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Molecular identification and genetic analysis for 24 turf-type *Cynodon* cultivars by Sequence-Related Amplified Polymorphism markers

Zhiyong Wang [a,b], Xuejun Yuan [a], Yiqi Zheng [a], Jianxiu Liu [a,*]

[a] Grass Research Centre, Institute of Botany, Jiangsu Province & Chinese Academy of Sciences, Zhongshanmenwai, Nanjing 210014, China
[b] College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

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ABSTRACT

Bermudagrass (*Cynodon* ssp.) germplasm is genetically diverse and widely distributed in the world. The study was conducted to identify and assess the molecular variation and relationship among 24 cultivars developed in China, Australia and the USA. Sequence-Related Amplified Polymorphism (SRAP) was applied to cultivars identification in this study for the first time. Thirty of the 90 SRAP primer combinations generated a total of 274 clearly bands encompassing 249 (91%) polymorphic. Each bermudagrass cultivar has its unique binary code and can be distinguished from the others. Three distinct clusters were obtained by unweighted pair-group method with arithmetic averages based on the polymorphic markers. Coefficients of genetic distance among the genotypes ranged from 0.57 to 0.97. The results demonstrated that SRAP marker is a stable molecular marker technique for the identification of bermudagrass cultivars and their genetic relationships.

1. Introduction

Bermudagrass (*Cynodon* spp.) is one of the most important warm-season turfgrass, mainly used for turf establishment of public green land, golf courses and sports fields in many countries. As a high quality turfgrass, bermudagrass is characteristics with recuperative potential, good color, high density and wear-tolerance (Beard, 1971). Currently, bermudagrass cultivars used in the turf industry mainly include common bermudagrass types with coarse texture and generally tetraploid ($2n = 4x = 36$) and hexaploid ($2n = 6x = 54$) [*C. dactylon* (L.) Pers.], and interspecific hybrid between common bermudagrass and African bermudagrass types [*C. transvaalensis* Burtt-Davy] with fine texture and generally diploid ($2n = 2x = 18$). The offspring of triploid hybrid bermudagrass ($2n = 3x = 27$) is mainly obtained from the hybridization of tetraploid common bermudagrass and diploid African bermudagrass, while all of the triploid hybrids are sterile, having essentially no pollen production or seed set, and must be clonally propagated (Powell et al., 1974). There are also small number of such hybrid bermudagrass cultivars as Patriot (Okc18-4) and Okc19-9, which were obtained from crossing between hexaploid common bermudagrass and diploid African bermudagrass. Bermudagrass could have both vegetative propagation as 'Tifton 10' and 'Nanjing', and seeded propagation as 'Cheyenne' and 'Jackpot'. The former are normally relatively fine, while the later are normally coarse.

Three commonly methods including morphological characteristics, isozyme electrophoresis patterns, and molecular marker are used for the identification of bermudagrass cultivars. Morphological characteristics are easily affected by environmental factors, which may restrict its utilization in cultivars identification (Tseng, 1962; Ma and Cai, 1996). Currently, morphological indexes may be used to easily distinguish the differences between tetraploid common bermudagrass and triploid hybrid bermudagrass, whereas the field observations reveal that these indexes are incompetent to discriminate the differences between the same tetraploid common bermudagrass cultivars and tetraploid hybrid bermudagrass cultivars. In addition, it is difficult to distinguish seed propagated bermudagrass cultivars from each other by their morphological traits. Isozyme electrophoresis patterns have been used to differentiate *Cynodon* cultivars, and some genotypes not easily differentiated by morphological traits could be distinguished by isozyme electrophoresis patterns (Dabo et al., 1990; Vermeulen et al., 1991; McMaugh, 1993). For example, Vermeulen et al. (1991) and Dabo et al. (1990) have used isozyme electrophoresis patterns to identify vegetative propagated genotypes of turf-type or forage-type *Cynodon* species. However, isozyme electrophoresis patterns was impossible to separate all genotypes of bermudagrass cultivars since the expression of some isozymes may be influenced by both environmental and developmental stage of the tested plants (Genkel et al., 1974; Vermeulen et al., 1991). Molecular marker is a preferred method for the

* Corresponding author. Tel.: +86 25 84347057; fax: +86 25 84347057.
E-mail address: turfunit@yahoo.com.cn (J. Liu).

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identification or comparison of plant cultivars since it could even distinguish between closely related genotypes (Nybom, 1994). The DNA-amplification Fingerprinting (DAF) technique has been used successfully to separate African bermudagrass, common bermudagrasses and their hybrid offspring (Caetano-Anollés et al., 1997), detected off-types obviously originating from sod contamination instead of somatic mutation, and identify the cultivars Tifway, Tifgreen, and Tifdwarf (Caetano-Anollés, 1998a), Yerramsetty et al. (2005) identified 13 out of the 17 cultivars by DAF, but Riviera, Princess, SW I-11, and Yukon) were practically indistinguishable using the DAF primers, while the MHP-DAF primers distinguished all cultivars readily. Ho et al. (1997) used the DAF technique to identify the cultivars of bermudagrass, and 10 of the 13 primers could not distinguish Wintergreen from Windsorgreen. Zhang et al. (1999) used the Amplified Fragment Length Polymorphism (AFLP) technique (Vos et al., 1995) to successful identify *C. transvaalensis* from hybrid bermudagrass (*C. dactylon* × *C. transvaalensis*); however, *C. dactylon* and *C. transvaalensis* could not be separated by AFLP.

Currently, several molecular markers were applied in the identification of bermudagrass cultivars. Restriction Fragment Length Polymorphism (RFLP) requires large amount of DNA, more elaborate detection procedures and long time, but produces less polymorphism; RAPD is convenient but with poor reproducibility; AFLP has good reproducibility and high polymorphism, but its operation is relatively elaborate and the expense is high. SRAP technique is to design the primers for amplification based on the facts of GC-rich exons and the AT-rich promoters and introns, and the polymorphism were produced due to the different sizes of introns, promoters and spacer regions in different organisms. Therefore, SRAP marker is able to present the codominant marker with a large amount of polymorphic loci and can be easily separated from the sequence. Importantly, it also has such advantages as Open Reading Frames (ORFs) of its amplification targets (Li and Quiros, 2001). Previous studies showed that SRAP marker was homogenously distributed in the genome and could produce higher polymorphism than those from ISSR, RAPD, and SSR (Budak et al., 2004a). The information given by SRAP markers was more concordant with the morphological variability and to the evolutionary history of the morphotypes than that of AFLP markers (Ferriol et al., 2003). At present, SRAP marker has been used in the studies on genetic diversity in *Brassica oleracea* (Li and Quiros, 2001), *Cucurbita pepo* (Ferriol et al., 2003), cotton (Lin et al., 2004) and other plants. As for the application of SRAP markers in the turfgrass, the previous reports mainly focused on the preliminarily study of the relationships among different species including bermudagrass, zoysiagrass (*Zoysia* spp.), centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack], buffalograss [*Buchloe dactyloides* (Nutt.) Englem] and other grasses (Budak et al., 2004c). Neither identification of cultivars nor analysis of genetic diversity in turfgrasses was reported except for the genetic diversity of buffalograss (Budak et al., 2004a,b,c).

Several studies have been conducted to examine the genetic relationships and molecular identification among vegetative or seed propagated bermudagrass cultivars (Caetano-Anollés et al., 1995; Caetano-Anollés, 1998b; Zhang et al., 1999; Yerramsetty et al., 2005), but no information has been published concerning identification and diversity among vegetative-type and seed-type bermudagrass cultivars, especially the released bermudagrass cultivars from different countries by SRAP marker.

The objectives of this study were to: (1) identify bermudagrass cultivars from USA, China, and Australia by constructing the SRAP fingerprint; (2) explore the genetic relationships among these cultivars by SRAP maker.

2. Materials and methods

2.1. Plant material

Three hybrid bermudagrass cultivars and 21 common bermudagrass cultivars were obtained from China, USA, and Australia. The bermudagrass cultivars were grown in the nursery at Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing, China. The seed-propagated or vegetative propagated bermudagrass cultivars were obtained from the suppliers listed in Table 1. Approximately 8 g healthy, mature and original seeds of seed-propagated cultivars were planted in the plot of 1 m × 1 m; and approximately 140 healthy stem segments of vegetative-propagated cultivars with two internodes were regularly planted in plot of 1 m × 1 m. The distances between the plots of different cultivars were 50 cm. The plots of these cultivars were trimmed around weekly or biweekly to prevent contamination of different

Table 1
Twenty four bermudagrass cultivars used in this study.

No.	Cultivars	Species	Source or reference	Chromosome no. (2*n*)	Selection type and propagation type
1	Primo	*C. dactylon*	USA (Khaleghi and Ramin, 2005)	–	Hybrid selection, seed propagation
2	Numex Sahara	*C. dactylon*	USA (Baltensperger, 1989)	–	Hybrid selection, seed propagation
3	Wintergreen	*C. dactylon*	Australia (McMaugh, 1993)	36	Hybrid selection, seed propagation
4	Windsorgreen	*C. dactylon*	Australia (McMaugh, 1993)	36	Systematic breeding, seed propagation
5	Santa Ana	*C. dactylon* × *C. transvaalensis*	Australia (Anonymous, 1972)	27	Hybrid selection, seed propagation
6	Common	*C. dactylon*	USA (Baltensperger et al., 1993)	36	Hybrid selection, seed propagation
7	Cheyenne	*C. dactylon*	USA (Samudio and Brede, 1998)	–	Hybrid selection, seed propagation
8	Pyramid	*C. dactylon*	USA (Cebeco Internation Seed Inc., Halsey, OR)	–	Hybrid selection, seed propagation
9	Sahara	*C. dactylon*	USA (Seeds West Inc., Roll, AZ)	18	Hybrid selection, seed propagation
10	Riviera	*C. dactylon*	USA (Oklahoma State University, Stillwater, OK)	36	Hybrid selection, seed propagation
11	Guymon	*C. dactylon*	USA (Taliaferro et al., 1983)	36	Hybrid selection, seed propagation
12	Panama	*C. dactylon*	USA (Fraser and Rose-Fricker, 2002)	–	Hybrid selection, seed propagation
13	Sydney	*C. dactylon*	USA (Seeds West Inc., Roll, AZ)	–	Hybrid selection, seed propagation
14	Yuma	*C. dactylon*	USA (Seeds West Inc., Roll, AZ)	–	Hybrid selection, seed propagation
15	MoHawk	*C. dactylon*	USA (Seeds West Ins., Roll, AZ)	–	Systematic breeding, seed propagation
6	Sundevi II	*C. dactylon*	USA (Samudio and Brede, 2002)	–	Hybrid selection, seed propagation
17	Jackpot	*C. dactylon*	USA (Samudio and Brede, 1997)	36	Hybrid selection, seed propagation
18	Patriot	*C. dactylon* × *C. transvaalensis*	USA (Oklahoma State University, Stillwater, OK)	36	Hybrid selection, seed propagation
19	Okc19-9	*C. dactylon* × *C. transvaalensis*	USA (Oklahoma State University, Stillwater, OK)	36	Hybrid selection, seed propagation
20	Yukon	*C. dactylon*	USA (Taliaferro et al., 2003)	36	Hybrid selection, seed propagation
21	Tifton 10	*C. dactylon*	USA (Hanna et al., 1990)	54	Systematic breeding, vegetative reproduction
22	Nanjing	*C. dactylon*	China (Liu et al., 2004)	–	Systematic breeding, vegetative reproduction
23	Xinnong No. 1	*C. dactylon*	China (Abulaiti et al., 2003a)	–	Systematic breeding, seed propagation
24	Kashi	*C. dactylon*	China (Abulaiti et al., 2003b)	–	Systematic breeding, seed propagation