GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic diversity among different physiological traits of *Sorghum bicolor* cultivars of subtropical origin

S.O. Bafeel

Department of Biology, College of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

Corresponding author: S.O. Bafeel
E-mail: bafeelso@gmail.com

Genet. Mol. Res. 14 (3): 9974-9984 (2015)
Received January 29, 2015
Accepted May 16, 2015
Published August 21, 2015
DOI http://dx.doi.org/10.4238/2015.August.21.3

ABSTRACT. The genetic diversity of Saudi locally growing sorghum (*Sorghum bicolor*) cultivars has not been thoroughly characterized. To understand the genomic patterns of diversification in Saudi sorghum cultivars (N = 7), random amplified polymorphic DNA (RAPD) was used as a rapid, inexpensive method for providing information regarding genomic variability below the species level. Six commercially available primers were initially used to select a single primer based on availability, universality, and its use with standard polymerase chain reaction (PCR) conditions. PCR-amplified molecular markers were reproducibly detected in Saudi cultivars. The single primer 2 produced clear bands and revealed variability among the cultivars. Seven tested cultivars were categorized into 2 major groups, indicating 2 genomogroups for the Saudi-cultivars. Five cultivars (S2, S3, S4, S5, and S6) showed identical banding patterns and were grouped in the same clade, although their panicles varied in size, shape, and color. Two cultivars (S1 and S7) showed different banding patterns. In this study, a single primer (P2) was used to demonstrate the effectiveness of genotype detection among sorghum cultivars. This is the first report describing genetic variation

among *S. bicolor* cultivars in Saudi Arabia. The commercial primer (P2) and PCR reaction mixture used in this study are readily available and can be used in sorghum improvement programs.

Key words: Genetic diversity; Random amplified polymorphic DNA; Cultivars; *Sorghum bicolor*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench; Poaceae, Grass) originated in Sub-Saharan Africa (Balota et al., 2008) and is the 5th most important cereal grain crop and staple food for millions of poor people in the semi-arid tropical regions of Africa and Asia (Haussmann et al., 2002). *Sorghum bicolor* ssp. *bicolo*r is the most important cultivated grain race. Drought tolerance is one of sorghum's most important traits, enabling the growth of sorghum in harsh environments (Pammi et al., 1994) such as the subtropical desert region of Saudi Arabia. Sorghum is recognized as a highly productive, drought-tolerant, C_4 cereal that provides humans with food, feed, fuel, fiber, and energy (Kimber et al., 2013). Sorghum evolved across a wide range of environments in Africa, exhibits a wide range of phenotypic diversity, and has been collected and conserved over the past 50 years. Numerous international and national collections of sorghum cultivars exist, and the major collections include in excess of 40,000 accessions. However, much of the diversity of sorghum remains uncharacterized (Kimber et al., 2013). Although sorghum is drought-tolerant, it requires approximately 30% less nitrogen fertilizer than corn to produce an equal amount of ethanol per acre under non-irrigated conditions. Therefore, sorghum may be used as a bioenergy source (Damasceno et al., 2014).

Genomic analysis of diverse populations can reveal the genetic basis of complex traits, including agroclimatic traits of crop species (Morris et al., 2013). Over the past decade, tremendous progress has been made in building the molecular and genomic foundation required to increase the understanding of sorghum genomic diversity, which can be used for crop improvement. Diverse, trait-specific promising accessions may be used in breeding programs for developing improved sorghum cultivars with a broad genetic base. Panicle width, weight, length, and grain yield per panicle are typically optimized for agricultural purposes. Among available polymerase chain reaction (PCR)-based techniques, random amplified polymorphic DNA (RAPD) is convenient and does not require prior knowledge of the DNA sequence to be amplified (Weder, 2002). Thus, the use of RAPD for identifying molecular markers for taxonomic and systematic analyses of plants (Bartish et al., 2000) as well as in plant breeding and the study of genetic relationships has increased (Ranade et al., 2001). The genetic diversity of sorghum assessed using methods such as RAPD has been reported (Pammi et al., 1994; Arya et al., 2006; Prakash et al., 2006; Iqbal et. al., 2010). Environmental effects on the production of some local grain sorghum cultivars grown in the central region of Saudi Arabia has been described previously (Refay, 1994); however, the genetic diversity of local Saudi sorghum cultivars has not been examined. Although the geographic region is similar (subtropical), the plants are exposed to different conditions. Whether these cultivars should be classified together remains unknown. In this study, we examined the genetic diversity of Saudi locally grown sorghum cultivars. In previous studies, primers and specific laboratory-based PCR conditions were developed, but difficult to implement. In this study, Saudi locally grown sorghum cultivars were collected. Commercially available standard RAPD primers and

PCR conditions were used to select a single effective primer. The genetic diversity of these local cultivars was determined using the selected single primer and standard conditions. The aim of this study was to determine the diversity of locally grown sorghum cultivars in an easy, cost-effective, rapid, and reproducible manner, which may help local farmers and breeding programs for crop improvement.

MATERIAL AND METHODS

Plant material and DNA extraction

Specimens (panicles of different cultivars of *S. bicolor*; Figure 1) were collected from sorghum-growing areas in Saudi Arabia (Jizan; latitude: 16°53'21"N; longitude: 42°33'03"E). Approximately 100 mg sorghum seeds were crushed in liquid nitrogen using a sterile mortar and pestle to obtain a fine powder. DNA was extracted from approximately 50 mg of fine powder following the protocol described for purifying total DNA from plant tissue (Bench Protocol: DNeasy Plant Mini) using the DNeasy plant mini kit (Qiagen, Hilden, Germany). Quality of the extracted DNA was determined using gel electrophoresis (HE 99X Max Submarine Unit Complete, GE Healthcare Life Sciences, Little Chalfont, UK) and a spectrophotometer (GeneQuant 1300 Classic, GE Healthcare Life Sciences). Isolated plant genomic DNA was preserved at -80°C.

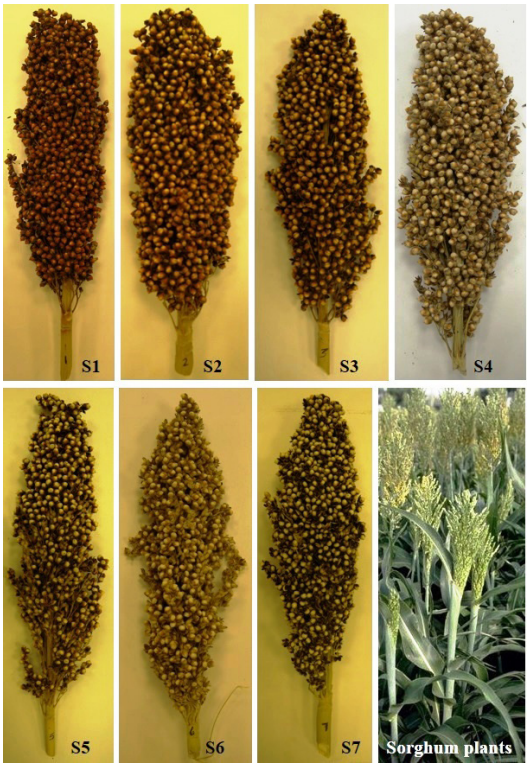

Figure 1. Panicles of the cultivars (S1-S7) of Saudi Arabian *Sorghum bicolor* (commonly known as Sorghum/Milo/Dohra Rafi'ah/Jowari). Sorghum plants were adapted from Schwartz (2010).

RAPD-PCR analysis

RAPD-PCR analyses were conducted using Illustra Ready-To-Go RAPD Analysis Kit (GE Healthcare Life Sciences; Code: 27-9502-01). The 25-µL PCR mixture contained a single Ready-To-Go RAPD analysis bead, 5 µL 25 pM single RAPD primer, 100 ng template DNA, and sterile distilled water. The bead contained thermo-stable polymerase (AmpliTaq™ DNA polymerase and Stoffel fragment), 0.4 mM of each dNTP, 2.5 µg bovine serum albumin and buffer containing 3 mM $MgCl_2$, 30 mM KCl, and 10 mM Tris, pH 8.3. Six commercially available primers (GE Healthcare Life Sciences) were initially used for the selection of a primer that would show variability among cultivars. Each primer was a 10-mer of arbitrary sequence: RAPD analysis primer 1 (P1) [5'-d(GGTGCGGGAA)-3']; 2 (P2) [5'-d(GTTTCGCTCC)-3']; 3 (P3) [5'-d(GTAGACCCGT)-3']; 4 (P4) [5'-d(AAGAGCCCGT)-3']; 5 (P5) [5'-d(AACGCG CAAC)-3'], and 6 (P6) [5'-d(CCCGTCAGCA)-3']. Primer 2 was selected as a workable primer based on preliminary results. PCR was performed using a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR conditions included 1 cycle of 95°C for 5 min, followed by 45 cycles of 95°C for 1 min, 36°C for 1 min, and 72°C for 2 min. A long (20 x 14 cm) 1% agarose gel using 1X TAE buffer containing 0.5 µg/mL ethidium bromide was used for the electrophoresis of the products. Gel images were obtained using Proxima C16 Phi+ (Isogen Life Science, Oudenrijn, Netherlands) UV transilluminator, and Opticom (version 3.2.5; OptiGo) imaging system.

Gel image analysis of the RAPD bands of the *Sorghum* cultivars was performed using 100-bp ladder (GE Healthcare Life Sciences; Code: 27400701) and the Total Lab TL100 1D software (version 2008.01). Amplified fragments of RAPD-PCR were scored as present (1) or absent (0). Only clear and major bands observed in duplicate tests were scored (Collard and Mackill, 2009). FreeTree (Version 0.9.1.50; Pavlíček et al., 1999) was used to determine genetic distances from Nei and Li's coefficient of similarity (Nei and Li, 1979). Dendrograms were constructed using the unweighted pair group method with arithmetic mean and bootstrap analysis with 1000 replicates.

RESULTS AND DISCUSSION

The panicles and grains of sorghum cultivars vary widely in shape and size, which can be used for racial classification. Head and grain characters of sorghum are useful to studying inheritance to enhance breeding and increase yield (Morakinyo, 1993). Sorghum panicles are made up of perfect flowers and are self-pollinating; however, outcrossing can sometimes be as high as 70% in some races in particular environments (Kimber et al., 2013). For example, the compact panicle and predominantly white seeds of the sorghum race duhra indicate an adaptation to low-rainfall environments with a low risk of grain mold (Mann et al., 1983). Panicles of *S. bicolor* cultivars (S1-S7) (Figure 1) were collected from Jizan (or Jazan), KSA, a province in the southwestern region of Saudi Arabia near the Red Sea (Figure 2). This area is known for the production of sorghum and millet, as these crops are drought- and heat-tolerant. Sorghum also grows very well in poor soils and has a short growing season, allowing for continuous growth; the Jizan, sorghum cultivation area in KSA is similar to the environment of the subtropical desert (El-Demerdash et al., 1994). The mean temperature is 29.6°C (85.3°F) and total annual precipitation averages 37.5 mm (1.5 in) (http://www.jizan.climatemps.com). Phenotypic variation among panicles of studied sorghum cultivars (S1-S7) are illustrated in

Figure 1. The cultivars had panicles consisting of deep to light brown and white seeds. Cultivars S1, S2, and S3 had brownish seeds. Cultivars S4 and S6 had more whitish and a few brownish seeds. Cultivars S5 and S7 had combinations of white and brownish seeds. A previous experiment involving the crossing of brown and white grain colors of sorghum varieties (F1; brown, BBYY/BbYy; white, bbyy) showed that gene Y was necessary for the expression of grain color in the F2 generation. An interaction between genes B and Y produced a brown grain color (B-Y-). The absence of a dominant Y allele resulted in white grain (B-yy), while yellow was represented by bbY- (Morakinyo, 1993). Physiological traits contributing to yield under specified environmental conditions would benefit genotypic selection for grain sorghum (Soltani et al., 2001). Diversity related to the plant's photoperiod (Clerget et al., 2004; Kouressy et al., 2008) and morpho-physiological characteristics in sorghum genotypes under various conditions have been reported previously (Vinodhana and Ganesamurthy, 2010; AghaAlikhani et al., 2012). To test genetic variation in commercially available Saudi cultivars, 6 RAPD standard primers (GE Healthcare) were used. These primers were chosen based on their easy availability and universality of the amplification of various organisms (animal, bacteria, fungi, and plants) (GE Healthcare, 2007). Among the 6 tested commercial primers (P1-P6), all primers successfully amplified the DNA (Figure 3). Based on the bands observed, the 6 primers could be grouped into 2 major categories. This grouping was supported by the 100% bootstrap value (Figure 4). P1, P5, and P6 belonged to group 1, while P2, P3, and P4 belonged to group 2. P1 and P5 showed similar banding patterns. However, P2 produced 3 clear bands and appeared to provide more variability than the other primers (P1, P3-P6), producing 1-2 bands. Thus, P2 was selected to test genetic variation among the 7 Saudi sorghum cultivars to achieve rapid and cost effective evaluation of genetic variations. The RAPD banding profile (duplicate data) of the examined 7 cultivars of *S. bicolor* for the selected primer P2 is shown in Figure 5. Thirty-one bands from the 7 cultivars were amplified using PCR. Of the 31 bands scored, 2 bands (1641 and 1243 bp) could be used to differentiate the selected cultivars. Band size ranged from 711-1717 bp. All 7 cultivars (S1-S7) produced 4 common bands (1717, 1516, 1143, and 711 bp) (Table 1; Figure 5). The number of amplified products produced per primer varied from 4-6, with an average of 4.4 bands per primer. Average mean pairwise similarities of the 7 cultivars was 92.2% (80-100%; Table 2) indicating a low level of genetic variability. This may be because the cultivars were isolated from the same geographic area. The results were in agreement with those of a previous study in which a low level of variation within 83 accessions based on an allozyme was observed because sorghum is a self-pollinating species. Most variation present in sorghum is attributable to differences in the geographic origin of the accessions rather than to racial differences (Morden et al., 1989).

The 7 tested cultivars were categorized into 2 major groups (Figure 6; 100% bootstrap value), indicating 2 genomogroups of Saudi locally grown cultivars. The results of this study agreed with those of a previous study that also defined 2 major RAPD groups for 29 *S. bicolor* cultivars (Iqbal et al., 2010). Five cultivars (S2, S3, S4, S5, and S6) showed an identical banding pattern and were grouped in the same clade, although their panicles varied in size, shape, and color (Figures 1 and 6). They shared 100% pairwise similarity in banding patterns (Table 2). Most (71%) Saudi sorghum cultivars belonged to this group, indicating that phenotypic variations were not the result of genetic variation, but may be related to the interaction between the environment and physiological factors (Romero et al., 2009). Cultivars S1 and S7 showed different banding patterns compared to the 5 cultivars and were grouped in a separate clade (Figure 6). However, S1 and S7 showed some differences. S1 produced a clear band

(1641 bp), which was not observed in S7 (Table 1; Figure 5). Panicles of these 2 cultivars (S1 and S7) were different colors; however, they appeared to be of the same origin.

Figure 2. Location of the sample collection area (Jizan/Jazan, KSA; latitude: 16°53'21"N, longitude: 42°33'03"E). Adapted from http://www.mapquest.com.

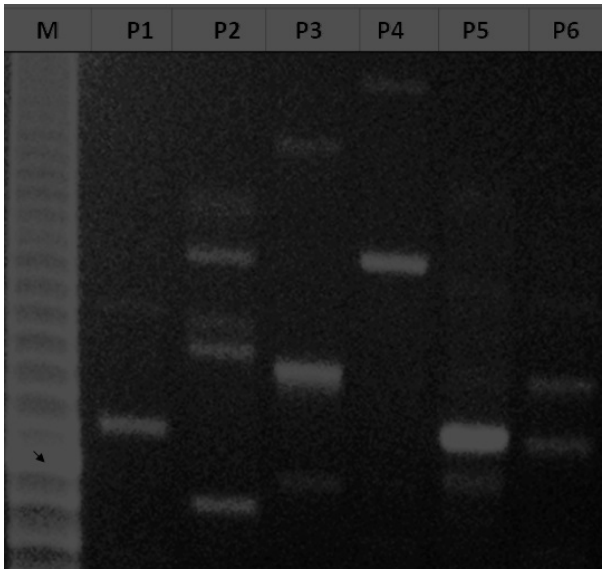

Figure 3. RAPD-PCR product profiles of different primers (P1-P6). *Lane M* = 100-bp molecular weight marker; Arrow indicates the 800-bp position. Sorghum-cultivar 2 (S2) was used to evaluate primer performance.

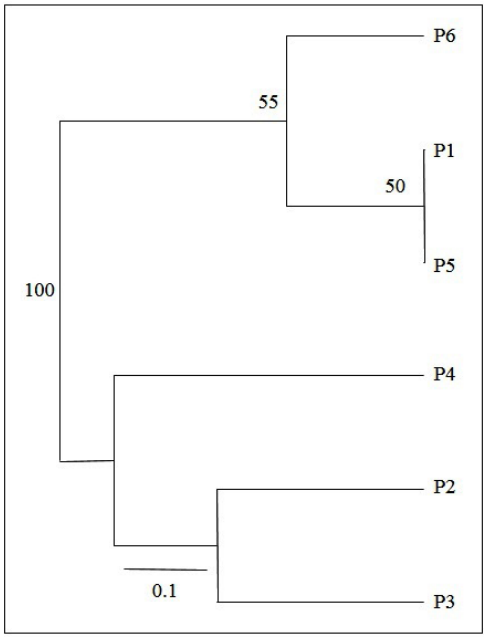

Figure 4. UPGMA dendrogram based on 6 RAPD primers (P1-P6). Bootstrap values (expressed as percentages of 1000 replications; >50% are shown at branch points). Scale bar represents 0.1 unit of Nei and Li's distance.

Table 1. RAPD-PCR product sizes for different sorghum cultivars (S1-S7).

Band No.	Sorghum cultivars						
	S1	S2	S3	S4	S5	S6	S7
1	1717	1717	1717	1717	1717	1717	1717
2	1641	-	-	-	-	-	-
3	1516	1516	1516	1516	1516	1516	1516
4	1243	-	-	-	-	-	1243
5	1143	1143	1143	1143	1143	1143	1143
6	711	711	711	711	711	711	711

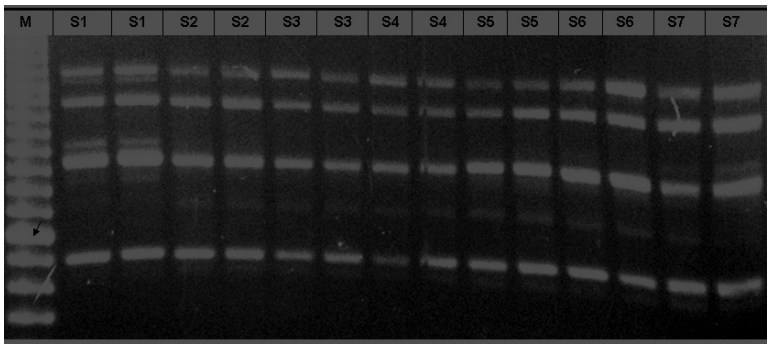

Figure 5. RAPD-PCR product profiles of different sorghum-cultivars (S1-S7). *Lane M* = 100-bp molecular weight marker; arrow indicates the 800-bp position.

Table 2. Pairwise comparisons of the Saudi *Sorghum* cultivars based on the proportion of shared bands (Nei and Li/Dice). Average, 92.2%.

Cultivar	S1	S2	S3	S4	S5	S6
	Similarity (%)					
S1						
S2	80					
S3	80	100				
S4	80	100	100			
S5	80	100	100	100		
S6	80	100	100	100	100	
S7	90.9	88.9	88.9	88.9	88.9	88.9

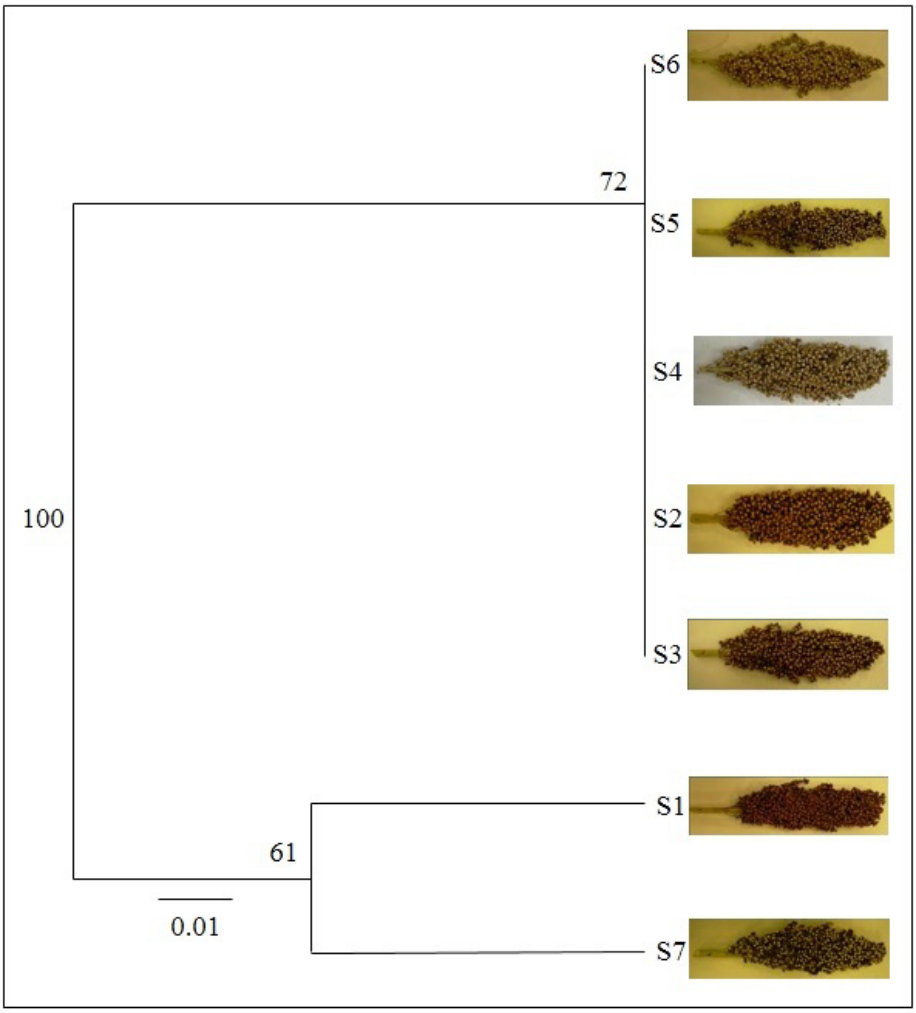

Figure 6. UPGMA dendrogram based on RAPD analysis of 7 sorghum cultivars (S1-S7). Selected RAPD-primer 2 was used. Bootstrap values (expressed as percentages of 1000 replications; >50% are shown at branch points). Scale bar represents 0.01 unit of Nei and Li's distance.

RAPD fingerprinting can be used to detect variety (Temiesak et al., 1993) and clonal variation in plant species (Wang et al., 2009). RAPD provides rapid results, is less time-consuming, inexpensive, and provides information regarding genomic variability below the species level (Williams et al., 1990). RAPD analysis reflects both coding and non-coding regions of the genome; therefore, RAPD primers can be used to distinguish taxa below the species level (Vanijajiva et al., 2005; Choo et al., 2009). Limitations of RAPD are related to reproducibility, designing appropriate primers, and amplifying RAPD-PCR products. The appropriate PCR conditions are critical for obtaining amplified products, particularly for plants (Jones et al., 1997). However, if the overall temperature profiles (especially the annealing temperature) in the PCR tubes are identical, RAPD fragments are likely to be reproducible (Penner et al., 1993; Skroch and Nienhuis, 1995). Primers and chemicals required for PCR and used in this study are readily commercially available. Therefore, the findings of this study may provide guidance for the identification and utilization of sorghum cultivars growing in an arid environment.

CONCLUSIONS

Standard conditions described by the ready to go RAPD analysis protocol resulted in reproducible detection of RAPDs in Saudi cultivars, even when using a single primer (P2; pairwise similarity level ranged from 80-100%). Reaction parameters, such as $MgC1_2$ concentration and primer annealing temperature, can significantly influence the yield and types of amplification products synthesized. In addition, the abundance of unexplained amplified DNAs increased when the number of PCR cycles exceeded 35 in a previous experiment (Pammi et al., 1994). Therefore, these parameters should remain constant in all reactions. Another study used 32 RAPD polymorphic primers for 29 sorghum germplasms (Iqbal et al., 2010). Eight RAPD primers for 32 genotypes of sorghum and conditions for reproducible amplification of RAPD markers in sorghum were also described previously (Pammi et al., 1994). This study used a single primer P2 and demonstrated the effectiveness of detecting the genotypes of sorghum cultivars. This is the first report describing the genetic variation among *S. bicolor* cultivars in Saudi Arabia. The commercial primer and PCR reaction mixture are readily available and can be used for selecting genomic variations based on parameters such as high yield, low fertilizer requirement, and environmental stress tolerance.

REFERENCES

AghaAlikhani M, Etemadi F and Ajirlo AF (2012). Physiological basis of yield difference in grain sorghum (*Sorghum bicolor* L. Moench) in a semi-arid environment. *ARPN J. Agric. Biol. Sci.* 7: 488-496.

Arya L, Sandhia GS, Singh SK, Rana MK, et al. (2006). Analysis of Indian sorghum [*Sorghum bicolor* (L) Moench] cultivars and lines using RAPD markers. *J. Plant. Biochem. Biotechnol*. 15: 97-101.

Balota M, Payne WA, Rooney WL and Rosenow DT (2008). Gas exchange and transpiration ratio in sorghum. *Crop. Sci.* 48: 2361-2371.

Bartish IV, Garkava LP, Rumpunen K and Nybom H (2000). Phylogenetic relationship and differentiation among and within population of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozyme. *Theor. Appl. Genet.* 101: 554-563.

Choo BK, Moon BC, Ji Y, Kim BB, et al. (2009). Development of SCAR markers for the discrimination of three species of medicinal plants, *Angelica decursiva* (*Peucedanum decursivum*)*, Peucedanum praeruptorum* and *Anthricus sylvestris*, based on the internal transcribed spacer (ITS) sequence and random amplified polymorphic DNA (RAPD). *Biol. Pharm. Bull*. 32: 24-30.

Clerget B, Dingkuhn M, Chantereau J, Hemberger J, et al. (2004). Does panicle initiation in tropical sorghum depend on day-to-day change in photoperiod? *Field Crops Res.* 88: 21-37.

Collard BCY and Mackill DJ (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene targeted markers in plants. *Plant Mol. Biol*. 27: 86-93.

Damasceno CM, Schaffert RE and Dweikat I (2014). Mining Genetic Diversity of Sorghum as a Bioenergy Feedstock. In: Plants and BioEnergy, Springer, New York, 81-106.

El-Demerdash MA, Hegazy AK and Zilay AM (1994). Distribution of the plant communities in Tihamah coastal plains of Jazan region, Saudi Arabia. *Vegetatio* 112: 141-151.

GE Healthcare (2007). Product Booklet. Ready to go RAPD Analysis Heads. GE Healthcare, UK.

Haussmann BI, Mahalakshmi V, Reddy BV, Seetharama N, et al. (2002). QTL mapping of stay-green in two sorghum recombinant inbreed populations. *Theor. Appl. Genet.* 106: 133-142.

Iqbal A, Sadia B, Khan AI, Awan FS, et al. (2010). Biodiversity in the sorghum (*Sorghum bicolor* L. Moench) germplasm of Pakistan. *Genet. Mol. Res.* 9: 756-764.

Jones CJ, Edwards KJ, Castaglione S, Winfield MO, et al. (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol. Breed.* 3: 381-390.

Kimber CT, Dahlberg JA and Kresovich S (2013). The Gene Pool of *Sorghum bicolor* and Its Improvement. In: Genomics of the Saccharinae, Plant Genetics and Genomics: 23 Crops and Models 11 (Paterson AH ed.). Springer Science+Business Media, New York, 23-40.

Kouressy M, Dingkuhn M, Vaksmann M and Heinemann AB (2008). Adaptation to diverse semi-arid environments of sorghum genotypes having different plant type and sensitivity to photoperiod. *Agric. For. Meteorol.* 148: 357-371.

Mann JA, Kimber CT and Miller FR (1983). The origin and early cultivation of sorghums in Africa. *Texas Agric. Exp. Stn. Bull.* 1454.

Morakinyo JA (1993). Inheritance of head and grain characters in *Sorghum bicolor* (L.) Moench. *Nig. J. Pure Appl. Sci.* 8: 245-252.

Morden CW, Doebley JF, and Schertz KF (1989). Allozyme variation in old world races of *Sorghum bicolor* (Poaceae). *Am. J. Bot* 76: 247-255.

Morris GP, Ramu P, Deshpande SP, Hash CT, et al. (2013). Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proc. Natl. Acad. Sci. U. S. A*. 110: 453-458.

Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A*. 76: 5269-5273.

Pammi S, Schertz K, Xu G, Hart G, et al. (1994). Random-amplified-polymorphic DNA markers in sorghum. *Theor. Appl. Genet*. 89: 80-88.

Pavlícek A, Hrdá S and Flegr J (1999). Free-Tree - freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia*. *Folia. Biol*. 45: 97-99.

Penner GA, Bush A, Wise R, Kim W, et al. (1993). Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. *Genome Res.* 2: 341-345.

Prakash SJ, Biji KR, Gomez SM, Murthy KG, et al. (2006). Genetic diversity analysis of sorghum (*Sorghum bicolor* L. Moench) accessions using RAPD markers. *Indian J. Crop Sci.* 1: 109-112.

Ranade SA, Farooqui N, Bhattacharya E and Verma A (2001). Gene tagging with random amplified polymorphic DNA (RAPD) marker for molecular breeding in plants. *Crit. Rev. Plant Sci.* 20: 251-275.

Refay YA (1994). Environmental effect on some local grain sorghum (*Sorghum bicolor*) cultivars production grown in central region of Saudi Arabia. *J. King Saud Univ. Agric. Sci.* 6: 281-288.

Romero G, Adeva C and Battad II Z (2009). Genetic fingerprinting: advancing the frontiers of crop biology research. *Phil. Sci. Lett.* 2: 8-13.

Schwartz HF (2010). Colorado State University, Bugwood.org. Available at [http://www.forestryimages.org/browse/detail.cfm?imgnum=5359959]. Accessed December 18, 2013.

Skroch P and Nienhuis J (1995). Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. *Theor. Appl. Genet*. 91: 1086-1091.

Soltani A, Rezai AM and Khajeh-Pour MR (2001). Genetic variability for some physiological and agronomic traits in grain sorghum (*Sorghum bicolor* L.). *JWSS-Isfahan Univ. Technol.* 5: 127-137.

Temiesak P, Ponpim Y and Harada T (1993). RAPD analysis for varietal identification in *Brassica*. *Kasetsart J. Nat. Sci.* 27: 37-42.

Vanijajiva O, Sirirugsa P and Suvachittanont W (2005). Confirmation of relationships among *Boesenbergia* (Zingiberaceae) and related genera by RAPD. *Biochem. Syst. Ecol.* 33: 159-170.

Vinodhana NK and Ganesamurthy K (2010). Evaluation of morpho-physiological characters in sorghum (*Sorghum bicolor*

(L.) Moench) genotypes under post-flowering drought stress. *Electronic J. Plant Breed.* 1: 585-589.
Wang Y, Wang S, Zhao Y, Khan DM, et al. (2009). Genetic characterization of a new growth habit mutant in tomato (*Solanum lycopersicum*). *Plant Mol. Biol. Rep.* 27: 431-438.
Weder JKP (2002). Influence of experimental conditions on the reproducibility of RAPD-PCR identification of legumes and cereals. *Lebensm. Wiss. Technol.* 35: 233-238.
Williams JG, Kubelik AR, Livak KJ, et al. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.

