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Assessment of Sorghum Genetic Resources for Genetic Diversity and Drought Tolerance Using Molecular Markers and Agro-morphological Traits

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Abstract: Forty sorghum genotypes were investigated for genetic diversity and drought tolerance. Diversity parameters were estimated using 16 Simple Sequence Repeats markers. For assessment of drought tolerance, the genotypes were field evaluated under normal and drought stress conditions for two seasons in three environments, in Sudan. In total, 98 SSRs alleles were detected with an average of 6.1 alleles per locus. The estimated polymorphic information contents ranged from 0.33 to 0.86. The genetic similarity ranged from 0.00 to 0.88 with a low mean of 0.32. The dendrogram, generated from the UPGMA cluster analysis, showed two main clusters differentiated into nine sub-clusters with close relationship to morphological characters and pedigree information. Mantel statistics revealed a good fit of the cophenetic values to the original data set (r = 0.88). The overall mean genetic diversity was 0.67. Significant differences were detected among genotypes under both normal and drought stressed conditions for all measured traits. Based on the relative yield, the most drought-tolerant genotypes were Arfa Gadamak, Wad Ahmed, El-Najada, Korcola, ICSR 92003 and Sham Sham. Drought

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caused five days delay in flowering, and the earliest genotypes were PI 569695, PI 570446, PI 569953, Dwarf White Milo and PI 569951.

Key words: Genetic diversity; drought tolerance; molecular markers: morphological traits; sorghum

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a very important food crop in the Sudan. It serves as a primary source of food for millions of people. Nearly 80% of the total grain production in the Sudan is obtained from sorghum. More than one third of the land under rain-fed agricultural production in the Sudan is devoted to sorghum production. The average yield of sorghum in Sudan is about 580 kg/ha compared to the average world yield of 1370 kg/ha. It is evident that there is an urgent need for development of improved sorghum cultivars to sustain production in drought prone areas. Sudan lies within the geographical range where sorghum was first domesticated (Mann *et al*. 1983) and where the largest genetic variation for both cultivated and wild sorghum is found (Doggett 1988).

Assessment of the genetic variability within cultivated crops has a strong impact on plant breeding and conservation of genetic resources (Simioniuc *et al.* 2002). It is particularly useful in the characterisation of individuals, accessions and cultivars, in determining duplications in genotypes collections, assigning lines to heterotic groups, landraces protection and for the choice of parental genotypes in breeding programmes (Russell *et al.* 1997). In the past, indirect estimates of similarity based on pedigree information, heterosis data and morphological traits have been widely used in many species including sorghum. However, phenotypic variation does not reliably reflect genetic

variation because of the role of environment interaction in determining phenotype (Graner *et al.* 1994). Now, the use of molecular markers, particularly DNA- based polymorhisms which detect variation at the DNA sequence level (Smith and Smith 1992), has become an increasingly useful and powerful tool in the assessment of genetic similarity and manipulation of important agronomic traits in breeding stocks (Lee 1995).

Drought stress adversely affects photosynthesis, growth and survival of plant species growing in semi-arid climates (Chaves *et al.* 2002). It is a serious agronomic problem and one of the most important factors contributing to crop yield loss in marginal areas and affecting yield stability in temperate areas (Sari-Gorla *et al*. 1999). In sorghum, drought is a major production constraint worldwide. Drought-response in sorghum has been characterised at both flowering and post-flowering stages resulting in a drastic reduction in grain yield (Rosenow 1987). In case of post-flowering drought stress, lodging further aggravates the problem resulting in total loss of crop yield in mechanised agriculture (Kebede *et al.* 2001). Therefore, assessment of genetic diversity and variability for drought stress is crucial to initiate knowledge-based breeding. Seetharama *et al.* (1990) stated that, regardless of the condition under which a cultivar is bred, only an empirical field test can provide satisfactory proof of the drought-tolerant cultivars.

The objectives of this study were (i) to assess genetic diversity among 40 sorghum genotypes, using SSR markers and (ii) to identify drought-tolerant genotypes, based on productivity and relative performance under drought stress conditions.

MATERIALS AND METHODS

Plant material

Forty sorghum genotypes were chosen from a total of 96 genotypes, based on genetic relationships analysed by Simple Sequence Repeats (SSRs) (Abu Assar *et al.,* 2005) and pedigree information.

Genetic diversity

Molecular analysis, using 16 polymorphic SSR primer pairs, was carried out in order to estimate the genetic relatedness. DNA isolation as well as SSR analyses were carried out as described by Abu Assar *et al.* (2005). Based on the SSR amplification products, genetic similarity (GS) was estimated by the following equation (Nei and Li 1979):

$$GS = \frac{2a}{2a+b+c}$$

where *a* refers to alleles shared between two genotypes, and *b* and *c* refer to alleles present in either one of the genotypes of a pair-wise genotypic comparison.

UPGMA-cluster analysis was carried out by using NTSys-pc program. The goodness of fit of the UPGMA-clustering in comparison to the computed similarity indices were estimated by Mantel (Mantel 1967) statistics. Polymorphic information content (PIC) for each of the SSRs was estimated by determining the frequency of alleles per locus as fallows:

$$PIC = 1-\sum X^2_{\ j}$$

where x_j is the relative frequency of the ith allele of the SSR loci.

The mean genetic diversity index (DI) across all loci was calculated according to Nei (1973) as fallows:

$$DI = n_a(1/n_l \sum_j (1-\sum_i x_{ij}^2))/(n_a-1)$$

where x_{ij} is the frequency of the *i*th allele of locus *j*; n_l is the number of genetic loci, and n_a is the number of genotypes.

Evaluation for drought tolerance

The 40 genotypes were evaluated for drought tolerance characteristics under field conditions. Field trials were executed at two locations in

Sudan {Gezira Research Station Farm (GRSF) at Wad Medani (Latitude $14^0$24´ North, Longitude $33^0$29´ East), and Rahad Research Station Farm (RRSF) at Rahad (Latitude 13^0 31´ North, Longitude 33^0 31´ East) during 2002 and 2003. The soil types of GRSF and RRSF are clays (Vertisols). Land preparation was done by disc ploughing and then harrowing, followed by levelling and ridging. The experiments were arranged in split- plot design with three replications. Water treatments were assigned to the main plots and genotypes to the subplots.

The material was sown on 25 July 2002 and on 30 June 2003 at Wad Medani and on 7 July 2003 at Rahad. Five seeds were sown per hill, 15 cm apart. The plot size was two rows, 3 m long and 80 cm apart. Seedlings were thinned to two plants per hill. All trials received standard cultural practices. Weed control was carried out manually, and neither herbicide nor insecticides were applied. Nitrogen fertilizer in the form of urea was applied at the rate of 86 kg N/ha, one month after germination. The experiment was watered immediately after sowing to ensure uniform germination. The amount of rains during the growing seasons was 207 mm in 2002 and 365 mm in 2003 at GRSF and 413 mm in 2003 at RRSF. Drought stress was applied by withholding irrigation water from the stressed plots throughout the season, while the other plots were fully irrigated. To avoid bird damage, 5 plants were randomly selected from each plot and their heads were covered with bags to obtain the average grain yield per plant. Data were recorded on vegetative and reproductive traits; namely, plant height, days to 50% flowering, grain yield per plant, growth rate, biomass per plant, harvest index and 1000-grain weight. The relative performance under drought stress was used as drought tolerance parameter:

$$\text{Relative performance (\%)} = \frac{\text{Performance under drought stress}}{\text{Performance under non-stress}} \times 100$$

Individual as well as combined analyses of variance were performed to examine differences among genotypes for all measured traits. In addition, correlation analysis was carried out for all measured traits and for drought tolerance parameters. Statistical analysis was carried out using SPSS 11.5. Data on the percentages of drought tolerance were analysed across the three environments.

RESULTS

Genetic relationships among sorghum genotypes

The 16 SSR primers, which cover all of the 10 linkage groups (A to J), were able to uniquely fingerprint each of the 40 genotypes (Table 1). Genetic similarity estimates ranged from 0.00 to 0.88 with a mean of 0.32. The dendrogram, generated from the UPGMA cluster analysis, showed two main clusters differentiated into 9 sub-clusters related to morphological characters and/or pedigree (Fig.1). The Mantel (1967) test showed a good fit of the cophenetic values to the original data set ($r = 0.88$).

The first cluster comprised cultivars, landraces and advanced breeding lines which were further subdivided into 7 sub-groups, whereas the second cluster consisted of gene bank accessions which were further subdivided into 2 sub-groups. The cluster from Red Mugud to Dwarf White Milo consisted of the three Mugud and two Milo landraces. This cluster is followed by one cluster of the Feterita landraces which were collected from El Gadarif State, covering the landraces from Feterita Eriana to Feterita Rass Girid. The cluster from El Najada to Tabat represented Hegiri group with the exception of Tabat. The cluster from Abu Teman to Dabar Habashi covered Milo group. Next to these clusters is a synthetic group (ICRISAT lines). The cluster from Dabar Baladi to LRB 6 represented Milo group. The second main cluster was sub-divided into two sub-clusters. The cluster from PI 569537 to PI 569953 and the second sub-cluster comprised two accessions, PI 569537 and PI 569853.

Table 1. Simple sequence repeats (SSR) and their PCR product range, number of alleles per locus and polymorphic information content in 40 sorghum genotypes

SSR ID	LG	Repeat type	Observed PCR product range	Number of alleles	PIC
SB6- 34	I	$[(AC)/(CG)]/_{15}$	218-243	3	0.33
SB5- 236	G	$(AG)_{20}$	173-193	6	0.70
SB4-121	D	$(AC)_{14}$	209-216	3	0.51
SB6- 57	C	$(AG)_{18}$	292-325	5	0.57
SB1-10	D	$(AG)_{27}$	230-311	9	0.86
SB 4-15	E	$(AG)_{16}$	106-132	4	0.40
SB 4- 32	E	$(AG)_{15}$	172-223	9	0.85
SB6-342	A	$(AC)_{25}$	281-300	4	0.68
SB 1-1	H	$(AG)_{16}$	251-270	6	0.68
SB5- 206	E	$(AC)_{13}/(AG)_{20}$	100-145	6	0.77
SB 4-72	F	$(AG)_{16}$	181-208	4	0.47
SB6- 84	B	$(AG)_{14}$	162- 224	9	0.73
SbAGB02	A	$(AG)_{35}$	95-145	7	0.54
SBAGF06	A	$(AG)_{35}$	100-179	11	0.82
SBAGH04	F	$(AG)_{39}$	127-155	6	0.73
SBKAFGKI	J	$(ACA)_9$	140-165	6	0.75

LG = linkage group; DI=diversity index; PIC = polymorphic information content

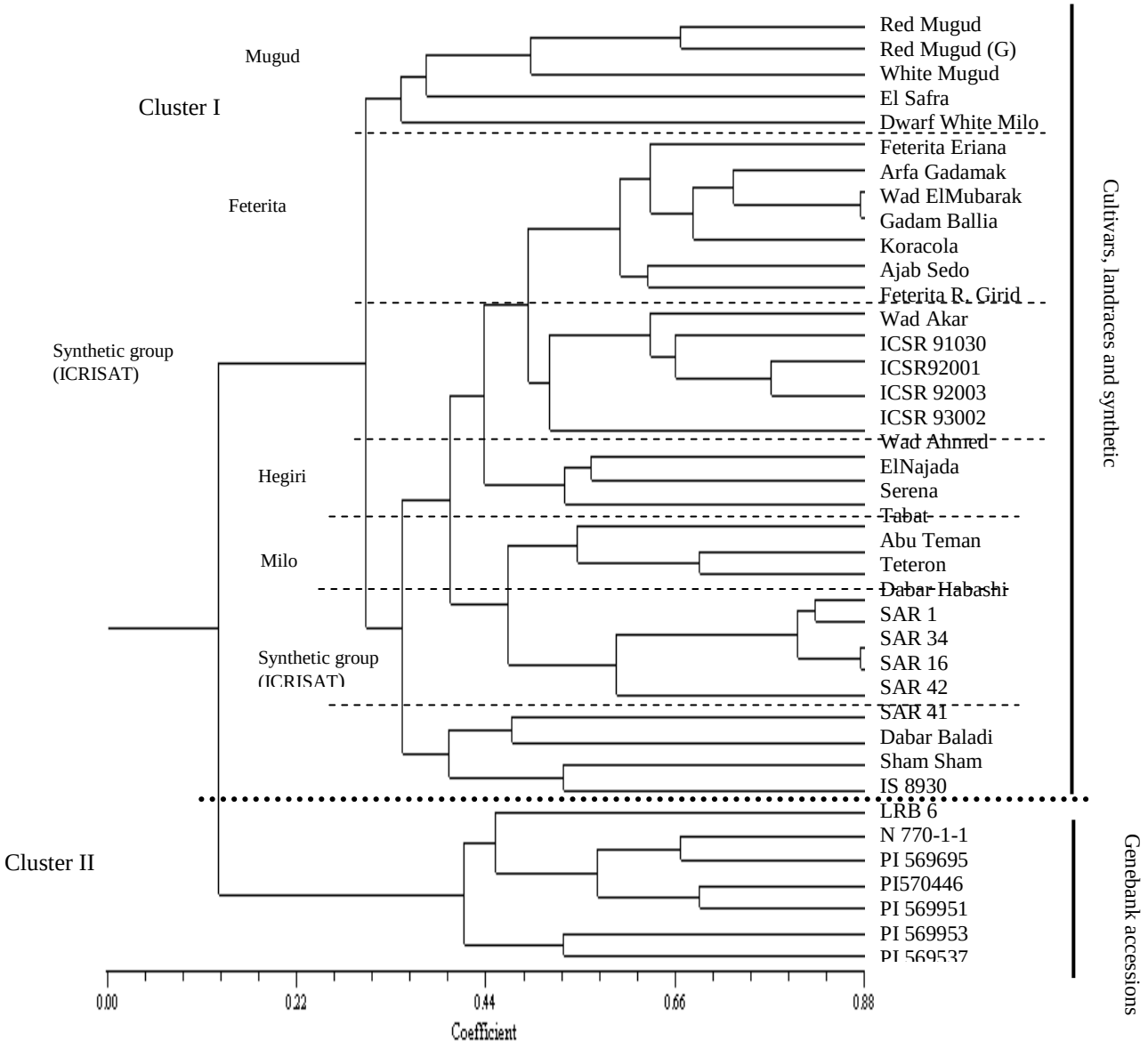

Fig. 1. Dendrogram generated by UPGMA cluster analysis showing the relationships among 40 sorghum

Genetic diversity

In total, 98 alleles were detected by the 16 SSR loci with an average of 6.1 alleles per locus and a range of 3 to 11 alleles per locus (Table 2). The size of the amplified fragments ranged from 95 to 325 bp. The estimated PIC values ranged from 0.33 to 0.86, and the overall mean genetic diversity was 0.67 (Table 2). The highest genetic diversity (DI=0.72) was found within the Milo group followed by Feterita, while the lowest genetic diversity (DI=0.47) was found within the Mugud group (Table 2).

Table 2. Genetic diversity within morphological groups of 40 sorghum genotypes from Sudan and ICRISAT

Category	No. of genotypes	Diversity index	Alleles/locus
Feterita	16	0.65	4.8
Milo	8	0.72	4.1
Synthetic	10	0.48	3.1
Hegiri	3	0.57	1.9
Mugud	3	0.47	1.9
All	40	0.67	6.1

Drought tolerance and agro-morphological traits

Significant differences were detected among the 40 genotypes for all measured traits (Table 3). Based on yield superiority under drought stress conditions, the best genotypes were Wad Ahmed, ICSR 92003, Feterita Eriana, El Najada and ICSR 91030. The lowest yields under stress conditions were given by PI 569953, Dwarf White Milo, Red Mugud and Wad ElMubarak (Fig. 2). According to relative performance, the best genotypes were Arfa Gadamak, Wad Ahmed, El-Najada, Koracola, ICSR 92003 and Sham Sham (Fig. 2).

Table 3. Ranges, means and standard errors for grain yield, days to 50% flowering, plant height, growth rate, 1000-grain weight, and biomass/plant and harvest index, of 40 sorghum genotypes, under stress conditions across three environments over 2 seasons (2002 and 2003) in 40 sorghum genotypes

Trait	Unit	Range	Mean	SE±
Grain yield (GY)	g	20 - 57	39	0.58**
Relative GY	%	52 - 85	75	0.67**
Days to 50% (DFF)	Days	61 - 94	75	0.59**
Relative DFF	%	102.9 - 111.0	107.2	0.34**
Plant height (PHT)	cm	119 - 265	183	2.67**
Relative PHT	%	81.1 - 91.9	86.2	0.55**
Growth rate (GR)	cm day^{-1}	1.6 - 3.4	2.5	0.04**
Relative GR	%	77.1 - 87.7	81.0	0.53**
1000-grain weight (TGW)	g	16.1 - 28.9	23	0.29**
Relative TGW	%	77.7 - 94.9	86.4	0.71**
Biomass (BM)	g	82.0 - 223.1	142.4	2.90**
Relative BM	%	61.6 - 87.8	74.5	0.76**
Harvest index (HI)	g	0.22-0.42	0.31	0.01**
Relative HI	%	73.7-132.8	102.1	0.01**

*, ** = significant at 0.01 and 0.05 levels of probability, respectively

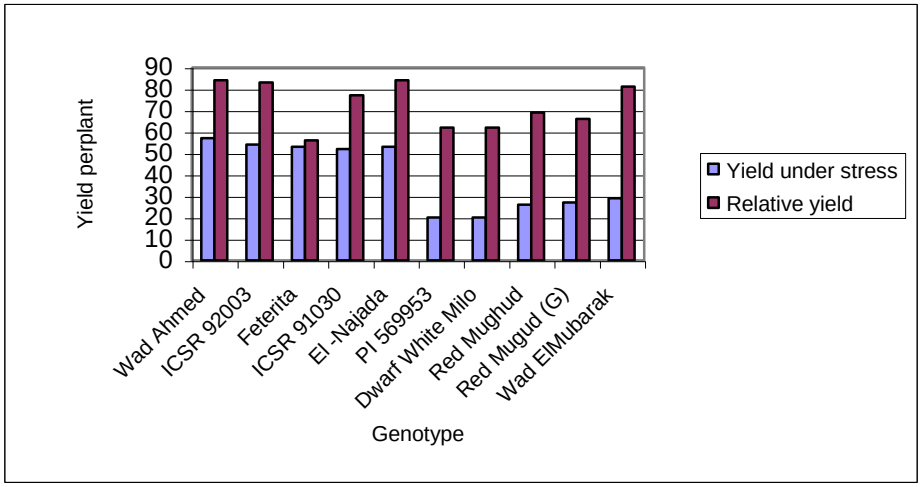

Fig. 2. Yield/plant (g) under drought-stress conditions, and relative yield (%) of the five high and low yielding sorghum genotypes

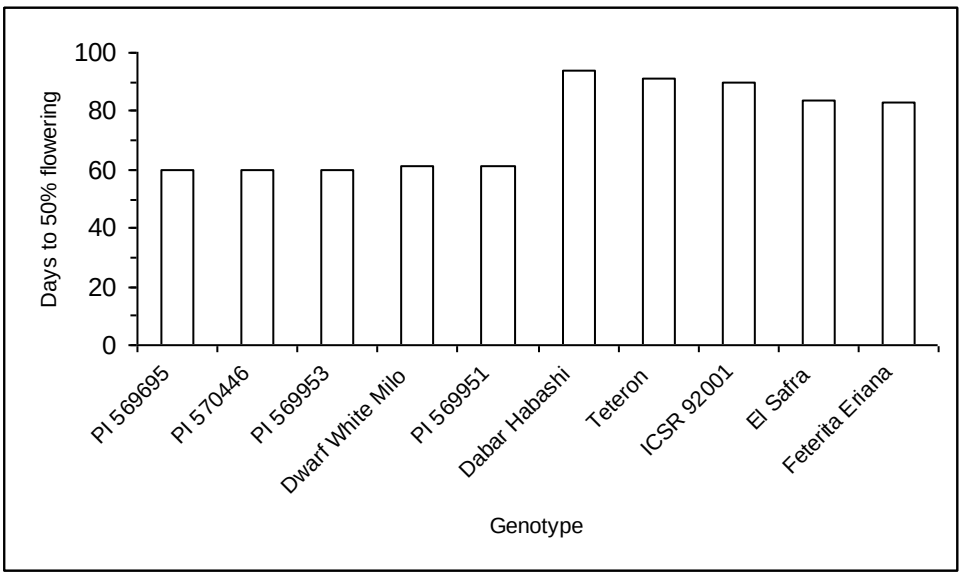

Fig.3. Days to 50% flowering of the five earliest and five latest sorghum genotypes

Drought stress caused, on average, 5 days delay in flowering. The earliest flowering genotypes were PI 569695, PI 570446, PI 569953, Dwarf White Milo and PI 569951, and the late maturing ones were Dabar Habashi, Teteron, ICSR92001, El-Safra and Feterita (Fig. 3).

Plant height also showed significant variation among the genotypes; the relative plant height ranged from 81.1% to 91.9% with an average of 86%. The relative growth rate ranged from 77.1% to 87.7% with an average of 81%. Drought stress caused an average of 13% reduction in the 1000-grain weight. Biomass was reduced by 25.5% (Fig. 2).

Correlation between grain yield and vegetative and reproductive traits

The yield/plant under field stress condition (YS) was positively and highly significantly correlated with yield under normal conditions (YN), days to 50% flowering (DFF), biomass (BM) and harvest index (HI) (Table 4). However, it had a significantly negative correlation with 1000 -grain weight (TGW). TGW was significantly and positively correlated with plant height and growth rate, but negatively and significantly correlated with YS and DFF. The growth rate exhibited significantly positive correlation with plant height and significantly negative correlation with yield under normal and stress conditions. The biomass/plant had a highly significantly positive correlation with DFF (Table 4). Harvest index showed a significantly positive correlation with yield under normal conditions, but significantly negative correlation with plant height and growth rate.

Table 4. Phenotypic coefficients of correlation among some vegetative and reproductive traits and drought tolerance parameters of 40 sorghum genotypes across three environments and averaged over three replications

	YN	YS	RY	DFF	PHT	TGW	GR	BM
YS	0.84**							
RY	-0.24	0.31						
DFF	0.52**	0.52**	0.07					
PHT	-0.05	0.08	0.04	0.18				
TGW	-0.26	-0.34*	-0.13	-0.37*	0.32*			
GR	-0.32*	-0.34*	0.01	-0.32*	0.96**	0.53**		
BM	0.65**	0.69**	0.13	0.69**	0.22	-0.28	-0.14	
HI	0.34*	0.47**	0.25	-0.14	-0.44**	-0.14	-0.36*	-0.28

*, ** = significant at 0.01 and 0.05 levels of probability, respectively

YN = yield under normal conditions; YS= yield under stress conditions; RY= relative yield; BM = biomass; HI harvest index; DFF= days to 50% flowering; PHT = plant height; TGW = thousand- grain weight

DISCUSSION

Genetic relationships and diversity

The 40 sorghum genotypes had unique SSRs fingerprints. All SSR markers were polymorphic, confirming their usefulness for genetic analysis. The range of pair-wise genetic similarity was very wide and resulted in low mean genetic diversity. Uptmoor *et al.* (2003) reported similar results in sorghum. The results on genetic relatedness and genetic diversity within sorghum accessions from Sudan and ICRISAT reflected clear differences between improved cultivars and gene bank accessions. The clustering of ICRISAT SAR and ICSR series in the same main cluster and two sub-clusters reflected pedigree relationships as well as common geographical origin (ICRISAT). The clustering of the landraces can be explained by the fact that both landraces belong to the Feterita group; and the cluster of gene bank accessions (Sudan collections) is an evidence for geographical origin.

The grouping of all Feterita, Mugud, Synthetic and Milo types separately into different sub-clusters conformed to morphological characteristics. Accordingly, these results suggest that the dendrogram based on the estimated genetic similarity, reflects pedigree and morphological relationships as reported by Ahnert *et al*. (1996) for sorghum inbred lines as well as geographic zones as reported by Hormaza (2002) in apricot (*Prunus armeniaca* L.) accessions. Ayana *et al.* (2000) reported a weak differentiation of Ethiopian and Eritrean sorghum accessions according to both agro-ecological adaptation zones and regions of origin.

The low value of diversity index (DI) within the Mugud group may be due to the small sample size (three landraces) and the close relationship that was revealed by the UPGMA analysis. The number of genotypes is much higher (10) in the synthetic group than in the Mugud group, but the DI is low as well. The reason, therefore, is the close pedigree relationship

(i.e., the synthetics are sister lines). When all 40 genotypes were taken into account, the overall DI became 0.67, which indicates that an increase in number of genotypes leads to increase in genetic diversity, resulting from a higher chance for genetic recombination, mutation, migration and gene flow, which are the most important evolutionary mechanisms for extending and maintaining genetic diversity.

The results of this study are in agreement with those of Djè *et al*. (2000) who also found high genetic diversity in accessions belonging to the race *bicolor* and/or originating from East Africa. The SSR data proved to be useful in identifying genetic relationships among a diverse collection of accessions, with the majority of the accessions clustering in concordance with pedigree relationships and/or morphological information.

Drought tolerance

The drought stress caused 25% reduction in yield, but there was clear variation among the tested genotypes. Many morphological traits play a great role in plant performance under drought stress conditions (Seetharama *et al.* 1990). Great variability was found among genotypes for days to 50% flowering, and drought stress caused 5 days delay in flowering. Early flowering genotypes can be useful in escaping late season drought.

Since yield stability in semi-arid and arid regions is strongly correlated with drought resistance, cultivars with specific adaptation to extreme stress condition and broad adaptation to climate differences between years are needed (Haussmann *et al*. 2000). Late flowering varieties tend to yield higher than early flowering ones (Ludlow and Muchow 1990), because early flowering cultivars mostly produce fewer assimilating organs (i.e., leaf area) which results in less production of assimilates.

Plant height showed highly significant differences among the genotypes, and drought stress reduced plant height by 14% and growth rate by 19%. The effect of drought under severe stress conditions is generally perceived

as a decrease in plant growth and photosynthesis rate and associated with changes in C and N metabolism (Sanchez *et al*. 2002). Stress caused 13% reduction in 1000-grain weight. Similar results were obtained in sorghum by Wenzel *et al.* (2001). On the other hand, under stress condition the harvest index increased by 2.1%, which may result from the reduction in producing assimilating organs under drought stress and indicates a more efficient water use regarding starch synthesis.

Seetharama *et al.* (1990) stated that, regardless of the condition under which a cultivar is bred, only an empirical field test can provide satisfactory proof of the drought-tolerant cultivars. From the positive and significant association between total biomass and harvest index, it appears that selection would be effective in simultaneously improving the yield under drought stress. This finding is in agreement with that of Blum *et al*. (1992) who reported the association of harvest index and above-ground dry mass of sorghum, suggesting that further improvement might be possible if higher harvest index, larger dry mass and early flowering could be combined in sorghum cultivars grown under drought conditions.

CONCLUSIONS

1. The SSR data are useful in identifying genetic relationships among genotypes.
2. The study revealed genetic variability for drought tolerance among the tested genotypes.
3. The clustering generated by the molecular markers, combined with the drought tolerance information, can be useful in selecting parents for crossing to breed drought tolerant varieties.

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تقييم الموارد الوراثية للذرة الرفيعة للتباين الوراثي و تحمل الجفاف باستعمال الواسمات ألجزيئية والصفات الزراعية المورفولوجية

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موجز البحث:- تمت دراسة 40 طرازا وراثيا من الذرة الرفيعة للتنوع الوراثي و تحمل الجفاف، و حددت معايير التنوع الوراثي باستخدام 16 من الواسمات الجزيئية (SSRs= المتكررات المتتابعة البسيطة) و لتقييم تحمل الجفاف اختبرت الطرز الوراثية تحت ظروف الحقل العادية والجفاف لموسمين وفى ثلاث بيئات بالسودان. تم التعرف على 98 من الأليلات بمتوسط قدرة 6.1 لكل موقع جيني. وتراوح المدى لمحتويات معلومات التعدد الظاهري بين 0.33 و 0.86. والتشابه الو راثي (GS) بين 0.00 و 0.88 بمتوسط قدره 0.32. أوضح الشكل التفريعي (Dendrogram) المنتج من تحليل الأزواج غير الموزونة (UPGMA) للمجموعات أن هنالك مجموعتين رئيسيتين تفرعت إلي تسع مجموعات فرعية ذات صلة وثيقة بالصفات المورفولوجية ومعلومات النسب. أوضحت معايير مانتل (Mantel) الإحصائية أن هنالك تطابقا جيدا للقيم المحسوبة مع المعلومات الأصلية

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(r=0.88). كان المتوسط العام لتنوع الوراثي 0.67، و ظهرت فرو قات معنوية بين الطرز الوراثية لكل الصفات المدروسة وذلك تحت الظروف العادية وإجهاد الجفاف. بناءً على الإنتاجية النسبية، كانت أكثر الطرز الوراثية تحملا لإجهاد الجفاف هي أرفع قدمك، و ود أحمد، و النجاضة، و كوركولا، و ICSR 92003، وشم شم، و تقع كل هذه الطرز الوراثية فى نفس المجموعة الرئيسية. أدى إجهاد الجفاف لتأخير الإزهار بمقدار خمسة أيام، وكانت الطرز الوراثية المبكرة في النضج هي: PI 56969، و PI 570446 ، وPI 569953 ، و المايلو القصير، و PI 569951.