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Genetic diversity of *Cynodon radiatus* assessed by sequence-related amplified polymorphism markers

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ABSTRACT

Sequence-related amplified polymorphism (SRAP) markers were used to assess the genetic relatedness among 33 *Cynodon radiatus* accessions from different regions of China. Fifteen primer combinations were used to amplify specific *C. radiatus* genomic sequences. A total of 382 SRAP fragments were generated, with fragment sizes ranging from 200 to 1800 bp. Genetic similarity coefficients among the 33 accessions ranged from 0.53 to 0.95, with an average of 0.70. Cluster analysis by two methods, the unweighted pair–group method with arithmetic averages and the principle coordinate analysis, separated the accessions into 3 distinct groups. This study shows that the SRAP technique is a reliable tool for differentiation of *C. radiatus* accessions and for determination of the genetic relationships among them.

1. Introduction

The genus *Cynodon* Richard which comprises 9 species and 10 varieties, originated from tropical or subtropical areas (Taliaferro, 1995), is geographically distributed, and is genetically diverse. *Cynodon radiatus,* genetically isolated from other *Cynodon* species, is the most morphologically distinct species of the genus (Harlan and de Wet, 1969).

In recent years, the use of molecular markers that show polymorphism at the DNA level has been shown to be a powerful tool for assessing genetic diversity. Numerous DNA markers have been used to estimate the genetic variation among *Cynodon* members (Zhang et al., 1999; Anderson et al., 2001; Roodt et al., 2002; Yerramsetty et al., 2005; Kang et al., 2008; Huang et al., 2010). Assefa et al. (1999) used DAF to assess the genetic relatedness among eight *Cynodon* taxa; they detected a few DAF polymorphisms among some *C. radiatus* accessions and found that genetic relatedness among the accessions was high and that *C. radiatus* was clearly separated from other species by numerous monomorphic bands.

These studies have proved the utility of DNA profiling in assessing the relatedness of *Cynodon* members, however, none has focused on assessing the variations within *C. radiatus* except Assefa et al. (1999).

Recently, a new marker system, sequence-related amplified polymorphism (SRAP) has been developed by Li and Quiros (2001), which aims at the preferable amplification of open reading frames (ORF). The SRAP is a simple and effective molecular marker technique. Due to its advantages, such as production of highly specific polymorphic fragments, easy manipulation, reliability, moderate throughput, and easy of sequence of selected bands, this approach has been successfully used for different purposes in several species, including *Brassica oleracea* (Li and Quiros, 2001), *Cucurbita pepo* (Ferriol et al., 2003), buffalograss [*Buchloe dactyloides* (Nutt.) Englem; Budak et al., 2004], elephant grass (*Pennisetum purpureum* Schumach; Xie et al., 2009), *Lactuca* sp. (van Treuren and van Hintum, 2009), and *Salvia miltiorrhiza* Bge (Song et al., 2010).

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Table 1
Geographical origins of 33 *C. radiatus* accessions investigated in the present study.

Accession	Origin	Longitude	Latitude	Elevation	Habitat
R01	Haikou, Hainan	109°23.7′	19°46.0′	13.7	Roadside
R02	Wanning, Hainan	110°28.0′	18°57.4′	31.2	Wasteland
R03	Chengmai, Hainan	110°00.1′	19°45.3′	94.3	Roadside
R04	Chengmai, Hainan	110°00.1′	19°45.3′	94.3	Roadside
R05	Lingao, Hainan	109°39.5′	19°55.1′	62.5	Wasteland
R06	Lingao, Hainan	109°31.3′	19°52.8′	0.7	Roadside
R07	Lingao, Hainan	109°31.3′	19°52.8′	0.7	Seaside
R08	Lingao, Hainan	109°38.5′	19°55.9′	159	Roadside
R09	Lingao, Hainan	109°44.7′	19°52.4′	40.6	Wasteland
R10	Danzhou, Hainan	109°23.7′	19°46.0′	13.7	Roadside
R11	Danzhou, Hainan	109°20.6′	19°36.6′	44.3	Roadside
R12	Danzhou, Hainan	110°28.1′	19°46.4′	14.2	Roadside
R13	Danzhou, Hainan	109°15.7′	19°51.2′	14.8	Wasteland
R14	Danzhou, Hainan	109°25.4′	19°38.8′	51.7	Roadside
R15	Baisha, Hainan	109°09.6′	19°34.9′	48	Roadside
R16	Baisha, Hainan	109°33.0′	19°06.4′	273.7	Roadside
R17	Changjiang, Hainan	108°57.0′	19°14.5′	54.2	Roadside
R18	Changjiang, Hainan	108°56.8′	19°22.3′	53.3	Sandy
R19	Changjiang, Hainan	109°02.5′	19°18.8′	140	Flat
R20	Dongfang, Hainan	108°50.9′	19°08.9′	58.3	Farmland
R21	Dongfang, Hainan	108°49.2′	19°06.3′	55.5	Ridge
R22	Dongfang, Hainan	108°55.7′	19°05.3′	139.8	Roadside
R23	Dongfang, Hainan	109°03.3′	18°50.0′	138.6	Sandy
R24	Ledong, Hainan	109°17.0′	18°34.5′	220.6	Wasteland
R25	Baoting, Hainan	109°41.3′	18°39.7′	322	Roadside
R26	Wuzhishan, Hainan	109°38.9′	18°47.6′	488.6	Beach land
R27	Linshui, Hainan	109°50.5′	18°23.0′	19	Seaside
R28	Linshui, Hainan	110°04.0′	18°24.5′	5.5	Seaside
R29	Sanya, Hainan	109°36.5′	18°16.6′	39.6	Roadside
R30	Heshan, Guangdong	112°55.9′	22°44.3′	29.2	Hillside
R31	Enping, Guangdong	112°17.9′	22°09.5′	37.4	Wasteland
R32	Jinghong, Yunnan	100°56.0′	21°51.5′	548.1	Roadside
R33	Hekou, Yunnan	103°33.6′	22°53.9′	162.2	Hillside

Although previous research has provided preliminary data regarding the genetic diversity of the genus *Cynodon*, none has focused on assessing the variations within *C. radiatus* accessions. This study was conducted to assess the genetic relatedness and diversity of 33 *C. radiatus* accessions collected from different regions of China based on SRAP markers.

2. Materials and methods

2.1. Plant materials

In the period 2006–2007, 33 native *C. radiatus* accessions were collected from the regions spanning the Southwest to the South of China (Table 1), of which 29 were collected from the Southernmost regions (Hainan island) of China between 18° and 19° N latitudes and between 108° and 110° E longitudes. Each accession was grown in 3 20-cm-diameter pots in a greenhouse under uniform conditions at the Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences (Hainan Island).

2.2. DNA extraction

Total genomic DNA was isolated following the modified hexadecyltrimethylammonium bromide (CTAB) DNA extraction procedure (Doyle and Doyle, 1990). The quality and quantity of genomic DNA were determined visually from the band

Table 2
The forward and reverse SRAP primers used in the present study.

Name	Forward primer(3′–5′)	Name	Reverse primer(3′–5′)
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me5	TGAGTCCAAACCGGAAG	Em3	GACTGCGTACGAATTGAC
Me7	TGAGTCCAAACCGGTAG	Em4	GACTGCGTACGAATTTGA
Me8	TGAGTCCAAACCGGTAA	Em5	GACTGCGTACGAATTAAC
Me9	TGAGTCCAAACCGGTCC	Em6	GACTGCGTACGAATTGCA
Me10	TGAGTCCAAACCGGTGC	Em7	GACTGCGTACGAATTCGA
Me11	TGAGTCCAAACCGGT	Em8	GACTGCGTACGAATTCAA