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Molecular Characterization of some Sorghum [*Sorghum bicolor* (L.) (Moench] Accessions from Sudan in Relation to Racial Classification*

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Abstract: The genetic diversity among a group of 20 sorghum accessions from Sudan were assessed with RAPD molecular marker. Out of 572 amplified PCR bands scored, 61.1% were polymorphic. Estimates of the genetic similarity among the accessions ranged from 60% to 93%. A dendrogram, constructed on the basis of similarly matrix data, categorized the accessions into two main groups: a major one comprising nine groups involving 14 accessions and a smaller one consisting of two groups involving four accessions. The race Caudatum and its hybrids and the Durra race and its hybrids were detected in 18 and 16 of the accessions tested, respectively, whereas the races Bicolor and Guinea were represented each by only one accession and Kafir by two accessions. There was agreement between the relationships revealed by molecular characterization and those reflected by racial classification in some cases but not in others. Thus, in future, to ensure a more complete and informative characterization of the germplasm., both molecular and morphological methods should be used.

Key words: Sorghum; races; genetic diversity; RAPD marker

INTRODUCTION

Assessment of genetic diversity within cultivated crops is crucial for effective utilization of crop genetic resources. It is particularly useful in the characterization of accessions (Smith and Smith 1992), in identifying duplicates in germplasm collections (Dean *et al*. 1999) and in the choice

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of parents in breeding programmes (Jordan *et al.*1997; Gupta *et al*. 1999).The study of genetic diversity among sorghums from Sudan attracts special interest as Sudan lies within the geographical region where sorghum is believed to have been domesticated and where the largest genetic variation in both cultivated and wild types is found (Doggett 1988).

In the past, indirect estimates of genetic diversity, based on phenotypic variability, have been widely used in many species including sorghum (Abu–el-Gasim and Kambal 1975; Teshome *et al*. 1997; Grenier *et al.* 2000). Phenotypic characters are relatively easy to measure in the field and some are of economic and adaptive value. However, phenotypic variability is subject to environmental variations and does not reliably reflect genetic variation.. Advances in molecular biology have led to the development of molecular markers (e.g., RAPD, SSR, RFLP) that provide a way to measure genetic variability without environmental influence. Surveys of genetic polymorphism in sorghum have been made by some workers including Menkir *et al*. (1997), Tuinstra and Agrama (2003), Uptmoor *et al.* (2003), Mehmood *et al.* (2008) and Abu Assar *et al.* (2009) using different molecular markers.

Different schemes have been used in sorghum classification, but most breeders prefer the simplified scheme proposed by Harlan and de Wet (1972). On the basis of this scheme, cultivated sorghum is classified into five basic races (Bicolor, Caudatum, Durra, Guinea, Kafir) and 10 hybrid races between them.

The objectives of the present study were (i) to classify a group of sorghum cultivars from Sudan on the basis of Harlan and de Wets' scheme (ii) to assess the genetic diversity among the cultivars through random amplified polymorphic DNA (RAPD) markers, and (iii) to determine to what extent the genetic relationships revealed by molecular characterization agree with those based on racial differentiation.

MATERIALS AND METHODS

Plant materials

The material studied consisted of 20 grain sorghum accessions, eighteen were native to Sudan and two (TxB623. and D.W. Milo) were introductions from U.S.A.

Racial identification

For racial identification, the material was grown under irrigation at Shambat (Lat. 15˚35́ N, Long. 32°31´E), Sudan. The experimental design was a randomized complete block with three replications. Each plot consisted of a single row, 5 m long with spacing of 70 cm between rows and 20 cm between plants along the row. The sowing date was 23 July, 2006.

At maturity, the race to which each accession belonged was identified on the basis of the scheme suggested by Harlan and de Wet (1972).

Molecular characterization

DNA extraction: Sorghum seedlings of the 20 sorghum accessions were raised in pots in the green house and seven days after sowing, the seedlings were harvested and air dried. Genomic DNA was isolated from individual seedlings following a standard CTAB extraction protocol (Dellaporta *et al.* 1983) with some modifications. One to two grammes of dry leaves were crushed in a mortar to a fine powder, mixed in 500µl CTAB buffer in 1.5 ml eppendrof tube and 5µl of RNase were added. The mixture was incubated at 65°C for 15 minutes in a water bath. An equal volume of chloroform: isoamyl-alcohol (24:1) was added, and the mixture was centrifuged at 13 000 rpm for 10 minutes. Three phases were separated, the upper phase which contains the nucleic acid was transferred to a new tube and an equal quantity of a mixture of phenol: chloroform: isoamyl-alcohol (1:24:1) was added. Another centrifugation was made for 10 minutes at 13 000rpm. The supernatant was transferred to another eppendrof tube, mixed with an equal volume of isopropanol and centrifuged at 13 000 rpm for 10 minutes. DNA was recovered as a pellet by centrifugation and washed with 70% ethanol. The pellets were dried, suspended in 100µl of TE buffer and stored at 4°C until used. The extracted DNA quality was determined according to the procedure

described by Williams *et al.* (1990), and its concentration was determined using UV-spectrophotometer at the wavelength 260 nm.

DNA amplification: Ten RAPD- primers (Table 1), obtained from Bexnet Co., Japan, were used to detect polymorphism among the twenty sorghum accessions. The PCR mixture contained 1µl (50 ng) genomic DNA, 1 µl primer, 0.5 µl dNTPs, 0.4µl Taq polymerase, 15.1µl ddH_2O (double distilled water) and 2 µl reaction buffer. The reaction of RAPD-PCR was carried out in a thermocycler machine. The annealing temperatures used are shown in Table 1. The RAPD-PCR reaction products were evaluated for polymorphism on 2% agarose gel. stained with ethidium bromide.

Band scoring and cluster analysis: Data were obtained from photographs of stained agarose gels by assigning one to visible bands and zero to absent bands according to the method described by Nei and Ii (1979). A dendrogram was constructed based on similarity matrix data by applying the UPGMA (Sneath and Sokal 1973). Cluster analysis was made using the software package NTSYS-PC version 2 (Rolf 1993).

Table 1. Primer code, sequence, and annealing temperature used in RAPD-PCR

Primer code	Primer sequence annealing	Annealing temp °C
A00	ATC AGC GCA CCA	39
A01	AGC AGC GCC CA	42
A02	GCCAGC GCC TCA	42
A03	TGCAGCGCC TCA	42
A04	GCC CCG TTA GCA	42
A06	ACT GGC CGA GG	45
A10	GCC TGC CTC ACG	45
A12	CTC CTG CTG TTG	39
A13	CTC AGC GAT ACG	39
A17	GGT TCG GGAATG	39

RESULTS AND DISCUSSION

Racial identification

Six races were detected in the material studied including two basic races, Caudatum and Durra, and four hybrid races (Table 2). Caudatum was detected in 18 of the accessions, confirming previous findings that it is the dominant race in Sudan (Harlan and de Wet 1972; Grenier *et al.* 2004). It has been described as a race with a great agronomic value (Doggett 1988), better adaptation to harsh conditions (Stemler *et al.* 1975), a wide range of response to changes in photoperiod (Grenier *et al.* 2000) and a source of genes for high yield and excellent seed quality (Rosenow and Dahlberg 2000). This may explain the marked contributions of Sudanese sorghums to sorghum improvement globally.

Table 2. Racial identification of 20 sorghum accessions from Sudan

Accession	Race	Accession	Race
Red Mugud	D	White Mugud	D
Najjad	DC	Karmaka	DC
Zinnari	DC	D.W. Milo	DC
PI 291027	GC	Deri	CB
Mabure	DC	Gishaish	C
Tetron	CK	Aklamoi	DC
Jebali	DC	Wad Ahmed	C
TxB 623	CK	Arfa Gadamak	DC
Abu Sabein Aliab	DC	Abu Sabein Robatab	DC
B2	C	B7	C

C=Caudatum; D=Durra; K=Kafir; B=Bicolor; G=Guinea

Twelve of the accessions belonged to the race Durra and its hybrids and most of these accessions were native to relatively dry areas in northern, eastern and western Sudan, confirming previews reports (Doggett 1988)

that Durras are adapted to dry areas. On the other hand, the Guinea race was detected in only one intermediate race, Guinea-Caudatum, in the accession PI291027 from southern Sudan. According to Harlan and de Wet (1972), the Guinea race is basically a west African race, usually grown in high rainfall areas and forests. Similarly, the Bicolor race was represented by one hybrid race, Bicolor-Caudatum, in the accession Deri, a widely grown cultivar in the Equatorial region of southern Sudan. Bicolor race is usually poorly represented in the grain types because of its poor grain quality. Kafir was detected in the intermediate race, Kafir-Caudatum, identified in the accessions Tetron and Tx 623 B which is an introduction from U.S.A. Harlan and de Wet (1972) stated that American sorghums are now entirely Kafir-Caudatums.

Molecular characterization

Seven of the ten primers tested produced polymorphic products resolvable by agarose gel electrophoresis. An example of the PCR products of the amplified fragments of twenty sorghum genotypes using two premiers (A04 and A17) is depicted in Figure 1.

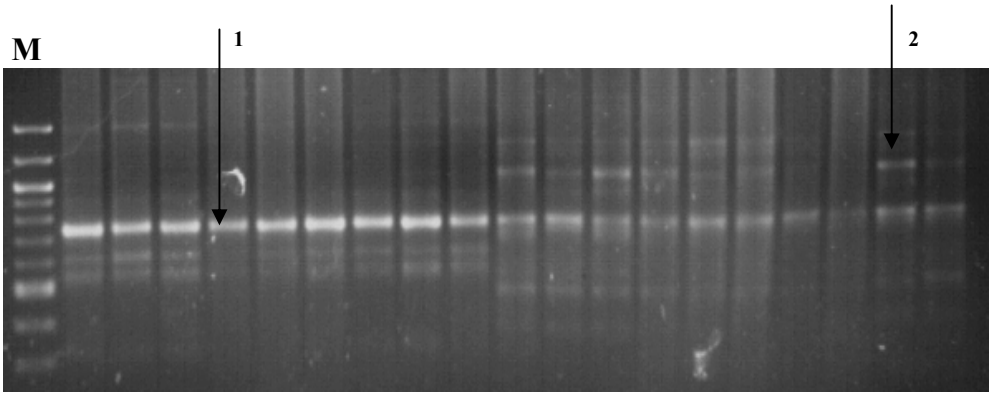

Fig 1. The PCR product of the amplified fragments of ten sorghum genotypes using two primers (A04 and A17).

M: Size marker (1000bp DNA ladder)

1: Common bands

2: Polymorphic bands

The total number of the amplified PCR bands scored was 512, of which 344 (67%) were polymorphic (Table 3). The number of bands per primer ranged from 49 to 94 with an average of 73.1 and the number of polymorphic bands per primer ranged from 22 to 74 with an average of 49.1. In a comparable RAPD study involving 10 sorghum cultivars from Pakistan and using 10 primers, the average number of polymorphic bands per primer was 7.5 (Mehmood 2008), indicating that in the present study the polymorphism detected was relatively abundant.

Table 3. Total number of bands, polymorphic bands and percentage of polymorphisms using seven primers in 20 sorghum genotypes

Primer code	Total number of bands	Number of polymorphic bands	Percentage of polymorphisms
A00	73	66	90.4
A01	49	38	77.6
A02	94	48	51.1
A04	77	74	96.1
A06	78	56	71.8
A12	80	40	50
A13	61	22	36.1
Total	512	344	67.2
Average	73.1	49.1	67.1

The UPGMA dendrogram constructed on the basis of the similarity matrix data is shown in Figure 2. The phylogenetic tree categorized the 20 sorghum accessions into two main clusters: a major one comprising nine groups involving 16 accessions and a minor one consisting of two groups involving four accessions. The genetic coefficient of similarity among the accessions ranged from 60% to 93%. This range is comparable to the range of 67.8%-95.6% reported by Mehmood *et al.* (2008) in a study

involving ten sorghum cultivars from Pakistan evaluated with PAPD markers using 10 primers. However, Abu-Assar *et al.* (2005) reported a wider range of 0%-97% which may be largely explained by the fact that their study involved a larger number of genotypes (96) from different geographical regions (Sudan, ICRISAT and U.S.A.).

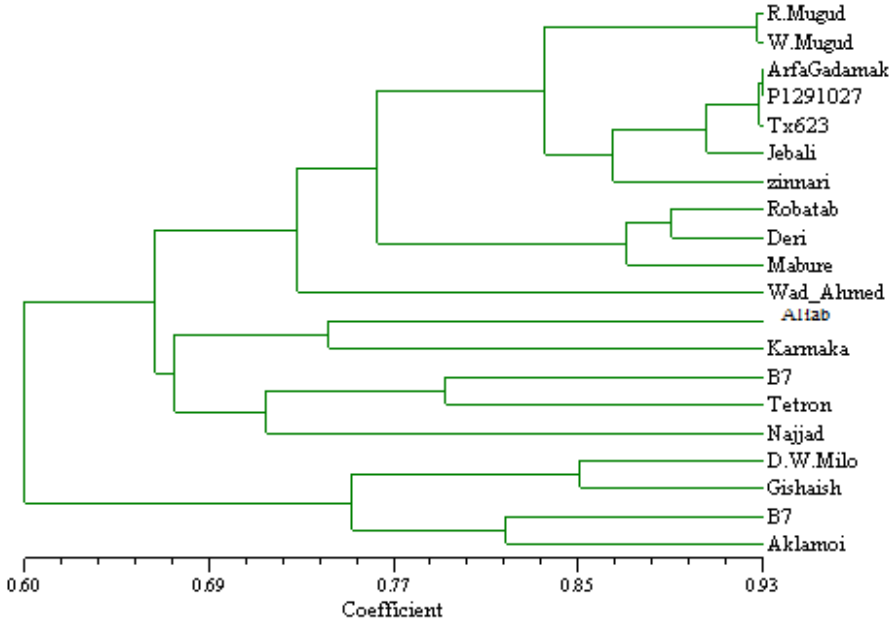

Fig 2. Dendrogram analysis of twenty sorghum genotypes based on data generated using ten RAPD primers

Comparison of relationships revealed by racial identification and molecular characterization

The dendrogram revealed a high similarity (92.9%) between the accessions Red and White Mugud. Both accessions belong to the same race (Durra), but they are grown under different ecological zones: rain fed and irrigated conditions, respectively. On the other hand, although the accessions Zinnari and Arfa Gadamak belong to the same hybrid race (Caudatum-Durra), they fell in different groups, probably, reflecting the importance of geographic distribution, as Zininari is a dominant landrace in the heavy clay soils of North Kordofan State, whereas Arfa Gadamak is

the main cultivar in the low rainfall areas of Gedarif State. The accessions Abu Sabein Robatab and Deri exhibited high similarity (89%) although they are from different ecological zones, north and south Sudan, respectively, but they have Caudam in common. Wad Ahmed fell in a separate group, showing some divergence from the nearest group, although they had Caudatum in common. This may be due to the fact that Wad Ahmed is not a landrace but a product of a formal breeding porogramme (Ibrahim *et al*. 1997).

Despite differences in ecological adaptation between Abu Sabein Aliab (from River Nile State) and Karmaka (from Nuba Mountains), the two accessions fell in one group with a similarity of 74.2% and belonged to the same hybrid race, Durra-Caudatum, indicating agreement between molecular characterization and racial classification. Contrary to expectation, Abu Sabein Aliab and Abu Sabien Robatab were categorized into different groups although they belong to the same race and are native to the same region (River Nile State). However, in a preliminary study involving ten sorghum accessions, these two accessions were categorized in one group. The accessions D.W. Milo and Gishaish fell in the same group with a similarity of 85%. Although they belonged to different races, they had Caudatum in common and both were early in flowering and dwarf in stature reflecting some agreement between morphological and molecular characterization.

In conclusion, the relationships revealed by molecular characterization are in agreement with categorization based on racial classification in some cases but not in others. Thus, as suggested by Rosenow and Dahlberg (2000) future studies of sorghum diversity should be based on both morphological and molecular methods, thereby ensuring a more complete and informative characterization of the germplasm.

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التوصيف الجزيئي لبعض سلالات الذره الرفيعة السودانية والعلاقة مع التصنيف العرقي*

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موجز البحث: تمت دراسة التنوع الوراثي في عشرين مدخلا من الذرة الرفيعة السودانية بطريقة الحمض النووي المتباين المتضاعف عشوائيا (RAPD). وقد أمكن التعرف على 512 حزمه، 67% منها متعددة الاشكال وقد تراوح التشابه الوراثي بين المداخيل من 60% إلى 93% . وقد قسم الرسم الشجري (Dendrogram)، المقام على أساس مصفوفة بيانات التشابه في المداخيل، إلى حزمتين رئيسيتين: الأولى مكونه من تسع مجموعات فرعيه من 12 مدخلا والثانية صغيره مكونه من مجموعتين فرعيتين من أربعة مداخيل. وقد تم التعرف على العرق كوديتم (Caudatum) وهجنه في 18 من المداخيل وعلى العرق درا (Durra) وهجنه في 16 من المداخيل بينما تم التعرف على العرقين بايكلر (Bicolor) وقينيا (Guinea) في مدخل واحد لكل منها وعلى العرق كافر (Kafir) في مدخلين. وكان هناك توافق بين العلاقات التي أوضحها التوصيف الجزيئي وتلك التي عكسها التصنيف العرقي في بعض الحالات. وخلصت الدراسة إلى أنه لضمان توصيف تام ومفيد للمصادر الوراثية في المستقبل ، لابد من إستخدام كل من الطرق الجزيئية والمورفولوجية.

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