Directional Breeding Generates Distinct Genetic Diversity in Hybrid Turf Bermudagrass as Probed with SSR Markers

Desalegn D. Serba , Tilin Fang , Yanqi Wu *

Posted Date: 29 May 2023

doi: 10.20944/preprints202305.1960.v1

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Article

Directional Breeding Generates Distinct Genetic Diversity in Hybrid Turf Bermudagrass as Probed with SSR Markers

Desalegn Serba [1], Tilin Fang [2] and Yanqi Wu [2,*]

[1] USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, AZ 85138, USA
[2] Plant and Soil Sciences Department, Oklahoma State University, Stillwater, OK 74078, USA
* Correspondence: yanqi.wu@okstate.edu

Abstract: Bermudagrass (*Cynodon* spp.) is one of the drought-resistant warm-season turfgrasses adapted to the southern and transitional zones in the United States. Multiple hybrid varieties have been developed and released for use as a turfgrass and others are in pipeline. Increasing genetic diversity of commercial varieties is vital to tackle stress tolerance. A DNA profiling study of 21 experimental selections from the Oklahoma State University (OSU) turfgrass breeding program and 11 cultivars was conducted using 51 simple sequence repeat (SSR) primer pairs that spread across the bermudagrass genome. Pairwise genetic relationship analysis among the genotypes using 352 polymorphic bands showed genetic similarity coefficients ranging from 0.59 to 0.89. Cluster analysis using the un-weighted paired group method with arithmetic average (UPGMA) method grouped the entries into six clusters. Correlation analysis identified different levels of pairwise genetic relationship among the entries that largely reflected parental relationship. Directional breeding and selection for cold hardiness or drought resistance created progeny that had distinct genetic diversity in the tested bermudagrasses. It is evident that an increase in genetic diversity of the existing cultivar pool with the release of one or more of the experimental selections for commercial use will strengthen and improve bermudagrass systems.

Keywords: *C. dactylon*; *C. transvaalensis*; interspecific hybrids; cluster analysis; genetic similarity

1. Introduction

Bermudagrass (*Cynodon* spp.) is one of the warm-season grasses well adapted to the tropical and subtropical, and warm climates [1]. Bermudagrass is comprised of cross-pollinated species with self-fertility of up to 3% [2,3]. The genus includes ploidy series of diploid, triploid, tetraploid, pentaploid, and hexaploid with a base chromosome number of x = 9 [4]. Common bermudagrass [*Cynodon dactylon* (L.) Pers.] and African bermudagrass [*C. transvaalensis* Burtt-Davy [5], and the hybrid between the two are widely used as turfgrasses in the United States [6,7]. Common bermudagrass contains both tetraploid (2n = 4x = 36) and hexaploid (2n = 6x = 54) [8] while African bermudagrass is a diploid (2n = 2x = 18). Interspecific hybrids between the two species are generally considered the most economically important and widely used in home lawns, sport fields and golf courses [9]. Intra- and inter-ploidy level crosses can produce viable seeds [7]. The *C. dactylon* var. *dactylon* has three races: a tropical race which forms loose turf with short stature; a temperate race which appears to be similar to tropical, but they are denser and more winter hardy; and a seleucidus race which has a very coarse texture, thick stolon and rhizomes and is much cold tolerant than the other two [10].

Bermudagrasses are widely used on athletic fields (football, baseball, and soccer), public parks, tennis courts, and golf courses, because of its drought and heat tolerance, high traffic tolerance and excellent recovery potential after wear damage [11]. Triploid interspecific turf bermudagrass hybrids (2n = 3x = 27) that are vegetatively propagated are popular in the turf industry, in part due to their aesthetic beauty and extreme uniformity on golf courses, sports fields, and home lawns [12]. Other characteristics that make bermudagrass an attractive choice include its perennial sod-forming nature,

 doi:10.20944/preprints202305.1960.v1

exceptional spreading ability, tolerance to low mowing, and adaptation to a wide range of growing conditions [13].

The amazing adaptability, aggressive, and dynamic nature of bermudagrass has long been recognized by scientists and it has become a species known all around the world [5]. Through evolution and population processes, bermudagrass has been able to spread across multiple environments and geographic locations [14,15]. It exhibits a wide range of features in terms of color, texture, density, vigor, and environmental tolerance, providing it with several advantages over other species [10,16]. These diverse characteristics give bermudagrass an immense edge, enabling it to withstand harsh environments, compete with other species, and colonize vast disturbed areas. As such, it stands out as a truly remarkable and cosmopolitan species, adapting and thriving across all sorts of challenging environments.

The remarkable improvements in bermudagrass throughout the past century have truly revolutionized the turf industry. Bermudagrass improvement methodologies have remarkably progressed from initial prospecting for superior plants among naturally occurring variations in the germplasm to the inclusion of scientific selective breeding that began in the mid-1900s [13]. The systematic development of new turf bermudagrass cultivars through breeding and selection has contributed to the development of numerous improved cultivars [13,17,18]. Incremental improvements in turf bermudagrass varieties were achieved by turfgrass enthusiasts searching for plants with superior characteristics relating to turf use [7,16]. The development of a sterile triploid interspecific hybrid 'Tiffine' [19], triggered the use of bermudagrasses on putting greens. Later, the development of another interspecific hybrid, 'Tifgreen', started the large-scale commercial use of bermudagrass on golf putting greens due to its improved putting quality, optimum leaf density, and canopy coverage at lower mowing heights [20,21]. With the creation of 'Tifgreen', golf course managers saw the possibilities of having immaculate putting greens and fairways all year long. As a survey data conducted in 2015 indicated, 34 % of the total golf course acreage (80 % of putting green) in the southern US has adopted bermudagrass due to its stress tolerance and maintenance requirements [22,23], thus redefining what is possible for modern day turf management. Not only could bermudagrass survive the scorching heat of the south, but it also performed extremely well at low mowing heights, leading to a consistent and visually pleasing golfing experience.

The Oklahoma State University (OSU) turfgrass breeding program has been at the forefront of developing new genotypes with enhanced traits. These experimental genotypes have undergone multi-environment field trials to assess turfgrass quality, winter survivability, and drought resistance [24]. Through careful evaluation, elite selections have been chosen for patent applications and commercial release, greatly enhancing the aesthetic value and stress tolerance of these varieties. Additionally, the identity and genetic diversity of the experimental selections have been studied, providing the opportunity to further broaden the genetic basis of the cultivars pool and continues to provide exceptional value to the industry.

In the past, genetic comparisons were largely based on morphological characteristics but were heavily influenced by the environment. As technology continues to evolve, researchers have looked for ways to make studying bermudagrass more efficient. The use of molecular markers like genomic and expressed sequence tags derived simple sequence repeat (SSR) markers have advantage over traditional morphological comparisons by providing environmentally neutral genetic differences among genotypes and have revolutionized how the species is studied. Many highly polymorphic, locus-specific, and reproducible SSR markers have been developed and widely used in bermudagrass [25]. A set of 11 highly polymorphic SSR markers, with an average of 12.8 bands per primer pairs (PPs) were utilized as a reliable tool for the identification of vegetatively propagated turf bermudagrass cultivars [26]. Nine SSR primer combinations amplified 88 bands and effectively detected genomic variation among bermudagrass accessions from various sources [27]. A total of 1003 validated SSR PPs developed from five genomic SSR libraries were used in the development of linkage mapping and QTL analysis for establishment rate in common bermudagrass [25]. Four SSR genomic libraries enriched with (CA)n, (GA)n, (AAG)n, and (AAT)n repeat motifs were sequenced to develop 1426 unique PPs in African bermudagrass [28]. EST-SSR markers were also used for

 doi:10.20944/preprints202305.1960.v1

genetic linkage mapping of bermudagrass [29]. With the advancement of marker technology, a more precise understanding of the genetic variations within the species and environmental influences have come to light; and a foundation for molecular breeding applications in bermudagrass has been laid.

As stated above, a genetic base of turf bermudagrass has been broadened to allow for the development of high-quality, adaptable cultivars to varying environments and end uses. The OSU has been conducting two directional breeding efforts: one for cold hardy cultivars development, and the other for drought resistant cultivars. The research done at OSU included characterizing the genetic diversity of new turf bermudagrass experimental selections and comparing them to standard cultivars using SSR markers [24]. By using this directional breeding technique, we were able to evaluate which cultivars may be more adaptable to colder climates or require less water in drought-prone areas. As this research is crucial for providing new turfgrass varieties for different regions and end-uses, the current study was conducted to characterize the genetic diversity and identity a set of new turf bermudagrass experimental selections of the breeding program against standard cultivars using SSR markers.

2. Results and Discussion

2.1. SSR Marker Polymorphisms

The PCR products of 51 out of the 54 SSR PPs showed good polymorphisms. Five of the SSR markers (CDCA5-463/464, CDCA5-505/506, CDCA7-611/612, CDGA5-1363/1364, and CDATG1-1891/1892) amplified two groups of bands on the 32 varieties, which were identified as two loci of markers. The size of amplified fragments varied from 100 to 500 bp. A total of 352 polymorphic bands were scored for the 51 PPs used, with 3 to 13 polymorphic bands for each PP, i.e., an average of 6 alleles per SSR PP (Table 1). Among the 51 PPs, the least amount of polymorphism was observed for CDCA5-473/474, CDGA3-1103/1104, CDGA2-961/962, CDCA5-461/462, and CDAAC2-2361/2362 with three alleles each. On the other hand, CDCA7-611/612 produced 13 polymorphic bands.

Table 1. Polymorphic bands for three SSR marker primer pairs selected from each of the 18 linkage groups (except LG10) in common bermudagrass [25].

	Marker one		Marker two		Marker three	
LG	**Marker**	**Bands**	**Marker**	**Bands**	**Marker**	**Bands**
LG1	CDGA5-1467/1468_160	7	CDCA5-505/506	10	CDCA7-689/690	5
LG2	CDGA5-1467/1468_120	5	CDGA1-865/866	5	CDCA3-259/260	6
LG3	CDGA5-1363/1364	11	CDGA2-961/962	3	CDCA5-437/438	4
LG4	CDCA5-473/474	3	CDCA1-81/82	-	CDATG1-1891/1892	12
LG5	CDGA5-1459/1460	6	CDCA7-641/642	7	CDATG1-1829/1830	-
LG6	CDGA1-895/896	7	CDCA7-611/612	13	CDAAC2-2361/2362	3
LG7	CDAAC6-2611/2612	6	CDCA5-475/476	7	CDATG5-2079/2080	7
LG8	CDCA5-501/502	8	CDATG4-2059/2060	7	CDGA4-1301/1302_175	9
LG9	CDCA7-703/704	9	CDCA1-7/8	5	CDGA3-1233/1234	7
LG10	CDCA5-471/472	5	CDGA1-829/830	13		-
LG11	CDCA2-179/180	9	CDGA1-827/828	7	CDGA3-1219/1220	5
LG12	CDGA3-1161/1162	8	CDCA5-463/464	12	CDGA2-1021/1022	7
LG13	CDCA5-431/432	5	CDGA3-1107/1108	-	CDGA2-1015/1016	7
LG14	CDGA4-1347/1348	11	CDGA3-1147/1148	9	CDCA1-123/124	7
LG15*	CDCA6-549/550	5	CDGA3-1195/1196	5	CDCA1-59/60	4
LG16	CDGA3-1103/1104	3	CDATG1-1905/1906	8	CDCA2-213/214	6
LG17	CDGA3-1187/1188	7	CDCA5-461/462	3	CDGA7-1697/1698	7
LG18	CDCA2-177/178	8	CDGA5-1359/1360	7	CDCA1-9/10	7

LG = Linkage group; * CDCA6-583/584 on LG15 was also used for genotyping.

SSR markers showed a high level of polymorphism among Cynodon dactylon accessions and touted as a reproducible marker system for genetic analysis [24,27], varietal identification or certification and genetic diversity analysis [24,30–32], and trait association [33,34]. SSR markers (also called microsatellites) are ubiquitous in the genome of different higher plant species with high polymorphism, length variation, and co-dominant Mendelian inheritance [35,36]. The amount of genome information derived from such markers and ease of genotyping make SSR markers a good

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choice for plant genetic studies. Consequently, numerous expressed sequence tag-derived simple sequence repeat (EST-SSRs) and genomic-SSRs were developed from bermudagrass genomic resources that can be used to assess genetic diversity, bermudagrass genotype fingerprinting, and differentiate contaminants from cultivars [28,29,37–39]. Furthermore, heterologous EST-SSR from sugarcane became useful sources of polymorphic markers in bermudagrass to facilitate diversity analysis, genetic mapping, QTL analysis, and marker assisted selection [40].

Among the 352 alleles, 46 were unique to the experimental selections while 28 were unique to the cultivars. Analysis of molecular variance (AMOVA) showed that the variation between the two groups (experimental selections and the commercial cultivars) was only 6% of the total variation based on 352 SSR alleles. The variation within the populations accounted for 94% of the total, indicating high genetic variation among the individual genotypes in each group (Table 2). Both variations between groups and within groups were statistically significant ($p < 0.05$). The notable genetic variations among the new experimental selections largely be attributed to differences in origins of parental germplasm. Previous studies of 157 natural bermudagrass germplasm using sequence-related amplified polymorphism (SRAP) markers reported 18% of total molecular variance attributed to diversity among subpopulations, while 82% of variance was associated with differences within subpopulations [41]. Genetic differentiation analysis of fifty-five wild accessions of bermudagrass using SSR marker also reported about 30% attributed to among and 70% within groups variations, respectively [42]. Our result is in agreement with previous reports in different sets of bermudagrass genotypes [24]. These higher frequency of within group than among group variations demonstrates the high heterozygosity due to outcrossing nature of bermudagrass.

Table 2. Analysis of molecular variance (AMOVA) for 21 bermudagrass experimental selections and 11 commercial cultivars using 352 polymorphic bands generated by 53 SSR primer pairs.

Source	df	Mean Sum of Square	Variance Component	Percentage
Among Pops	1	106.65	3.46	6
Within Pops	30	56.71	56.71	94
Total	31	58.32	60.17	100

2.2. Genetic diversity and relatedness

Genetic similarity coefficients (GSC) among the 32 bermudagrass entries were analyzed using all the 352 polymorphic bands. Pairwise genetic similarity coefficient estimate based on the SSR markers ranged from 0.59 to 0.89 for the 32 entries (Table 3). The highest genetic similarity coefficient of 0.89 was observed between experimental selections OSU2053 and OSU2073 (0.89) followed by 10 × 18 and 12 × 19 (0.88). The least genetic similarity was observed between the commercial cultivars Tifton10 and TifTuf (0.59) followed by Tifton10 and TifGrand (0.60). Commercial cultivars such as U3, Tifton10, and Patriot are distantly related to the other commercial cultivars with genetic similarity coefficients ranging from 0.60 to 0.61. Among the experimental selections, OKC7018, OSU1433, and OSU1656 showed the least genetic similarity with most of the other selections (0.62 to 0.67).

Table 3. Genetic similarity of 21 bermudagrass experimental selections and 11 commercial cultivars using 352 polymorphic bands generated by 53 SSR primer pairs.

	OKC7018	OSU1318	OSU1433	OSU1605	OSU1656	OSU1657	OSU1675	OKC1873	OKC1876	OSU2053	OSU2073	OSU2081	19 × 13	6 × 2	10 × 18	12 × 19	14 × 2	14 × 16	15 × 8	15 × 10	18 × 14	Latitude36	NorthBridge	Tahoma31	Patriot	Tifway	TifTuf	TifGrand	Tifton10	Bimini	Celebration
OSU1318	0.74	1																													
OSU1433	0.75	0.75	1																												
OSU1605	0.69	0.73	0.73	1																											
OSU1656	0.70	0.70	0.71	0.73	1																										
OSU1657	0.68	0.71	0.71	0.75	0.85	1																									
OSU1675	0.69	0.72	0.72	0.73	0.83	0.84	1																								
OKC1873	0.63	0.69	0.66	0.69	0.68	0.66	0.69	1																							
OKC1876	0.63	0.68	0.67	0.66	0.68	0.66	0.69	0.85	1																						
OSU2053	0.66	0.70	0.71	0.70	0.69	0.70	0.66	0.80	0.83	1																					
OSU2073	0.65	0.69	0.69	0.71	0.69	0.70	0.68	0.79	0.80	0.89	1																				
OSU2081	0.67	0.67	0.68	0.70	0.70	0.70	0.65	0.80	0.80	0.85	0.85	1																			
19 × 13	0.66	0.68	0.67	0.71	0.75	0.76	0.78	0.68	0.69	0.66	0.68	0.67	1																		
6 × 2	0.68	0.71	0.69	0.71	0.70	0.71	0.70	0.79	0.80	0.86	0.86	0.84	0.70	1																	
10 × 18	0.63	0.70	0.66	0.71	0.66	0.69	0.67	0.73	0.72	0.70	0.74	0.72	0.72	0.73	1																
12 × 19	0.62	0.68	0.64	0.68	0.67	0.69	0.68	0.73	0.73	0.70	0.72	0.70	0.73	0.73	0.88	1															
14 × 2	0.67	0.67	0.70	0.70	0.69	0.67	0.69	0.80	0.82	0.79	0.80	0.82	0.67	0.80	0.76	0.74	1														
14 × 16	0.62	0.67	0.64	0.68	0.67	0.70	0.69	0.71	0.69	0.70	0.72	0.72	0.71	0.73	0.81	0.86	0.71	1													
15 × 8	0.66	0.67	0.65	0.69	0.66	0.68	0.69	0.79	0.82	0.78	0.81	0.81	0.68	0.82	0.72	0.71	0.85	0.73	1												
15 × 10	0.64	0.64	0.66	0.66	0.65	0.65	0.62	0.74	0.76	0.81	0.81	0.77	0.63	0.80	0.69	0.68	0.80	0.70	0.78	1											
18 × 14	0.62	0.70	0.65	0.70	0.66	0.67	0.70	0.73	0.71	0.69	0.72	0.71	0.72	0.73	0.84	0.86	0.76	0.84	0.73	0.70	1										
Latitude36	0.69	0.71	0.74	0.67	0.71	0.68	0.74	0.67	0.65	0.66	0.66	0.66	0.72	0.67	0.68	0.67	0.68	0.64	0.64	0.66	0.70	1									
NorthBridge	0.71	0.73	0.72	0.70	0.72	0.72	0.74	0.64	0.62	0.70	0.69	0.67	0.69	0.69	0.66	0.67	0.68	0.63	0.62	0.66	0.70	0.87	1								
Tahoma31	0.73	0.72	0.73	0.69	0.69	0.69	0.69	0.67	0.64	0.68	0.67	0.65	0.69	0.68	0.70	0.71	0.69	0.65	0.62	0.64	0.69	0.72	0.73	1							
Patriot	0.69	0.66	0.68	0.67	0.68	0.66	0.67	0.63	0.61	0.64	0.65	0.64	0.66	0.67	0.64	0.64	0.67	0.65	0.64	0.64	0.66	0.69	0.69	0.67	1						
Tifway	0.67	0.69	0.67	0.70	0.68	0.68	0.67	0.73	0.73	0.73	0.73	0.74	0.69	0.77	0.74	0.74	0.75	0.75	0.74	0.74	0.73	0.66	0.63	0.67	0.63	1					
TifTuf	0.67	0.72	0.70	0.68	0.68	0.67	0.66	0.74	0.74	0.75	0.70	0.73	0.69	0.74	0.73	0.74	0.70	0.73	0.74	0.73	0.76	0.69	0.67	0.67	0.64	0.80	1				
TifGrand	0.66	0.71	0.70	0.70	0.73	0.69	0.68	0.73	0.75	0.74	0.77	0.76	0.69	0.77	0.71	0.72	0.75	0.76	0.79	0.73	0.74	0.68	0.64	0.66	0.63	0.82	0.84	1			
Tifton10	0.68	0.64	0.67	0.65	0.66	0.64	0.66	0.64	0.61	0.66	0.64	0.65	0.61	0.65	0.61	0.61	0.66	0.60	0.62	0.62	0.63	0.67	0.68	0.69	0.79	0.61	0.59	0.60	1		
Bimini	0.69	0.67	0.66	0.67	0.68	0.66	0.66	0.64	0.63	0.66	0.68	0.67	0.67	0.69	0.68	0.70	0.65	0.66	0.64	0.67	0.67	0.65	0.66	0.69	0.64	0.74	0.68	0.69	0.64	1	
Celebration	0.64	0.67	0.70	0.65	0.67	0.68	0.66	0.67	0.71	0.70	0.69	0.70	0.63	0.69	0.70	0.69	0.70	0.68	0.69	0.68	0.69	0.67	0.67	0.66	0.63	0.70	0.71	0.72	0.63	0.68	1
U3	0.66	0.65	0.65	0.69	0.71	0.68	0.67	0.64	0.63	0.65	0.67	0.66	0.69	0.65	0.66	0.65	0.64	0.62	0.64	0.60	0.65	0.66	0.68	0.66	0.63	0.64	0.64	0.69	0.64	0.65	0.67

doi:10.20944/preprints202305.1960.v1

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The dendrogram generated using 352 polymorphic bands at 0.70 genetic similarity level showed six clusters formed of 32 genotypes (Figure 1). Each of cluster #1 and cluster #2 consisted of two genotypes. Patriot and Tifton 10 formed cluster #1 with 0.78 similarity between them and distantly related to the rest of the genotypes. The result was not surprising as Tifton 10 was a parent for Patriot [43]. Bimini and Celebration formed cluster #2, implying certain relatedness. Cluster #3 encompassed 13 experimental genotypes and three cultivars (Tifway, TifGrand, and TifTuf). The OSU experimental selections were progeny from crosses among drought tolerant parents. Cluster #4 contained only U3 bermudagrass, indicating the unique genetic makeup of the cultivar. Cluster #5 included five experimental genotypes, which were derived from crosses of cold hardy parents. Cluster #6 was formed with three experimental genotypes (OKC7018, OSU1318, and OSU1433) and three commercial cultivars (Latitude 36, NorthBridge and Tahoma 31). These six genotypes shared one African bermudagrass parent, but the other cold hardy common bermudagrass parents. In Figure 1, it is obvious that non-cold hardy genotypes were grouped in two neighboring clusters #2 and #3 while cold hardy genotypes were grouped in clusters #4, #5, and #6, indicating breeding and selection for winter survival or drought resistance has profoundly modified genetic constitutions of those bermudagrasses.

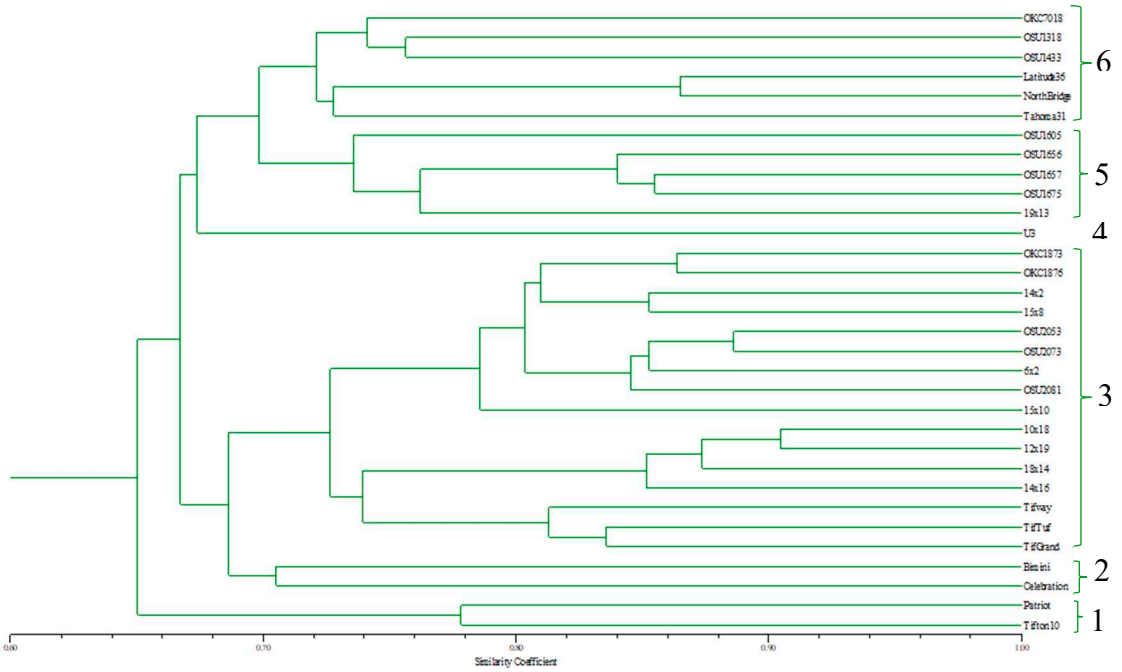

Figure 1. A UPGMA dendrogram of 21 bermudagrass experimental selections and 11 cultivars based on Jaccard similarity coefficients generated from 51 simple sequence repeat markers.

Alike a previous report demonstrating diversity among another set of bermudagrass experimental selections and genetic difference from commercial cultivars [24], our results revealed perceptible genetic difference from commercial cultivars as well as genetic diversity among the experimental selections that will have a potential to broaden genetic base if released for commercial production. Genetic diversity assessment of bermudagrass using ISSR marker reported the degree of relationship among the genotypes follows some degree of geographical origin, ploidy level, and some morphological characteristics [44]. A study conducted on the population structure of 296 bermudagrass genotypes from different latitudes using 153 expressed sequence tag-derived simple sequence repeat (EST-SSR) marker stated more genetic diversity in higher ploidy level and lower latitudes in China [15]. They also reported population structure to follow latitudes with notable admixture and no isolation by distance. A recent genetic diversity and population structure study of common bermudagrass collection using EST-SSR markers reported notable population differentiation along longitude gradients caused by low gene flow [45]. The population structure along the latitude gradient implies the existence of genetic variation for cold adaptation among

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bermudagrass germplasm while the longitudinal gradient implies population isolation. Evolutionary adaptation to a range of climatic and edaphic conditions contributed for the high magnitudes of genetic diversity and marker polymorphism [46]. These results imply the existence of a wide genetic diversity in bermudagrass, polyploidy speciation, and an adaptive evolution.

Directional breeding specifically targets a trait or traits of interest to improve for an intended use or alleviate a priority problem. Drought tolerance and winter survivability are the two important objective traits for OSU turf bermudagrass breeding. As water is the primary input for growth and survival of turfgrass, breeding for drought-resistant turfgrass is pivoted on water conservation by the turfgrass industry [47,48]. Similarly, reduced winter injury is an important factor for hybrid bermudagrass use in the transition zone [49,50]. Therefore, developing bermudagrasses hybrids with better freeze tolerance is another key priority area for the OSU bermudagrass breeding program.

A recent study of bermudagrass germplasm for cold hardiness and freezing tolerance under field and laboratory condition, reported a range of variability for reduced winterkill, spring green-up, and genetic sources for cold hardiness [50]. It is obvious that the prevalence of genetic variability among germplasm determines the success of the breeding program. The molecular variability observed among the hybrid selections warranted the success of the directional breeding practice.

Correlation among the genotypes based on the 352 markers alleles amplification profile was also assessed (Figure 2). Three entries OKC7018, OSU1318, and OSU1433, which formed a separate cluster as shown in the dendrogram are also not significantly correlated with any of the experimental selections and commenrcial cultivars. On the contrary, OSU1605 was closely related with OSU2073, 15 × 8, and TifTuf. Similarly, OSU1656 was correlated with OSU2081, 14 × 16, and 18 × 14. There is also strong correlations among OSU1675, 19 × 13, and 14 × 16; OKC1876 with OSU2081 and 15 × 10. Among the cultivars, NorthBridge and Bimini, Patriot and Tifton10, and Patriot and Celebration were much correlated. TifTuf and TifGrand are slightly correlated with some of the experimental selections. The genetic correlation can be used to describe traits in the same individual or the same trait in different individuals [51]. In our study, we assessed the correlation of genotypes with different parental background and pedigrees based on SSR marker polymorphism. The correlation matrix revealed different level of genetic relationship among the experimental selections and with the commercial cultivars.

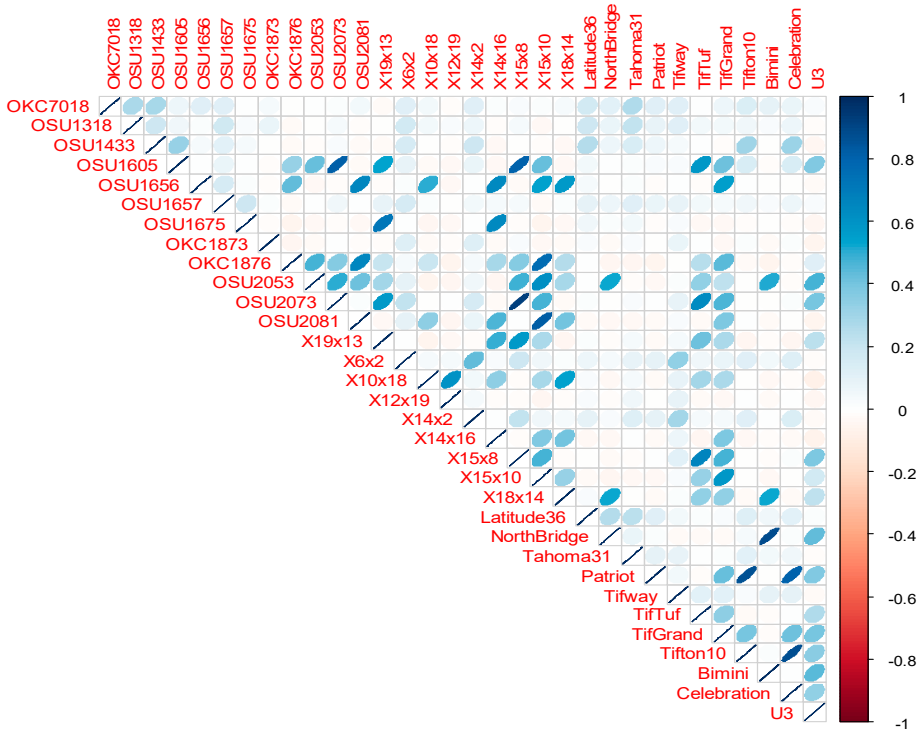

Figure 2. Correlations among 21 bermudagrass experimental selections and 11 cultivars assessed using 52 simple sequence repeat markers alleles amplifications.

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The principal component analysis (PCA) performed revealed the structure of covariation between the experimental selections and commercial cultivars (Figure 3). PC1, which explained 17.8% of the variability in the data set, was driven largely by 15 × 10 and OSU2073. PC2 explained 11% of the variability and was largely contributed by OSU1656 and OSU2081. The analysis demonstrated how the covariation between the experimental selections and commercial cultivars were influencing the data set and revealed new insights into their relationship.

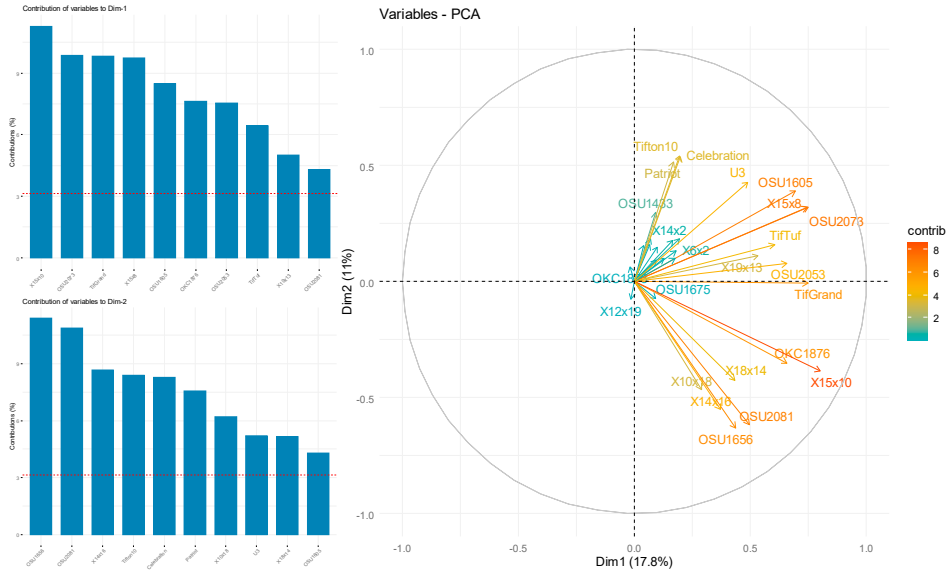

Figure 3. Principal component analysis (PCA) of 21 bermudagrass experimental selections and 11 cultivars using 352 polymorphic alleles amplified with 52 simple sequence repeat markers and percent contribution of different genotypes (Selections that has numerical cross identification are preceded by 'X' in this figure only).

The outcome of the research on this set of bermudagrass genotypes showed remarkable polymorphisms with regards to SSR markers. Clustering the accessions with the UPGMA method, using Jaccard's similarity coefficient as input, formed six clusters of unique genetic signatures. Interestingly, the correlation analysis using marker data highlighted different levels of relatedness between the genotypes which is largely attributed to their parental background. This revelation gives significant insight on the directional breeding and selection that have occurred in this bermudagrass breeding program. As the research indicates, one or more of these genotypes could make an ideal addition to the commercial cultivar pool, and thus introduce even more genetic diversity.

3. Materials and Methods

3.1. Materials

A total of 32 bermudagrass genotypes, comprising of 21 experimental selections and 11 cultivars were investigated for genetic diversity (Table 4). The OSU turf bermudagrass breeding program attempted to combine high turfgrass quality and cold hardiness in interspecific hybrids since the 1980s. This study included eight cold hardy selections (OKC7018, OSU1318, OSU1433, OSU1605, OSU1656, OSU1657, OSU1675, 19 × 13) and six cold hardy commercial cultivars (Latitude 36, NorthBridge, Tahoma 31, Patriot, Tifton 10, and U3). Since 2010, the OSU program received funding sponsored by the USDA Specialty Crop Research Initiative to develop drought resistant interspecific hybrids. Fourteen drought resistant selections (OKC1873, OKC1876, OSU2053, OSU2073, OSU2081, 6 × 2, 10 × 18, 12 × 19, 14 × 2, 14 × 16, 15 × 8, 15 × 10, 18 × 14) and five commercial cultivars (Tifway, TifGrand, TifTuf, Bimini, Celebration) were included in this study.

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Table 4. List of 21 experimental selections (No. 1–21) and 11 cultivars (No. 21-32) of turf type bermudagrass (Cynodon spp.) used for genotyping.

Entry	Name	Species	Origin/Reference
1	OKC7018	*C. dactylon* x *C. transvaalensis*	OAES§
2	OSU1318	*C. dactylon* x *C. transvaalensis*	OAES
3	OSU1433	*C. dactylon* x *C. transvaalensis*	OAES
4	OSU1605	*C. dactylon* x *C. transvaalensis*	OAES
5	OSU1656	*C. dactylon* x *C. transvaalensis*	OAES
6	OSU1657	*C. dactylon* x *C. transvaalensis*	OAES
7	OSU1675	*C. dactylon* x *C. transvaalensis*	OAES
8	OKC1873	*C. dactylon* x *C. transvaalensis*	OAES
9	OKC1876	*C. dactylon* x *C. transvaalensis*	OAES
10	OSU2053	*C. dactylon* x *C. transvaalensis*	OAES
11	OSU2073	*C. dactylon* x *C. transvaalensis*	OAES
12	OSU2081	*C. dactylon* x *C. transvaalensis*	OAES
13	19 × 13	*C. dactylon* x *C. transvaalensis*	17-4200 nursery
14	6 × 2	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
15	10 × 18	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
16	12 × 19	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
17	14 × 2	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
18	14 × 16	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
19	15 × 8	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
20	15 × 10	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
21	18 × 14	*C. dactylon* x *C. transvaalensis*	20-8100 nursery
22	OKC 1119 (Latitude 36®)	*C. dactylon* x *C. transvaalensis*	[52]
23	OKC 1134 (NorthBridge®)	*C. dactylon* x *C. transvaalensis*	[53]
24	OKC 1131 (Tahoma 31®)	*C. dactylon* x *C. transvaalensis*	[54]
25	Patriot	*C. dactylon* x *C. transvaalensis*	[55]
26	Tifway	*C. dactylon* x *C. transvaalensis*	[56]
27	DT-1 (TifTuf®)	*C. dactylon* x *C. transvaalensis*	[57]
28	ST-5 (TifGrand®)	*C. dactylon* x *C. transvaalensis*	[58]
29	Tifton 10	*C. dactylon*	[59]
30	Bimini	*C. dactylon* x *C. transvaalensis*	(Parsons & Lehman, 2007)
31	Riley's Super Sport (Celebration®)	*C. dactylon*	[61]
32	U3	*C. dactylon*	[62]

§ OAES = Oklahoma Agricultural Experiment Station.

3.2. Genomic DNA extraction

Healthy young leave tissues were sampled from each of the 32 plants grown in separate pots (the pot size: 15 cm in diameter and 15 cm in depth) in a greenhouse on the Oklahoma State University Agronomy Research Station, Stillwater, OK. DNA was isolated following a modified phenol–chloroform extraction method [30,63]. DNA samples were quantified using a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, Waltham, MA) and diluted to 10 ngmL-1 for polymerase chain reaction (PCR).

3.3. SSR markers PCR amplification, gel electrophoresis, and data analysis

A total of 54 SSR marker primer pairs (PPs) were used for genotyping in this study (Table 2). Three SSR markers were selected from each of the linkage groups of common bermudagrass (except

LG3, LG10, and LG 13) [25]. PCR reactions were conducted with two replications on each genotype following the procedure described by Fang et al. (2015).

Two plates of PCR products labeled with 700 and 800 nm florescent dye were pooled and loaded into a 6.5% KB Plus gel and separated by running in a LI-COR 4300 DNA Analyzer (LI-COR, Lincoln, NE). Bands that were amplified by a given SSR primer pairs from the gel were visually scored as present (1) or absent (0) for all the samples. To ensure the accuracy of the results, only reproducible and consistent SSR fragments were scored. Amplified fragments of different sizes were considered as different alleles.

Genetic similarity coefficients using the simple matching (SM) coefficient [64] were calculated among all possible pairs with the SIMQUAL option using NTSYS-pc version 2.2 [65]. The similarity matrix was used in cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) [66] with sequential, agglomerative, hierarchical, and nested (SAHN) clustering algorithm [67] to obtain a dendrogram. Secondly, the principal components analysis (PCA) was performed to show the differentiation of the genotypes in a two-dimensional array of eigenvectors using the DCENTER and EIGEN modules of NTSYS-pc.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Initial 54 SSR primer pairs and 16 backup primer pairs screened.

Author Contributions: Conceptualization, Y.W. and T.F.; methodology, T.F.; investigation, T.F.; resources, Y.W.; data curation, T.F. and D.S.; writing—original draft preparation, D.S.; writing—review and editing, D.S., T.F. and Y.W.; visualization, T.F. and D.S.; supervision, Y.W.; project administration, Y.W.; funding acquisition, Y.W. and D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by NAME OF FUNDER, grant number XXX" and part of USDA-ARS National Program 215: Pastures, Forage and Rangeland Systems. Mention of a trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or any part herein. USDA is an equal opportunity provider and employer.

Data Availability Statement: The data generated in this study are presented in the article.

Conflicts of Interest: The authors declare no conflict of interest.

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