1163 means.

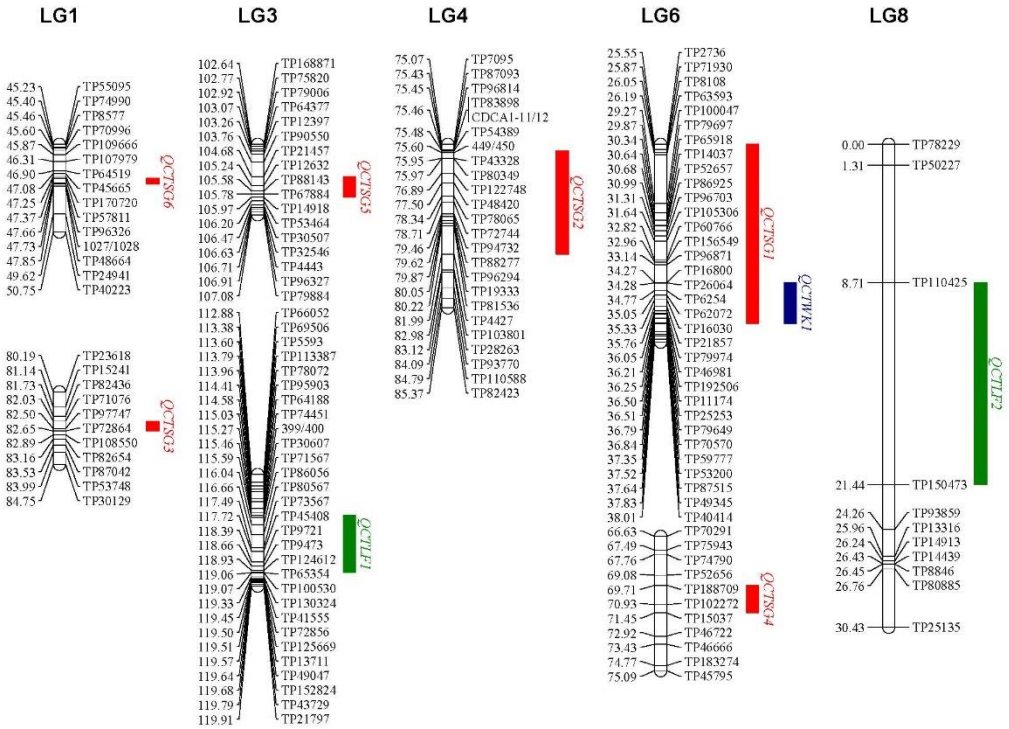

Fig. 5 The locations of peak QTL for spring greenup (SG), winterkill (WK), and leaf firing (LF) on the segments of OKC1163 linkage groups (LG) 1, 3, 4, 6, and 8.

Table 1. Descriptive statistics of winter survivability (winterkill, spring greenup visual and digital measurements), drought response (leaf firing) recorded in the OKC1163 S_1 population over three years.

Trait	Dataset	S_1 population						Parent mean
		Mean	Max.	Min.	SD	Skewness	Kurtosis	
WK[a]	5/11/2018	11.6	100.0	0.0	20.3	3.3	10.6	4.3
	5/15/2019	11.4	100.0	0.0	19.2	3.5	13.1	6.3
	5/10/2020	16.6	95.0	1.0	17	2.5	7.0	4.3
SG[b]	3/25/2018	2.4	4.0	1.0	0.6	0.1	-0.3	3.0
	4/2/2018	2.9	5.0	1.0	0.8	-0.5	1.0	3.7
	4/12/2018	4.7	7.0	1.0	1.2	-1.2	2.0	5.7
	4/16/2019	3.2	6.0	2.0	0.8	0.5	0.3	3.3
	4/22/2019	3.9	7.0	2.0	1.1	0.4	-0.1	4.3
	4/27/2019	5.1	8.0	2.0	1.3	-0.2	-0.5	6.0
	4/7/2020	1.5	4.0	1.0	0.6	1.0	0.3	2.3
	4/12/2020	2.3	5.0	1.0	0.8	0.8	0.7	3.7
	4/23/2020	3.4	7.0	1.0	1.2	0.3	-0.4	5.0
SGPGC[c]	3/26/2018	8.7	31.0	0.0	4.8	1.0	2.1	11.4
	4/3/2018	22.1	59.7	0.0	10.9	0.5	0.6	30.9
	4/13/2018	37.5	77.9	0.0	14.4	-0.5	0.7	47.5
	4/17/2019	12.9	37.0	0.5	7.1	0.6	0.1	16.3
	4/26/2019	44.5	84.4	0.5	18.6	-0.2	-0.8	54.9
	4/17/2020	9.3	39.6	0.0	6.2	1.4	2.9	16.7
	4/28/2020	45.2	97.8	1.3	20.6	0.2	-0.4	66.9
LF[d]	8/6/2019	6.8	9.0	3.0	1.2	-1.1	1.3	7.7
	8/26/2020	7.1	9.0	3.0	1.0	-1.0	1.5	8.0

SD, standard deviation.

[a]WK = winterkill was rated on a scale from 0-100% where 0 = no winterkill and 100% = completely winterkill.

[b]SG = spring greenup was rated a scale from 1-9 where 1 = dormant turf and 9 = fully green turf.

[c]SGPGC = Spring greenup percent green cover measured by digital image analysis calculating the percent live cover on a scale from 0-100% where 0 = no green cover and 100% = whole plot is green.
[d]LF = leaf firing was rated on a scale from 1-9 during drydown where 1 = all leaves fired and 9 = no leaf firing.

Table 2. Analysis of variance and variance component estimates of winter survivability and drought response traits.

Source of variation	SG[a]		SGPGC		WK		LF	
	P > *F*	Variance component	*P* > *F*	Variance component	*P* > *F*	Variance component	*P* > *F*	Variance component
Replication	0.01488		0.1965		0.0065		0.04	
Year	0.235		0.9496		<.0001		<.0001	
Date(Year)	<.0001		<.0001					
Genotype	0.0022	0.07196	0.0013	13.5631	<.0001	68.9984	<.0001	0.5849
Genotype*Replication	<.0001	0.168	<.0001	28.1187	<.0001	96.7327	0.1559	0.0399
Genotype*Year	<.0001	0.1887	<.0001	19.2225	<.0001	28.3678	0.0277	0.0665
Genotype*Date(Year)	1	0	<.0001	15.7694				
Residual		0.5334		102		149.24		0.5312
H		0.36		0.41		0.54		0.81

[a]For trait acronyms, see Table 1.

Table 3. Pearson correlation coefficients among the winter survivability and drought response traits within each of three years.

Year	Trait[a]	SG	SGPGC	WK
2018	SGPGC	0.86****		
	WK	-0.81****	-0.67****	
2019	SGPGC	0.83****		
	WK	-0.35***	-0.55****	
	LF	0.11	0.25**	-0.11
2020	SGPGC	0.75****		
	WK	-0.70****	-0.77****	
	LF	0.32***	0.45****	-0.43****

Significant correlations are indicated by *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.
[a]For trait acronyms, see Table 1.

Table 4. Pearson correlation coefficients between phenotypic values of the same traits, spring greenup (SG), SG percent green color (SGPGC), winterkill (WK), and leaf firing (LF) under drought stress in two or three years.

Trait[a]	Season	2019	2020
SG	2018	0.21*	0.26**
	2019		0.49****
SGPGC	2018	0.43****	0.10
	2019		0.46****
WK	2018	0.65****	0.28**
	2019		0.32***
LF	2020	0.70****	

Significant correlations are indicated by *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.
[a]For trait acronyms, see Table 1.

Table 5. QTL for spring greenup (SG), spring greenup percent green color (SGPGC), winterkill (WK), and leaf firing (LF) were detected in the S_1 population of OKC1163 African bermudagrass.

Dataset[a]	QTL	LG	LOD	Peak[b]	Locus	PVE	Dominate	Additive	LOD threshold[c]
SG 03/25/18	*QCTSG1*	6	4.51	36.05	TP79974	17.3%	0.67	0.57	4.0
	QCTSG2	4	3.37	77.50	TP48420	11.0%	0.25	-0.11	
SG 04/02/18	*QCTSG1*	6	4.12	36.84	TP70570	16.0%	0.81	0.78	4.8
SG 04/12/18	*QCTSG1*	6	6.58	36.84	TP70570	24.3%	1.63	1.62	5.5
SG 04/16/19	*QCTSG3*	1	3.75	82.50	TP97747	14.6%	-0.30	-0.19	3.8
	QCTSG4	6	3.02	70.93	TP102272	10.2%	0.36	-0.06	
SG 04/22/19	*QCTSG3*	1	5.15	82.50	TP97747	13.8%	-0.36	-0.26	4.0
	QCTSG2	4	3.77	81.99	TP4427	9.8%	0.23	-0.36	
SG 04/29/19	*QCTSG2*	4	5.56	81.99	TP4427	20.9%	0.42	-0.56	4.0
	QCTSG3	1	3.55	82.50	TP97747	11.0%	-0.39	-0.27	
SG 04/07/20	*QCTSG5*	1	5.38	47.66	TP96326	20.3%	-0.17	-0.29	4.1
SG 04/13/20	*QCTSG1*	6	6.03	29.27	TP100047	19.8%	0.55	0.13	4.1
	QCTSG6	3	5.76	105.78	TP67884	18.8%	0.44	0.08	
SG 04/23/20	*QCTSG6*	3	7.04	105.78	TP67884	22.9%	0.80	0.05	4.0
	QCTSG1	6	5.42	29.27	TP100047	17.0%	0.87	0.40	
SGPGC 03/26/18	*QCTSG2*	4	4.33	77.50	TP48420	16.7%	0.02	-0.02	4.5
SGPGC 04/03/18	*QCTSG2*	4	4.51	77.50	TP48420	17.3%	0.05	-0.04	4.1
SGPGC 04/13/18	*QCTSG1*	6	4.31	35.33	TP16030	16.6%	0.17	0.16	4.3
SGPGC 04/17/19	*QCTSG2*	4	4.33	81.99	TP4427	16.7%	0.02	-0.03	4.0
SGPGC 04/26/19	*QCTSG2*	4	4.97	81.99	TP4427	15.8%	0.04	-0.07	3.9
	QCTSG1	6	4.00	35.76	TP21857	12.4%	0.19	0.15	
SGPGC 04/17/20	*QCTSG2*	4	3.73	77.50	TP48420	12.1%	-3.31	-0.22	4.1
SGPGC 04/28/20	*QCTSG6*	3	5.58	105.24	TP12632	16.1%	12.39	-1.62	4.0
	QCTSG1	6	3.75	25.55	TP2736	10.4%	11.8	1.33	
WK 05/11/18	*QCTWK1*	6	10.42	36.84	TP70570	35.6%	-33.32	-35.43	8.1
WK 05/15/19	*QCTWK1*	6	5.87	36.05	TP79974	20.5%	-25.16	-21.20	6.2
WK 05/10/20	*QCTWK1*	6	9.29	34.27	TP16800	30.3%	-26.10	-20.82	5.3
LF 08/06/19	*QCTLF1*	3	11.41	119.06	TP65354	32.5%	0.31	-0.83	4.4

	QCTLF2	8	6.26	13.71	TP150473/TP110425	15.7%	-0.44	-1.00	
LF 08/26/20	*QCTLF1*	3	6.41	115.46	TP30607	20.2%	0.38	-0.49	4.2
	QCTLF2	8	5.71	14.71	TP150473/TP110425	17.6%	-0.26	-0.73	

QTL, quantitative trait loci; LG, linkage group; LOD, logarithm of the odds; PVE, phenotypic variation explained.
[a]For trait acronyms, see Table 1. [b]Peak is the QTL maximum LOD position on the linkage group.

Table 6. Means and associated standard deviations of two SNP markers that closely linked to the major spring greenup (SG), winterkill (WK), and leaf firing (LF) QTLs in the S1 population.

			SG[a]			SGPGC		
QTL	Marker	Genotype	2018	2019	2020	2018	2019	2020
			Mean ± SD			Mean ± SD		
QCTSG1	TP16030	A (hh)	3.3±0.4	4.0±0.7	2.8±0.5	36.7±7.0	42.0±12.6	44.8±15.2
		H (hk)	3.4±0.5	4.2±0.7	2.5±0.5	38.3±10.7	45.7±13.1	46.4±13.6
		B (kk)	1.4±0.4	2.5±0.1	1.3±0.2	5.5±2.5	8.6±0.8	14.7±5.3
	TP70570	A (hh)	3.3±0.4	4.0±0.7	2.3±0.5	39.3±7.2	41.7±11.4	44.8±15.2
		H (hk)	3.4±0.6	4.2±0.7	2.5±0.5	37.4±11.4	45.5±13.3	46.1±13.8
		B (kk)	1.0±0.0	2.3±0.0	1.1±0.0	3.0±0.0	9.5±0.0	9.4±0.0
QCTSG2	TP48420	A (hh)	3.3±0.5	4.3±0.7	2.5±0.6	36.7±9.1	47.8±12.9	45.9±15.9
		H (hk)	3.5±0.4	4.1±0.7	2.4±0.5	40.1±9.3	45.0±12.6	46.1±14.1
		B (kk)	3.0±0.7	3.6±0.7	2.2±0.4	31.3±11.7	33.3±12.8	41.8±14.2
	TP4427	A (hh)	3.3±0.5	4.1±0.8	2.5±0.7	38.3±10.5	43.6±17.3	46.8±19.3
		H (hk)	3.3±0.6	4.1±0.7	2.4±0.5	37.5±10.9	43.3±12.8	43.9±12.7
		B (kk)	3.4±0.4	4.0±0.6	2.3±0.4	36.3±7.7	43.9±10.6	46.7±12.5
			WK					
			2018	2019	2020			
			Mean ± SD					
QCTWK1	TP70570	A (hh)	9.2±8.8	11.6±13.4	17.2±12.8			
		H (hk)	11.4±14.4	10.0±11.6	14.9±9.5			
		B (kk)	86.7±0.0	35.0±0.0	65.0±0.0			
	TP16800	A (kk)	9.6±9.0	10.0±8.8	17.4±12.5			
		H (hk)	10.9±14.3	11.4±14.6	14.6±9.3			
		B (hh)	55.9±30.5	42.2±21.7	53.1±8.5			
			LF					
			2019	2020				
			Mean ± SD					
QCTLF1	TP65354	A (kk)	5.8±1.2	6.6±0.9				
		H (hk)	6.9±0.8	7.2±0.8				
		B (hh)	7.5±0.5	7.4±0.5				
	TP30607	A (hh)	5.8±1.2	6.5±0.9				
		H (hk)	6.9±0.9	7.2±0.7				
		B (kk)	7.4±0.5	7.4±0.5				

QTL, quantitative trait loci; SD standard deviation.

[a]For trait acronyms, see Table 1. Due to the insignificant genotype by date within the year interaction, the SG genotype means for each marker were averaged by year. The SGPGC means for each marker were calculated using the dataset from the last SGPGC measurements in each year.

CHAPTER IV

GENETIC VARIABILITY AND QTL MAPPING OF MORPHOLOGICAL AND REPRODUCTIVE TRAITS IN AFRICAN BERMUDAGRASS

ABSTRACT

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) has naturally crossed with and been artificially used to cross with common bermudagrass (*C. dactylon* Pers. var. *dactylon*) in developing F_1 hybrid turf cultivars. African bermudagrass has some unique morphological characteristics that contribute to F_1 hybrid cultivars quality improvement. However, the molecular basis of its morphological variation is unknown. Accordingly, the objectives of this study were to estimate the heritability and identify quantitative trait loci (QTL) associated with morphological and reproductive traits. A first-generation self-pollinated (S_1) population of 109 individuals of OKC1163 African bermudagrass was evaluated for plant height, leaf blade width and length, stem internode diameter and length, and inflorescence prolificacy in a replicated field trial over three seasons (2018-2020). The broad-sense heritability estimates ranged from 0.32 (plant height) to 0.74 (inflorescence prolificacy). Twenty-five QTL were identified and nine of them were recurrent QTL. QTL co-locations were found among morphological traits and between morphological and reproductive traits, partially explained the significant correlation. The findings provide critical genetic information and resources toward

understanding the molecular genetic basis associated with the morphological and reproductive traits and shed light towards marker-assisted selection for breeding new turf bermudagrass cultivars.

INTRODUCTION

Among the eight species of the genus *Cynodon* (L.) Rich., two that have significant values for turf use are common bermudagrass (*C. dactylon* var. *dactylon*) and African bermudagrass (*C. transvaalensis*)[1,2]. African bermudagrass origins in the southwestern Transvaal and the northern part of the central Cape Province of South Africa[3,4]. African bermudagrass is important to the turfgrass industry because it can serve as a parent to artificially cross with common bermudagrass to create interspecific F_1 hybrid (*C. dactylon* × *C. transvaalensis*) cultivars[5]. African bermudagrass contributes genes for aesthetic properties to hybrid cultivar's morphological traits such as fine leaf blades and high sod density[3]. Many interspecific hybrid bermudagrass cultivars have excellent turfgrass quality and stress tolerance[2]. Long-term industry-standard 'Tifway' and most recently released Latitude 36®, NorthBridge®, TifTuf® and Tahoma 31® have been commercially successfully used on golf courses, sports fields, home lawns, and municipal parks[5,6,7,8,9].

African bermudagrass is morphologically recognizable to be different from other species in *Cynodon*. Its linear-lanceolate leaf blades are rarely wider than 2 mm, it often has yellowish-green color with sparsely hairs. Stolons are fine, slender, with shortened internodes[10]. Flowering culms can be as high as 10 cm[11]. African bermudagrass inflorescences are small, usually 2 to 4 cm long, with 2 to 4 (normally 2) racemes in a whorl[10]. Flowering is prolific during the spring and fall with few seedheads occurring during the summer months and seed shattering at maturity is common for African bermudagrass[2]. Even de Wet and Harlan[10] described that African bermudagrasses were very uniform in appearance, but variation exists in morphological characteristics.

Gerken[12] evaluated African bermudagrass genotypes and found differences for shoot density, color, and turf quality under putting green management. Kenworthy et al.[13] reported that the broad-sense heritability estimates of turfgrass performance, morphological, and reproductive traits were moderate to high. They indicated African bermudagrass population improvement is possible through recurrent selection except for genetic color, raceme number, seed number, and percent seed set due to low additive genetic variation[13].

Linkage maps are one of the most important tools in genetic and genome studies. Bethel et al.[14] constructed a framework linkage map of African bermudagrass using an interspecific hybrid population between *C. dactylon* 'T89' x *C. transvaalensis* 'T574'. The linkage map was constructed using 77 single-dose restriction fragments (SDRFs) markers spanning 973.4 cM[14]. Harris-Shultz et al.[15] and Khanal et al.[16] enriched this African bermudagrass linkage map framework by adding expressed sequence tag-derived simple sequence repeat (EST-SSR) and simple sequence repeat (SSR) markers, respectively. Subsequently, Khanal et al.[17] reported 11 quantitative trait loci (QTL) for morphological traits such as leaf length, leaf width, plant height, stolon internode length, and the longest stolon length in African bermudagrass. Using single nucleotide polymorphisms (SNP) markers produced by next-generation sequencing technology, recently, a high-density African bermudagrass linkage map was constructed with 1,278 markers with an average marker spacing of 0.69 cM[18]. A higher resolution linkage map is more favorable in mapping and discovering QTL. Accordingly, the objectives of this study were to quantify the genetic variability and detect the genomic regions associated with morphological traits and reproductive trait in African bermudagrass.

RESULTS

The means, ranges, distributions, and descriptive statistics for IP, LBW, LBL, SIL, SID, and PH are given in Table 1 and Fig. 1 to 6 by each dataset due to significant genotype by year or genotype by date within the year interaction for most traits (Table 2). Compared to the parent, this S_1 population on average showed decreased plant size (i.e. narrower leaf blade, shorter leaf blade, shorter internode, smaller internode size, and shorter canopy height), indicating inbreeding depression. The progeny means of LBW, SIL, and PH in 2019 were numerically higher than the mean values in 2018. The progeny means of SIL in 2020 were numerically higher than the mean values in 2019. However, the progeny means of SID in 2020 was numerically lower than the mean values in 2019.

The ANOVA results of all the traits studied are shown in Table 2. According to the ANOVA results, significant ($P < 0.05$) genotype effects were found for all the traits, suggesting segregation of genes underpinning the phenotypes, which were desirable for QTL mapping. Highly significant ($P < 0.0001$) genotype effects were found for IP, LBW, LBL, and SIL. The effects of replication were significant ($P < 0.05$) for all the traits except for LBL and SID. The effects of genotype by replication interactions were significant for all traits except for LBW and SID. Year effects were significant ($P < 0.05$) for all traits that data were collected in multiple years except for SID. The genotype by year interactions were significant for all traits except for LBW and SID.

The variance components estimate and broad-sense heritability for all studied traits are presented in Table 2. The variances for the SID genotype by replication was negative estimated by the TYPE III sum of squares. In this case, the variance for genotype by replication was considered to be zero since the negative variance value was

logically impossible. For the traits even with highly significant genotype effects, the genetic variances were not the largest. The error variances were the largest component of all the traits.

The broad-sense heritability estimates for all traits ranging from 0.32 to 0.74 (Table 2). Morphological trait LBW, LBL, and SIL had moderate to high broad-sense heritability. The PH and SID had low to moderate broad-sense heritability. The high broad-sense heritability for LBL (0.73) may inflate due to only one-year data was collected. Reproductive trait IP had the highest broad-sense heritability (0.74) among all the traits.

Correlation Analysis

Due to significant genotype by year interactions for most of the traits, data were analyzed by year. Significant correlation coefficients between morphological and reproductive traits are presented in Table 3. LBW was significantly positively correlated with SIL in both 2018 and 2019. In 2019, significant correlations were found between all the traits except between PH and IP and between PH and SID. In 2020, significant correlation was found between IP and SID. Subsequently, correlations for each trait were calculated between years (Table 4). Significant correlations were found for all the traits among different years. The highest correlation was for IP between 2019 and 2020. Stem internode diameter has the lowest significant correlation (0.26) compared to other morphological traits. For the traits collected in three seasons, the correlations between the first and the last season were lower than the correlation between neighbor seasons.

QTL Analyses

Inflorescence Prolificacy

Four QTLs were identified on LGs 1 and 3, explaining 11.7 to 21.2% phenotypic variation (Table 5, Fig 7 & 8). *QCTIP1* on LG3 with peak LOD values located between positions 100.83 to 113.96 cM, was consistently identified in all three seasons. The percentage of phenotypic variation explained by *QCTIP1* ranged between 14.2 to 21.2%, showing negative additive effects (i.e., increasing IP). The IP means and associated standard deviations for three respective genotypes of the two markers flanking *QCTIP1* are given in Table 6. The homozygous genotypes A of both markers consistently produced more seedhead while homozygous genotypes B produced less seedhead except using marker TP16800 in 2019 (Table 6). *QCTIP2*, located on LG1 between 34.31 and 36.32 cM, was also identified in 2018 and 2019, explaining 11.7 to 14.7% phenotypic variation. The intra-locus genetic effect of *QCTIP2* was positive additive (i.e., reducing IP) and negative dominance (increasing IP). *QCTIP3* was detected on LG3 at 49.16 cM in 2019 and 39.48 in 2020, explaining 13.8 and 11.7% phenotypic variation, respectively. *QCTIP4*, on LG1 between 74.03 and 83.53 cM was identified in both 2019 and 2020.

Plant Height

Two QTL associated with PH were identified on LG6 (Table 5, Fig 7 & 8). In 2018, *QCTPH1* was detected on 70.71 cM that resided in the 1.2 cM interval between makers TP102272 and TP188709, and it explained 27.4% phenotypic variance. While in 2019, *QCTPH1* was detected on marker TP74779 at position 76.08 cM, explaining 20.3% phenotypic variation. *QCTPH1* expressed negatively additive (decreasing plant height) and partially positively dominant (increasing plant height) intra-locus genetic effects in

both seasons. Another QTL, *QCTPH2*, was only identified in 2019. This QTL was located at position 36.05 cM on LG6, explaining 10.0% phenotypic variation. Unlike *QCTPH1*, *QCTPH2* showed a positive additive intra-locus genetic effect.

Leaf Blade Width

Three QTL associated with LBW were identified (Table 5, Fig 7 & 8). In 2018, the position of peak LOD of *QCTLBW1* was located at 108.46 cM on LG3, resided in the 1.9 cM interval between TP62635 and TP83433, explaining 32.9% phenotypic variation. In 2019, the peak LOD of *QCTLBW1* was located at 106.204 cM near marker TP53464, accounting for 21.8% phenotypic variation. *QCTLBW1* expressed negative additive genetic effects in both seasons while its dominance was inconsistent in the two years. *QCTLBW2* on LG6 was detected in both years. In 2018, *QCTLBW2* was located at 36.049 cM near marker TP70570, explaining 16% phenotypic variation. In 2019, *QCTLBW2* was on position 36.844 cM and accounted for 18% phenotypic variation. *QCTLBW3* was only detected in 2019. This QTL was detected on 79.16 cM on LG1, explaining 10.7% phenotypic variation. While *QCTLBW1* showed negative additive effect, both *QCTLBW2* and *QCTLBW3* showed positive additive effects. The LBW means and associated standard deviations for three respective genotypes of the two *QCTLBW1* linked markers are given in Table 6. The homozygous genotypes A of both markers consistently had finer leaf texture than the genotypes B and H in both years (Table 6).

Stem Internode Length

Seven QTL associated with SIL were identified based on data collected from three seasons (Table 5, Fig 7 & 8). In 2018, *QCTSIL1* and *QCTSIL2* were identified,

accounting for 12.2 and 9.7% of phenotypic variation, respectively. *QCTSIL3*, *QCTSIL4*, and *QCTIL5* were identified in 2019, explained 8 to 16.1% of phenotypic variation. *QCTSIL2*, *QCTSIL6*, and *QCTSIL7* were identified in 2020, accounted for 14.7 to 21.5% of phenotypic variation. *QCTSIL2* was the only repeated QTL associated with SIL and it explained large phenotypic. Amongst the seven QTL, *QCTSIL1*, *QCTSIL5*, and *QCTSIL6* showed positive additive effects and the rest of QTL showed negative additive effects. *QCTSIL2* showed a negative additive effect in 2018 but positive additive effect in 2020. The SIL means and associated standard deviations for three respective genotypes of the two *QCTSIL2* linked markers are given in Table 6. The heterozygous genotypes H of both markers consistently had shorter stem internode length than the respective genotypes A and B in three years (Table 6).

Stem Internode Diameter

Four QTL associated with SID were identified in two seasons (Table 5, Fig 7 & 8). In 2019, *QCTSID1* was detected on LG6 at 82.06 cM, explaining 10.5% phenotypic variation. *QCTSID2* was detected both in 2019 and 2020. In 2019, the peak location of *QCTSID2* was located at 103.26 cM on LG3, while in 2020, the location was 118.39 cM. *QCTSID2* explained 9.7 and 21.4% of phenotypic variation in 2019 and 2020, respectively. *QCTSID3* was identified in 2020 on LG1 with peak LOD at 44.55 cM, explaining 10.7% phenotypic variation. *QCTSID4*, located on LG4 at 71.08 cM, explaining 8.6% phenotypic variation. *QCTSID4* was only detected in 2020. The SID means and associated standard deviations for three respective genotypes of the two *QCTSID2* linked markers are given in Table 6. The homozygous genotypes A of both

markers consistently had thinner stem internode than the respective genotypes B and H in both years (Table 6).

Leaf Blade Length

Five QTL associated with LBL were identified (Table 5, Fig 7 & 8). *QCTLBL1* and *QCTLBL4* were located on LG6, explaining 8.0 to 13.1% phenotypic variation. *QCTLBL2* was located on LG5 at 79.41 cM, accounting for 10.4% phenotypic variation. *QCTLBL3* was identified on LG1 at 36.25 cM, explaining 8.3% phenotypic variation. On LG3, *QCTLBL5* was identified at position 61.82 cM, explaining 7.7% phenotypic variation. All the QTL showed positive additive effects except for *QCTLBL2*.

DISCUSSION

African bermudagrass is a native species to a small geographic region in South Africa. The species has unique morphological characteristics, including fine leaf texture, thin and short internodes, which are relevant to the development of modern turf bermudagrass cultivars. In the past century, the species has been introduced to the USA and used in the development of widely adopted interspecific hybrid turf bermudagrass cultivars[19]. When the genomes of African bermudagrass and common bermudagrass are combined through hybridization some progeny exhibit desirable turf qualities, encompassing fine leaf blades and high sod density. In the present study, we combinatorially analyzed morphological traits and IP data collected across two to three seasons and a pre-existing highly dense genetic map in a self-pollinated African bermudagrass population[18]. The major findings included the identification of 25 QTL in the population and the colocalizations of multiple QTL, which partially validated the correlations between these traits. Khanal et al.[17] reported 11 QTL for morphological traits of an African bermudagrass genotype 'T574' based on in an F_1 population from interspecific hybridization between common bermudagrass and African bermudagrass for morphological traits. The morphological traits included plant height, internode length, and leaf blade length and width. Unfortunately, due to no shared DNA markers between the two genetic maps[17,18], the QTLs identified in the two studies cannot be compared and validated each other. Therefore, it is needed to develop a whole genome sequence for African bermudagrass, which would serve as a platform for accurate molecular investigations, such as a comparison between research findings by Khanal et al.[17] and that from this study.

Heritability and Correlation Analyses

In this African bermudagrass S_1 population, a wide range of broad-sense heritability was found for morphological and reproductive traits. The broad-sense heritability for morphological traits SIL and LBL was similar to the heritability estimates reported by Kenworthy et al.[13], suggesting that the SIL and LBL phenotypic expression were mostly influenced by genetic factors even under different evaluation conditions. However, the broad-sense heritability for PH in this study was much lower than what was reported by Kenworthy et al.[13] Turfgrasses plant height is a combination of both stem and leaf extension and is affected by stem and leaf angle[20]. Although the trial was mowed once after spring greenup to allow establishing a relatively uniform surface, it has been observed that the canopy height was inconsistent within the plot. The plant height is more vulnerable to environmental conditions compared to leaf and stem traits in the field[13,21]. The heritability estimate for this trait in this study was low, even five measurements were averaged to minimize the error within each plot. The broad-sense heritability for SIL, LBW, and LBL were lower than the values for common bermudagrass reported by Wofford and Baltensperger[22] but much greater than the heritability values of 0.03 for SIL and 0.25 for LBW reported by Guo et al.[23]. Wu et al.[24] reported that genetic effects strongly influenced the SID in common bermudagrass. Genetic effects still control the SID in African bermudagrass even our result for African bermudagrass SID heritability was lower than common bermudagrass. The heritability of reproductive trait IP was moderate to high (0.60), lower than the common bermudagrass IP heritability reported by Guo et al.[23] and Wu et al.[24]. Inflorescence appearance in a high quality turf will decrease the aesthetic value and consequently needs increased mowing management.

Most of the correlations among the morphological traits were significant, especially for leaf and stem traits. It is not surprising that a highly significant ($P <$ 0.0001) correlation was between LBL and LBW, suggesting that longer leaf blades tend to be wider. The significant ($P < 0.05$) correlation between LBL and LBW was also found in common bermudagrass[24]. The significant correlation between LBL and LBW allows bermudagrass breeders to select high turfgrass quality (fine-texture) and less mowing required (shorter leaf blade length) genotypes for residential and landscape uses. The correlation between SIL and SID was less consistent compared to the correlation between LBL and LBW. In 2019, low SIL and SID correlation was found, and in 2020, the correlation became negative. Wu et al.[24] reported the significant but low to moderate correlation between SIL and SID in common bermudagrass. Similar to leaf traits, bermudagrasses with longer stems usually have thicker stems. Bermudagrasses with shorter SIL will produce more crowns per unit area, resulting in more growing points for leaves[25]. Therefore, SIL could be a selection index for density. Stem internode length also relates to mowing quality. Genotype with shorter stem internode length can withstand lower mowing height to avoid scalping. Ultra-dwarf bermudagrass used on golf course putting green have characteristically shorter stem internodes and leaf blades that can withstand height of cut at 3 mm. The highly significant correlation between LBL and SIL suggests that the genes that control leaf and stem size may tightly be linked or the same genes affect different traits. Plant height was significant correlated with most morphological traits but not SID in 2019. However, no correlation was found between PH and morphological traits in 2018, suggesting plant aging might affect morphologic

expression. Reproductive trait IP was significantly correlated with all the leaf and stem traits.

QTL Analyses

Genetic maps are a useful tool in understanding genetic basis for and detecting QTL associated with traits of interest, which is promising in improving selection efficiency in plant breeding. QTL analysis has been widely used to identify molecular markers linked to morphological and reproductive traits in crops[26,27,28,29,30,31,32,33]. However, the only genomic information associated with morphological traits of African bermudagrass was reported by Khanal et al.[17] Taking the advantage of next-generation sequencing technology, a high-density African bermudagrass genetic map of 1,278 markers that cover all nine chromosomes has been recently reported[18], providing a powerful foundation for QTL analysis of traits of interest in African bermudagrass. As the recent map was developed from an S_1 population, it is conducive for investigating main allelic effects within a locus.

A total of 25 QTL associated with one reproductive and five morphological traits were identified in this study. Consistency with the high (0.74) broad-sense heritability found for IP, all of the four QTL associated with IP were recurrent QTL, suggesting these QTL represent the true genomic regions that associated with IP. Using the markers closely linked to the QTL associated with IP it is possible to select turf cultivars with reduced IP, which will improve turf bermudagrass aesthetic quality.

Two QTL associated with PH were identified in this study. The major effect QTL was recurrent over environments (i.e., year) even with a low to moderate heritability estimate. Plant height of African bermudagrass is a quantitative trait[13,17]. A large number

of QTL associated with PH were identified in bermudagrass[17], rice[21,34,35], sorghum[29], and foxtail millet [*Setaria italica* (L.) P. Beauv.][36]. Khanal et al.[17] reported two QTL for African bermudagrass PH, one on LG 7a-2/b-I & 5a/b and another on LG 6a/b, corresponding to African bermudagrass LGs 1 and 3 in the current study, respectively. However, no QTL associated with PH was located on LGs 1 and 3 in the present study, suggesting that PH segregation expression may be influenced by different loci in different populations.

Three QTL were identified for LBW, two of them were recurrent QTL identified on LGs 3 and 6, explaining from 39.8 to 48.9% combined phenotypic variation. Another QTL, *QCTLBW3* on LG1 was only detected in 2019. Khanal et al.[17] identified three QTL associated with LBW on two African bermudagrass LGs 3a/b and 7a-2/b-I & 5a/b, which correspond to African bermudagrass LGs 1 and 3 in the current study, respectively. The consistent findings reinforced that African bermudagrass LGs 1 and 3 carried QTLs for LBW. Leaf plays a significant role in photosynthesis and can be used to determine yield potential[37]. Mapping QTL associated with flag leaf width has been conducted in rice[30,32,38,39], and QTL have been found on all 12 rice chromosomes. The recurrent LBW QTL found on African bermudagrass LGs 3 and 6 were primarily corresponded to rice chromosomes 1 and 6, respectively, indicating the similarity in genetic basis regarding LBW between rice and African bermudagrass.

Seven QTL were detected for SIL on LGs 1, 3, 4, 5, and 6. *QCTIL2* was the only recurrent QTL associated with SIL. Although six QTL were not consistently detected in three seasons, the broad-sense heritability for SIL was moderate to high, suggesting the recurrent QTL may be the major effect QTL. The remaining non-recurrent QTL

associated with SIL may have minor effects. The inconsistency QTL detection was likely due to different environmental conditions in each year caused significant gene by environment interaction[47]. Khanal et al.[17] reported four QTL associated with African bermudagrass stolon internode length on four LGs. Although stolons and stems are different in morphology, the internode length QTL were found at the same corresponding LGs in the current study except for LG5. However, we could not know whether these QTL were located at the same genomic regions in each LG due to different maker systems and population used to construct the genetic maps.

There were four QTL associated with SID identified in the current study. One of the QTL, *QCTID2* on LG3, was a recurrent QTL. Stem internode diameter is an important agronomic trait correlated to lodging resistance contributing to high-yield[29,31,33]. Shehzad and Okuno[29] found that the QTL associated with sorghum culm diameter were located on chromosomes 1, 3, 4, and 5. Wang et al.[31] discovered three QTL associated with foxtail millet SID were identified on chromosome 2, 5, and 9. Zhu et al.[33] reported three QTL associated with the first rice basal internode diameter on chromosomes 1, 3, and 10.Based on our unpublished data, rice chromosome 1, sorghum chromosome 4, and foxtail millet chromosome 5 all correspond to LG3 in this study. The correspondences among grass species reinforce that *QCTSID2* on LG3 is a true genomic region for African bermudagrass SID.

QTL Co-Localization

There were five genomic regions on LGs 1, 3, 5, and 6 where QTLs were identified for two or more traits. Two genomic regions on LG1, one around 35 cM, harbored *QCTIP2*, *QCTLBL3*, and *QCTSIL7*. Another genomic region on LG1 around 80

cM was co-located for *QCTIP4, QCTSIL1*, and *QCTLBW3*. On LG3, a region around 108 cM was identified to have *QCTIP1, QCTLBW1, QCTSIL2*, and *QCTSID2*. *QCTSIL3* and *QCTLBL2* were co-located on LG5 at position 79.41 cM. Additionally, one genomic region on LG6 around 36 cM was co-located for *QCTLBW2, QCTSIL6, QCTLBL1*, and *QCTPH2*. Significant positive correlations and co-localized QTL between morphological traits are common phenomena, especially between leaf and stem traits[29,31,33]. In the current study, the QTL associated with leaf and stem traits were overlapped on multiple African bermudagrass LGs. Khanal et al.[17] reported that QTL colocalization between LBL and LBW was not observed in bermudagrass. We found that LBL and LBW was highly significant correlated ($P < 0.0001$), and that one co-located QTL was observed. This discrepancy may due to Khanal et al.[17] measured the leaves from the first node was immature since LBL phenotypic expression was more reliable on mature leaves[23,24]. Consistency with the findings that significant correlations were found between PH, LBW, and LBL in 2019. A QTL co-location was found among PH, LBW, and LBL only in 2019, but not in 2018. The major recurrent PH QTL was not able to overlap with any other morphological traits QTL detected in this study, suggesting African bermudagrass PH was controlled by different loci. Several QTL co-locations were observed between morphological traits and reproductive trait IP in the current study. It has been well documented that the QTL co-location among yield, leaf, and stem related traits in rice[26,30,32], wheat[27], switchgrass[28], and sorghum[29]. Apparently, yield, leaf, and stem related traits share pleiotropic or closely linked genetic determinants among different species in the grass family.

Besides QTL co-locations observed between morphological and reproductive traits, QTL co-locations have also been found between adaptive and morphological traits[18,41]. Establishment rate (ER) is an important adaptive trait that is not only related to recovery from stress (i.e., traffic, diseases, drought, and cold) damages but also critical to sod production since vegetatively-propagated turf-type bermudagrass can only be vegetatively reproduced. Yu et al.[18] reported four QTLs associated with ER, and three of them were co-localized with either one or multiple LBW, LBL, SIL, SID, and even PH QTL, suggesting the similar genetic control mechanisms. Winter survival *QCTWK1* and *QCTLBW2* were co-located on LG6 near 36 cM[18]. The positive correlation between LBW and winter survival has been reported in winter barley (*Hordeum vulgare* L.)[42]. Wu et al.[24] found that both leaf length and width positively correlated with winter survival in common bermudagrass. Consistent with the opinion given by Stefaniak et al.[43], bermudagrass cold tolerance improvement is difficult because it is not only a quantitative trait but also negatively correlated with desirable trait such as fine leaf texture. It is well accepted that cold acclimation improves freeze tolerance that partially contributes to the winter hardiness in warm-season grasses[44,45,46,47]. Transcriptomic analysis showed that common bermudagrass cold tolerance genotypes selectively expressed low-temperature sensing and signaling-related genes, functional proteins, and transcription factors when subjected to low and freeze temperature stress[48]. MicroRNA319 (*miR319*), a family of endogenous small non-coding RNAs can regulate transcription factors, has been found able to increase leaf blade width and enhance cold tolerance in rice[49,50] and *Arabidopsis*[51], may explain the QTL co-location between winter survival and LBW QTL. Liu et al.[52] reported the overexpression of *miR319* promoted the leaf width that resulted in a

significant increase in biomass yield in switchgrass. Consistent with the findings in the current study that LBW is significantly correlated with PH. QTL co-location has been observed among LBW, PH, and ER. Other than cold tolerance, biomass yield, *miR319* was also found to suppress the flower development in *Arabidopsis*[53]. This finding in *Arabidopsis* similar to our result that African bermudagrasses with coarse leaf texture usually have less inflorescences present, reinforced by the evidence that the QTL co-location between IP and IBW. Zhou et al.[54] report that the transgenic creeping bentgrass (*Agrostis stolonifera* L.) overexpression of a rice *miR319* gene showed larger plant size (i.e. larger leaves, longer stem, and more biomass) and improved drought tolerance. Field observations support that African bermudagrass genotypes with coarse leaf texture showed better drought response, which is reinforced by the evidence that LBW and leaf firing QTL were colocalized on LG3[19]. Although the peak LOD position of *QCTLBW1* and the primary leaf firing QTL *QCTLF1* were more than 10 cM away using MQM approach. They can consider overlapped because in the initial QTL analyses using IM, the LOD values for both LBW and LF in this genomic region were greater than their LOD threshold values.

Use of The Markers Linked to QTL in Selection

Current turf bermudagrass breeding programs use hybridization and selection in the development of new cultivars improved in turfgrass quality and resistance to biotic and abiotic stresses. This investigation identified and characterized significant QTLs for morphological traits and IP that are related to turf performance. The molecular markers closely linked to the QTL can be used in selection process of improving turfgrass quality traits. As proposed by Wu et al. (2008), using two markers flanking a major QTL

simultaneously will precisely assist the selection process. Once the whole genome sequence of African bermudagrass is available, the markers TP62635 and TP16800 can be converted to breeder friendly polymerase chain reaction (PCR) based markers to screen the inheritance of morphological and reproductive characteristics in breeding materials.

CONCLUSION

African bermudagrass has played an essential role in the development of interspecific F1 hybrid turf cultivars, especially in the improvement of morphological traits that are directly related to the turfgrass quality. Using an S_1 progeny population of African bermudagrass in this study, we analyzed phenotypic and genotypic variation for leaf blade length and width, stem internode length and width, and inflorescence prolificacy. There were significant genetic components for the traits as indicated by their heritability. Using a pre-existing high-density linkage map and the phenotypic data collected in the population, QTL analysis identified twenty-five significant genomic regions associated with the traits. Many QTL were colocalized on LGs 1, 3, and 6, revealing the genetic mechanisms for the significant phenotypic correlations between these traits characterized by other studies and this study. The stable QTL have potential to be used in fine mapping to identify genes in the future. The markers flanking the QTL can be used in the selection of high quality F1 hybrids towards new cultivars.

MATERIALS AND METHODS

Plant Materials and Field Experiment

The detailed information for plant materials, experimental design, methods of establishing were reported by Yu et al.[18]. Briefly, 109 'OKC1163' progenies were transplanted to field plots on the Oklahoma State University Agronomy Research Station, Stillwater, OK, in June 2017. The experimental design was a randomized complete block with three replications. Plot size was 0.9 by 0.9 m separated by 0.3 m alleys between plots. Field managements in 2017 were as reported by Yu et al.[18]. In 2018, 107 kg ha^{-1} of urea (46-0-0, N-P-K) (Chouteau Lime Co., Pryor, OK) was applied in the second week of May. Another 107 kg ha^{-1} of urea (46-0-0, N-P-K) was applied in the second week of August to promote growth. In 2019, based on the soil test, 289 kg ha^{-1} of 17-5-5 (N-P-K) (Chouteau Lime Co., Pryor, OK) was applied in the second week of May and 107 kg ha^{-1} of urea (46-0-0, N-P-K) was applied in the middle of August to promote the growth. Plots were mowed at 2.5 cm in late March in 2018 and 2019 to remove the dormant canopy for regrowth in spring. Then plots were sprayed with 2.24 kg a.i. ha^{-1} glyphosate and 0.25% (v/v) nonionic surfactant and a subsequent application of 2.3 kg ha^{-1} a.i. oxadiazon (Ronstar FLO, Bayer Environmental Science, Cary, NC) for pre-emergence control. After most plots greenup, the trial was mowed again at 3.8 cm in the second week of May 2018 and the first week of May 2019 to achieve a relatively uniform growth for sample collection. In the middle of August in 2019, plots were mowed at 3.8 cm to remove the wilted canopy for recovering from drought stress. Alleys were sprayed with 2.24 kg a.i. ha^{-1} Glyphosate (Roundup, Monsanto, St. Louis, MO) and 0.25% (v/v) nonionic surfactant to prevent excessive stolon growth to avoid contamination.

Data Collection

Plant heights (PH) were measured in the first week of September 2018 and the second week of August 2019. The heights from the ground to the top of the leaves at five random spots in each plot were measured using a tape measure. In early July of 2018, 2019, and 2020, full-length stems were collected randomly from five different spots in each plot and placed in a Ziploc bag. Bags were temporarily placed into a chest filled with ice packs to keep samples fresh during collection and then stored in a freezer at −20°C for subsequent measurements[24]. Leaf blade width (LBW), leaf blade length (LBL), stem internode length (SIL), and stem internode diameter (SID) were measured on the defrosted samples with a digital caliper. Leaf blade width and length were measured on the leaves originating from the third stem node. Internode length and diameter were measured on the internode between the second and the third nodes. Due to the leaf senescence, LBL data were not collected in 2018 and 2020. Leaf blade width was measured on samples collected from 2018 and 2019. Inflorescence prolificacy (IP) ratings were taken on May 10, 2018, May 29 and June 8, 2019, and June 4, 2020. A scale of 1 to 9 was used, where 1 = most abundant seedheads while 9 = no seedheads[55].

Statistical Analysis

Analysis of variance (ANOVA) for PH, LBW, LBL, SIL, and SID were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) MIXED Procedure (PROC MIXED). Variance components were estimated using TYPE III method of moments estimation[56]. All sources of variation were considered as random effects because year and rating date within the year were not chosen based on expected environmental conditions[57] and the information on traits performance for this S_1 population was unknown. Pearson

correlation coefficients for traits between years and within a year were calculated using the PROC CORR.

The broad-sense heritability (*H*) for LBL was calculated using the following formula: $H= \sigma_G^2/ (\sigma_G^2+ \sigma_E^2/R)$. The broad-sense heritability (*H*) for LBW, SIL, SID, and PH were calculated using the following formula: $H= \sigma_G^2/ (\sigma_G^2+ \sigma_{GY}^2/Y+ \sigma_E^2/RY)$. The broad-sense heritability (*H*) for IP was calculated using the following formula: $H=\sigma_G^2/(\sigma_G^2+\sigma_{GY}^2/Y+\sigma_{GR}^2/R+\sigma_{GDY}^2/DY+\sigma_E^2/RDY)$, where σ_G^2, σ_{GY}^2, σ_{GR}^2, σ_{GDY}^2, and σ_E^2 represent the variance of genotype, the variance of genotype by year, the variance of genotype by replication, the variance of genotype by date within the year, and the error variance, respectively. *R*, *Y*, and *D* respectively represents the number of replications, the number of years, and the number of rating dates[58].

Linkage Map and QTL Analysis

The detailed information about population genotyping, linkage analysis and linkage map construction were reported by Yu et al.[18]. QTL analyses were performed using MapQTL 6.0[59]. The mean values of traits from each rating date were analyzed separately. The genome-wide LOD threshold was calculated by a 1,000 permutation test and determined at a significant *p*-value of 0.05. The Interval mapping (IM) method was initially selected to detect QTL with one cM mapping step size. Markers with the peak LOD values greater than the threshold LOD in each linkage group (LG) were selected as cofactors. Then multiple QTL model (MQM) was used to detect QTL, as this approach gives more analysis power[60]. The MQM approach was reiterated until the selected cofactors consistently have the highest LOD value. Graphics showing the positions of QTL were generated by using MapChart 2.2[61].

REFERENCES

1. Harlan, J. R. *Cynodon* species and their value for grazing and hay. *Herb. Abstr.* **40**, 233–238 (1970).
2. Taliaferro, C. M. *Bermudagrass (Cynodon (L.) Rich)*. p. 235– 256. In Casler M.D. & Duncan R. (ed.) *Turfgrass biology, genetics, and breeding*. Sleeping Bear Press, Chelsea, MI (2003).
3. Harlan, J. R. et al. Geographic distribution of the species of *Cynodon* L.C. Rich (Gramineae). *East Afr. Agric. Forest. J.* **36**, 220–226. (1970a).
4. Harlan, J. R. et al. A guide to the species of *Cynodon* (Gramineae). *Bull. Okla. Agric. Exp. Stn.* **B-673** (1970b)
5. Burton, G. W. A history of turf research at Tifton. *U.S. Golf Assoc. Green Section Record* **29**, 12–14 (1991).
6. Schwartz, B. M. et al. 'DT-1', a drought-tolerant triploid turf bermudagrass. *HortScience* **53**, 1711–1714 (2018).
7. Wu, Y. Q. et al. Northbridge turf bermudagrass. USPP 24,116. *U.S. Patent and Trademark Office* (2013).
8. Wu, Y. Q. et al. Latitude 36 turf bermudagrass. USPP 24,271. *U.S. Patent and Trademark Office* (2014).
9. Wu, Y. Q. et al. Bermudagrass plant named 'OKC 1131'. USPP 2019/0364715 P1. *U.S. Patent and Trademark Office* (2019).

10. de Wet, J. M. J. & Harlan, J. R. Biosystematics of *Cynodon* L.C. Rich. (Gramineae). *Taxon* **19**, 565–569 (1970).
11. Juska, F. V. & Hanson, A. A. Evaluation of bermudagrass varieties for general purpose turf. *USDA-ARS, Agric. Handbook* No. 270 (1964).
12. Gerken, D. A. Evaluation of African bermudagrass (*Cynodon transvaalensis*) as a putting surface. *Oklahoma State Univ.*, Stillwater, OK (M.S. thesis) (1994).
13. Kenworthy, K. E. et al. Genetic Variation in *Cynodon transvaalensis* Burtt-Davy. *Crop Sci.* **46**, 2376–2381 (2006).
14. Bethel, C. M. et al. A framework linkage map of bermudagrass (*Cynodon dactylon* × *transvaalensis*) based on single-dose restriction fragments. *Theor. Appl. Genet.* **112**, 727–737 (2006).
15. Harris-Shultz, K. R. et al. Development, linkage mapping, and use of microsatellites in bermudagrass. *J. Amer. Soc. Hort. Sci.* **135**, 511-520 (2010).
16. Khanal, S. et al. SSR-enriched genetic linkage maps of bermudagrass (*Cynodon dactylon* x *transvaalensis*), and their comparison with allied plant genomes. *Theor. Appl. Genet.* **130**, 819–839 (2017).
17. Khanal, S. et al. Molecular Dissection of Quantitative Variation in Bermudagrass Hybrids (*Cynodon dactylon* x *transvaalensis*): Morphological Traits. *G3: Genes, Genomes, Genet.* **9**, 2581–2596 (2019).
18. Yu, S. et al. Genetic and QTL mapping in African bermudagrass. *Plant Genome*, doi.org/10.1002/tpg2.20073 (2020).
19. Taliaferro, C. M. Out of Africa—a new look at "African" bermudagrass. *U.S. Golf Assoc. Green Section Record* **30**, 10-12 (1992).

20. Kenworthy, K. E. et al. Growth habit determination of genotypes of African bermudagrass. *HortScience* **42**, 1513-1516 (2007).

21. Li, Z. et al. QTL× environment interactions in rice. I. Heading date and plant height. *Theor. Appl. Genet.* **108**, 141-153 (2003).

22. Wofford, D., & Baltensperger A. Heritability estimates for turfgrass characteristics in bermudagrass. *Crop Sci.* **25**, 133-136 (1985).

23. Guo, Y. et al. Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. *Crop Sci.* **57**, 82-88 (2017).

24. Wu, Y. Q. et al. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. *Crop Sci.* **47**, 1985–1994 (2007).

25. Karcher, D. E. et al. Recovery of bermudagrass varieties from divot injury. *Appl. Turfgrass Sci.* **2** doi: 10.1094/ATS-2005-0117-01-RS (2005).

26. Cai, J. et al. QTLs for rice flag leaf traits in doubled haploid populations in different environments. *Genet. Mol. Res.* **14**, 6786–6795 (2015).

27. Fan, X. et al. QTLs for flag leaf size and their influence on yield-related traits in wheat (*Triticum aestivum* L.). *Mol. Breed.* **35** doi: 10.1007/s11032-015-0205-9 (2015).

28. Lowry, D. B. et al. QTLs for biomass and developmental traits in switchgrass (*Panicum virgatum*). *Bioenerg. Res.* **8**, 1856-1867 (2015).

29. Shehzad, T. & Okuno K. QTL mapping for yield and yield-contributing traits in sorghum (*Sorghum bicolor* (L.) Moench) with genome-based SSR markers. *Euphytica* **203**, 17-31 (2015).

30. Wang, P. et al. Fine mapping a major QTL for flag leaf size and yield-related traits in rice. *Theor. Appl. Genet.* **123**, 1319-1330 (2011).

31. Wang, J. et al. A high-density genetic map and QTL analysis of agronomic traits in foxtail millet [*Setaria italica* (L.) P. Beauv.] using RAD-seq. *PloS one* **12**: e0179717 (2017).

32. Yue, B. et al. QTL analysis for flag leaf characteristics and their relationships with yield and yield traits in rice. *Acta Genetica Sinica* **33**, 824-832. (2006).

33. Zhu, L. et al. Differential expression of lodging resistance related QTLs in rice (*Oryza sativa* L.). *Plant Sci.* **175**, 898-905 (2008).

34. Huang, N. et al. Association of quantitative trait loci for plant height with major dwarfing genes in rice. *Heredity* **77**, 130–137 (1996).

35. Li, Z. et al. Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **91**, 374–381 (1995).

36. Xue, C. et al. Characterization and Fine Mapping of *SiDwarf2* (D2) in Foxtail Millet. *Crop Sci.* **56**, 95–103 (2016).

37. Tsukaya, H. Mechanism of leaf-shape determination. *Annu. Rev. Plant Biol.* **57**, 477–496 (2006).

38. Wang, P. et al. Clustered QTL for source leaf size and yield traits in rice (*Oryza sativa* L.). *Mol. Breed.* **29**, 99-113 (2012).

39. Zhang, B. et al. Genetic analysis of flag leaf size and candidate genes determination of a major QTL for flag leaf width in rice. *Rice* **8** doi: 10.1186/s12284-014-0039-9 (2015).

40. Johnson, E. B. et al. Fractionation, stability, and isolate-specificity of QTL for resistance to *Phytophthora infestans* in cultivated tomato (*Solanum lycopersicum*). *G3: Genes, Genomes, Genet.* **2**, 1145–1159 (2012).

41. Kimball, J. A. et al. Linkage analysis and identification of quantitative trait loci associated with freeze tolerance and turf quality traits in St. Augustinegrass. *Mol. Breed.* **38** doi.org/10.1007/s11032-018-0817-y (2018).

42. Hensleigh, P. et al. Natural selection on winter barley composite cross XXVI affects winter survival and associated traits. *Crop Sci.* **32**, 57-62 (1992).

43. Stefaniak, T. R., et al. The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. *Crop Sci.* **49**, 1489–1495 (2009).

44. Anderson, J. A. et al. Evaluating freeze tolerance of bermudagrass in a controlled environment. *HortScience* **28**, 955–955 (1993).

45. Fry, J. D. et al. Freezing tolerance and carbohydrate content of low temperature acclimated and non-acclimated centipedegrass. *Crop Sci.* **33**, 1051–1055 (1993).

46. Qian, Y. et al. Freezing tolerance of six cultivars of buffalograss. *Crop Sci.* **41**, 1174–1178 (2001).

47. Zhang, X. et al. Metabolic defense responses of seeded bermudagrass during acclimation to freezing stress. *Crop Sci.* **46**, 2598-2605. 2006.

48. Chen, L. et al. A transcriptomic analysis of bermudagrass (*Cynodon dactylon*) provides novel insights into the basis of low temperature tolerance. *BMC Plant Boil.* **15**, 216-237 (2015).

49. Wang, S. et al. *MicroRNA319* positively regulates cold tolerance by targeting *OsPCF6* and *OsTCP21* in rice (*Oryza sativa* L.). *PloS one* **9**, e91357 doi:10.1371/journal.pone.0091357 (2014).

50. Yang, C. et al. Overexpression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*Oryza sativa* L.). *Plant Cell Environ.* **36**, 2207-2218 (2013).

51. Song, J. et al. miR394 and its target gene LCR are involved in cold stress response in *Arabidopsis*. *Plant Gene* **5**, 56-64 (2016).

52. Liu, Y. et al. Heteroexpression of *Osa-miR319b* improved switchgrass biomass yield and feedstock quality by repression of *PvPCF5*. *Biotechnol. Biofuels* **13**, 1-13 (2020).

53. Nag, A. et al. miR319a targeting of *TCP4* is critical for petal growth and development in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **106**, 22534-22539 (2009).

54. Zhou, M. et al. Constitutive expression of a *miR319* gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. *Plant physiol.* **161**, 1375-1391 (2013).

55. Morris, K. N. & Shearman, R. C. NTEP Turfgrass Evaluation Guidelines. [Online]. Available at http://www.ntep.org/pdf/ratings.pdf. *National Turfgrass Evaluation Program*. Beltsville, MD (2000).

56. Chang, D. et al. Quantitative trait loci mapping for tillering-related traits in two switchgrass populations. *Plant Genome* **9** doi: 10.3835/plantgenome2016.01.0010 (2016).

57. Gordon, I. L. et al. Variance of heritability ratios estimated from phenotypic variance components. *Biometrics* **28**, 401–415 (1972).

58. Hallauer, A. R., & Miranda, J. B. Quantitative genetics in maize breeding. *Iowa State Univ. Press*, Ames, IA. (1981).

59. Van Ooijen, J. W. MapQTL 6: Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma B.V., Wageningen, the Netherlands (2006).

60. Dong H. et al. QTL mapping for reproductive maturity in lowland switchgrass populations. *BioEnergy Res.* **8**, 1925–1937 (2015).

61. Voorrips, R. E. MAPCHART: software for the graphical presentation of linkage maps and QTLs. *J. Heredity* **93**, 77–78 (2002).

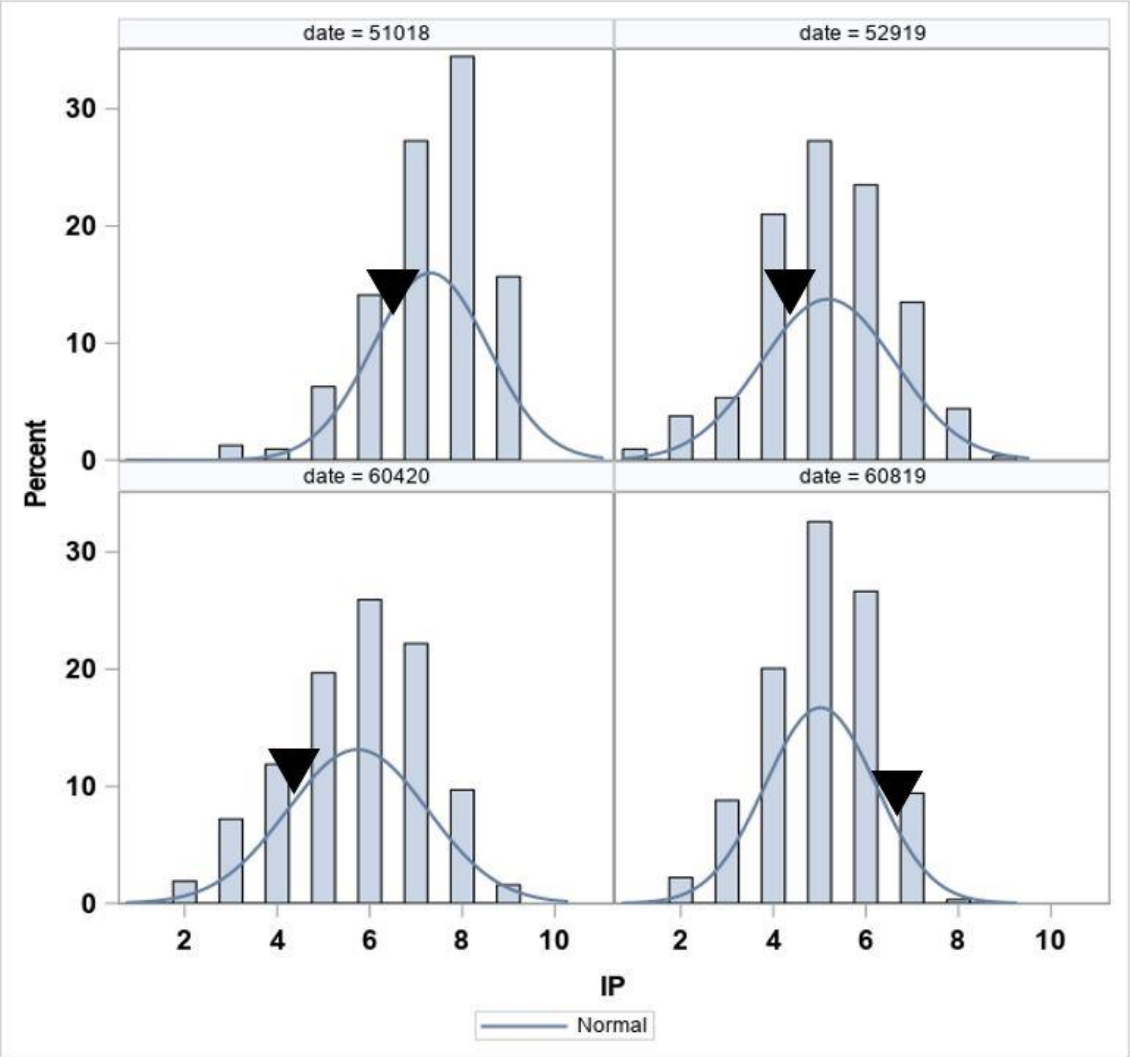

Fig. 1 Distribution of inflorescence prolificacy (IP) for the OKC1163 S_1 population evaluated from 2018 to 2020. Solid triangles indicate OKC1163 means.

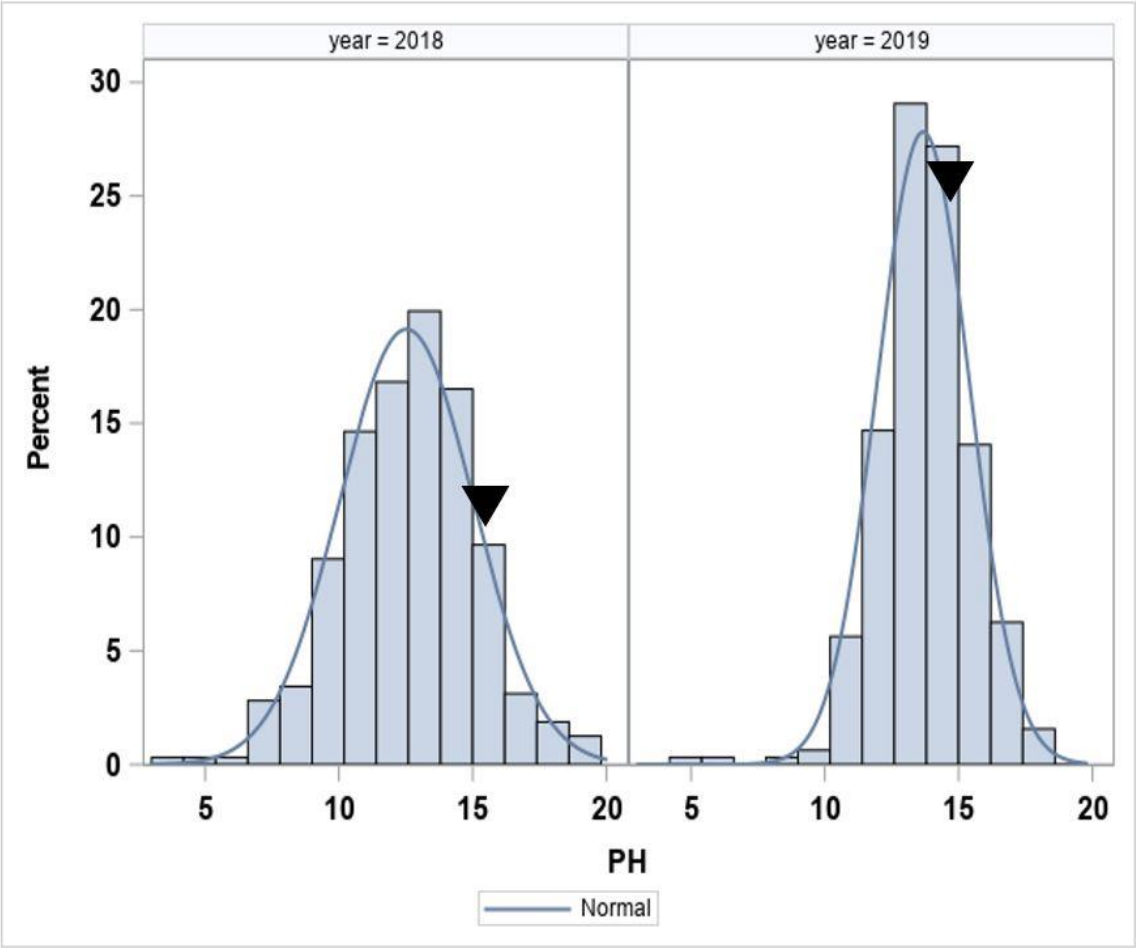

Fig. 2 Distribution of plant height (PH, cm) for the OKC1163 S_1 population evaluated in 2018 and 2019. Solid triangles indicate OKC1163 means.

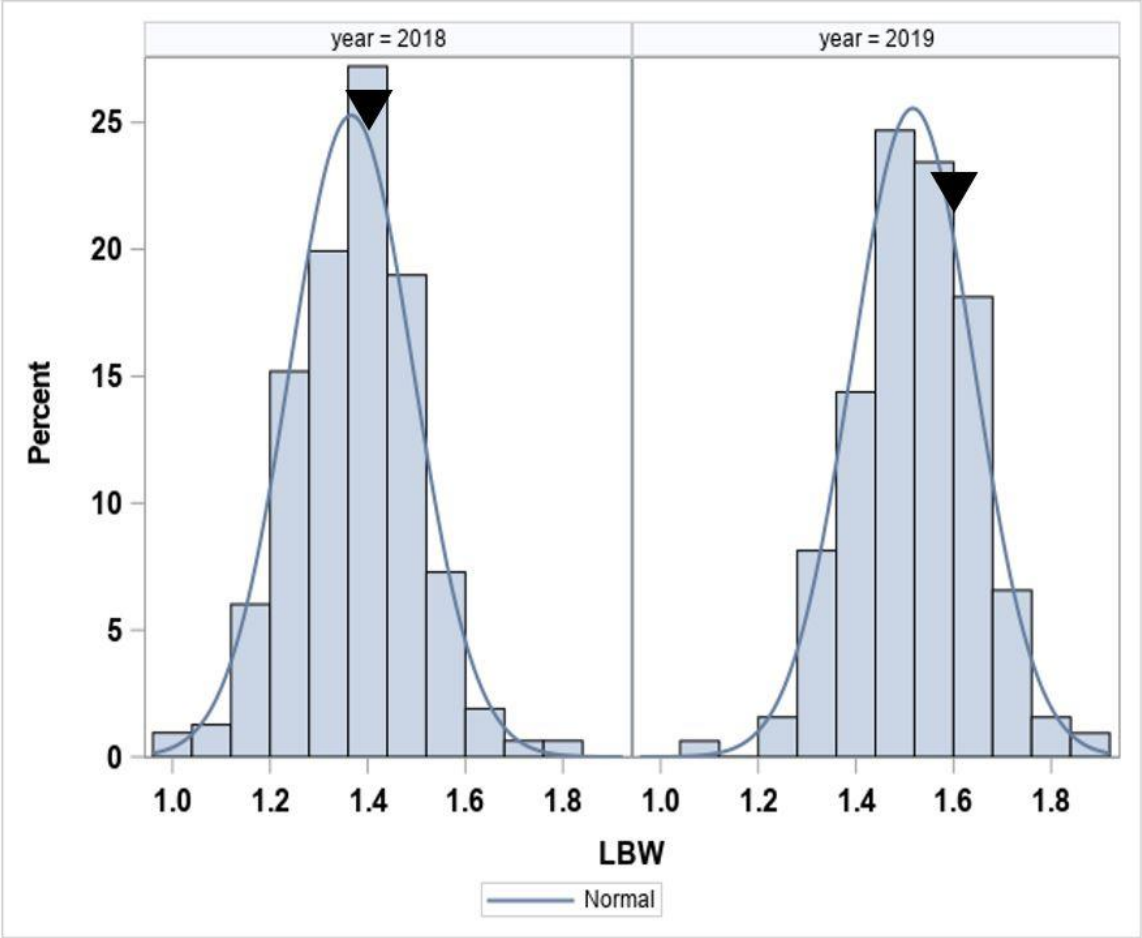

Fig. 3 Distribution of leaf blade width (LBW, mm) for the OKC1163 S_1 population evaluated in 2018 and 2019. Solid triangles indicate OKC1163 means.

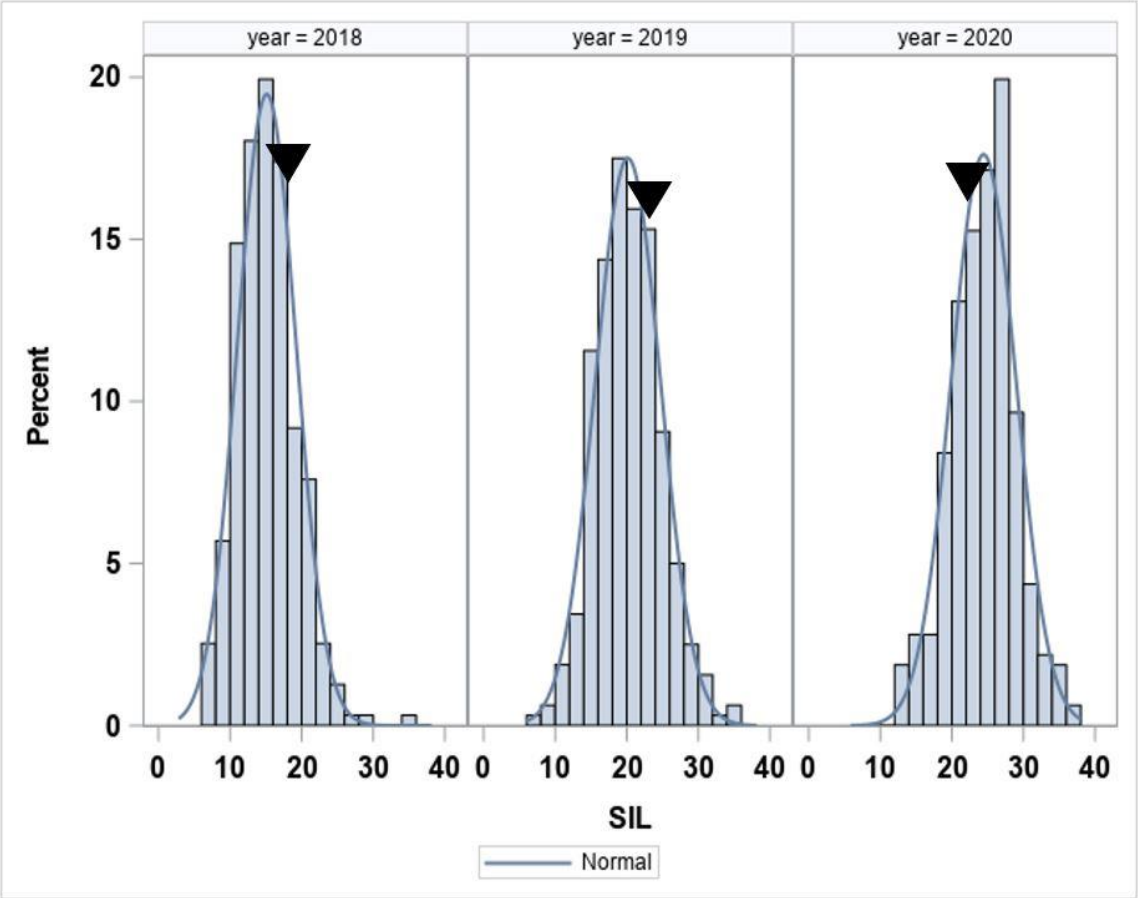

Fig. 4 Distribution of stem internode length (SIL, mm) for the OKC1163 S_1 population evaluated from 2018 to 2020. Solid triangles indicate OKC1163 means.

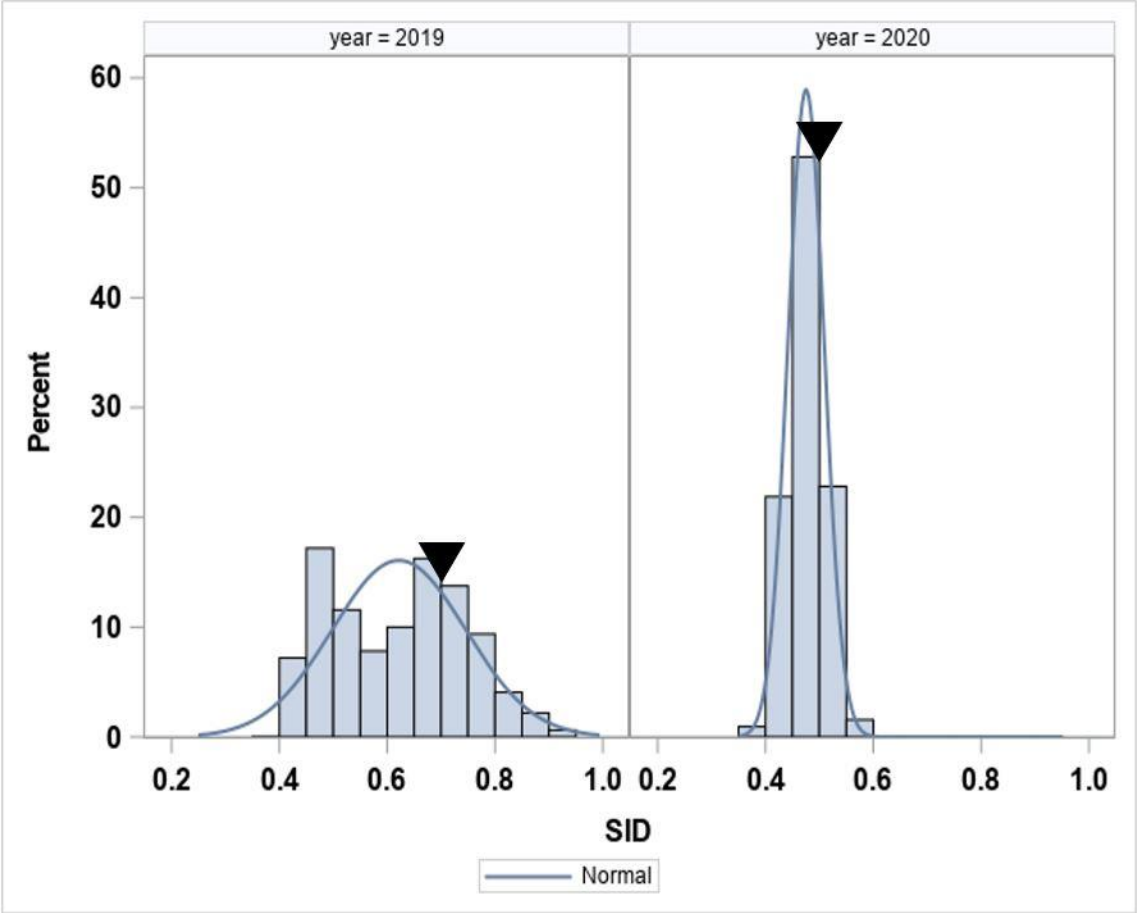

Fig. 5 Distribution of stem internode length (SIL, cm) for the OKC1163 S1 population evaluated in 2019 and 2020. Solid triangles indicate OKC1163 means.

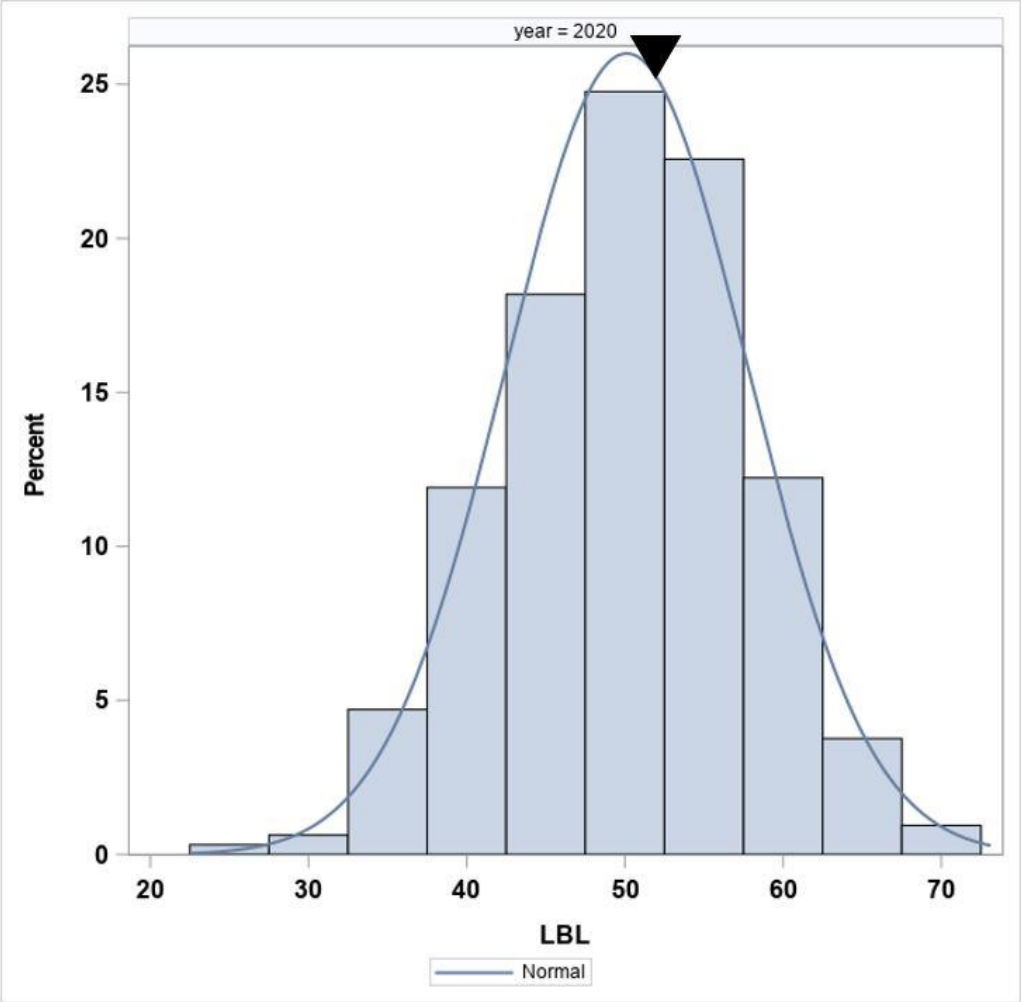

Fig. 6 Distribution of leaf blade length (LBL, mm) for the OKC1163 S1 population evaluated in 2020. Solid triangle indicates OKC1163 mean.

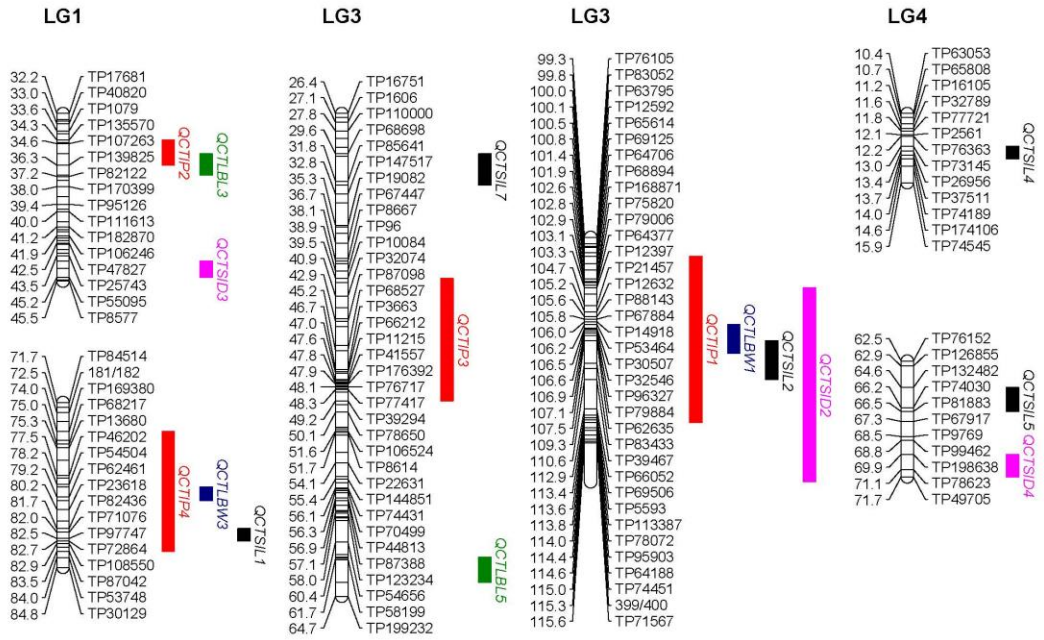

Fig. 7 The locations of QTL peaks for inflorescence prolificacy (IP), plant height (PH), leaf blade width (LBW), stem internode length (SIL), stem internode diameter (SID), and leaf blade length (LBL) on the right side of each of OKC1163 linkage groups (LG) 1, 3, and 4.

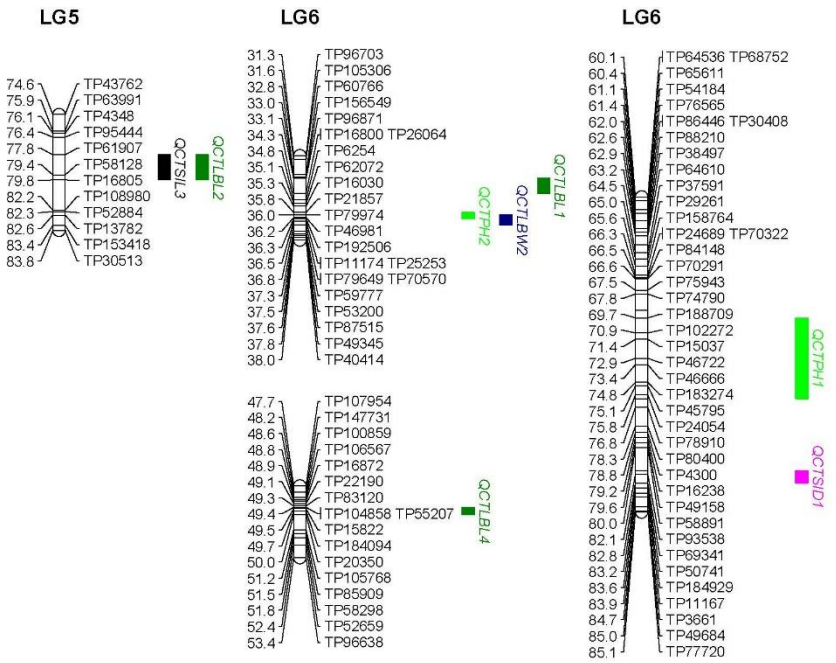

Fig. 8 The locations of QTL peaks for inflorescence prolificacy (IP), plant height (PH), leaf blade width (LBW), stem internode length (SIL), stem internode diameter (SID), and leaf blade length (LBL)on the right side of each of OKC1163 linkage groups (LG) 5 and 6.

Table 1. Descriptive statistics of inflorescence prolificacy (IP), plant height (PH), leaf blade width (LBW), stem internode length (SIL), stem internode diameter (SID), and leaf blade length (LBL) collected in the OKC1163 S_1 population.

Trait	Dataset	S_1 population						Parent
		Mean	Max.	Min.	SD	Skewness	Kurtosis	
IP[a]	51018	7.3	9.0	3.0	1.3	-0.8	0.8	6.0
	52919	5.2	9.0	1.0	1.5	-0.2	0.1	4.3
	60819	5.0	8.0	2.0	1.2	-0.3	-0.3	4.3
	60420	5.7	9.0.	2.0	1.5	-0.3	-0.4	6.7
PH (cm)[b]	101518	12.5	19.6	3.3	2.5	-0.1	0.5	15.5
	72519	13.7	18.4	5.2	1.7	-0.6	2.4	14.3
LBW (mm)[c]	62918	1.4	1.8	1.0	0.1	0.0	0.5	1.4
	62819	1.5	1.9	1.1	0.1	-0.1	0.4	1.6
SIL (mm)[d]	62918	15.1	35.4	6.2	4.1	0.7	1.6	17.5
	62819	20.2	35.3	7.8	4.6	0.3	0.5	23.1
	61220	24.5	37.0	12.1	4.6	-0.2	0.2	23.6
SID (mm)[e]	62819	0.6	0.9	0.4	0.1	0.1	-1.0	0.7
	61220	0.5	0.6	0.4	0.0	0.1	0.2	0.5
LBL (mm)[f]	62819	50.1	70.3	23.4	7.7	-0.2	-0.1	53.2

SD standard deviation.

[a]IP = inflorescence prolificacy was rated on a scale from 1-9 where 1 = most abundant seedhead and 9 = no seedhead.

[b]PH = plant height was measured from ground to the top of leaves from five random spots in each plot.

[c]LBW = leaf blade width was measured on the leaf on the third stem node from five random samples collected in each plot.

[d]SIL = stem internode length was measured between the second and the third nodes from five random samples collected in each plot.

[e]SID = stem internode diameter was measured on the internode between the second and the third nodes from five random samples collected in each plot.

[f]LBL = leaf blade length was measured on the leaf on the third stem node from five random samples collected in each plot.

Table 2. Analysis of variance of inflorescence prolificacy (IP) collected in three years (2018, 2019, and 2020), plant height (PH), leaf blade width (LBW), stem internode length (SIL), and stem internode diameter (SID) collected in two years (2018 and 2019), and leaf blade length (LBL) in 2019, in OKC1163 S_1 population.

Source of variation	IP[a]		PH		SIL		SID		LBW		LBL	
	P > F	Variance component	P > F	Variance component	P > F	Variance component	P > F	Variance component	P > F	Variance component	P > F	Variance component
Replication	<.0001		0.0004		0.0003		0.4753		<.0001		0.1559	
Year	0.0364		<.0001		<.0001		0.1676		<.0001			
Date(Year)	0.0626											
Genotype	<.0001	0.4757	0.0177	0.4989	<.0001	4.415	0.0361	0.0003	<.0001	0.0052	<.0001	27.9922
Genotype*Replication	<.0001	0.1540	<.0001	0.9667	0.0221	1.059	0.7650	0.0000	0.2754	0.0004		
Genotype*Year	0.0085	0.0100	0.0001	0.6691	<.0001	2.174	0.1232	0.0002	0.1905	0.0005		
genotype*date(Year)	0.0096	0.1179										
Residual		0.8897		2.4063		11.533		0.0029		0.0093		30.6670
H		0.74		0.32		0.65		0.34		0.73		0.73

[a]For trait acronyms, see Table 1.

Table 3. Within-year Pearson correlation coefficients among plant height (PH), leaf blade width (LBW), leaf blade length (LBL), stem internode length (SIL), stem internode diameter (SID), and inflorescence prolificacy (IP) evaluated in the OKC1163 S_1 population during three years.

Season	Trait	PH	LBW	SIL	IP	SID
2018	LBW	0.09				
	SIL	0.17	0.48****			
	IP	-0.15	0.15	-0.07		
2019	LBW	0.27**				
	SIL	0.38***	0.31**			
	IP	0.09	0.45****	0.32***		
	SID	0.01	0.49****	0.23*	0.23*	
	LBL	0.30**	0.46****	0.47****	0.21*	0.45****
2020	IP			-0.16		
	SID			-0.12	0.26**	

Significant correlations are indicated by *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.
[a]For trait acronym, see Table 1.

Table 4. Pearson correlation coefficients for plant height (PH), leaf blade width (LBW), stem internode length (SIL), stem internode diameter (SID), and inflorescence prolificacy (IP) among seasons.

Trait	Season	2019	2020
PH[a]	2018	0.36***	
LBW	2018	0.59****	
SIL	2018	0.56****	0.31***
	2020	0.41****	
IP	2018	0.43***	0.28**
	2020	0.73****	
SID	2020	0.26**	

Significant correlations are indicated by *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.
[a]For trait acronyms, see Table 1.

Table 5. QTL for inflorescence prolificacy (IP), plant height (PH), leaf blade width (LBW), stem internode length (SIL), stem internode diameter (SID), and leaf blade length (LBL) identified in the African bermudagrass OKC1163 S_1 population.

Trait dataset	QTL	LG	LOD	Peak	Locus	PVE.	Dominate	Additive	LOD threshold[b]
IP[a] 051018	*QCTIP1*	3	7.08	109.68	TP12115	21.2%	-0.09	-0.65	4.0
	QCTIP2	1	4.15	36.25	TP139825	11.7%	-0.31	0.41	
IP 052919	*QCTIP1*	3	7.12	113.96	TP78072	21.0%	0.42	-0.80	4.0
	QCTIP2	1	5.25	34.31	TP135570	14.7%	-0.38	0.61	
IP 060819	*QCTIP1*	3	5.65	100.84	TP69125	14.5%	0.24	-0.48	4.0
	QCTIP3	3	5.42	49.16	TP39294	13.8%	-0.12	0.49	
	QCTIP4	1	4.55	83.53	TP87042	11.4%	0.12	0.43	
IP 060420	*QCTIP1*	3	5.76	106.47	TP30507	14.2%	0.30	-0.65	
	QCTIP4	1	5.46	74.03	TP169380	13.4%	-0.01	0.70	
	QCTIP3	3	4.86	39.48	TP10084	11.7%	-0.06	0.60	
PH 101518	*QCTPH1*	6	7.58	70.71	TP102272/TP188709	27.4%	0.50	-1.40	4.1
PH 072519	*QCTPH1*	6	5.37	76.08	TP74779	20.3%	0.64	-0.65	4.3
	QCTPH2	6	3.17	36.05	TP79974	10.0%	1.36	1.44	
LBW 062918	*QCTLBW1*	3	11.04	108.46	TP62635/TP83433	32.9%	-0.07	-0.07	4.0
	QCTLBW2	6	5.92	36.84	TP70570	16.0%	0.13	0.13	
LBW 062819	*QCTLBW1*	3	8.28	106.20	TP53464	21.8%	0.06	-0.06	3.9
	QCTLBW2	6	7.04	36.05	TP79974	18.0%	0.13	0.13	
	QCTLBW3	1	4.44	79.16	TP62461	10.7%	0.05	0.05	
SIL 062918	*QCTSIL1*	1	4.37	82.03	TP71076	12.2%	2.27	0.11	3.9
	QCTSIL2	3	3.53	107.46	TP62635	9.7%	2.00	-0.47	
SIL 062919	*QCTSIL3*	5	7.38	79.41	TP58128	16.1%	-1.34	-1.75	4.0
	QCTSIL4	4	3.97	13.42	TP26956	8.0%	1.81	-0.82	
	QCTSIL5	4	3.96	66.21	TP74030	8.0%	1.38	0.97	
SIL 061220	*QCTSIL2*	3	9.24	110.55	TP39467	22.0%	2.70	1.33	4.0
	QCTSIL6	6	7.52	36.79	TP79649	17.0%	5.82	5.33	
	QCTSIL7	3	6.67	29.83	TP66977	15.0%	1.72	-1.49	
SID 062819	*QCTSID1*	6	3.00	82.06	TP93538	11.0%	0.03	-0.01	4.0
	QCTSID2	3	2.78	103.26	TP12397	10.0%	0.03	0.01	
SID 061220	*QCTSID2*	3	6.99	118.39	TP9721	21.4%	0.01	-0.02	3.9
	QCTSID3	1	3.74	44.55	TP72679	10.7%	-0.04	0.03	

	QCTSID4	4	3.07	71.08	TP78623	8.6%	-0.01	0.01	
LBL 062819	*QCTLBL1*	6	6.39	34.27	TP16800	13.1%	7.65	7.04	4.0
	QCTLBL2	5	5.23	79.41	TP58128	10.4%	-3.12	-2.16	
	QCTLBL3	1	4.27	36.25	TP139825	8.3%	2.76	1.96	
	QCTLBL4	6	4.11	49.67	TP184049	8.0%	4.27	0.16	
	QCTLBL5	3	3.95	61.82	TP69811	7.7%	0.72	2.63	

QTL, quantitative trait loci; LG, linkage group; LOD, logarithm of the odds; PVE, phenotypic variance explained.

[a]For trait acronyms, see Table 1.

[b]Genome-wide LOD threshold determined using 1000 permutations at $P < 0.05$.

Table 6. Means and associated standard deviations of leaf blade width (LBW), stem internode length (SIL), stem internode diameter (SID), and inflorescence prolificacy (IP) in an S_1 population of two SNP markers that closely linked to major QTL of LBW, SIL, SID, and IP on LG3.

Marker	Genotype	LBW [a]		SID		SIL			IP		
		2018	2019	2019	2020	2018	2019	2020	2018	2019	2020
TP62635	A	1.27±0.06	1.43±0.08	0.61±0.05	0.46±0.02	12.95±2.47	17.87±2.37	24.65±2.96	6.75±1.20	3.48±0.84	4.72±1.09
	H	1.39±0.07	1.54±0.09	0.64±0.05	0.48±0.02	16.05±2.68	20.89±2.76	25.73±3.14	7.21±0.74	5.08±0.82	5.82±1.09
	B	1.39±0.10	1.54±0.09	0.61±0.04	0.48±0.02	14.69±3.59	20.53±3.69	22.27±3.61	7.91±0.67	5.18±0.82	6.18±1.00
TP16800	A	1.26±0.05	1.46±0.11	0.60±0.04	0.47±0.02	13.15±2.15	18.81±3.12	24.76±2.59	6.61±1.74	4.73±0.94	5.13±1.38
	H	1.38±0.07	1.54±0.09	0.64±0.05	0.48±0.02	15.97±2.75	20.82±2.71	25.54±3.16	7.18±0.79	5.18±0.82	5.92±1.03
	B	1.39±0.10	1.53±0.09	0.61±0.04	0.48±0.02	14.48±3.84	20.34±3.69	22.63±3.93	8.06±0.47	5.02±0.87	5.99±1.09

[a]For trait acronyms, see Table 1.

CHAPTER V

SYNCHRONOUS ASSESSMENT OF GENETIC VARIABILITY FOR WINTER SURVIVABILITY AND DROUGHT RESPONSE IN TURF-TYPE BERMUDAGRASS

ABSTRACT

Winterkill is a major concern for turf-type bermudagrasses (*Cynodon dactylon* and *C. dactylon* × *C. transvaalensis)* when cultivated in the U.S. transition zone. Water scarcity is a widespread issue facing most of the turf industry. So far, winter survivability and drought resistance of turf-type bermudagrasses have not been evaluated concurrently under field conditions, although it would be valuable for new cultivars to have both traits. Therefore, the objectives of this study were to estimate reliability for spring greenup (as an indicator of winter survivability) and drought response in turf-type bermudagrass. Seventy-seven experimental genotypes and seven cultivars were planted in a randomized complete block design with three replications in Goodwell, OK. The reliability estimates for drought response ranged from low to moderate ($0.20 \leq i^2 \leq 0.63$). The estimates for two spring greenup phenotypes were low (0.27 and 0.08), indicating poor reliability of this response and that winter survivability and drought resistance may be evaluated in separate trials.

INTRODUCTION

Turf-type bermudagrass (*Cynodon* spp.) has a dense canopy, vigorous growth, excellent drought resistance, and excellent tolerance to heat and wear stresses. These desirable traits make turf-type bermudagrass the most widely used species on athletic fields, golf courses, residential lawns, and recreation parks in the U.S. southern and transition zones (Beard, 1973; Taliaferro et al., 2004a). When bermudagrass is grown in the transition zone, particularly in the northern portion of the region, a high risk of winterkill exists (Taliaferro et al, 2004a). Winterkill is characterized as the loss of a turfgrass stand due to low temperature stress. Winterkill typically results in substantial expense to a turf facility due to direct cost of re-establishing turf and loss of revenue for an extended period. Efforts have been made to develop turf-type bermudagrass cultivars with improved winter survivability. Since the 1960s, bermudagrass germplasm have been collected from around the world to conduct biosystematics investigation and genetic research at Oklahoma State University. Some of this collection has since been used in improving bermudagrass winter survivability (Harlan and de Wet, 1969; Taliaferro et al., 2004b). 'Patriot' ($2n=4x=36$) released by Oklahoma State University (OSU) in 2002 was an interspecific hybrid with high turfgrass quality and improved winter survivability (Taliaferro, 2004b). Subsequently, three additional interspecific F_1 bermudagrass ($2n=3x=27$) cultivars (Latitude 36®, Northbridge®, and Tahoma 31®) have been released from the OSU bermudagrass breeding program (Wu et al., 2013a; Wu et al., 2014; Wu et al., 2019).

As urban and suburban areas expand, large areas of previously non-irrigated lands are being converted to irrigated lawns and landscapes. The increasing irrigation demand

has exacerbated water scarcity issues, especially in summer. The use of drought resistant turfgrass species and cultivars is a sound management strategy for water conservation (Carrow, 1995; Marcum et al., 1995). Turfgrass consumers in the transition zone need new turf-type bermudagrass cultivars that combine high visual quality, sufficient winter hardiness, and improved drought resistance.

Controlled environment experiments have commonly been used to estimate the lethal freezing temperature to reach 50% mortality (LT_{50}) (Anderson et al., 1993, 2002, 2003) or the physiological response to low temperature stress (Fontanier et al., 2020; Munshaw et al., 2006; Zhang et al., 2006) of selected turfgrasses. Field trials have also been widely used to evaluate spring greenup rate (i.e. post winter dormancy regrowth in spring) (NTEP, 2017; Wu and Anderson, 2011), which is negatively correlated with winterkill and thus a good proxy for winter survivability (Wu et al., 2007). Prior reports have shown substantial variation in spring greenup rate in common bermudagrass (Dunne et al., 2019; Guo et al., 2017; Stefaniak et al., 2009; Wu et al., 2007; Wu et al., 2013b) and African bermudagrass (Dunne et al., 2019; Kenworthy et al., 2006).

Drought resistance is a complex trait that involves the mechanisms of escape, avoidance, and tolerance (Levitt, 1980). Considerable efforts have been devoted to understanding how bermudagrasses use avoidance and tolerance mechanisms in response to water deficit stress (Amgain et al., 2018; Du et al., 2012; Fuentealba et al., 2015; Hays et al., 1991; Katuwal et al., 2020; Su et al., 2013; Yurisic, 2016). Chalmers et al. (2008) reported ‘Premier’ had the lowest turfgrass quality and was most susceptible to leaf firing injury among bermudagrasses at the end of a 60-day drought period under field conditions in San Antonio, TX, while ‘TexTurf’ bermudagrass had the best leaf firing

ratings. Steinke et al. (2011) reported that ‘Celebration’ bermudagrass had the best drought response and took more than 50 days to decrease to 50% green cover in a field trial. Richardson et al. (2010) found that bermudagrasses took over 40 days without water before losing significant live green cover. Yu (2017) found that bermudagrass cultivar ‘TifTuf’ maintained 90% live green cover while ‘Tifway’ only had 34% left after 72 days of acute drought stress.

Drought response and winter survivability have not been evaluated concurrently in the same trial. Therefore, the objectives of this study were to evaluate winter survivability and drought response in turf-type bermudagrass; and to estimate reliability for the traits.

MATERIALS AND METHODS

Plant Materials, Experimental Design and Field Management

Eighty-four bermudagrass genotypes, including 77 experimental genotypes and seven cultivars (Table 1), were tested in a field trial. Plant materials were propagated from sprigs into 2.5 cm diameter cone-tainers in a greenhouse for five weeks before transplanting into a nursery located at OSU Panhandle Research and Extension Center in Goodwell, OK (36 ^{o}59' N, 101^{o}60' W; 6b USDA plant cold hardiness zone) on June 28, 2017. The site has an average summer monthly rainfall of 55 mm and an average annual low temperature of -7.2 ^{o}C. The experimental design was a randomized complete block with three replications. The plot size was 1.8 m x 1.8 m with 0.9 m alleys between plots. Four plugs were transplanted in each plot. Ronstar FLO (oxadiazon, Bayer Environmental Science, Cary, NC) was applied at 2.3 kg ha^{-1} of active ingredient (a.i.) one day after planting for pre-emergence weeds control. The soil type was a Richfield clay loam with a pH of 8.2. Nitrogen was applied at 107 kg ha^{-1} of 46-0-0 (N-P-K) (Chouteau Lime Co., Pryor, OK) at planting and again on August 17, 2017. Alleys were periodically sprayed with 2% glyphosate (Tenkoz, Inc. Alpharetta, GA) and 0.25% (v/v) nonionic surfactant (Winfield Solutions, LLC. St. Paul, MN) to kill excessive growth that might cause cross-contamination.

In early March of 2018 and 2019 (before spring greenup), plots were mowed at 2.5 cm to remove the dormant canopy. Plots were sprayed with 2% (v/v) glyphosate and 0.25% (v/v) nonionic surfactant and a subsequent application of 2.3 kg ha^{-1} a.i. Ronstar FLO for pre-emergence control. Fertilizer was applied as 107 kg ha^{-1} of 46-0-0 (K-P-K) in the first week of May and again in early July each year. The trial was mowed at 3.8 cm

weekly with a rotary mower. BioAdvanced insect, disease, and mite control (Tau-fluvanlinate and Tebuconazole, SBM Life Science Crop, Cary, NC) ready-to-use pesticide was spot sprayed to control spider mites (*Tetranychus urticae* C. L. Koch) in a few plots in each year.

Approximately 2.5 cm of irrigation was applied weekly using an overhead irrigation system to prevent any drought stress during the establishment period. After plots reached approximately 100% coverage, irrigation was withheld to initiate an ambient drought period. The ambient drought period began for each year on July 17, 2018 and July 6, 2019, respectively. Mowing was discontinued when some genotypes showed drought stress symptoms. The ambient drought period concluded on October 4, 2018 and October 3, 2019, respectively. After ambient drought was concluded, irrigation was resumed for recovery until the first frost.

Data Collection

Spring Greenup

Spring greenup (SG) is a measure of the transition from winter dormancy to active spring growth. The visual rating of SG was based on a 1 to 9 rating scale, with 1 being no green color and 9 being fully green in a plot. Spring greenup percent green cover (SGPGC) was calculated from images were taken with a digital camera (Canon Powershot G1X, Canon U.S.A., Inc., Melville, NY) mounted on a custom-built lightbox with battery-powered four compact fluorescent bulbs as light sources (Richardson et al., 2001). Images were analyzed by Turf Analyzer (Karcher et al., 2017) calculating the ratio of green pixels out of total pixels. All the SG and SGPGC data were collected every week or two until some plots fully greenup.

Drought Responses

Turfgrass quality (TQ) is a function of color, green cover, density, leaf texture, uniformity, and abiotic and biotic factors (Morris and Shearman, 2000). Visual ratings of TQ were made on a scale of 1-9, with 1 representing represented completely dead or dormant turf, 9 being outstanding turf, and 6 being minimally acceptable turf. Leaf firing (LF) was defined as the amount of browning on the turfgrass canopy due to drought injury. Visual ratings of LF were made on a 1-9 scale, with 1 being completely straw-colored leaves and 9 being completely green leaves. Percent green cover (PGC) was collected using the same methods as described for SGPGC. Canopy temperature (CT) was measured using an infrared thermometer (Fluke 568, Fluke Corporation, Everett, WA), holding the instrument at the same height pointing to the center of the plot without creating shade. Each drought response measurement was collected biweekly before the presence of severe drought stress. Once drought stress became apparent, each measurement was collected with greater frequency as was feasible. Environmental data including rainfall and temperature were obtained from an onsite weather station (Brock et al., 1995).

Data Analysis

Year, rating date, and genotype were considered random because year and rating date within the year were not chosen based on expected environmental conditions (Gordon et al., 1972), and the information of drought response and winter survivability of these bermudagrass genotypes was unknown before the beginning of the study. Random effects data included repeated measures. These data were analyzed using the MIXED procedure SAS 9.4 (SAS Institute Inc., Cary, NC). Variance components were estimated

using TYPE III method of moments estimation (Chang et al., 2016; Dong et al., 2015). SAS/GLM was used to calculate mean values and standard deviations for phenotypic data collected at each rating date since significant genotype by year and genotype by date within the year interactions existed. Fisher's protected LSD was used to test at the P = 0.05 significance level to separate the genotype means. Pearson correlation coefficients were calculated among collected phenotypic data using SAS/CORR procedure. Since the testing materials were from different breeding backgrounds that do not represent a reference population, reliability would be appropriate compared to heritability (Bernardo, 2002). The reliability (i^2) for data collected in one year was calculated by:

$$i^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GR}^2/R + \sigma_{GD}^2/D + \sigma_E^2/RD)$$

where σ_G^2 stands for the variance of genotype, σ_{GR}^2 for the variance of genotype by replication, σ_{GD}^2 for the variance of genotype by date, σ_E^2 for the error variance, R equals the number of replications, and D equals the number of rating dates (Bernardo, 2002). The reliability (i^2) for data collected in more than one year were calculated by:

$$i^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GY}^2/Y + \sigma_{GR}^2/R + \sigma_{GDY}^2/DY + \sigma_E^2/RDY)$$

where σ_G^2 is the variance of genotype, σ_{GY}^2 the variance of genotype by year, σ_{GR}^2 the variance of genotype by replication, σ_{GDY}^2 the variance of genotype by date within the year, σ_E^2 the error variance, R the number of replications, Y the number of years, and D the number of rating dates.

RESULTS AND DISCUSSION

Drought Response

In 2018, plots were subjected to ambient drought for 79 days during which they received 151.4 mm of rainfall (Oklahoma Mesonet, 2019). The estimated evapotranspiration (ET) for warm-season turfgrass was 336.8 mm (Oklahoma Mesonet, 2019). In 2019, plots were subjected to ambient drought for 89 days during which they received 40.2 mm of rainfall while the estimated ET was 414.3 mm. Subtracting rainfall from ET, the estimated water deficit for warm-season turfgrass increased by 47.8% in 2019 compared to 2018.

The variance components estimation and reliability for drought response measurements are given in Table 3. Significant ($P < 0.001$) genotypic variances were detected in TQ and CT. The genotype by year interactions were highly significant ($P < 0.0001$) for all measurements, suggesting that genotypes did not consistently respond over the two years. The variances of date within the year were highly significant ($P < 0.0001$) for all measurements, and the variances of genotype by date within the year interactions were all significant in all drought response measurements, indicating that genotypes responded differently at different water-deficit levels or environmental conditions (Table 3).

In 2018, at the end of the ambient drought period, the TQ rating ranged from 2 to 6, LF ratings ranged from 4 to 9, and PGC ranged from 16.5 to 96.6% (Table 1). In 2019, many of the genotypes demonstrated numerically lower TQ, LF, and PGC compared to their 2018 values due to more severe water deficit stress. However, the top-performing genotypes from 2018 were largely unchanged, indicating some superior bermudagrass

genotypes were consistent with maintaining green canopy under a prolonged period of water deficit in both years. The differences in drought response measurements observed in the two years likely were due to the different environmental conditions and the accumulated effects. Bermudagrass responded differently to the apparent soil moisture levels, suggesting varying defense mechanisms to water deficit stress

Although the effect of genotype was highly significant ($P < 0.0001$) for TQ and CT, the genetic variance components were not the greatest, indicating the genetic component played a role in drought response, but the environment can also alter the drought response. The reliability estimate for TQ was 0.64, indicating that 64% of the observed variation in TQ was attributed to the genetic difference under drought stress. High (0.7 to 0.76) TQ broad-sense heritability was also reported in non-stressed African bermudagrass and zoysiagrass (*Zoysia* spp.) (Kenworthy et al., 2006; Schwartz et al., 2009). The variances of genotype for LF and PGC were not significant, resulting in a low genotype variance compared to other variance components. LF and PGC are direct indicators of canopy drought response, and CT can be used to predict drought stress five days before symptoms occur (Hong et al., 2019). The reliability estimates for LF, PGC, and CT were low at 0.24, 0.20, and 0.33, receptively. When reliability was estimated by year, the genotype effects were significant ($P < 0.01$) for LF and PGC, and the reliability values were higher compared to analysis using the combined data (Table 3). This suggests that different environments in each year and the environment by genotype interactions affected the drought response. Plant response to drought stress depends on drought avoidance and drought tolerance mechanisms (Levitt, 1980). Drought avoidance includes lower ET rates (Amgain et al., 2018) and the ability to establish a deep and

extensive root system to take up water from the soil profiling (Yurisic, 2016), while drought tolerance is the ability to produce organic acids, amino acids, and sugars to maintain osmotic pressure under drought stress (Du et al., 2012). Because this study was conducted under ambient drought, rainfall could temporarily alleviate drought stress, thus being more similar to chronic stress than acute stress. In addition to favoring genotypes having drought resistance, these conditions may favor those genotypes with a fast drought recovery rate, and possibly illustrate the interaction between drought resistance and drought recovery traits. The large interaction variances between genotypes and the environment are attributed to the low LF, PGC, and CT reliability. Schwartz et al. (2009) reported large error variance and low broad-sense heritability were common for stress-related traits in zoysiagrass, which was consistent with our findings in this study. Kenworthy et al. (2006) reported increased heritability for genetic color when using a normalized difference vegetation index sensor compared to using visual ratings. In contrast, the sensor-based measurements in the present study did not result in a higher reliability estimate than visual rating measurements. This suggests that variations caused by error and interaction effects can be better captured by sensor-rated measurement than visual rating for stress-related traits.

Spring Greenup

The annual low temperature was -15.5 ^{o}C in each winter, and the annual low soil temperature at 10.2 cm was -4.4 ^{o}C in 2017-18 and -2.2 ^{o}C in 2018-19 (Oklahoma Mesonet, 2019). In 2018, at the end of the spring greenup period, the SG rating ranged from 3 to 9, and PGC ranged from 29.6 to 99.9% (Table 1). In 2019, at the end of the spring greenup period, many of the genotypes demonstrated numerically lower SG and

SGPGC and some genotypes showed higher SG and SGPGC compared to their values in 2018, suggesting some genotypes may gain winter hardiness along with maturity and greenup earlier even with shorter regrowth period after dormancy. The testing materials means, ranges, and standard deviations for SG and SGPGC in each rating date are presented in Table 4.

The SG and SGPGC analyses showed that genotypic variances were not significant using combined data (Table 5). The genotype variance estimates were lower than the variances of other components except for the genotype by replication interaction variance for SGPGC. When analyzed by year, the genotype effects of SG and SGPGC were highly significant ($P < 0.0001$), and the genotype variances were the largest among all the components in each year. Genotype by year interactions and the genotype by date within the year interactions were highly significant ($P < 0.0001$), suggesting genotypes responded differently over the two years and different dates within each year.

The reliability estimates for SG and SGPGC were 0.08 and 0.27, respectively (Table 5). The bermudagrass spring greenup reliability in this study was much lower than common bermudagrass (0.77 to 0.91) reported by Stefaniak et al. (2009) and Guo et al. (2017) and African bermudagrass (0.89) reported by Kenworthy et al. (2006). The low SG and SGPGC reliability in this study could likely be attributed to the large genotype by year interaction and error variances, which were likely due to testing drought response as far into the season as early October. The apparent lack of a sufficient drought recovery period prior to winter dormancy likely influenced SG and SGPGC uniquely each year, which caused the low reliability estimate. To test how drought injury influenced the SG and SGPGC reliability estimation, data were analyzed by year, which resulted in a

reliability estimate greater than 0.7 each year (Table 5). This suggests that even with dramatic differences in SG between 2018 and 2019 values, the relative SG performance was consistent within each year. Genotypes having different SG values each year were likely more affected by drought stress in summer 2018 than those having similar SG in each year. Winter desiccation of plant tissues, particularly the crown, has often been attributed to increased winterkill in turfgrasses (Beard, 1973). Localized dry spot on sand-based putting greens during the late fall has also been reported as increasing winterkill or delaying SG (Deboer et al., 2017; Deboer et al., 2019). No reports have previously attributed summer drought stress and associated tissue desiccation to subsequent winterkill. It is reasonable to assume that drought injuries on tissues or organs of some bermudagrass genotypes, especially crown cause more winter injury in 2019 than the only low temperature kill effect in 2018. However, for some genotypes with little or no drought injuries, the metabolites produced from osmotic adjustments such as proline and sugar may facilitate the cold acclimation process that helps bermudagrass gaining winter hardiness that causes earlier spring greenup (Du et al., 2012; Zhang et al., 2006). These hypotheses can be supported by some genotypes showed earlier spring greenup and some showed delayed greenup in this study. Schwartz et al. (2009) reported low to moderate spring greenup heritability in zoysiagrass when subjected to stresses such as disease caused by *Bipolaris* spp., glufosinate herbicide application and mole cricket damage that influence the spring greenup performance in next spring. Thus, in order to accurately quantify spring greenup reliability, stresses such as drought and diseases should be avoided in summer, or stress-related injuries need full recovery before

going dormant in the fall. Although the reliability will decrease, testing and selection in a multi-stressed environment is a reliable way to identify superior genotypes.

Correlation Analysis

Highly significant ($P < 0.0001$), moderate to high positive correlations were found within spring greenup and drought response measurements (Table 6). Canopy temperature was negatively correlated with other drought response measurements with highly significant ($P < 0.0001$) values ranging from -0.65 to -0.78, indicating CT can be a useful parameter in describing drought response. Interestingly, low to moderate (0.23 to 0.43) but significant ($P < 0.05$) correlations were found between spring greenup and most drought response measurements (0.23 to 0.43), suggesting the genotypes with better drought response greened up earlier. Guo et al. (2017) also reported the moderate to high (0.66) correlation between SG and turf density. The correlations between spring and drought response measurements suggested the potential of identifying genotypes with improved drought response and winter survivability.

CONCLUSION

Substantial genetic variability was characterized in this set of 84 turf-type bermudagrass genotypes. Moderate to high reliability estimates were found in TQ for drought response. The results suggested that the selection of these traits should be repeatable under drought stress. Lower reliability for drought response measurements LF, PGC, and CT suggested that these measurements can be affected by environment and genotype by environment effects. The low SG and SGPGC reliability estimates caused by drought stress, and genotype by environment interactions indicated that winter survivability selection was not reliable when tested in the same trial with drought stress. Therefore, testing winter survivability and drought response may be conducted in separate trials.

REFERENCES

Anderson, J.A., Taliaferro, C.M., Martin, D.L., 1993. Evaluating freeze tolerance of bermudagrass in a controlled environment. HortScience 28, 955.

Anderson, J.A., Taliaferro, C.M., 2002. Freeze tolerance of seed-producing turf bermudagrasses. Crop Sci. 42, 190–192.

Anderson, J.A., Taliaferro, C.M., Martin, D.L., 2003. Longer exposure durations increase freeze damage to turf bermudagrasses. Crop Sci. 43, 973–977.

Amgain, N.R., Harris, D.K., Thapa, S.B., Martin, D.L., Wu, Y.Q., Moss, J.Q., 2018. Evapotranspiration rates of turf bermudagrasses under nonlimiting soil moisture conditions in Oklahoma. Crop Sci. 58, 1409–1415.

Beard, J.B., 1973. Turfgrass: Science and culture. Prentice-Hall, Englewood Cliffs, NJ.

Bernardo, R., 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Brock, F.V., Crawford, K.C., Elliott, R.L., Cuperus, G.W., Stadler, S.J., Johnson, H. L., Eilts, M.D., 1995. The Oklahoma Mesonet: A technical overview. J. Atmos. Oceanic Technol. 12, 5–19.

Carrow, R.N., 1995. Drought resistance aspects of turfgrasses in the southeast: evapotranspiration and crop coefficients. Crop Sci. 35, 1685–1690.

Chalmers, D.R., Steinke, K.S., White, R., Thomas, J.C., Fipps, G., 2008. Evaluation of sixty-day drought survival in San Antonio of established turfgrass species and cultivars. Final report to the San Antonio Water System & the Turfgrass Producers of Texas. pp. 1–60.

Chang, D., Wu, Y.Q., Liu, L., Lu-Thames, S., Dong, H., Goad, C.L., Bai, S., Makaju, S, Fang, T., 2016. Quantitative trait loci mapping for tillering-related traits in two switchgrass populations. Plant Genome 9, doi: 10.3835/plantgenome2016.01.0010.

DeBoer, E.J., Karcher, D.E., McCalla, J.H. Richardson, M.D., 2017. Effect of late-fall wetting agent application on winter survival of ultradwarf bermudagrass putting greens. Crop Forage Turfgrass Management: e20035, doi.org/10.1002/cft2.20035.

DeBoer, E.J., Richardson, M.D., McCalla, J.H. Karcher, D.E., 2019. Reducing ultradwarf bermudagrass putting green winter injury with covers and wetting agents. Crop Forage Turfgrass Management 5, doi.org/10.2134/cftm2019.03.0019.

Dong, H., Thames, S., Liu, L., Smith, M.W., Yan, L., Wu, Y.Q., 2015. QTL mapping for reproductive maturity in lowland switchgrass populations. BioEnergy Res. 8, 1925–1937.

Du, H., Wang, Z., Yu, W., Huang, B., 2012. Metabolic responses of hybrid bermudagrass to short-term and long-term drought stress. J. Amer. Soc. Hort. Sci. 137, 411–420.

Dunne, J.C., Tuong, T.D., Livingston, D.P., Reynolds, W.C., Milla-Lewis, S.R., 2019. Field and laboratory evaluation of bermudagrass germplasm for cold hardiness and freezing tolerance. Crop Sci. 59, 392–399.

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

Fontanier, C.H., Moss, J.Q., Gopinath, L., Goad C.L., Su, K., Wu, Y.Q., 2020. Lipid composition of three bermudagrasses in response to chilling stress. J. Amer. Soc. Hort. Sci. 145, 95–103.

Fuentealba, M.P., Zhang, J., Kenworthy, K.E., Erickson, J.E., Kruse, J., Trenholm, L.E., 2015. Root development and profile characteristics of bermudagrass and zoysiagrass. HortScience 50, 1429–1434.

Gordon, I.L., Byth, D.E, Balaam, L.N., 1972. Variance of heritability ratios estimated from phenotypic variance components. Biometrics 28, 401–415.

Guo, Y., Wu, Y.Q., Moss, J.Q., Anderson, J.A., Zhu, L., 2017. Genetic variability for adaptive, morphological, and reproductive traits in selected cold-hardy germplasm of common bermudagrass. Crop Sci. 57, 1–7.

Harlan, J.R., De Wet, J., 1969. Sources of Variation in *Cynodon dactylon* (L). Pers. Crop Sci. 9, 774–778.

Hays, K., Barber, J., Kenna, M., McCollum, T., 1991. Drought avoidance mechanisms of selected bermudagrass genotypes. HortScience 26, 180–182.

Hong, M., Bremer, D.J., van der Merwe, D., 2019. Thermal imaging detects early drought stress in turfgrass utilizing small unmanned aircraft systems. Crop Sci. 59, 1–16.

Karcher D.E., Purcell C.J., Richardson M.D., Purcell L.C., Kenneth W.H., 2017. New Java program to rapidly quantify several turfgrass parameters from digital images. CSSA Oral presentation. https://scisoc.confex.com/crops/2017am/webprogram/Paper109313.html.

Katuwal, K.B., Schwartz, B., Jespersen, D., 2020. Desiccation avoidance and drought tolerance strategies in Bermudagrasses. Environ. Exp. Bot. 171, doi.org/10.1016/j.envexpbot.2019.103947

Kenworthy, K.E., Taliaferro, C.M., Carver, B.F., Martin, D.L., Anderson, J.A., Bell, G.E., 2006. Genetic variation in *Cynodon transvaalensis* Burtt-Davy. Crop Sci. 46, 2376–2381.

Levitt, J., 1980. Response of plants to environmental stresses. 2nd ed. Academic Press, New York.

Marcum, K.B., Engelke, M., Morton, S.J., White, R.H., 1995. Rooting characteristics and associated drought resistance of zoysiagrasses. Agron. J. 87, 534–538.

Morris, K.N., Shearman, R.C., 2000. NTEP Turfgrass Evaluation Guidelines. [Online]. Available at http://www.ntep.org/pdf/ratings.pdf. National Turfgrass Evaluation Program. Beltsville, MD.

Munshaw, G.C., Ervin, E.H., Shang, C., Askew, S.D., Zhang, X., Lemus, R.W., 2006. Influence of late-season iron, nitrogen, and seaweed extract on fall color retention and cold tolerance of four bermudagrass cultivars. Crop Sci. 46, 273–283.

National Turf Evaluation Program (NTEP), 2017. National bermudagrass test. 2013–2017 data. Final Report. July 21. 2020. http://www.ntep.org/reports/bg13/bg13_18-14f/bg13_18-14f.htm

Oklahoma Mesonet, 2019. Past Data. July 21. 2020. http://www. mesonet.org.

Richardson, M.D., Karcher, D.E., Purcell, L.C., 2001. Quantifying turfgrass cover using digital image analysis. Crop Sci. 41, 1884–1881.

Richardson, M.D., Karcher, D.E., McCalla, J., 2010. Drought tolerance of 15 bermudagrass cultivars. Arkansas Turfgrass Report 2009, Ark. Ag. Exp. Stn. Res. Ser. 579, 112–115.

Schwartz, B.M., Kenworthy, K.E., Engelke, M., Genovesi, A.D., Quesenberry, K.H., 2009. Heritability estimates for turfgrass performance and stress response in *Zoysia* spp. Crop Sci. 49, 2113–2118.

Stefaniak, T.R., Rodgers, C., Van Dyke, R., Williams, D., Phillips, T., 2009. The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. Crop Sci. 49, 1489–1495.

Steinke, K., Chalmers, D., Thomas, J., White, R., 2011. Bermudagrass and buffalograss drought response and recovery at two soil depths. Crop Sci. 51, 1215–1223.

Su, K., Moss, J.Q., Martin, D.L., Zhang, G., Wu, Y.Q., 2013. Bermudagrass drought tolerance associated with dehydrin protein expression during drought stress. J. Amer. Soc. Hortic. Sci. 138, 277–282.

Taliaferro, C.M., Rouquette, Jr. F.M., Mislevy, P., 2004a. Bermudagrass and stargrass. In Moser, Z.Z., Burson, B.L., Sollenberger, L.E. (Eds.), Warm-season (C_4) grasses. ASA Monogr. No. 45. ASA, CSSA, and SSSA, Madison, WI. pp. 417–475.

Taliaferro, C.M., Martin, D.L., Anderson, J.A., Anderson, M.P., Guenzi, A.C., 2004b. Broadening the horizons of turf bermudagrass. U.S. Golf Assoc. TERO Online 3, 1–9.

Wofford, D.S., Baltensperger, A.A., 1985. Heritability estimates for turfgrass characteristics in bermudagrass. Crop Sci. 25, 133–136.

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

Wu, Y.Q., Anderson, J.A., 2011. Genetic improvement of cold hardiness in bermudagrass. In: Pessarakli, M. (Eds.), Handbook of Plant and Crop Stress (3rd edition). CRC Press of Taylor & Francis Group, Boca Raton, FL. pp 851-865.

Wu, Y.Q., Martin, D.L., Taliaferro, C.M., Anderson, J.A., Moss, J.Q., 2013a. Northbridge turf bermudagrass. USPP 24,116. U.S. Patent and Trademark Office.

Wu, Y.Q., Samuels, T., Tan, C., Moss, J.Q., Martin, D.L., Guo, Y., Adhikari, L., 2013b. Phenotypic selection for winter survivability and molecular characterization in turf bermudagrass populations. Int. Turfgrass Soc. Res. J. 12, 335–341.

Wu, Y.Q., Martin, D.L., Taliaferro, C.M., Anderson, J.A., Moss, J.Q., 2014. Latitude 36 turf bermudagrass. USPP 24,271. U.S. Patent and Trademark Office.

Wu, Y.Q., Martin, D.L., Moss, J.Q., Walker, N.R., Fontanier, C.H., 2019. Bermudagrass plant named 'OKC 1131'. USPP 2019/0364715 P1. U.S. Patent and Trademark Office.

Yu, S., 2017. Advanced turf-type bermudagrass and zoysiagrass experimental genotypes show marked variation in responses to drought stress under field conditions. M.S. thesis, Oklahoma State Univ., Stillwater, OK.

Yurisic, C.A., 2016. Rooting characteristics and antioxidant pigment responses of three hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy] cultivars exposed to drought. M.S. thesis. Univ. of Tennessee, Knoxville, TN.

Zhang, X., Ervin, E.H., LaBranche, A.J., 2006. Metabolic defense of seeded bermudagrass during acclimation to freezing stress. Crop Sci. 46, 2598–2605.

Table 1. Eight-four turf bermudagrasses and their last rating means of turfgrass quality (TQ), leaf firing (LF), percent green cover (PGC), and canopy temperature (CT) under drought stress, spring greenup (SG), spring greenup percent green cover (SGPGC), in 2018 and 2019.

Entry	Genotype	TQ[b]		LF[c]		PGC[d]		CT[e]	SG[f]		SGPGC[g]	
number[a]	ID	2018	2019	2018	2019	2018	2019	2019	2018	2019	2018	2019
1	OKC1302[h]	4.7	4.0	7.7	5.3	74.5	58.0	36.9	5.0	5.0	70.3	67.9
2	OSU1221[i]	4.0	3.3	7.0	5.7	64.9	54.4	40.3	4.3	3.3	50.9	46.3
3	OSU1257	4.0	3.7	8.0	5.7	76.3	44.7	40.1	6.3	5.7	93.0	77.7
4	OSU1310	4.3	3.7	8.0	6.0	91.2	62.2	37.1	5.0	4.7	71.0	60.1
5	OSU1318	5.5	5.3	8.0	7.7	95.0	90.0	37.7	7.0	5.0	93.1	81.2
6	OSU1337	4.3	4.0	7.3	7.3	87.4	79.5	35.2	8.0	4.3	97.7	61.5
7	OSU1402	4.3	3.3	7.7	5.7	75.8	41.3	39.7	4.3	5.3	66.9	62.4
8	OSU1403	4.0	2.0	7.7	3.3	75.6	24.4	41.8	4.7	5.7	56.8	64.5
9	OSU1406	4.0	3.7	7.3	5.7	64.7	55.2	36.9	7.3	5.0	93.8	66.3
10	OSU1408	4.0	3.3	7.3	5.3	72.1	47.6	38.6	5.0	4.7	64.8	37.2
11	OSU1409	5.0	4.3	8.0	7.3	83.4	72.6	37.1	4.0	5.0	53.9	67.3
12	OSU1412	4.0	4.0	7.0	7.0	65.6	61.0	38.3	5.7	4.3	76.8	63.7
13	OSU1415	4.7	3.7	7.7	5.3	60.5	56.4	37.3	4.7	6.0	53.3	65.8
14	OSU1417	5.0	4.3	8.0	7.0	85.9	70.3	37.6	5.0	6.7	48.9	72.0
15	OSU1418	4.0	3.0	6.7	4.7	55.3	39.0	43.7	4.0	3.7	53.4	50.6

16	OSU1420	5.0	4.3	7.7	8.0	86.5	86.4	35.1	4.7	4.7	63.4	61.0
17	OSU1423	5.0	5.0	8.0	7.7	89.4	84.9	34.7	5.0	6.0	69.7	79.7
18	OSU1425	5.0	4.0	8.0	6.3	84.7	64.6	35.7	5.0	5.7	70.7	74.2
19	OSU1433	4.0	3.0	7.0	5.0	67.5	49.7	40.0	4.0	5.0	55.1	60.6
20	OSU1435	4.0	3.7	8.7	6.0	68.5	47.3	38.0	5.3	5.3	81.6	62.0
21	OSU1439	4.3	4.7	7.3	7.7	83.6	88.1	36.1	8.0	3.7	95.3	57.9
22	OSU1604	5.0	4.7	7.7	7.7	70.0	65.6	40.4	8.7	6.3	99.3	84.5
23	OSU1606	4.0	3.7	7.7	6.7	53.8	48.4	39.1	8.7	5.0	96.8	65.4
24	OSU1607	4.7	6.0	7.7	8.7	92.5	96.3	37.6	7.0	6.0	94.5	91.1
25	OSU1610	2.7	3.3	6.0	6.0	53.4	57.3	39.0	5.3	3.3	71.1	52.5
26	OSU1611	2.0	2.3	4.7	3.3	45.9	49.0	45.6	4.3	3.7	38.0	63.1
27	OSU1617	4.3	3.3	8.3	5.0	74.7	48.1	39.0	4.7	7.3	65.3	89.1
28	OSU1625	4.3	4.3	7.7	7.7	75.0	80.2	37.4	8.0	5.0	98.1	68.2
29	OSU1629	4.3	4.0	7.7	7.0	70.9	42.4	39.3	7.0	7.7	67.2	93.8
30	OSU1631	5.0	4.0	8.0	6.7	79.4	46.2	38.6	6.0	6.0	75.8	79.7
31	OSU1640	4.0	5.0	8.0	8.3	63.6	80.2	39.2	7.0	4.7	92.9	63.7
32	OSU1641	3.7	4.3	7.3	6.7	63.4	47.5	39.8	7.3	5.7	97.8	74.2
33	OSU1656	2.7	3.0	4.7	5.7	32.8	41.6	40.4	6.3	4.0	85.7	54.5
34	OSU1664	2.7	4.3	5.7	8.3	39.4	69.3	38.8	8.0	2.7	96.5	31.4

35	OSU1674	3.3	2.7	5.0	3.3	35.8	37.3	44.5	6.0	3.3	75.1	46.0
36	OSU1682	4.0	3.7	7.0	5.7	71.6	49.4	38.9	6.7	5.0	98.1	67.1
37	OSU1695	5.0	4.7	8.3	6.7	74.2	54.5	41.0	7.0	4.7	87.5	58.8
38	OC2	3.0	3.0	6.0	5.0	57.0	61.3	40.8	4.7	5.0	62.8	68.2
39	OC8	3.7	5.0	7.7	8.0	59.8	74.9	37.7	8.0	4.3	99.0	58.4
40	OC12	3.0	3.0	7.3	5.3	38.3	30.3	41.4	5.0	5.0	76.7	57.2
41	OC15	4.3	4.7	7.3	7.7	75.9	68.7	37.1	7.0	5.0	94.8	65.3
42	OC19	5.0	5.3	8.0	7.7	85.5	74.8	34.1	7.3	5.7	74.4	66.8
43	OC21	4.3	4.3	6.7	6.3	66.0	66.3	38.3	6.0	3.7	83.0	53.7
44	OC22	3.7	2.7	6.0	5.3	55.3	42.4	40.9	5.7	3.7	86.2	51.1
45	OC23	3.7	3.0	7.0	4.7	71.4	43.6	39.3	5.0	5.3	54.8	68.6
46	OC26	5.0	4.3	7.7	7.3	87.6	66.8	35.7	4.0	4.3	58.8	69.5
47	OC27	4.3	3.0	7.3	5.0	87.3	51.1	39.8	3.7	3.7	39.1	55.3
48	OC30	5.0	5.7	7.7	7.7	73.7	86.3	34.3	5.7	5.3	84.9	73.4
49	OC32	4.0	4.0	8.3	7.3	73.0	70.8	36.3	6.0	4.3	84.1	58.3
50	OC34	3.7	3.3	7.7	5.3	63.2	56.3	37.9	4.0	4.0	64.8	47.6
51	OC35	4.3	4.7	8.0	7.0	76.0	71.1	34.1	5.3	4.3	83.0	57.0
52	OC37	5.0	3.7	8.0	6.0	91.4	59.2	34.9	5.3	4.7	63.6	61.1
53	OC38	4.7	3.7	7.7	6.0	77.2	65.8	38.9	4.3	5.0	50.0	69.7

54	OC39	4.3	5.0	7.7	8.0	68.0	84.1	36.1	3.7	4.0	43.1	60.0
55	OC43	4.0	4.3	7.7	8.0	81.8	73.5	36.2	6.0	6.3	71.1	76.5
56	OC45	4.0	4.3	8.0	7.7	82.2	84.7	35.6	5.3	5.0	69.9	65.7
57	OC52	4.3	4.0	8.0	7.7	83.4	87.7	38.5	4.0	5.7	52.5	86.7
58	OC57	3.7	4.0	7.0	7.0	72.1	67.2	39.1	6.0	3.3	82.1	50.0
59	OC61	4.3	5.0	7.3	8.0	61.3	74.0	37.2	7.3	4.7	98.4	64.1
60	OC64	3.7	3.3	8.0	6.7	50.5	40.5	40.5	7.3	4.3	98.3	53.6
61	OC68	4.3	4.0	8.0	7.3	70.4	62.7	36.6	6.0	5.3	69.8	70.4
62	OC69	4.7	5.7	7.7	7.7	61.6	77.9	37.1	6.3	5.3	91.2	72.5
63	OC72	4.0	3.7	7.7	6.0	64.0	49.0	42.3	5.7	4.7	90.6	65.9
64	OC76	4.7	4.3	7.7	6.7	72.5	67.2	37.4	8.3	5.0	98.8	71.0
65	OC77	4.0	4.0	8.0	6.3	62.6	58.1	38.5	4.7	4.7	70.9	57.3
66	OC85	3.7	4.0	6.3	6.7	63.1	73.9	36.1	8.0	4.7	97.3	78.9
67	OC87	4.0	4.3	7.3	7.7	62.6	80.1	37.3	6.7	5.3	96.2	78.6
68	OC88	3.3	3.3	6.7	6.3	67.2	63.8	39.4	5.3	4.3	76.1	62.0
69	OC94	4.3	4.7	8.0	6.7	72.3	60.4	38.3	7.3	6.0	86.8	75.7
70	OC95	3.3	2.3	6.0	3.7	39.3	24.6	42.5	6.0	5.3	72.7	62.7
71	OC96	4.7	3.7	7.3	5.3	66.5	57.0	39.8	5.7	3.3	63.0	36.1
72	OC97	4.0	4.7	6.7	7.3	65.8	87.9	34.7	7.0	4.7	94.8	55.0

73	OC99	4.3	3.7	7.3	7.3	59.2	53.2	38.0	7.0	6.0	86.2	82.4
74	3x7	4.7	4.7	8.0	8.0	80.1	74.9	37.7	8.0	5.3	99.0	76.8
75	19x9	4.7	4.0	8.3	7.0	80.2	60.6	40.5	6.7	4.3	80.3	64.0
76	4x16	4.7	3.0	7.0	4.3	62.9	41.9	40.7	4.7	5.0	57.9	61.7
77	Tilin #5[j]	3.7	4.0	6.7	7.3	50.9	86.0	36.2	3.7	5.3	35.3	71.0
78	Latitude 36	4.3	2.3	6.7	4.0	72.8	39.9	45.3	6.3	5.0	83.4	76.6
79	NorthBridge	3.7	2.7	6.7	3.7	54.0	38.1	41.1	5.7	4.7	69.2	60.4
80	Tahoma 31	5.0	3.3	7.7	5.3	74.9	52.1	39.5	8.0	5.7	94.8	63.5
81	TifTuf	5.3	6.3	9.0	8.7	92.3	93.7	35.2	4.3	5.7	62.1	79.3
82	Tifway	4.0	5.0	6.7	7.3	39.8	81.3	35.9	3.0	4.3	33.2	52.5
83	U3	3.7	3.3	7.7	5.7	69.9	52.4	38.6	4.3	4.3	58.9	57.6
84	Astro	3.7	3.3	6.3	6.0	46.0	72.6	38.2	4.0	4.0	46.0	62.6
	LSD[k]	0.9	1.5	1.0	2.7	19.6	35.8	5.7	1.2	1.2	15.7	13.5

[a]Entry number 1 to 77 are experimental genotypes and 78 to 84 are standard cultivars.
[b]TQ = Turfgrass quality was rated on a scale from 1-9 where 1 = dead or dormant turf, 6 = minimal acceptable turf, and 9 = excellent turf.
[c]LF = Leaf firing was rated from 1-9 during dry down where 1 = all leaves fired and 9 = no leaf firing.
[d]PGC= Percent green cover measured by digital image analysis calculating the percent live cover on a scale from 0-100 where 0= no green cover and 100= whole plot is green.
[e]CT = Canopy temperature measured by an infrared thermometer.
[f]SG = Spring greenup was rated a scale from 1-9 where 1 = dormant turf and 9 = fully green turf.
[g]SGPGC = Spring greenup percent green cover measured by digital image analysis calculating the percent live cover on a scale from 0-100 where 0 = no green cover and 100 = whole plot is green.
[h]Cite from Amgain et al. (2018).
[i]Cite from Yu (2017).
[j]Cite from Fang et al. (2015).

[k]LSD = least significant difference at the $p = 0.05$ level.

Table 2. Means, ranges, and standard deviations for turfgrass quality, leaf firing, percent green cover, and canopy temperature of 84 bermudagrass genotypes.

	Turfgrass quality[a]											
	2018						2019					
	7/18	8/3	8/14	8/30	9/19	10/4	7/10	7/16	7/27	8/10	8/21	9/11
Mean	6.2	6.2	5.7	5.5	5.1	4.2	6.8	6.3	5.9	5.3	4.0	3.9
Maximum	8.0	8.0	7.0	7.0	6.0	6.0	8.0	8.0	8.0	7.0	7.0	7.0
Minimum	4.0	4.0	3.0	4.0	4.0	2.0	5.0	4.0	3.0	3.0	1.0	1.0
SD	0.8	0.7	0.7	0.6	0.5	0.8	0.6	0.7	0.8	0.8	1.1	1.2
	Leaf firing[b]											
	2018						2019					
	7/18	8/3	8/14	8/30	9/19	10/4	7/10	7/16	7/27	8/10	8/21	9/11
Mean	9.0	9.0	8.9	8.7	8.6	7.4	9.0	8.9	8.7	8.1	6.4	6.4
Maximum	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Minimum	9.0	8.0	7.0	7.0	6.0	4.0	9.0	6.0	5.0	3.0	2.0	2.0
SD	0.0	0.1	0.3	0.5	0.7	0.9	0.0	0.4	0.6	1.2	1.8	1.9
	Percent green cover[c]											
	2018						2019					
	7/18	8/3	8/14	8/30	9/19	10/4	7/10	7/27	8/10	8/21	9/11	
Mean	99.5	98.4	98.5	97.1	95.9	68.9	98.8	97.3	88.8	69.2	61.9	
Maximum	99.9	99.9	99.9	99.8	99.8	96.6	99.9	99.9	99.8	99.4	98.4	
Minimum	96.7	73.5	83.6	79.9	48.6	16.5	96.4	65.4	6.9	1.2	2.9	
SD	0.4	2.6	1.9	3.6	6.9	0.9	0.6	4.5	17.5	30.5	25.2	

	Canopy temperature[d]			
	2019			
	7/10	7/27	8/10	8/21
Mean	34.8	33.6	34.6	38.4
Maximum	39.9	41.9	43.2	49.8
Minimum	30.7	29.4	31.1	32.2
SD	1.9	2.6	2.4	3.8

[a]Turfgrass quality was rated on a scale from1-9 where 1 = dead or dormant turf, 6 = minimal acceptable turf, and 9 = excellent turf.
[b]Leaf firing was rated on a scale from 1-9 during dry down where 1 = all leaves fired and 9 = no leaf firing.
[c]Percent green cover measured by digital image analysis calculating the percent live cover on a scale from 0-100 where 0 = no green cover and 100 = whole plot is green.
[d]Canopy temperature measured by an infrared thermometer.

Table 3. Analysis of variance and variance component estimates of turfgrass quality, leaf firing, percent green cover, and canopy temperature of 84 bermudagrass genotypes.

Measurement	Year	σ_G^2	*P* > *F*	σ_{GxY}^2	*P* > *F*	σ_{GxD}^2	*P* > *F*	σ_{GxR}^2	*P* > *F*	σ_E^2	i^2
Leaf firing	2018	0.063	<.0001			0.099	<.0001	0.023	<.0001	0.110	0.67
	2019	0.397	<.0001			0.131	<.0001	0.725	<.0001	0.905	0.56
	Combined	0.059	0.0863	0.230	<.0001	0.052	0.0003	0.195	<.0001	0.696	0.24
Turfgrass quality	2018	0.129	<.0001			0.077	<.0001	0.092	<.0001	0.158	0.71
	2019	0.226	<.0001			0.094	<.0001	0.184	<.0001	0.324	0.72
	Combined	0.122	<.0001	0.072	<.0001	0.067	<.0001	0.086	<.0001	0.307	0.64
Percent green cover	2018	7.595	<.0001			22.716	<.0001	2.560	0.0001	29.981	0.55
	2019	34.030	0.0044			25.384	0.0002	113.610	<.0001	198.970	0.38
	Combined	6.280	0.1380	23.840	<.0001	15.130	<.0001	31.260	<.0001	131.360	0.20
Canopy temperature	2019	0.538	0.0154			0.421	0.0062	1.978	<.0001	4.094	0.33

[a]σ_G^2, genotype variance; σ_{GY}^2, genotype by year variance; σ_{GR}^2, genotype by replication variance; σ_{GDY}^2, genotype by date within the year variance; σ_E^2, error variance; i^2, reliability.

Table 4. Means, ranges, and standard deviations for spring greenup and spring greenup percent green cover of 84 bermudagrass genotypes.

	Spring greenup[a]						
	2018			2019			
	5/1	5/21	5/31	4/19	5/1	5/11	5/22
Mean	1.9	4.5	5.8	1.7	2.4	3.8	4.9
Maximum	4.0	8.0	9.0	3.0	5.0	6.0	8.0
Minimum	1.0	3.0	3.0	1.0	1.0	2.0	2.0
SD	0.5	1.2	1.5	0.5	0.6	0.7	1.1

	Spring greenup percent green cover[b]				
	2018			2019	
	5/2	5/21	5/31	5/1	5/22
Mean	9.6	42.6	75.1	23.5	64.9
Maximum	32.1	94.4	99.9	70.1	97.7
Minimum	1.6	9.8	26.9	3.1	21.8
SD	5.3	18.6	19.8	9.6	13.7

[a]Spring greenup was rated a scale from 1-9 where 1 = dormant turf and 9 = fully green turf.
[b]Spring greenup percent green cover measured by digital image analysis calculating the percent live cover on a scale from 0-100 where 0 = no green cover and 100 = whole plot is green.

Table 5. Analysis of variance and variance component estimates of spring greenup and percent spring green cover of 84 bermudagrass genotypes.

Measurement	Year	σ_G^2	*P* > *F*	σ_{GxY}^2	*P* > *F*	σ_{GxD}^2	*P* > *F*	σ_{GxR}^2	*P* > *F*	σ_E^2	i^2
Spring greenup	2018	0.624	<.0001			0.365	<.0001	0.108	<.0001	0.259	0.77
	2019	0.187	<.0001			0.116	<.0001	0.093	<.0001	0.203	0.71
	Combined	0.02	0.365	0.412	<.0001	0.195	<.0001	0.049	<.0001	0.288	0.08
Percent spring	2018	116.38	<.0001			62.59	<.0001	24.778	<.0001	50.953	0.77
green cover	2019	62.555	<.0001			23.957	<.0001	34.342	<.0001	18.653	0.7
	Combined	19.499	0.0731	73.73	<.0001	44.741	<.0001	12.376	<.0001	54.957	0.27

[a]σ_G^2, genotype variance; σ_{GY}^2, genotype by year variance; σ_{GR}^2, genotype by replication variance; σ_{GDY}^2, genotype by date within the year variance; σ_E^2, error variance; i^2, reliability.

Table 6. Pearson correlation coefficient between the drought response and winter survivability traits of 84 bermudagrass genotypes collected from 2017 to 2019.

Traits	SGPGC[a]	SG	PGC	TQ	LF
SG[b]	0.90****				
PGC[c]	0.27*	0.23*			
TQ[d]	0.39***	0.43****	0.75****		
LF[e]	0.43****	0.40****	0.81****	0.74****	
CT[f]	-0.22*	-0.19	-0.77****	-0.65****	-0.78***

[a]SGPGC = spring greenup percent green cover, [b]SG = spring greenup, [c]PGC = percent green cover, [d]TQ = turfgrass quality, [e]LF = leaf firing, [f]CT = canopy temperature.
* Indicating significance at the probability of 0.05.
** Indicating significance at the probability of 0.01.
*** Indicating significance at the probability of 0.001.
**** Indicating significance at the probability of 0.0001.

VITA

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Doctor of Philosophy

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