ISSN 1996-0700

Asian Journal of Biotechnology

Knowledgia
SCIENTIFIC
A Place to Publish Outstanding Research

http://knowledgiascientific.com

Asian Journal of Biotechnology 3 (3): 262-268, 2011
ISSN 1996-0700 / DOI: 10.3923/ajbkr.2011.262.268
© 2011 Knowledgia Review, Malaysia

Genotypic Diversity Among Wild Populations of Buffelgrass (*Cenchrus ciliaris* L. Link) in Al-Qassim Region

A. Al-Soqeer
Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Al-Qassim University, Saudi Arabia

ABSTRACT

Buffelgrass (*Cenchrus ciliaris* L. Link) is an African agamospermous grass that has been widely introduced into arid regions of the world to improve rangelands for cattle production and as a result, it has invaded adjacent habitats. *Cenchrus ciliaris* is apomictic, a condition that is normally associated with low genetic variation within populations, but even moderate levels of genetic variation among populations could account for differences in invasiveness. To assess genotypic variation in populations of *C. ciliaris* from Al-Qassim region, we used Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat markers (ISSRs) to examine genetic variation in populations from the five areas. Analysis of the 10 selected RAPD primers among the five buffelgrass populations included in this study generated 75 bands, 11 of which were polymorphic (14.6%). In ISSR analysis, a total of 34 bands were observed, with 5.6 bands per primer. The genetic similarity coefficients among all buffelgrass ranged from 0.89 to 0.97. The dendogram constructed with UPGMA analysis revealed two clusters. Cluster I contained Buraydah-1 (C1) and Buraydah-2 (C2) and Al-Shamasia (C5) populations. Cluster II contained Al-Molida (C3) and AL-Qaraa (C4) populations. The results showed low differences in genotypic diversity among buffelgrass populations in Al-Qassim region.

Key words: *Cenchrus ciliaris*, genetic diversity, buffelgrass populations, molecular markers

INTRODUCTION

Buffelgrass (*Cenchrus ciliaris* (L.) Link syn. *Pennisetum ciliare* L.) is an apomictic, warm-season, perennial forage grass grown throughout the arid and semi-arid tropics. It is an aposporous apomict, in which most genotypes reproduce by either obligate or facultative (sexual and apomictic) apomixis. Most buffelgrass cultivars have resulted from selecting and increasing superior apomicitic ecotypes. These natural accessions contain both desirable and undesirable characteristics and are limited by adaptations to the local in which they were selected (Bashaw and Funk, 1987). Rare sexual genotypes have facilitated the production of buffelgrass hybrids (Bashaw and Funk, 1987).

Buffelgrass has several advantages as a model for genetic mapping studies of polyploidy, perennial forage grasses. It is protogynous, which allows hybridizations to be made without hand emasculations of florets. Obligate apomictic buffelgrass genotypes can be maintained long-term as seeds instead of vegetative plants. The high levels of heterozygosity in buffelgrass result in adequate levels of DNA polymorphism for genetic mapping in hybrids between most genotypes. Genetic mapping of hybrid buffelgrass populations was first accomplished by Gustine *et al.* (1997) and the genome map (Jessup *et al.*, 2003) subsequently placed molecular markers across a majority of the buffelgrass genome, distinguished between genomic regions with disomic and tetrasomic inheritance and included markers capable of screening genotypes for apomictic reproduction.

Therefore, information about genetic diversity within and between genotypes of buffelgrass is an important factor to study and to classify genotypes into different heterotric groups. The relatively narrow range of morphological traits and limited number of polymorphic isoenzyme systems are not adequate to discriminate all the genotypes of any given species. Furthermore, many phenotypic traits are developmentally regulated or influenced by the genotype by environment interaction. However, the advent of DNA-based genetic markers, such as Restriction Fragment Length Polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) has become more efficient, reliable and useful (Caetano-Anolles *et al*., 1991; Nybom, 1994). Bulk segregant analysis coupled with RAPD has been successfully employed earlier to find markers liked to apomixes in buffelgrass (Gustine *et al*., 1997). Also, Inter Simple Sequence Repeat (ISSR) are a new type of DNA markers which involve the use of microsatellite sequences directly in the Polymerase Chain Reaction (PCR) for DNA amplification (Sanchez de la Hoz *et al*., 1996). ISSRs have been used successfully in genome mapping a variety of crop species including maize, rice, barley, wheat and turfgrass (Wolff and Morgan-Richards, 1998; Saghai-Maroof *et al*., 1994; Ben El Maati *et al*., 2004; Al-Humaid *et al*., 2004). ISSR have been proposed as a new source of genetic markers which overcomes the technical limitations of Restriction Fragment Length Polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD). The objective of this study was to investigate genetic diversity of buffelgrass grown in five different locations in Al-Qassim region using RAPD and ISSR markers.

MATERIALS AND METHODS

Population sampling: Five populations of *C. ciliaris* located in Al-Qassim region (The Central of Saudi Arabia), were sampled during September of 2007. Sampling tried to cover the current distribution of buffelgrass (wild populations) in Al-Qassim region (Buraydah-1, Buraydah-2, Al-Molida, AL-Qaraa and Al-Shamasia). Leaf tissue was collected from 50 plants, using 10 individuals per population. To increase the likelihood of picking leaves from different clones, leaf samples were taken from individuals at least 10 m apart. Samples were immediately stored in liquid nitrogen and once in the lab, they were transferred to an ultra cold freezer (-80°C). In this study, populations were defined as discrete groups of plants covering an area of at least 1 ha.

DNA extraction: Bulk leaf samples from 10 plants of each of buffelgrass population were used. The bulk sample of leaves was first ground into fine powder with liquid nitrogen. Genomic DNA was extracted adding 260 μL of CTAB extraction buffer and 975 μL of STE buffer. The mixture was then agitated and subsequently centrifuged at 12,000 rpm for 8 min. The supernatant was eliminated and the precipitated was resuspended in 250 μL of CTAB buffer and 750 μL of STE buffer. Samples were centrifuged at 12,000 rpm for 8 min, the supernatant was eliminated and the precipitated was resuspended in 600 μL of 2× CTAB buffer. The samples were treated with ribonuclease 7000 μ mL^{-1} (4 μL $sample^{-1}$) at 37°C for 20 min to digest RNA. After this treatment, 25 μL of proteinase-K (20 mg mL^{-1}) was added and incubated at 65°C for 30 min. The samples were placed on ice for 15 min. DNA was isolated using chloroform: octanol 24:1 separation (adding 600 μL $sample^{-1}$ and homogenising), centrifuged at 9000 rpm for 12 min and the supernatant was transferred to a fresh centrifuge tube. DNA was precipitated with 600 μL of cold isopropanol (-20°C) and maintained for 12 h at -20°C. After that time, samples were centrifuged at 12,500 rpm for 7 min and the supernatant was eliminated. The pellet was washed agitating gently for 5 min with 70% ethanol (-20°C). The pellet was dried, resuspended in 100 μL of PCR grade water and stored at 4°C. DNA concentration of each extract was quantified using an Eppendorf biophotometer.

RAPD analysis: RAPD analysis was run in a thermal cycler (Thermolyne Amplitron).The reaction mixture (25 μL) will be contained 1x PCR buffer with Mg Cl_2 (50 mM K Cl, 10 mM Tris-HCl (pH 9.0), 2 mM Mg Cl_2 and Triton X-100), 200 μM each of dATP, dCTP, dGTP and dTTP, 30 ng template DNA, 50 pM of oligonucleotide primer (Operon Tech., CA, USA) and 1.5 unit of Taq polymerase. The mixtures was subjected to the following conditions: hold at 94°C for 2 min, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final hold at 72°C for 5 min. PCR products will be visualized along with a DNA marker on 2% agarose gel with 1X TAE buffer and was detected by staining with the ethidium bromide.

ISSR assay: The ISSR-PCR method was carried out according to Nagaoka and Ogihara (1997). Amplification was carried out in 25 μL reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM Mg Cl_2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 50 pmol of ISSR primers and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron).

Data analysis: RAPD and ISSR data were scored for presence (1), absence (0) or as a missing observation (9) and each band was regarded as a locus. Two matrices, one for each marker, were generated. The Genetic Similarities (GS) were calculated according to Nei and Li (1979):

$$GS = 2\ Nij/(Ni+Nj)$$

where, Nij is the number of bands present in both genotypes i and j, Ni is the number of bands present in genotypes i and Nj is the number of bands present in genotype j. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes, was constructed using the unweighted pairgroup method with arithmetic average (UPGMA) though the software NTSYS-pc version 2.11 (Rohlf, 2000).

RESULTS

Identification and evaluation of RAPD and ISSR markers for diversity estimates: In RAPD analysis, fifteen primers of arbitrary nucleotide sequence were used to amplify DNA segments from five populations of buffelgrass. The number of amplification bands per primer varied between 0 and 10. Ten primers out of 15 were selected for further analysis based on the intensity, size and number of amplified products (Table 1). Analysis of the 10 selected primers among the five

Table 1: RAPD primers with the number of amplified products, polymorphic fragments among buffelgrass populations

Primers	Sequence 5` to 3`	Amplified products	Polymorphic fragments
OP-A01	CAGGCCCTTC	7	1
OP-A02	TGCCGAGCTG	8	0
OP-A03	AGTCAGCCAC	6	1
OP-A08	GTGACGTAGG	6	0
OP-A10	GTGATCGCAG	9	2
OP-A13	CAGCACCCAC	8	1
OP-A16	AGCCAGCGAA	6	2
OP-A17	GACCGCTTGT	7	0
OP-A18	AGGTGACCGT	10	3
OP-A20	GTTGCGATCC	8	1

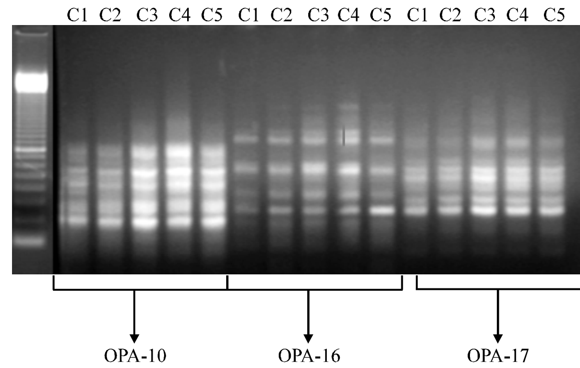

Fig. 1: Polymorphism revealed using RAPD primers (OPA-10, OPA-16 and OPA-17) to amplify genomic DNA purified from buffelgrass populations (left to right) Buraydah-1(C1) and Buraydah-2 (C2) Al-Molida (C3), AL-Qaraa (C4) and Al-Shamasia (C5). The first lane is 1 kbp ladder DNA marker

Table 2: ISSR primers with the number of amplified products and polymorphic fragments

Primers	Sequence 5` to 3`	Amplified products	Polymorphic fragments
P02	(ATCG)4	0	0
D12	(GA)6CG	5	1
D14	(CAC)3GC	5	0
D24	(CA)6CG	6	3
HB 13	(GAG)3GC	7	1
HB 14	(CTC)3GC	11	1

buffelgrass populations included in this study generated 75 bands, 11 of which were polymorphic (14.6%). Examples of polymorphism are shown in Fig. 1. Polymorphism between populations can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming sites too distant to support amplification; and insertions or deletions that change the size of the amplified product (Williams *et al.*, 1990).

In ISSR analysis, a total of 34 bands were observed, with 5.6 bands per primer (Table 2). Six out of 34 bands (17.6%) were polymorphic. The number of amplification bands per primer varied between 0 and 11. The trinucleotide repeats (CTC)n primer had more bands than (GAG)n and (CAC)n primers and dinucleotide repeats (CA)n and (GA)n primers (Table 2). On the other hand, tetranucleotide repeats did not amplify with DNA of buffelgrass populations. This might indicate that di- and trinucleotide-based ISSR-PCR markers could provide potential marker in buffelgrass genome.

Genetic diversity: The pair-wise genetic distance estimates of the buffelgrass populations in this study were analyzed and are given in Table 3. The genetic similarity coefficients among all buffelgrass ranged from 0.89 to 0.97. Maximum similarity was observed between: Buraydah-1 and Buraydah-2 populations (0.95) and Buraydah-2 and Al-Shamasia populations (0.97). Buraydah-2 population was quite distinct from AL-Qaraa population. Cluster analysis was conducted to generate a dendogram elucidating for relationships among buffelgrass populations. The dendogram constructed with UPGMA analysis revealed two clusters (Fig. 2). Cluster I contained Buraydah-1

Table 3: Simple matching coefficients of similarity determined from analysis using RAPD and ISSR loci

Buffelgrass populations	C-1	C-2	C-3	C-4	C-5
Buraydah-1 (C-1)	1				
Buraydah-2 (C-2)	0.95	1			
Al-Molida (C-3)	0.91	0.92	1		
AL-Qaraa (C-4)	0.89	0.9	0.93	1	
Al-Shamasia (C-5)	0.92	0.97	0.92	0.91	1

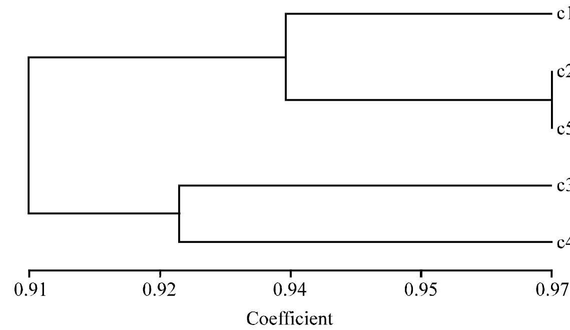

Fig. 2: Dendrogram constructed from similarity coefficients and showing the clustering of buffelgrass populations

(C1) and Buraydah-2 (C2) and Al-Shamasia (C5) populations. Cluster II contained Al-Molida (C3) and AL-Qaraa (C4) populations.

DISCUSSION

The molecular data from this study indicate that buffelgrass is generally genetically invariant in all five populations studied. The low level of genetic variation is perhaps not surprising given the presumed apomictic breeding system of buffelgrass (Poulin *et al.*, 2005). Most apomictic species, however, have some sexual capability and this breeding system is usually associated with populations of coexisting, genetically variable clones (Richards, 1997). The molecular data presented here indicate that genetic factors are unlikely to be driving the variation in buffelgrass populations in the central of Saudi Arabia. Populations of buffelgrass are most likely completely apomictic and somatic mutations are probably rare, although rare sexual recombination or somatic mutation cannot be ruled out (Poulin *et al.*, 2005). Also, Gutierrez-Ozuna *et al.* (2009) found that the levels and distribution of genotypic and genetic diversity among pasture and roadside populations fall within the range.

Several factors may affect levels of genotypic diversity among populations of buffelgrass (Gutierrez-Ozuna *et al.*, 2009). Founder events during colonization and the frequency of sexual reproduction (Hignight *et al.*, 1991) may affect levels of variation within populations while patterns of migration may affect levels of differentiation. Gutierrez-Ozuna *et al.* (2009) estimated the genetic diversity among Buffelgrass (*C. ciliaris*) populations. They found that 27 reproducible bands generated with three primers, 16 (59.26%) were polymorphic. While, in the current study reported that 17.6% of bands were polymorphic. Similar to my results were reported by Poulin *et al.* (2005) using Inter-Simple Sequence Repeat markers (ISSRs) to examine genetic variation in *Pennisetum setaceum* populations from the three areas (Arizona, California and Hawaii). They found that

screening of 16 primers revealed no genetic variation within any population or between any geographical areas. The current study reported that the trinucleotide repeats (CTC)n primer had more bands than (GAG)n and (CAC)n primers and dinucleotide repeats (CA)n and (GA)n primers. However, the repeats $(GA)_n$ and $(CA)_n$ were the most abundant in rice (Nagaraju *et al.*, 2002) and date palm (Trifi *et al.*, 2000) genomes.

REFERENCES

Al-Humaid, A., M.I. Motawe, M.Y. Abdalla and F. Mana, 2004. Detection of genetic variation and Fusarium resistance in turfgrass genotypes using PCR-based markers (ISSR and SCAR). Int. J. Food Agric. Environ., 207: 225-229.

Bashaw, E.C. and C.R. Funk, 1987. Apomictic Grasses. In: Principles of Cultivar Development: Crop Species, Fehr, W.R. (Eds.). Vol. 2. Macmillan Publ. Co., New York, pp: 40-82.

Ben El Maati, F., M. Jlibene and M. Moummi, 2004. Study of the polymorphism of common wheat using ISSR markers. J. Food Agric. Environ., 3: 121-125.

Caetano-Anolles, G., B.J. Bassam and P.M. Bresshoff, 1991. DNA amplification fingerprinting: A strategy for genome analysis. Plant Mol. Biol. Rep., 9: 294-307.

Gustine, D.L., R.T. Sherwood and D.R. Huff, 1997. Apospory-linked molecular markers in buffelgrass. Crop Sci., 37: 947-951.

Gutierrez-Ozuna, R., L.E. Eguiarte and F. Molina-Freaner, 2009. Genotypic diversity among pasture and roadside populations of the invasive buffelgrass (*Pennisetum ciliare* L. Link) in north-western Mexico. J. Arid Environ., 73: 26-32.

Hignight, K.W., E.C. Bashaw and M.A. Hussey, 1991. Cytological and morphological diversity of native apomictic buffelgrass, *Pennisetum ciliare* (L.) link. Botanical. Gazette, 152: 214-218.

Jessup, R.W., B.L. Burson, Y.W. Wang, C. Chang and Z. Li *et al.*, 2003. Segmental allotetraploidy and allelic interactions in buffelgrass (*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.) as revealed by genome mapping. Genome, 46: 304-313.

Nagaoka, T. and Y. Ogihara, 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Applied Genet., 94: 597-602.

Nagaraju, J., M. Kathirvel, R.R. Kumar, E.A. Siddiq and S.E. Hasnain, 2002. Genetic analysis of traditional and evolved Basmati and non-basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci., 99: 5836-5841.

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA., 76: 5269-5273.

Nybom, H., 1994. DNA fingerprinting-A useful tool in fruit breeding. Euphytica, 77: 59-64.

Poulin, J., S.G. Weller and A.K. Sakai, 2005. Genetic diversity does not affect the invasiveness of fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii. Diversity Distribut., 11: 241-247.

Richards, A.J., 1997. Plant Breeding Systems. Chapman and Hall, London, New York.

Rohlf, F.J., 2000. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System-User's Manual. Version 2.1, Applied Biostatistics Inc., New York.

Saghai-Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang and R.W. Allard, 1994. Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations and population dynamics. Proc. Natl. Acad. Sci. USA., 91: 5466-5470.

Sanchez de la Hoz, M.P., J.A. Davila, Y. Loarce and E. Ferrer, 1996. Simple sequence repeat primers used in polymerase chain reaction amplifications to study genetic diversity in barley. Genome, 39: 112-117.

Trifi, M., A. Rhouma and M. Marrakchi, 2000. Phylogenetic relationships in Tunisian date palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. Agronomie, 20: 665-671.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Wolff, K. and M. Morgan-Richards, 1998. PCR markers distinguish plantago major subspecies. Theor. Applied Genet., 96: 282-286.