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Genetic diversity of Eritrean sorghum landraces assessed with simple sequence repeat (SSR) markers

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Abstract A precise high-throughput approach was used to characterize diversity in 28 Eritrean sorghum landraces, and to compare this diversity to representative samples of the world sorghum collection. Pools of simple sequence repeat (SSR) markers were sized and scored on automated DNA-sizing gels. An exceptionally high level of diversity was observed among the 28 Eritrean landraces, compared to other sorghum germplasms, in both the number and size range of SSR alleles. Individual landraces were found to carry a high level of within-population diversity and heterozygosity, and between-population diversity was equally high. Eritrean sorghums could be clustered into 7–10 major subgroups, with most (but not all) classifications agreeing with descriptions by farmers. Clustering did not concur particularly well with the major classification system applied in Eritrea, highland versus lowland. Eight of the Eritrean landraces grouped with other sorghums in the world collection, particularly those from Ethiopia/Sudan and India or of the durra and caudatum races, but most Eritrean sorghums clustered in a separate subgroup. These results indicate that a great deal of germplasm diversity and genetic novelty are available in Eritrean sorghums, and that SSR markers can contribute to the wise use of this diversity for sorghum study and improvement.

Keywords DNA markers · Germplasm diversity · Microsatellites · *Sorghum bicolor*

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Introduction

Effective plant-breeding and crop-improvement programs depend on the availability of crop genetic diversity. In the search for diverse breeding material, farmer varieties or landraces (locally adapted populations bred through traditional methods of direct selection) are usually the major sources of genetic variation. The tendency for a crop's diversity to be high in its region of origin has long been established (Vavilov 1926; Doggett 1965). Collections made in such areas can be important sources of material for crop improvement.

The Eastern African region (Abyssinia), to which Eritrea belongs, has been described as one of the centers of diversity (Vavilov 1926) and a possible area of domestication for sorghum (Doggett 1965). Worede (1988) reports the existence of high genetic variability of crops such as sorghum and other crops like wheat and teff in Abyssinia. Recent studies using molecular markers have also suggested that the Central and North-Eastern regions of Africa were the principal area or areas of sorghum domestication (Deu et al. 1994). Cultivated sorghums in Eritrea reveal diverse morphological and physiological characteristics [Ministry of Agriculture MOA 1999; Araya et al. 1997]. Wild sorghum can be found growing in the western lowlands of Eritrea (Tessenei, Golidge and Aligidir) (MOA 1999). Sorghum landraces in Eritrea have not been subjected to any systematic selection or breeding apart from traditional farming practices.

Sorghum is an important crop in many tropical countries. It is a staple food in some dry parts of the world. In Eritrea, sorghum is the major crop both in terms of production and food, and occupies on average 43% of the cultivated land (Cliffe 1988; MOA 1999). Sorghum is produced on a subsistence basis under rainfed conditions in traditional farming systems. Based on their growth zones within the country, farmers in Eritrea recognize two groups of sorghums called 'lowland' and 'highland' types. Lowland sorghums grow mainly at an altitude of 100–1,900 m with average annual rainfall range of

200–600 mm (MOA 1999). The highland sorghums grow at altitudes of 2,000–3,000 m with an average annual rainfall of 500 mm.

In recent years, there has been a considerable loss of sorghum landraces and associated genetic variability in Eritrea and other dryland areas, because of the replacement of landraces with new varieties, particularly those with superior tolerance for deteriorating biotic and abiotic environmental conditions. Evaluation of germplasm diversity can indicate which landraces carry the greatest genetic novelty, and are thus most-suitable for rescue, agronomic evaluation, and possible future use in crop improvement. Molecular tools, especially those employing DNA markers, have proven to be a robust and cost-effective technology for the assessment of sorghum genetic diversity (Deu et al. 1994; Oliveira et al. 1996; Yang et al. 1996). Most recently, a set of 15 microsatellite or simple sequence repeat (SSR) markers has been developed for sorghum that allows a very high rate (and low cost) of sorghum genotype assessment (Dean et al. 1999; Djé et al. 2000; Grenier et al. 2000; Smith et al. 2000).

SSRs are tandem repeats of di-, tri-, or a higher number of nucleotide units in the DNA of plants and animals. SSRs are abundantly distributed throughout the nuclear genomes of all studied plant species, which makes them useful both for genetic mapping and for the study of natural populations. SSRs have several advantages over other DNA markers such as RFLPs, RAPDs or AFLPs. The advantages include uniform genome coverage, high levels of polymorphism, co-dominance, and specific PCR-based assays (Pejic et al. 1998). As a result, simple sequence repeat diversity has been used as a successful tool in genotyping and studying the genetic diversity of many plant species. In addition, they are useful for pedigree analysis because they represent single loci and can uniquely define genotypes. Studies have shown that SSR loci give good discrimination between closely related individuals in some cases even when only a few loci were employed (Powell et al. 1996; Scotti et al. 1999; Kong et al. 2000). The analysis of SSRs has been automated, thereby facilitating data exchange among researchers (Saghai Maroof et al. 1994; Powell et al. 1996). Finally, SSR markers have been shown to provide the highest level of information per single marker when used to detect genetic similarity among maize inbred lines (Pejic et al. 1998).

In this study, we provide an analysis of diversity in 28 Eritrean landraces of sorghum, using a high-throughput SSR-based strategy developed by Kresovich and coworkers (Dean et al. 1999; Smith et al. 2000). We also used these SSRs to compare the genotypes of the Eritrean sorghums to 32 sorghums from different parts of the world, thereby uncovering high levels of genetic novelty and diversity both within Eritrean sorghums and in comparison to worldwide sorghum diversity.

Materials and methods

Plant material

The seed sources and plant materials for this study consisted of 28 sorghum accessions provided by the Ministry of Agriculture (MOA) of Eritrea. The Eritrean sorghum accessions are farmers' varieties and were collected directly from farmers' fields. Table 1a shows their places of collection and some features of the accessions as characterized by the farmers that provided the materials (Araya et al. 1997; MOA 1999). In addition, 32 inbred lines derived from accessions in the world collection accessions (Table 1b) were obtained as previously described (Oliveira et al. 1996; Yang et al. 1996).

DNA preparation

Genomic DNA from Shanqui Red (a Chinese sorghum) and the Eritrean sorghums was prepared from fresh leaves collected from 3–6 week-old plants, as described by Dellaporta (1994). Five plants were sampled from each accession. The genomic DNA for the world collection was prepared as described previously (Oliveira et al. 1996; Yang et al. 1996).

SSR primers

The 15 SSR markers used in this study were kindly provided by M. Hopkins (USDA-ARS, Griffin, Ga.) and S. Kresovich (Cornell University, Ithaca, N.Y.). The markers consist of three sets of five multiplex primer pairs, carefully selected for their compatibility during PCR and their easy resolution on single lanes of acrylamide gels. The forward primer of each set was labeled with 6-carboxyfluorescein (FAM), terachloro-6-carboxyfluorescein (TET) or hexachloro-6-carboxyfluorescein (HEX). The details of these markers have been provided elsewhere (Dean et al. 1999; Smith et al. 2000) (see Table 2). The SSRs used are distributed widely across the sorghum linkage groups (A–I), thus giving a comprehensive coverage of the sorghum genome.

PCR amplification

Three sets of multiplex PCR reactions per sample were set up for the amplification process. In a 20-μl reaction, 5 pmol of each primer, 0.25 mm of dNTP, 1U of *Taq* DNA Polymerase (Promega) and 1 μl (20–50 ng) of DNA were used. Reactions were run in a Perkin Elmer 9600 thermocycler with an initial denaturation step of 4 min at 95 °C, followed by 25 cycles of 95 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min, and a final extension at 72 °C for 10 min (Dean et al. 1999). For PCR fragment-size determinations, 0.5 μl of an internal size standard (Genescan-500, TAMRA) was mixed with 1 μl of the PCR product and 0.5 μl of formamide:dye (5:1). The mixture was warmed at 95 °C for 5 min and loaded onto a 4% acrylamide:bisacrylamide (19:1) gel that contained 8 M urea. Electrophoresis was carried out on an ABI-377 Prism sequencer (Applied Biosystems) at 3,000 V for 3 hours in 1 × TBE buffer.

Data analysis

PCR fragment sizes of the SSR loci were read using the GeneScan 2.1 software (Applied Biosystems). Allele sizes were determined using the Genotyper 2.1 software (Applied Biosystems). Where a PCR product was not obtained, data for the specific locus and plant were treated as missing. In all cases, analysis tolerance of missing data was set to a 5% level per locus. Accessions Zangeda and Koden had only four plants each. Only individual DNA samples were analyzed for each of the 31 inbreds derived from the world population accessions.

Table 1a Eritrean sorghum accessions used in the study. Key: B = Beer (local) C = Caudatum Co = Compact D = Durra Dr = Dropping E = Elliptic F = Fodder Ft = Feterita G = *Geat* H = Highland I = *Injera* K = *Kitcha* L = Lowland Lo = Loose O = Oval S = Semi V = Very

Ref no.	Local name	Acc. no.[a]	Collection site	Growth altitude	Head type	End use	Race
1	Antilu (Kinaara)	1,302	Barentu	L	VLo	I,K,B,G	C/D[c]
2	Chimro Bicha(I)	1,325	Tukumbia	L	CoLo	I,K,B	C/D[c]
3	Chimro Bicha(II)	1,329	Augaro	L	VLo	I,B	C/D[c]
4	Chimro Tsada(III)	1,333	Shambuko	L	VLo	I,B	C/D[c]
5	Duruta Keih(K)	1,300	Augaro	L	CoO	G	C(Ft)[b]
6	Duruta Tsada(T)	1,301	Augaro	L	Co	G	C(Ft)[b]
7	Gunseber	1,335	Shambuko	L	VLoDr	I,K,B	C(Ft)[b]
8	Hugurtay	1,319	Goluj	L	SCoO	I,G	C/D[c]
9	Kina Biba(B1)	1,330	Augaro	L	SCoE	I,G	C/D[c]
10	Kina Biba(B2)	1,303	Barentu	L	VE	I,G	C/D[c]
11	Kina Dirga(Bazenay)(D)	1,331	Augaro	L	SCoE	I,K,B	C(Ft)[b]
12	Karakora	1,306	Goluj	L	SCoE	I,B	C(Ft)[b]
13	Wedi Aker Hatsir(H)	1,337	Shambuko	L	CoE	I,B	C/D[c]
14	Wedi Aker Newih(N)	1,338	Shambuko	L	SCoE	I,K,B	C/D[c]
15	Wedi Ferej(A)	1,334	Shambuko	L	SCo	I,K,B	C(Ft)[b]
16	Wedi Ferej(B)	1,336	Shambuko	L	SCoE	I,K,B	C(Ft)[b]
17	Wedi Arba	1,332	Shambuko	L	Co	I,K,B	C/D[c]
18	Adeni	So-265	Tsorona	H	SLo	I,B	C/D[c]
19	Amal	So-266	Tsorona	H	Co	I,K,B	C/D[c]
20	Amge	So-064	Tsorona	H	SCoE	I,K,	C/D[c]
21	Daguya	So-259	Mendefera	H	SLo	B	C/D[c]
22	Gumbilu	So-263	Mayayni	H	SCoE	I,B	C/D[c]
23	Hele Keih(K)	So-1,313	Tsorona	H	CoO	I,B	C(Ft)[b]
24	Hele Tsada(T)	So-1,312	Tsorona	H	CoO	I,K,B	C(Ft)[b]
25	Letemehret Hatsir(H)	So-238	Elabered	H	SCoE	I,B	C/D[c]
26	Letemehret Newih(N)	So-239	Elabered	H	SCoE	I,B	C/D[c]
27	Zangeda	So-267	Senafe	H	VLoDr	B	
28	Koden(Hatsir)	1,318	Debub	H	CE	I	

Sources: [a] MOA 1999
[b] Stenhouse and Tesfamicael 1998
[c] Harlan and de Wet 1972, Mann et al. 1983

Table 1b World sorghum accessions used in the study. Source: Oliviera et al. 1996

No.	Reference	Name	Origin	Race
29	B1437	IRAT 1437	Burkina Faso	Virgatum(V)
30	B1455	IRAT 1455	Burkina Faso	Aethiopicum(V)
31	B249	IRAT 249	Burkina Faso	Guinea(G)
32	CA7094	IS 7094	Central African Republic	Caudatum(C)
33	China	Shanqui Red	China	Bicolor(B)
34	ET3722	IS 3722	Ethiopia	Durra(D)
35	I5275	IS 5275	India	Caudatum bicolor
36	I6451	IS 6451	India	Durra
37	I8309	IS 8309	India	Durra bicolor
38	Ma14351	IS 14351	Malawi	Guinea(conspicuum)
39	Ma1484	IRAT 1484	Malawi	Guinea(conspicuum)
40	Mal379	IRAT 379	Mali	Guinea Shallu
41	Ni557	IRAT 557	Niger	Guinea (gambicum)
42	Nig3620	IS 3620	Nigeria	Guinea
43	Pak8346	IS 8346	Pakistan	Durra
44	Sa1243	IRAT 1243	South Africa	Kafir(K)
45	Sa14331	IS 14331	South Africa	Guinea(roxburghii)
46	Sa3137	IS 3137	South Africa	Guinea
47	Sa3142	IS 3142	South Africa	Kafir
48	Sa3151	IS 3151	South Africa	Caudatum
49	Sa9334	IS 9334	South Africa	Kafir
50	SL19467	IS 19467	Srilanka	Guinea
51	Su3534	IS 3534	Sudan	Guinea
52	Su9708	IS 9708	Sudan	Caudatum
53	Su9746	IS 9746	Sudan	Caudatum
54	Su9926	IS 9926	Sudan	Caudatum bicolor
55	Su9954	IS 9954	Sudan	Durra Caudatum
56	Tan1291	IS 1291	Tanzania	Bicolor
57	Tha10266	IS 10266	Thailand	Caudatum Kafir
58	USA829	IS 829	USA	Bicolor Kafir
59	USA91051	M91051	USA	Caudatum
60	Z2840	IS 2840	Zimbabwe	Kafir

Statistical analyses on the PCR fragment sizes were performed using the Arlequin software package, version 2000 (Schneider et al. 2000). In the analysis of molecular variance (AMOVA) tolerance was set to a 5% level of missing data per locus. Fragment-size data were also converted into relative repeat-number data in order to utilize parameters that use the stepwise mutation model for microsatellite data (Slatkin, 1995). These analyses were undertaken with the assumption that all size variation was caused by variation in microsatellite repeat number, and not by variation in other sequences within the amplified segment. This assumption would tend to underestimate (rather than overestimate) the actual degree of polymorphism, but would also apply equally to all samples investigated. The genetic variation of each locus was measured in terms of the number of alleles, the observed heterozygosity and gene diversity (Nei 1987) as H (gene diversity) = $n/n–1$ $(1-\sum P_i^2)$ where P_i is the frequency of the ith allele, and n is the number of samples. Genetic distance matrices were calculated for each pair of accession using the parameters F_{ST} (Reynolds et al. 1983), R_{ST} (Slatkin 1995) and Nei's pairwise differences. To visualize the relationship among the different accessions, dendrograms from the randomized input of the distance matrices data were constructed using the neighbor program of the PHYLIP computer package (Felsenstein 1993). Several trees were generated using different matrices (F_{ST}, R_{ST} and Nei's pairwise genetic distances) and a consensus tree was generated from the results of these analyses. As shown by Dean et al. (1999), neither bootstrap analysis nor confidence intervals were needed because the cluster analysis was applied only to group accessions.

Results

Polymorphism and allelic richness

Each of the 15 primer pairs that we employed gave amplification products in almost all of the plants. As an indication of polymorphism, the number of alleles and their frequency was analyzed. All 15 marker loci were polymorphic. In total, 208 putative alleles (different fragment sizes) were observed for the Eritrean and world collections (Table 2). The number of alleles per locus observed range from 7 (for Sb4-121) to 28 (for Sb5-206) with an average of 13.9 alleles per locus. The highest observed frequency of an individual allele per locus was 0.52. Of the total number of alleles, 56.7% are shared between the Eritrean and world population, 33.7% are unique to the Eritrean lines and 9.1% are unique to the world lines (Table 2). The observed heterozygosity among the Eritrean sorghums ranged from 0.7% (Sb4-121) to 26.5% (Sb6-42) with an average of 13.9% per locus (Table 2).

Genetic diversity in Eritrean sorghums

Analysis of molecular variance (AMOVA) of the total genetic variation among the 28 Eritrean accessions showed all variance components to be significant ($P < 0.001$). Differences between accessions accounted for 50.4% of the variation while within-accession diversity accounted for the other 49.6% (Table 3). When the data from the Eritrean sorghums were analyzed on the basis of the local classification of the landraces into highland and lowland varieties, the group item was significant but accounted for only 3.8% of the total variation. A significant portion of the variation was between (47.6%) and within (48.6%) accessions.

The data provided significant variation, so that relatedness trees could be constructed. Several cluster trees were assembled using the matrices of F_{ST}, F_{ST}, and and Nei's pairwise genetic distances. Figure 1 shows a single tree derived from a consensus of these different cluster trees, representing the observed clustering of the Eritrean

Table 2 Characteristics of the 15 SSR loci analyzed

Set	SSR locus	Repeat motif	Linkage group	A_R*[a]	Obs. size range(bp)	A_T[b]	A_{SH}[b]	A_{ER}[b]	A_{WO}[b]	H_o[c]	H_e[c]
1	Sb4-121	$(AC)_{14}$	D	4–5	214–228	7	7	–	–	0.007	0.74
	Sb4-15	$(AG)_{16}$	E	6	119–135	8	6	1	1	0.029	0.65
	Sb4-32	$(AG)_{15}$	E	7–8	172–216	11	9	2	–	0.159	0.79
	Sb5-236	$(AG)_{20}$	G	7–8 16**	162–222	15	8	7	–	0.221	0.86
	Sb6-342	$(AC)_{25}$	A	4	270–294	9	6	2	1	0.089	0.82
2	Sb6-36	$(AG)_{19}$	C	5–6 10***	163–199	13	7	3	3	0.073	0.48
	Sb1-1	$(AG)_{16}$	H	10–11	241–277	12	6	5	1	0.152	0.72
	Sb1-10	$(AG)_{27}$	D	7–8	242–488	20	8	9	3	0.133	0.83
	Sb5-256	$(AG)_8$	C	3 1***	162–214	15	6	9	–	0.252	0.81
	Sb6-84	$(AG)_{14}$	F	5	174–212	16	8	7	1	0.183	0.91
3	Sb6-42	$(AG)_{26}$	–	5–6	168–216	19	12	4	3	0.265	0.90
	Sb6-57	$(AG)_{18}$	C	3	283–313	13	9	3	1	0.131	0.81
	Sb5-206	$(AC)_{13}/(AG)_{20}$	E	7–8	92–156	28	14	12	1	0.209	0.94
	Sb4-72	$(AG)_{16}$	B	3–4	182–208	12	5	5	2	0.097	0.80
	Sb6-34	$[(AC)/(CG)]_{15}$	I	5	186–208	10	7	1	2	0.080	0.58

[a] A_R = No. of alleles in previous studies: *Dean et al. 1999, ** Djé et al. 2000, *** Smith et al. 2000; [b] A_T = Total no. of observed alleles, A_{sh} = Shared Alleles, A_{ER} = Alleles unique to Eritrean sorghums, A_{WO} = Alleles unique to World sorghums; [c] H_O = Observed heterozygosity, H_E = Expected Hetrozygosity

Table 3 Summary of AMOVA results (values given are % variation)

Source of variation	Groups/or accessions	Variance components[a]					
		Among groups/or accessions	Among accessions/ within groups	Within accessions	F_{ST}	F_{SC}	F_{CT}
Eritrean	1	50.4		49.6	0.50	–	–
Eritrean (Highland vs Lowland)	2	3.8	47.6	48.3	0.52	0.49	0.038
Eritrean	7	16.9	34.8	48.7	0.51	0.42	0.17
Eritrean	10	21.4	29.9	48.7	0.51	0.38	0.21
Eritrean vs World	–	10.2	45.0	40.9	0.59	0.57	0.09
All (Eritrean+World)	–	59.1		40.9	0.59	–	–

[a] All items were significant ($P < 0.001$)

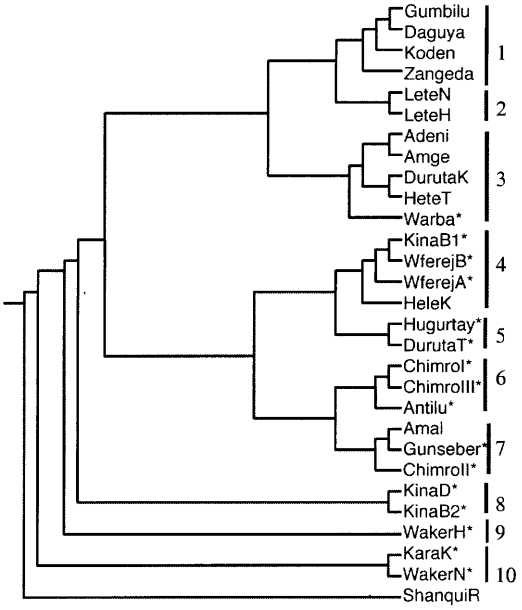

Fig. 1 Dendrogram showing relationships among Eritrean landraces (*= lowland types)

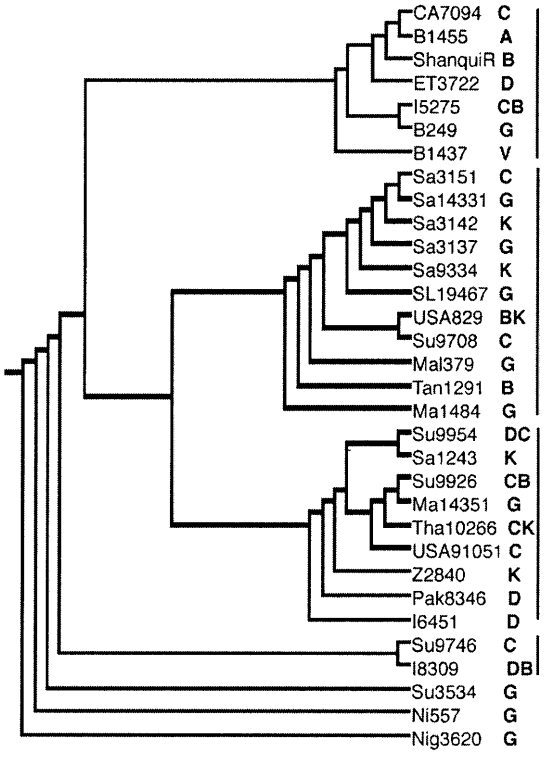

Fig. 2 Dendrogram showing relationships among the World Sorghum Accessions. A = Aethiopicum, B = Bicolor, C = Caudatum, D = Durra, G = Guinea, K = Kafir, V = Virgatum

accessions using the PHYLIP neighbor joining program. Distance-matrix data that employ a Chinese sorghum (Shanqui Red) as the outgroup were used. All accessions were distinctly placed in this dendrogram, and showed clustering into seven or ten smaller groups. Investigation of the data by AMOVA, employing the visualized groups, increased the among-groups item from 3.8% to 16.9% (when seven groups were considered) and to 21.4% (when ten groups were considered) (Table 3).

Although overall differentiation between the highland and lowland sorghum was not particularly strong or consistent in the 28 Eritrean landraces, the data in Fig. 1 indicate that many highland landraces are closely related (clusters one, two, and three out of the ten cluster designations). The greatest degree of diversity within the Eritrean populations was found to be associated with the lowland sorghums, which dominate clusters 4–9 (Fig. 1). A considerable degree of genetic differentiation (F_{ST} = 0.5) was observed among the accessions. The measured degree of inbreeding (F_{SC} = 0.41–0.49) is low, in agreement with the high levels of observed heterozygosity. The degree of relatedness between the lowland and highland sorghums is very low (F_{CT} = 0.09). However, when the landraces are grouped on the similarities based on marker-loci dendrograms, landraces tend to group into clusters with a of higher degree of relatedness (F_{CT} = 0.13–0.20).

Genetic differences between the Eritrean and world collections

Hierarchical analysis of molecular variance (AMOVA) (Table 3), performed on the data from the 60 surveyed

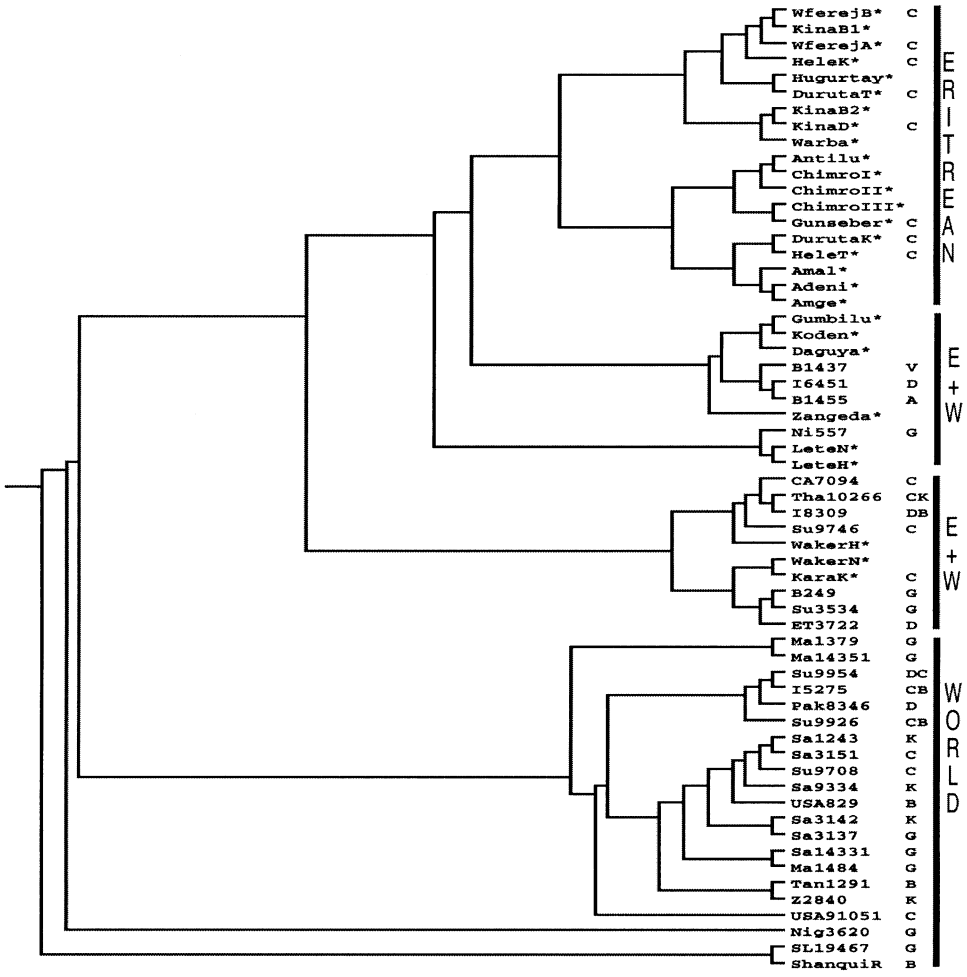

Fig. 3 Dendrogram showing relationships among Eritrean (*) and World Sorghums. A = Aethiopicum, B = Bicolor, C = Caudatum, D = Dura, G = Guinea, K = Kafir, V = Virgatum

populations, showed all variation components to be highly significant ($P < 0.001$). Of the total variation, 10.2% could be attributed to the difference between the Eritrean and the world accessions. However, the highest percentage (45%) of SSR variability was attributed to variability among populations and the within-population (40.9%) differentiation. The variance of the within-populations item comes mainly from within the Eritrean landraces.

Dendrograms generated from distance matrices of world sorghums alone (Fig. 2) showed three major clusters, with one cluster largely composed of South African sorghums. Beyond this observation, major clustering by race or region of collection was not observed. The wild sorghums B1455 (*Sorghum aethiopicum*) and B1437 (*Sorghum virgatum*) cluster in the same group in all trees. As noted previously (Oliveira et al. 1996), a subset of the guinea sorghums appeared to be the most-different from the other sorghums, and from each other.

Genetic-distance matrix data from all the comparisons of the 60 total accessions were used to visualize the relationships among the accessions (Fig. 3). Distinct clustering of the Eritrean sorghums is seen in the top two major clusters and those of the world sorghums in the bottom cluster was evident. Mixed groups of both Eritrean and world sorghums can also be seen in the middle clusters. The Eritrean accessions Wediaker Hatsir, Wediaker Newih and Karakora group in a cluster with an Ethiopian sorghum (ET3722) and two Sudanese sorghums (Su3534 and Su9746).

Discussion

The diversity present in Eritrean sorghum landraces was initially suggested by their morphological variation, their different growth properties, and their varied uses. Sorghum is the staple diet of rural populations in Eritrea and several other Central, East and West African nations. Its flour is used to make a pancake-like soft bread called *Injera* or its two-layered alternative *Hanza*, unleavened bread called *Kitcha*, porridge (*Geat*), and a beer called *Siwa*. The seeds are used for making snacks by boiling or by popping (*Imbeba*) as with popcorn. Some sorghum types have a high sugar content and their fresh sweet stalks are used for chewing as with sugar cane. The straw is used as feed for animals and in some areas for construction. There are even medicinal uses of sorghum.

As reported in a number of genetic-diversity studies on other species and populations, SSR loci were able to uniquely identify each of our Eritrean landraces. Fragment sizes obtained from this study were usually across a wider size range than those previously reported in studies of the same loci in other sorghum varieties (Brown et al. 1996; Dean et al. 1999; Djé et al. 2000). Although

allelic information may be inconclusive because same-size PCR products can be seen for different alleles (Schlotterer 1998), the number of observed alleles for most of the loci was also greater in this study. This suggests that Eritrean sorghums may be exceptionally polymorphic, providing more size variation within 28 landraces than seen in hundreds of landraces from other sorghums in the world collection (Brown et al. 1996; Dean et al. 1999; Djé et al. 2000; Grenier et al. 2000).

The results from the analysis of variance suggest that the grouping of the Eritrean sorghums into highland and lowland varieties does not maximize the variance, and that smaller groups better explain the data. When closely examined, the smaller clusters observed consist of a group of highland and lowland accessions only within the clusters. Most of the landraces given the same name or similar identification characters by farmers were grouped together, or more or less agree with their classification. The dendrogram clusters elucidate some of the recorded observations by the Eritrean Ministry of Agriculture (MOA 1999). For instance, Wedi Arba and Duruta (Keih and Tsada), Wedi Ferej (A and B) and Hele (Tsada and Keih), Wedi Aker (Newih and Hatsir) and Karakora are said to be similar from their morphological charateristics and this speculation is reflected in the clusters. In addition, Letemehret Hatsir and Letemehret Newih, sharing the same name but differing in plant height, are found in the same cluster. On the other hand, Zangeda and Kina Biba (B1 and B2), considered to be the same landrace but given different names in the highlands and lowlands of Eritrea, do not group together. A second apparent example of non-concurrence is provided by Hele Tsada and Hele Keih, sharing the same name but differing in seed color, that are found in different clusters. However, this is in accordance with their recognition as quite different landraces by Eritrean farmers.

It is interesting that the lowland landraces appear to be much-more diverse than the highland landraces. The lowland sorghums grow over a broader area, often near wild sorghums. Also, Eritrean lowland sorghums grow in an area bordering the Sudan, providing an additional opportunity for the introgression of foreign genetic material.

Compared to the 32 accessions from the world collection that were analyzed in this study, three clusters of Eritrean sorghums grouped with members of the world collection, while the other six clusters were not grouped with any members of the world collection. One of the three Eritrean clusters that grouped with non-Eritrean accessions was an exclusively highland cluster (cluster 1, Fig. 1) that grouped with the only two *Sorghum verticilliflorum* accessions (B1437 and B1455, Fig. 3). These two weedy species are often found near sorghum fields in many parts of Africa (Harlan and de Wet 1972; Mann et al. 1983), so it is possible that the highland races in this cluster have a significant recent introgression of portions of these weed genomes. The other prominent clustering of Eritrean sorghums is between a lowland group (cluster 9, Fig. 1) and a diverse group of world accessions (Karakora, Wediaker H, Wediaker N, Fig. 3). The varied world accessions in this cluster are enriched for durra and caudatum varieties, and also for materials from Ethiopia, Sudan and India compared to the world accession-sampling performed. This was not totally unexpected, as Eritrea is a close neighbor to Ethiopia and Sudan, and has an ancient tradition of trade with the Indian subcontinent (Mann et al. 1983). Moreover, Eritrean sorghums have the morphological characteristics appropriate to members of the durra and caudatum races (Harlan and de Wet 1972).

The F_{ST} (0.50) and F_{SC} (0.55) values observed in this study, although lower than reported for other sorghum populations, are significant. Djé et al. (2000) report F_{ST} = 0.68 for landraces on the basis of only three different SSR loci. The Eritrean sorghums show a lower level of allelic fixation than in previously reported landrace populations. Apparently, this is due to a reduced level of inbreeding and hence high levels of heterozygosity. Small F_{CT} values show the small degree of relatedness among the Eritrean and world accessions. This may be explained by the fact that there is little selection for uniformity exercised by the farmers in the collection areas (B. Ghebru, personal interview of local farmers; Araya et al. 1997; MOA 1999). It also indicates that there are high levels of genetic diversity still in existence in Eritrean sorghums and that this diversity requires attention in terms of germplasm conservation.

The F_{ST} values observed in this study are relatively low, indicating a reduced degree of allelic fixation. New alleles may be generated because of outcrossing and subsequent intralocus recombination, including gene conversion. Because local farmers practice little selection, effective population sizes stay large, thereby decreasing the opportunity for fixation of any alleles.

The lower informativeness of the loci in the portion of the world collection that we sampled was expected because the material was inbred within the Bennetzen laboratory prior to the preparation of DNA samples (Oliveira et al. 1996; Yang et al. 1996). Less within-accession variation was expected and, in fact, all plants were homozygous for each locus tested. Hence, the greater variability present within the Eritrean accessions was partly due to the fact that 28 landraces were sampled as 138 individual plants (five for all landraces except two that were sampled with four plants each), many turning out to be highly heterozygous. The 32 world varieties, however, were sampled as essentially 32 inbred plants. Despite this difference, the within-landrace and between-landrace variation in the Eritrean sorghums is exceptional by any criteria.

This study provides a first detailed analysis and quantification of genetic diversity in Eritrean landraces of sorghum. The data also reaffirm the power of SSR markers to distinctly group closely related landraces. Several authors have indicated that SSR technology is highly cost-effective (Smith et al. 2000) and that this technology could easily be employed in resource-poor countries. It could provide efficient and fast screening for both germplasm conservation and crop improvement.

Our data demonstrate that landraces of Eritrean sorghums contain a great deal of genetic diversity as indi-

cated by the observed number of alleles, far beyond that observed in any other sorghum germplasm source of comparable sample number. Because these materials prevail in farmers' varieties, conservation strategies in Eritrea should focus on these resources. Many SSR loci will be significantly linked to important agronomic traits. Hence, Eritrean sorghums deserve broader characterization at both molecular and agricultural levels, including the molecular mapping of important traits (Lin et al. 1995; Peng et al. 1999; Lan and Paterson, 2000) that can be used in future crop improvement.

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