African Journal of Biotechnology Vol. 10(75), pp. 17106-17115, 28 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1422
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic relationships of bermudagrass (*Cynodon dactylon* var. *dactylon*) from different countries revealed by sequence-related amplified polymorphism (SRAP) analysis

Zhiyong Wang[1,2,3], Li Liao[3], Xuejun Yuan[4], Aigui Guo[1] and Jianxiu Liu[1]*

[1]Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, China.
[2]Key Laboratory of Protection and Development Utilization of Tropical Crop Germplasm Resources (Hainan University), Ministry of Education , Haikou 570228, China.
[3]College of Agronomy, Hainan University, Danzhou 571737, China.
[4]College of Life Science, Qiongzhou University, Sanya 572000, China.

Accepted 31 October, 2011

Bermudagrass [*Cynodon dactylon* (L.) Pers.] germplasm is genetically diverse and widely distributed between 45°N and 45°S latitudes in the world. This study was conducted to assess genetic variation and relatedness of 33 Chinese accessions of common bermudagrass (*C. dactylon* var. *dactylon*) and 22 cultivars developed in China, Australia and the USA by sequence-related amplified polymorphism (SRAP) markers. 30 primer combinations generated a total of 274 clearly scorable bands encompassing 268 that were polymorphic. Percentage of polymorphic loci (PPL) for the domestic and introduced accessions was 93 and 83%, respectively. Cluster analysis by unweighted pair-group method with arithmetic averages (UPGMA) based on the polymorphic markers indicated three distinct clusters. Genetic similarity coefficient (GSC) among the genotypes ranged from 0.57 to 0.97. Genetic diversity estimate, He, for the domestic and introduced accessions were 0.26 and 0.24, respectively. The results of this molecular characterization will be valuable for breeding new bermudagrass cultivars in the future.

Key words: *Cynodon dactylon* (L.) Pers., germplasm, sequence-related amplified polymorphism (SRAP), genetic relationship.

INTRODUCTION

Bermudagrass (*Cynodon* sp.) is the most important member of the Cynodonteae tribe and the Chloridoideae sub-family, within the grass (Poaceae) family (Clayton and Renvoize, 1986). Common bermudagrass is widely distributed between 45°N and 45°S latitudes, penetrating to approximately 53°N latitude in Europe (Harlan and de Wet 1969). Evidence from biosystematic studies of *Cynodon dactylon* var. *dactylon* suggested that it was a Eurasian grass until recent times (Harlan and de Wet 1969, Harlan, 1970a, b), and that a geographic area extending from West Pakistan to Turkey was a center of evolutionary activity for the taxon. Harlan (1970a) stated that the aggressive weedy races now widely distributed likely emerged from that center. Although *C. dactylon* (L.) Pers. is widely distri-buted in China, little information is available regarding the magnitude of genetic variation within the Chinese indigenous *Cynodon* (Wu et al., 2006), especially the genetic variation in Chinese bermudagrass accessions and introduced cultivars of different countries.

Several efforts have been made to examine genetic relationships between bermudagrass accessions and cultivars by using molecular markers to detect poly-morphism in DNA extracted from bulked plant samples.

*Corresponding author. E-mail: turfunit@yahoo.com.cn. Tel/Fax: +086-25-84347057.

Zhang et al. (1999) used amplified fragment length polymorphisms (AFLPs) to differentiate bermudagrass genotypes and determine genetic relationships among them, and Karaca et al. (2002) utilized the same marker to detect the genetic diversity among forage Bermudagrass cultivars. Wu et al. (2005, 2006) quantified the genetic variation of *Cynodon transvaalenis* and its relatedness to hexaploid *C. dactylon*, and analyzed the genetic relatedness of Chinese *Cynodon* accessions within and among different ploidy levels by AFLPs.DNA amplification fingerprinting (DAF) has been used to examine the relatedness of *Cynodon* cultivars from Australia (Ho et al., 1997), to assess the phylogenetic relationships among *Cynodon* species (Assefa et al., 1999) and hybrid offspring of different species (Caetano-Anollés et al., 1995, 1997), and to analyze the genetic relationships among off-types associated with vegetative propagated cultivars 'Tifgreen' and 'Tifdwarf' (Caetano-Anollés et al., 1998). Etemadi et al. (2006) demonstrated that randomly amplified polymorphic DNA (RAPD) could detect genetic diversity and certain ploidy levels of bermudagrass accessions, and little correlation was found between morphological characteristics and molecular analysis. Gulsen et al. (2009) demonstrated that different polyploidy *Cynodon* accessions could have higher diversity by different molecular markers. In China, Liu et al. (2007) and Yi et al. (2008) quantified the genetic diversity of bermudagrass accessions in southwestern provinces by inter simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) molecular markers, respectively.

SRAP targets open reading frames (ORFs), which is the constitutive part of the functional genes, so SRAP may have more direct relationship with functional genes than other marker systems. As SRAP targets ORFs, and simple sequence repeat (SSR) targets SSRs, a genetic map with high-intensity that reflected the distribution of both ORFs and SSRs could be constructed when the two marker techniques were used together. In addition, when compared with other commonly used molecular markers, SRAP is more stable, reproducible and informative than RAPD and less complicated than AFLP.

When compared with SSR, SRAP primers can be used in almost all plants, which avoid the tedious work for developing the primers. Therefore, SRAP markers system has been used to investigate genetic diversity in plant species, including *Brassica* (Li and Quiros, 2001), cotton (Lin et al., 2004), turfgrass (Budak et al., 2004a, b), and other plant species. The results show that this marker is homogenously distributed in the genome and could produce higher polymorphism and more abundant information than those from ISSR, RAPD and SSR (Ferriol et al., 2003; Budak et al., 2004a). As for the application of SRAP markers in turfgrass, previous reports mainly focused on the investigations on the relationships among bermudagrass, zoysiagrass (*Zoysia* sp.), centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack], buffalograss [*Buchloe dactyloides* (Nutt.) Englem] and other grasses (Budak et al., 2004c; Yi et al., 2008). However, the degree to which bermudagrass accessions or cultivars from different countries or regions are genetically interrelated is unknown. An estimation of genetic diversity in those accessions or cultivars will provide important information for genetic diversification in breeding programs.

The objectives of this research were: (1) to assess the value of SRAP marker system for its ability to distinguish bermudagrass accessions; and (2) to describe the genetic variation of bermudagrass accessions from different countries or regions and to study their genetic relationships.

MATERIALS AND METHODS

Plant materials included 55 bermudagrass accessions, including 33 wild *C. dactylon* var. *dactylon* accessions and 22 introduced cultivars originating from 4 countries (China, Australia, USA and India). The indigenous Chinese bermudagrass accessions were collected from 19 provinces ranging from tropical Hainan Island to the temperate climatic region around Xinjiang region (Table 1). All indigenous Chinese accessions were determined to be *C. dactylon* based on morphological characteristics, as described by Harlan et al. (1970a). Distinguishing characteristics of *C. dactylon* include racemes arranged in one whorl on inflorescences and subequal glumes with at least 3/4 of the length of spikelets.

The bermudagrass accessions were grown in the nursery at Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing, China. The seeded or vegetatively propagated bermudagrass cultivars were obtained from the suppliers listed in Table 1. All the wild accessions were collected by gathering healthy stolons from their origins (Table 1). Approximately 8 g healthy, mature and original seeds of seed-propagated cultivars were planted in plots of 1 × 1 m^2; and approximately 140 healthy stem segments of vegetatively-propagated cultivars with two internodes were regularly planted in plots of 1 × 1 m^2. The distances between the plots of different cultivars were 50 cm. The plots of these cultivars were trimmed weekly or biweekly to prevent contamination of different cultivars. The plots were frequently mowed at a height of 50 mm to ensure the turf health and supply newly expanded leaf blades. Necessary irrigation, fertilization and fungicide application were made to ensure healthy turf.

Primer selection, amplification and detection

Genomic DNA of each bermudagrass accession was extracted from fresh and healthy leaves with sodium dodecyl sulfate (SDS) method (Wang et al., 2009). DNA concentrations of the samples were determined by λDNA with a known concentration as a reference, and were finally adjusted to 50 ng μL^{-1} for PCR amplification (Wang et al., 2009). 90 SRAP primer combinations were applied for PCR amplification and analysis of 55 accessions (Table 2). Primers were excluded from the study if their banding patterns were difficult to score or failed to amplify in all accessions. Of these, 90 primer pairs and 30 primer combinations (Table 3) were selected for their consistent amplifications and clear banding patterns.

The reaction system for amplification was the same as the previously established protocols (Wang et al., 2009). Each 20 µl PCR reaction mixture consisted of 2 µl of 1 × buffer (10 mM Tris-HCl pH 8.3, 5 mM KCl), 50 ng genomic DNA, 1.25 mM of $MgCl_2$, 260 µM of each dNTP (Invitrogen), 0.2 µM primer (Invitrogen) and1.0 units of Taq DNA polymerase (Promega). The amplifications

Table 1. Identification and source of 33 *C. dactylon* var. *dactylon* accessions and 22 commercial *C. dactylon* cultivars.

Identification	Origin or reference	Identification	Origin or reference
Wintergreen	McMaugh, 1993	C069	Wuhu, Anhui
Windsorgreen	McMaugh, 1993	C262	Haikou, Hainan
C134	Yangling, Shanxi	C461	Yongzhou, Hunan
C135	Nanjing, Jiangsu	C158	Shenzhen, Guangdong
C113	Sanzhi, Hunan	C672	Lanzhou , Gansu
C788	Tianjing, China	C737	Shaoxing, Zhejiang
Common	Baltenspergen et al.,1993	C810	Jiangning, Jiangsu
C173	Taiwan, China	C291	Yangjiang, Guangdong
Cheyenne	Samudio and Brede, 1998	Primo	Khaleghi , 2005
Pyramid	Cebeco Internation Seed Inc. Halsey, OR	Numex Sahara	Baltensperger, 1989
C174	New Delhi, India	C431	Baise, Guangxi
Sahara	Ho et al., 1997	C610	Chengdu, Sichuang
C177	Xichang, Sichuan	C615	Chongqing, China
Riviera	Oklahoma State University, Stillwater , OK	C716	Handan, Hebei
Guymon	Taliaferro et al., 1983	C180	Danba, Sichuang
Panama	Fraser and Rose-Fricker, 2002	C638	Tongbai, Henan
Sydney	Seeds West Inc. Roll, AZ	C704	Sangqiu, Henan
Yuma	Seeds West Inc. Roll, AZ	C574	Longling, Yunnan
MoHawk	Seeds West Ins. Roll, AZ	C432	Liuzhou, Guangxi
Sundevi II	Samudio and Brede, 2002	C386	Yingde, Guangdong
Jackpot	Samudio et al., 1997	C394	Wuzhou, Guangxi
C189(1)	Sanya, Hainan	C359	Ruyuan, Guangdong
Yukon	Taliaferro et al., 2003	Nanjing	Liu et al., 2004
Yangjiang	Jiangsu Province and Chinese Academy of Sciences, Nanjing	Xinnong No.1	Abulaiti et al., 2003a
Tifton 10	Hanna et al., 1990	Kashi	Abulaiti et al., 2003b
C088	Jurong, Jiangsu	C224	Baisha, Hainan
C089	Jurong, Jiangsu	C224M	Nanjing, Jiangxu
C134M	Nanjing, Jiangxu		

were performed with TC-412 (TECHNE Company, UK). The protocol of PCR amplification was as follows: 4 min of denaturing at 94℃, 1 min of degenerated at 94℃, 1 min annealed at 37℃ and 10 s of elongated at 72℃. In the following 35 cycles, the annealing temperature was increased to 50℃, with a final elongation step of 7 min at 72℃. 10 µl of amplified products were fractionated on 8.0% non-denatured polyacrylamide gels using a Hoefer vertical-gel apparatus (JY-SCZ6). Gels consisted of acrylamide (19 acrylamide: 1 bisacrylamide) in 1×TBE buffer (90 mM Tris-boracic acid, 2 mM EDTA; pH 8.0). Gels were 0.75 mm in thickness and 16 × 18 cm in dimension. Electrophoresis conditions were held at 200 V for 2.5 h at room temperature. The gel was then subjected to rapid silver staining for detection (Wang et al., 2009).

Data analysis

Each SRAP band was visually coded as present (1) or absent (0). The distance matrix and dendrogram were constructed using the numerical taxonomy multivariate analysis system (NTSYS-pc) version 2.1 (Exeter Software, Setauket, N. Y.) software package. Genetic polymorphism (P-5%), Nei's gene diversity (*He*) and Shannon's information index were used to compute Nei's standard genetic distance coefficients (Nei and Li, 1979) and to construct a UPGMA dendrogram within the SAHN module of the NTSYS program (Sneath and Sokal, 1973).

RESULTS

SRAP polymorphisms

The 30 pairs screened from 90 combinations were selected to amplify the genomic DNA of 55 accessions. The PCR products of all primer pairs showed polymorphism, with 6 to 12 polymorphic bands for each primer pair. The size of amplified fragments varied from 100 to 500 bp. Figure 1 shows an example of the results of amplification using primer combination Me4-Em7 with 55 accessions. A total of 274 bands were produced by 30 primer combinations, among which 98% were polymorphic, with 8.9 polymorphic bands per primer combination on average.

Based on the origins of the bermudagrass accessions, they were divided into domestic and introduced

Table 2. The forward and reverse SRAP primer information for this study.

Name	Forward primer (5'- 3')	Name	Reverse primer (5'- 3')
Me1	TGA GTC CAA ACC GGA TA	Em1	GAC TGC GTA CGA ATT AAT
Me2	TGA GTC CAA ACC GGA GC	Em2	GAC TGC GTA CGA ATT TGC
Me3	TGA GTC CAA ACC GGA AT	Em3	GAC TGC GTA CGA ATT GAC
Me4	TGA GTC CAA ACC GGA CC	Em4	GAC TGC GTA CGA ATT TGA
Me5	TGA GTC CAA ACC GGA AG	Em5	GAC TGC GTA CGA ATT AAC
Me6	TGA GTC CAA ACC GGA CA	Em6	GAC TGC GTA CGA ATT GCA
Me7	TGA GTC CAA ACC GGA CG	Em7	GAC TGC GTA CGA ATT CAA
Me8	TGA GTC CAA ACC GGA CT	Em8	GAC TGC GTA CGA ATT CAC
Me9	TGA GTC CAA ACC GGA GG	Em9	GAC TGC GTA CGA ATT CAG
—	—	Em10	GAC TGC GTA CGA ATT CAT

Table 3. The 30 SRAP primer combinations used in this study.

S/N	Primer combination	S/N	Primer combination	S/N	Primer combination
1	Me1 - Em2	11	Me3 - Em1	21	Me5 - Em8
2	Me1 - Em4	12	Me3 - Em3	22	Me5 - Em9
3	Me1 - Em5	13	Me3 - Em7	23	Me5 - Em10
4	Me1 - Em7	14	Me3 - Em10	24	Me6 - Em1
5	Me1 - Em10	15	Me4 - Em7	25	Me6 - Em8
6	Me2 - Em1	16	Me5 - Em1	26	Me6 - Em10
7	Me2 - Em2	17	Me5 - Em2	27	Me7 - Em3
8	Me2 - Em3	18	Me5 - Em3	28	Me7 - Em9
9	Me2 - Em4	19	Me5 - Em4	29	Me8 - Em5
10	Me2 - Em9	20	Me5 - Em7	30	Me8 - Em8

accessions. The results show that Percentage of polymorphic loci (PPLs) for domestic and introduced accessions were 83 and 93%, respectively, and *He* were 0.24 and 0.26, respectively (Table 4).

Genetic diversity and relatedness

About 268 polymorphic bands were analyzed for genetic similarity coefficient (GSC) among the 55 bermudagrass accessions. The genetic diversity was relatively high among the accessions in this study. GSCs based on the SRAP data ranged from 0.57 to 0.97 from the 55 accessions. The lowest similarity coefficient (0.57) was between C158 and 'Xinnong No.1'. Accession C158 was collected in Shenzhen, Guangdong province of China. 'Xinnong No.1' was introduced from Xinjiang, China. The highest similarity coefficient was 0.97, detected between 'Cheyenne' and 'Pyramid', and 'Common' and C173. The three cultivars ('Cheyenne', 'Pyramid' and 'Common') were introduced from USA. Accession C173 was from Taiwan province of China.

Cluster analysis based on the GSCs separated the 55 bermudagrass accessions into three major groups: A, B and C (Figure 2). The variation patterns of the bermudagrass accessions appear to be associated with geographic origin. 'Wintergreen' and 'Kashi' were found to span the extremes of the dendrogram while the other accessions were distributed in between (Figure 2). Cluster A contained 'Wintergreen' and 'Windsorgreen' (Figure 2).

The GSC between 'Wintergreen' and 'Windsorgreen' is 0.80. 'Wintergreen' and 'Windsorgreen' are from Nsygan, NSW, Austrilian and 'Windsorgreen' were the offspring of 'Wintergreen' by radiation mutation (McMaugh, 1993).

Cluster B contained 48 accessions, making it the largest group. This cluster consisted of the majority of the wild accessions, which are two Chinese cultivars ('Yangjiang' and 'Nanjing') and 13 USA cultivars (Figure 2). The GSCs in the cluster ranged from 0.57 to 0.97. The 48 accessions were separated into four subgroups: I, II, III, and IV.

Subgroup I contained C134, C134M, C135, C610, C174, C672 and C788. Accessions C134, C135, C672, C174, C788 and C610 were collected from Shanxi, Jiangsu, Gansu, India, Tianjing and Sichuang, respectively. Accession C134M was the offspring of C134 radiation mutation (Table 1).

Subgroup II included 15 cultivars and 21 wild accessions. These were scattered into various sub-subgroups

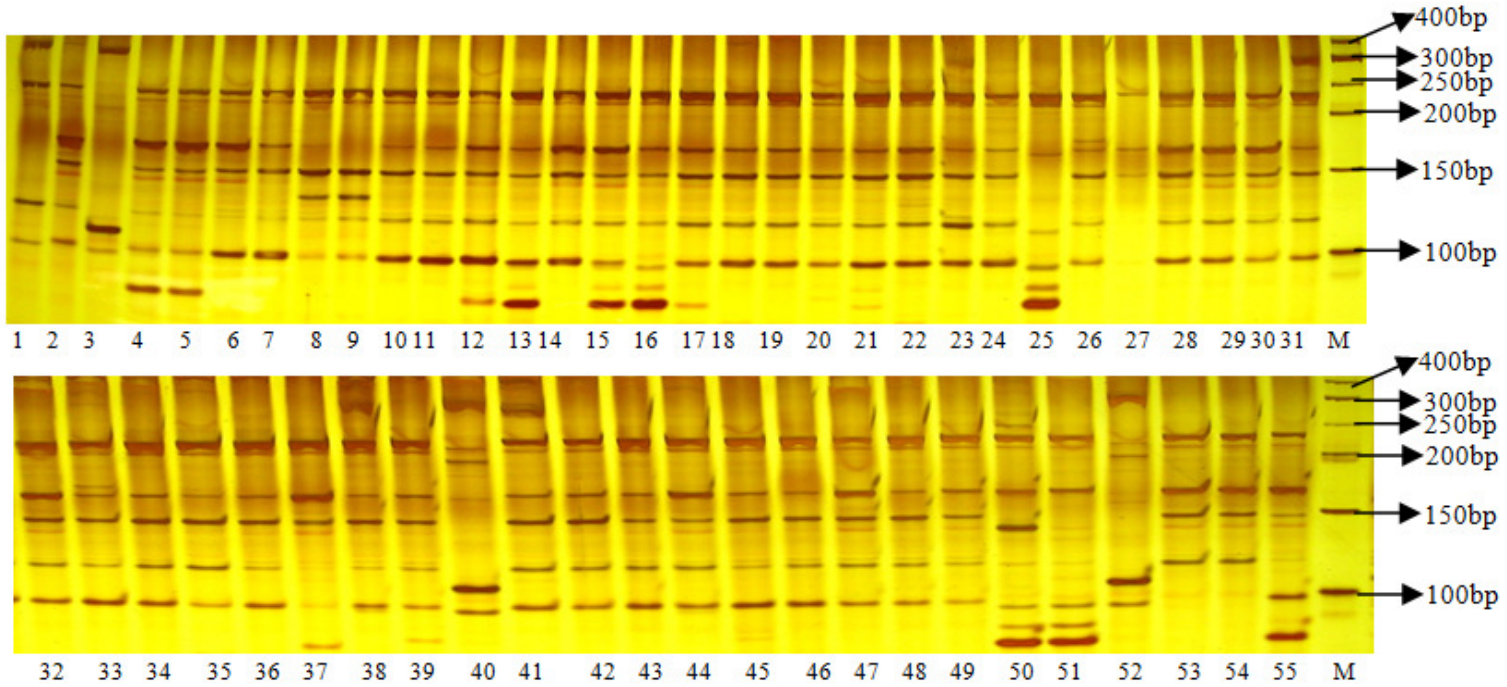

Figure 1. PCR amplification of bermudagrass genomic DNA from 55 genotypes. Lanes: 1, 'Wintergreen'; 2, 'Windsorgreen'; 3, C134; 4, C135; 5, C113; 6, C788; 7, 'Common'; 8, C173; 9, C174; 10, 'Cheyenne'; 11, 'Pyramid'; 12, 'Sahara'; 13, C177; 14, 'Riviera'; 15, 'Guymon'; 16, 'Panama'; 17, 'Sydeny'; 18, 'Yuma'; 19, 'Mohawk'; 20, 'Sundevi II'; 21, 'Jackpot'; 22, C189-1; 23, 'Yukon'; 24, 'Yangjiang'; 25, 'Tifton 10'; 26, C088; 27, C089, 28, C069; 29, C262; 30, C461; 31, C158; 32, C672; 33, C737; 34, C810; 35, C291; 36, 'Primo'; 37, 'Numex Sahara'; 38, C431; 39, C610; 40, C615; 41, C716; 42, C180; 43, C638; 44, C704; 45, C574; 46, C432; 47, C386; 48, C394; 49, C359; 50, 'Nanjing'; 51, 'Xinnong No.1'; 52, 'Kashi'; 53, C224; 54, C224M; 55, 134M; M, 50 bp marker (Promega). One sequence-related amplified polymorphism (SRAP) primer combination, Me4-Em7 was assayed. The DNA samples were fractionated in 8% non-denaturing acrylamide gels stained with silver.

(Figure 2), and the same zone or nearly regions tended to have higher GSCs and cluster into the same sub-subgroups or neighbor sub-subgroups. As shown in Figure 1, most accessions were collected from western, eastern and southern provinces of China, inclu-ding Jiangsu, Anhui, Shanghai, Guangxi and other neighboring provinces. However, six accessions (C177, C180, C574, C638, C704 and C716), which are from Sichuang, Henan and Hebei, formed sub-subgroups with accessions of eastern or southern regions. The GSC of 'Cheyenne' and 'Pyramid' cultivars was 0.97. Moreover, the ampli-fied bands from the two cultivars were almost identical as shown in Figure 1. In this subgroup, 'Yangjiang' was the mutant C291, which is from Yangjian, Guangdong.

Subgroup III contained C189 (1), C158, C224 and C224M, which were all from southern provinces of China. Accessions C224 were collected from Hainan province, C224M was the offspring of C224 radiation mutation and C158 was collected from Guangdong province.

Subgroup IV only contained C615, which was collected from a football field in Chongqing, China (Figure 2).

Cluster C contained five cultivars, which were

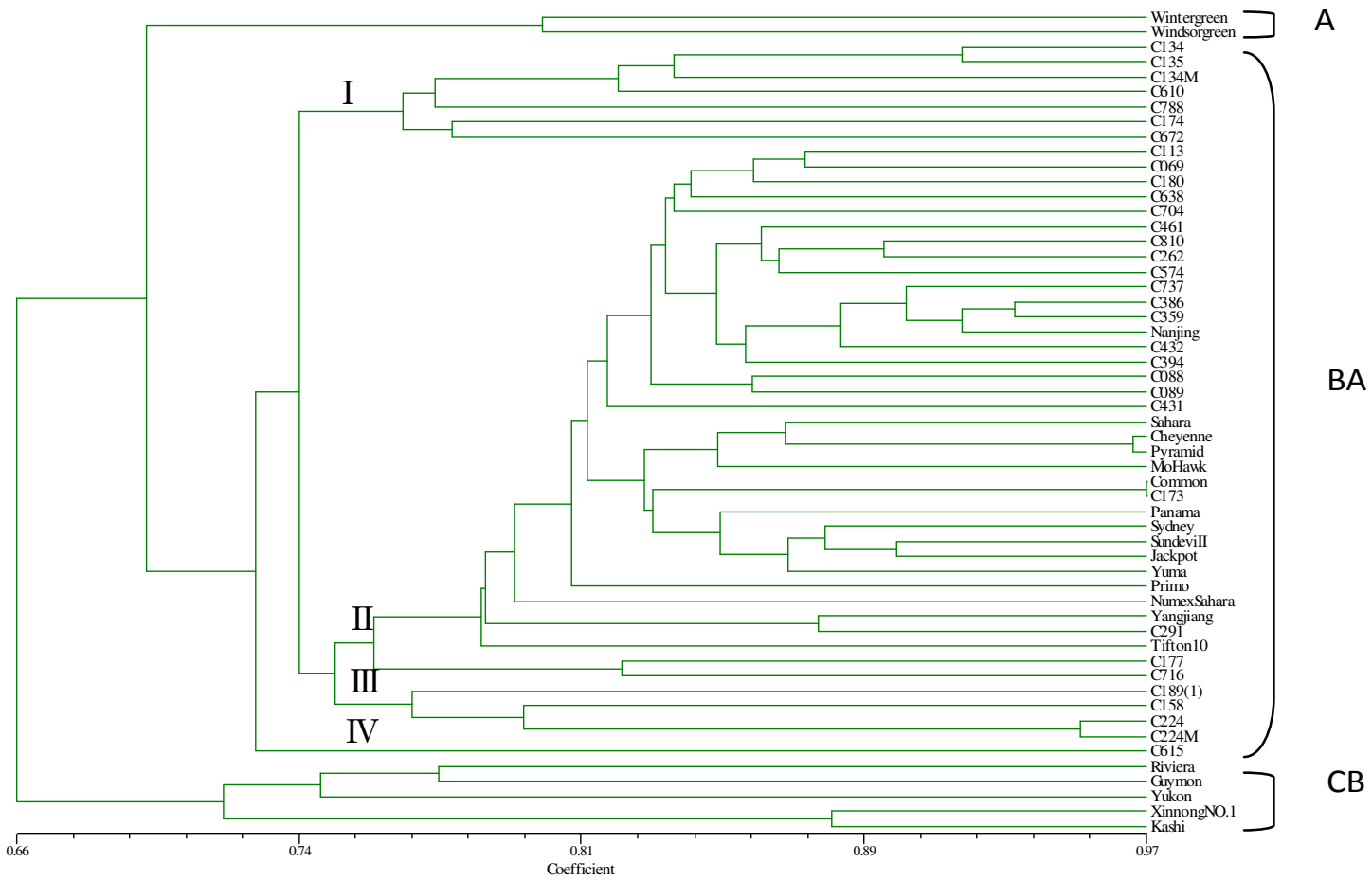

Figure 2. Dendrogram of 55 *C. dactylon* var. *dactylon* produced by UPGMA clustering method based on the genetic similarity matrix derived from 274 SRAP markers.

Table 4. Diversity of domestic and abroad bermudagrass germplasm.

Region	Number of sample	NPL	PPL (%)	He	I
Domestic	38	255	93	0.26	0.37
Abroad	17	229	83	0.24	0.38
Total	55	268	97	0.25	0.39

NPL, Number of polymorphic loci; PPL, percentage of polymorphic loci; He, Nei's gene diversity; *I*, Shannon's information index

from Oklahoma State University, Stillwater, OK, USA ('Riviera', 'Yukon' and 'Guymon') and Xinjiang, China ('Xinnong No.1' and 'Kashi'), and the GSCs among the five cultivars ranged from 0.60 to 0.77 and averaged as 0.68.

DISCUSSION

SRAP marked advantages, polymorphism and stability

Currently, several molecular marker systems have been applied to the analysis of the genetic relationships of plants (Botstenin et al., 1980; Tautz, 1989; Williams et al., 1990; Vos et al., 1995). Each PCR-based marker technique has its own advantages and disadvantages. For instance, RAPD provides a simple PCR-based molecular tool for the evaluation of genetic variation, but its poor consistency and low reproducibility limit its use (Roodt et al., 2002). AFLP technology is now widely used for genomic fingerprinting due to its high multiplexing ratio (Vos et al., 1995; Zhang et al., 1999; Karaca et al., 2002). However, AFLP is complex, requires multiple steps, and has pseudo-polymorphism when methylation-sensitive restriction enzymes are used (Li and Quiros,

2001). SRAP marker is a new and useful molecular marker system for mapping and gene tagging in *Brassica* (Li and Quiros, 2001) and *Cucurbita* (Ferriol et al., 2003). It targets coding sequences and results in the identification of a number of co-dominant markers. SRAP is based on two-primer amplifications where the primers are 17 or 18 nucleotides long. Primers consist of a core sequence of 13 or 14 bases, where the 5´-most 10 or 11 bases are non-specific, followed by the sequence CCGG in the forward primer and AATT in the reverse primer. The core sequence is followed by three selective nucleotides at the 3´ end of each primer (Li and Quiros, 2001). SRAP is more reproducible than RAPD and less complicated than AFLP. Ferriol et al. (2003) reported that the information obtained from SRAP markers was more concordant with the morphological variations and the evolutionary history of the morphtypes than that found with AFLP markers. Budak et al. (2004c) comparative analysis of buffalograsses based on phylogenentic relationship using ISSRs, SSRs, RAPDs and SRAPs, showed that SRAP marker was homogenously distributed in the genome and could produce high polymorphism and more abundant information than ISSR, RAPD and SSR, and PPLs for SRAP, ISSR, RAPD and SSR was 95, 81, 79 and 87%, respectively. In this study, the PCR reaction mixture suitable for bermudagrass was set up according to the SRAP mixture of *Brassica* (Li and Quiros, 2001) with a few modifications (Wang et al., 2009). 30 selected from 90 SRAP primer combinations generated a total of 274 clear bands encompassing 268 (PPL = 97%) polymorphic.

Genetic diversity of domestic and introduced bermudagrass accessions

Cynodon sp. originated in the old world where centers of diversity are distributed in parts of Africa and Eurasia (Harlan, 1970a), during the course of long-term evolution. *C. dactylon* (L.) Pers. is widely distributed in the world through natural and man-made-introduction and its aggressive growth characters, such as very strong vegetative propagation and anti-interference capability. A number of researchers have employed RAPD (Roodt et al., 2002), ISSR (Liu et al., 2006), DAF (Anderson et al., 2001), AFLP (Wu et al., 2004; 2006) and SRAP (Wang et al., 2009) to quantify genetic diversity of *Cynodon* sp. The result shows that the genetic variation of bermudagrass is significant. Wu et al. (2004, 2006) has analyzed bermudagrass accessions collected from Europe, Oceania, Africa and Asia, to detect the genetic diversity, and to quantify the genetic variation of Chinese Bermudagrass accessions GSCs and PPLs among four continents and Chinese accessions ranged from 0.53 to 0.98 and 75%, and 0.65 to 0.99 and 61%, respectively. In this study, GSCs and PPL of 55 accessions ranges from 0.57 to 0.97 and 97%, respectively. Within the Chinese indigenous accessions, GSCs and PPL ranges from 0.57 to 0.96 and 93%, respectively. This study indicate similarity in the scope of the GSC between Chinese indigenous accessions and four continents; this may be related with different geographical environment of China, which contained tropical, subtropical, temperate and other climate zones.

This study shows that PPLs of introduced and domestic accessions are 83 and 93%, respectively, and the PPL of 55 accessions is 97%. Wu et al. (2006) study showed that the PPL of Chinese indigenous accessions was only 61.1%, the main difference may be caused by different materials or molecular markers. In our study, the materials contains USA, China, Australia and India accessions, Chinese indigenous accessions were collected from 19 provinces ranging from tropical to temperate climatic region (Table 1). These regions have a relatively unique climate, such as the Xinjiang region, the southwestern region and the Yangtze River region. In Wu et al. (2006) study, the materials contained different regions of China, except for the northwest and Xinjiang region of China. However, these regions, especially Xinjiang region, are relatively unique geographic areas and the difference of bermudagrass morphological is also significant (data not reported). In addition to these reasons, this study not only used many domestic and introduced cultivars, but also used a number of wild accessions, which were collected from 19 representational provinces. Therefore, the genetic diversity of 55 bermudagrass accessions is very rich in this study.

Relationship of domestic and introduced accessions

Thirty SRAP primer pairs generated 274 bands, the average number of bands of per primer pair was 9.1 (Table 2). Cluster analysis by UPGMA separated the 55 accessions into distinct major groups: A, B and C. The results show that Group A includes Australian cultivars, that is ‘Wintergreen’ and ‘Windsorgreen’, ‘Windsorgreen’ is the offspring of ‘Wintergreen’ ^{60}Co irradiation derivative (McMaugh, 1993). Australia has a unique climate conditions and the effect of the island, so that there are high genetic distance between bermudagrass accessions of Australia and those of China or USA, which could lead them to cluster different groups. Similarly, Wu et al. (2004) observed that accessions originating from Africa, Australia, Asia and Europe were separately clustered based on AFLPs.

In this study, five cultivars, ‘Yangjing’, ‘Nanjing’, ‘Kashi’, ‘Tifton 10’ and ‘Xinnong No.1’, were collected from China. However, these cultivars did not belong to a group. ‘Tifton 10’, ‘Nanjing’ and ‘Yangjiang’ were collected from Shanghai, Jiangsu and Guangdong, respectively, which all belonged to the Yangtze River Delta region. Therefore, they had similar genetic background and were clustered together (Group B).

In this cluster, the accession C173 and 'Common' have the highest GSC (0.97), the morphological traits of accession C173 and 'Common' in the field plots were very similar (data not shown). Accession C173 was collected from Taiwan, with probably mutation of 'Common'. Caetano-Anollés (1998b) analogously reported that triploid bermudagrass had genetic instability based on DAF and arbitrary signatures from amplification profiles (ASAP). Therefore, common bermudagrass may also easily produce similar phenomenon. Accession C615 in subgroup IV of Group B, may have unique climate in Chongqing, China or escaped out of an unknown cultivar (Figure 2)'Cheyenne' and 'Pyramid' in the field were similar in this subgroup, and previous data revealed that they were both seed propagated cultivars (Samudio and Brede, 1998). The field on-spot record indicated that 'Pyramid' had excellent texture, delicate leaves and dark green color, but the 'Pyramid' used in this investigation had coarse texture and light green color, indicating that this cultivar might be contaminated. 'Cheyenne' and 'Pyramid' were adjacently planted in the nursery, which might give rise to cross-contamination. The GSC of 'Cheyenne' and 'Pyramid' was also 0.97, which further confirmed that Pyramid had been contaminated. The amplified bands from the two cultivars were almost identical as shown in Figure 1, indicating that this conjecture was reasonable.

In Group C, 'Rivera', 'Guymon' and 'Yukon' are from the same region and showed similar ecotype adaptability and high cold resistant, which led to clustering of different group with other USA cultivars. Yerramsetty et al. (2005) reported that many bermudagrass cultivars ('Jackpot', 'Mohawk', 'Pyramid', 'Numex Sahara' and other cultivars) were closely related to 'Common' by DAF and MHP-DAF (Minihairpin-DAF) markers. However, 'Yukon' was least genetically related to 'Common' (Tallaferro et al., 1983, 2003). The five cultivars in Group C all exhibited relatively strong cold resistance (data not reported), and were bred in cold regions. In fact, there is a wide zone existing between Xinjiang and other southern provinces, there is no distribution of bermudagrass, which perhaps resulted in distinct differences between accessions of Xinjiang and the other region on the morphological and molecular of characteristics (data not reported). Therefore, Xinjiang cultivars could solely cluster a group. In this context, Australia's cultivars ('Wintergreen' and 'Windsorgreen') also have similar characteristics and led to cluster obviously different groups. Therefore, this showed that there is a wide genetic diversity among genotypes within *C. dactylon*. The results could be useful for ecotype selection in a breeding program.

The dendrogram resolved by SRAP markers in this study shows that most accessions from the same areas could form a subgroup, but a few accessions were not accorded. For example, there were a few accessions of southeast of China which have not clustered together. This showed that there was incomplete direct relationship between the origin of the accessions and molecular clusters, although the accessions were collected from different regions or discrete genetic differentiation within a region (Table 1). The reasons might be as follows: firstly, this might be genetic overlap occurring in the bermudagrass accessions from two different regions; secondly, this might be as a result of the open pollination behavior of *Cynodon* plants. Cross-pollination results in gene flow between natural populations, which probably prevents formation of distinctly differentiated genetic groups; thirdly, this might be as a result of the different countries or regions to exchange germplasm resources; lastly, the ploidy might lead to this result. Wu et al. (2006) reported that southeast provinces might be a unique climate type, which are relative to the frequency of occurrence of hexaploid and pentaploid of *C. dactylon* cytotypes. Gulsen et al. (2009) and Kang et al. (2007) had similar result by analyzing Turkey and Korean *Cynodon* accessions. On the basis of this study, we will further analyze *Cynodon* accessions of ploidy, in order to study the depth relationship among them, because ploidy distribution might be due to environmental effects, or the evolutionary and historical development of genotypes (Johnson and Riordan, 1998).

The results from this study show that the SRAP technique measure sufficient polymorphism for DNA typing, and may be a powerful tool for the genetic dissection of the bermudagrass genome. Chinese wild accessions and introduced cultivars all have maintained a broad degree of genetic diversity. Substantial genetic variation still exists within the Chinese bermudagrass gene pool, and selection within the gene pool is still feasible. The genetic relationships and distinctiveness obtained for the bermudagrass accessions in this study based on SRAPs should facilitate the selection of less related germplasm for intercrossing. At the same time, we would integrate molecular marker with many agronomical traits such as turf quality, cold resistance, drought resistance, acid resistance and other resistance, which could be useful to determine optimal breeding strategies, to ensure sustainable breeding programs in the future for various uses such as turf, forage, soil stabilization, and remediation, as well as to understand evolution of this warm season grass.

ACKNOWLEDGEMENTS

The authors are grateful to Zhongchun Jiang (PhD), from the Department of Plant Sciences, College of Agriculture and Technology of New York State University for the paper review. This work was supported by the National Natural Science Foundation of China (Grant No. 31060266), Hainan Provincial Natural Science Foundation (Grant No. 310031), and the project (Grant No. BM2009905) . The authors would like to extend their sincere thanks to the editors and referees for their valuable comments to improve this manuscript.

Abbreviations

SRAP, Sequence-related amplified polymorphism; **PPL,** percentage of polymorphic loci; **UPGMA,** unweighted pair-group method with arithmetic average; **ISSR,** inter simple sequence repeat; **SSR**, Simple sequence repeats; **RAPD,** random amplified polymorphic DNA; **GSC,** genetic similarity coefficient; **ORF,** open reading frames; **AFLP,** amplified fragment length polymorphisms; **He,** Nei's gene diversity.

REFERENCES

Abulaiti Shi DS, Yang ZM, Li PY, Zhao Q, Sun ZJ (2003a). Xinnong No.1. Pratacultural Sci. 20(9): 30-31.

Abulaiti, Shi DS, Zhao Q, Li PY, Sun ZJ (2003b). Kashi. Pratacultural Sci. 20(5): 57-58.

Anderson MP, Taliaferro CM, Martin DL, Anderson CS (2001). Comparative DNA profiling of U-3 turf bermudagrass strains. Crop Sci. 41: 1184-1189.

Assefa S, Taliaferro CM, Anderson MP, de los Reyes BG, Edwards RM (1999). Diversity among *Cynodon* accessions and taxa based on DNA amplification fingerprinting. Genome 42: 465-474.

Baltensperger AA (1989). registration of 'Numex Sahara' bermudagrass. Crop Sci. 29: 1326.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of genetic linkage map in man using restriction length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Bethel CM, Sciara EB, Estill JC, Bowers JE, Hanna W, Paterson AH (2006). A framework linkage map of bermudagrass (*Cynodon dactylon* × *transvaalensis*) based on single-dose restriction fragments. Theor. Appl. Genet. 112: 727-737.

Budak H, Shearman RC, Parmaksiz I, Dweikat I (2004c). Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRS, RAPDs, and SRAPs. Theor. Appl. Genet. 109: 280-288.

Budak H, Shearman RC, Gaussoin RE, Dweikat I (2004b). Application of sequence-related amplified polymorphism markers for characterization of turfgrass species. Hort. Sci. 39: 955-958.

Budak H, Shearman RC, Parmaksiz I (2004a). Molecular characterization of buffalograss germplasm using sequence-related amplified polymorphism markers. Theo. Appl. Genet. 108: 328-334.

Caetano-Anollés G (1998b). Genetic instability of bermudagrass (*Cynodon*) cultivars 'Tifgreen' and 'Tifdwarf' detected by DAF and ASAP analysis of accessions and off-types. Euphytica, 101: 165-173.

Caetano-Anollés G (1998a). DNA analysis of turfgrass genetic diversity. Crop Sci. 38: 1415-1424.

Caetano-Anollés G, Callahan LM, Gresshoff PM (1997). The origin of bermudagrass (*Cynodon*) off-types inferred by DNA amplification fingerprinting. Crop Sci. 37: 81-87.

Caetano-Anollés G, Callahan LM, Williams PE, Weaver KR, Gresshoff PM (1995). DNA amplification fingerprinting analysis of bermudagrass (*Cynodon*): Genetic relationships between species and interspecific crosses. Theor. Appl. Genet. 91: 228-235.

Clayton WD, Renvoize SA (1986). Genera Graminum-grasses of the world. Kew Bulletin. Additional Series XIII. Royal Botanic Gardens, Kew.

Ferriol M, Pico B, Nuez F (2003). Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theo. Appl. Genet. 107: 271-282.

Fraser ML, Rose-Fricker CA (2002). Registration of 'Panama' bermudagrass. Crop Sci. 42: 308.

Hanna WW, Burton GW, Johnson AW (1990). Registration of 'Tifton 10' turf bermudagrass. Crop Sci. 30: 1355-1356.

Harlan JR (1970a). *Cynodon* species and their value for grazing or hay. Herbage Abs. 40: 233-238.

Harlan JR, de Wet JMJ (1969). Sources of variation in *Cynodon dactylon* (L.) Pers.. Crop Sci. 9: 774-778.

Harlan JR, de Wet JMJ, Rawal KM (1970b). Origin and distribution of the seleucidus race of *Cynodon dactylon* (L.) Pers. var. *dactylon* (Gramineae). Euphytica 19: 465-469.

Ho CY, McMaugh CY, Wilton AN, McFarlane IJ, Mackinlay AG (1997). DNA amplification variation within cultivars of turf-type Couch grasses (*Cynodon* sp.). Plant Cell Rep. 16: 797-801.

Johnson PG, Riordan TP (1998). Ploidy level determinations in buffalograss clones and populations. Crop Sci. 38: 478-482.

Karaca M, Saha S, Zipf A, Jenkins JN, Lang DJ (2002). Genentic diversity among forage bermudagrass (*Cynodon* sp.): Evidence from chloroplast and nuclear DNA fingerprinting. Crop Sci. 42: 2118-2127.

Li G, Quiros CF (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103: 455-461.

Lin ZX, Zhang XL, Nie YC (2004). Evaluation of application of a new molecular marker SRAP on analysis of F2 segregation population and genetic diversity in cotton. Acta Genetic Sinica 31: 622-626.

Liu JX, Liu YD, He SA, Chen SL, Chen ZY (2004). The breeding of *Cynodon dactylon* cv. Nanjing. Pratacultural Sci. 21(11): 84-85.

Liu W, Li XQ, Zhang F, Ma X, Fan Y (2007). Genetic diversity of bermudagrass accessions in south-west by ISSRs molecular markers and geographic proverance. Acta Prataculturae Sinica 16(3): 55-61.

McMaugh P (1993) Couch grass (turf). Aust. Plant Varieties J. 6: 23.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. PNAS, 76: 5269-5273.

Khaleghi E, Ramin AA (2005). Study of the effects of salinity on growth and development of lawns (*Lolium perenne* L. *Festuca arundinacea* and *Cynodon dactyl.on*). J. Sci. Technol. Agric. Nat. Res. 9: 57-68.

Etemadi N, Sayed-tabatabaei BE, Zamanni Z, Razmjoo K, Khalighi A, and H. Lessani (2006). Evaluation of diversity among *Cynodon dactylon* (L.) Pers.. Int. J. Agri. Biol. 32: 117-122.

Gulsen O, Sever-Mutlu S, Mutlu N, Tuna M, Karaguzel O, Sheraman RC, Riordan TP, Heng-Moss TM (2009). Polyploidy creates higher diversity among *Cynodon* accessions as assessed by molecular markers. Theor. Appl. Genet. 118: 1309-1319.

Kang SY, Lee GJ, Lim KB, Lee HJ, Park IS, Chung SJ, Kim JB, Kim DS, Rhee HK (2007). Genetic diversity among Korean bermudagrass (*Cynodon* sp.) ecotypes characterized by morphological, eytological and molecular approach. Mol. Cells, 25: 163-171.

Samudio SH, Brede AD (1997). Registration of 'Jackpot' bermudagrass. Crop Sci. 37: 1380.

Samudio SH, Brede AD (1998). Registration of 'Cheyenne' Bermudagrass. Crop Sci. 38: 537-538.

Samudio SH, Brede AD (2002). Registration of 'sundevil II' bermudagrass. Crop Sci. 42: 670-671.

Sneath PHA, Sokal RR (1973). Numerical taxonomy. Freeman, San Francisco.

Taliaferro CM, Aharing RM, Richardson WL (1983). Registation of 'Guymon' bermudagrass. Crop Sci. 23: 1219.

Taliaferro CM, Martin DL, Anderson JA, Anderson MP, Bell GE, Guenzi AC (2003). Registration of 'Yukon' bermudagrass. Crop Sci. 43: 1131-1132.

Tautz D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. 17: 6463-6471.

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Wang ZY, Yuan XJ, Zheng YQ, Liu JX (2009). Molecular identification and genetic analysis for 24 turf-type Cynodon cultivars by Sequence-Related Amplified Polymorphism markers. Scientia Hortcult. 17: 79-85.

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Wu YQ, Taliaferro CM, Bai GH, Anderson MP (2004). AFLP analysis of *Cynodon dactylon* (L.) Pers. var. *dactylon* genetic variation. Genome, 47: 689-696.

Wu YQ, Taliaferro CM, Bai GH, Anderson MP (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 YQ, Taliaferro CM, Bai GH, Martin DL, Anderson JA, Anderson MP, Edwards RM (2006). Genetic analysis of Chinese *Cynodon* accessions by flow cytometry and AFLP markers. Crop Sci. 46: 917-926.
Yi YJ, Zhang XQ, Huang LK, Ling Y, Ma X, Liu W (2008). Genetic variety of wild bermudagrass germplasm using SRAP marker. Heredity Chines 30(1): 94-100
Yerramsetty PN, Anderson MP, Taliaferro CM, Martin DL (2005). DNA fingerprinting of seed bermudagrass cultivars. Crop Sci. 45: 772-777.
Zhang LH, Ozias-Akins P, Kochert G, Kresovich S, Dean R, Hanna W (1999). Differentiation of bermudagrass (*Cynodon* sp.) genotypes by AFLP analysis. Theor. Appl. Genet. 98: 895-902.