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RFLP diversity in cultivated sorghum in relation to racial differentiation

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Abstract Careful assessment of the comparative diversity for molecular markers and for potentially-useful morpho-agronomic traits is paramount to the analysis of a genome through the mapping of favorable genes. Sorghum (*Sorghum bicolor* ssp. *bicolor*) varieties are traditionally classified into five races on the basis of morphological traits, especially panicle and grain traits. Isozyme diversity has provided a new insight into genetic diversity, and showed a marked geographic structure. We performed RFLP analysis on 94 varieties, chosen to represent the main cross combinations (race × geographic origin), using 35 maize probes that detect polymorphism with at least one of the two restriction enzymes *Hin*dIII and *Xba*I. A total of 50 polymorphic probe-enzyme combinations yielded 158 polymorphic bands. The bicolor race appeared highly variable and included many rare markers. Among the other races multivariate analysis of the data differentiated six clusters corresponding, by decreasing magnitude of divergence, to: the margaritiferum types (a sub-race of race guinea); the guinea forms from western Africa; race caudatum; race durra; race kafir; and the guinea forms from southern Africa.The apparent geographic differentiation was related to the contrasting distribution of these races and to a higher similarity between races localized in southern Africa. The data agree with the current hypotheses on sorghum domestication but reveal associations between neutral markers and traits probably highly subjected to human selection. Whether such associations will be observed with other useful traits, and to what extent they are maintained by genetic linkage, is worth exploring.

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Introduction

Information about genetic diversity and relationships among landraces is essential for the improvement of agricultural plants and the management of their genetic resources. Various markers, essentially morphological and isozymic markers, are traditionally used for that purpose. Despite the relatively-small number of loci involved, they have allowed efficient varietal classification for a large array of crops.

In sorghum (*Sorghum bicolor* ssp. *bicolor)*, traditional cultivars were classified by Harlan and De Wet (1972) into five main races (bicolor, caudatum, durra, guinea, kafir) and ten intermediates (e.g., bicolor-caudatum, durra-kafir), mainly on the basis of spikelet and grain morphological criteria. This differentiation was documented with quantitative data by Chantereau et al. (1989).

Numerical analyses of isozyme diversity were recently performed (Morden et al. 1989; Ollitrault et al. 1989; Aldrich et al. 1992; Dégremont 1992) and compared to the scheme based on morphology. No evident congruence was found between the classical racial classification of Harlan and De Wet (1972) and the distribution of allele frequencies. At the most, concordance between the enzymatic differentiation and the racial classification appeared in particular geographic areas.

A recent study (Aldrich and Doebley 1992) evaluated RFLP diversity in the nuclear and chloroplast genomes of sorghum using 56 accessions. This study confirmed the earlier hypothesis that central-northeastern Africa is the most likely principal area of domestication of sorghum. Comparison of the nuclear and cytoplasmic genomes suggested that introgression between wild and cultivated forms occurs.

The aim of the research reported here is to study nuclear RFLP diversity in cultivated sorghum, using a sam-

Table 1 Accessions of sorghum included in this study with their seed source, their country of origin, racial classification, and classification based on isozymes

Accession number	Seed Source[a]	Geographic origin	Racial classification[b]		Isozyme classification[c]	
			(A)	(B)	(C)	(D)
		Western Africa				
IS 2156	1	Nigeria	B	B		
IS 10194	1	Burkina Faso	G-B	B		
IS 20145	1	Senegal	B	B		
		Central and eastern Africa				
IS 3450	1	Sudan	B	B		
IS 10816	1	Chad	G	B		
IS 12169	1	Ethiopia	B	B		
IS 12179	1	Ethiopia	B	B		
		Southern Africa				
IS 23178	1	Zambia	B	B		
IS 23254	1	Zambia	B	B		
		Asia				
IS 1237	1	India	B	B		
IS 1596	1	India	G	B		
IS 3780	1	China	G-B	B		
		America				
IS 5	1	Mexico	B	B	n. c.	
IS 13	1	USA	B	B	n. c.	
IS 21	1	USA	B	B	n. c.	
		Western Africa				
IS 10882	1	Nigeria	G-C	C		
509	2	Niger	C	C	II	
552	2	Niger	C	C	I	
1123	2	Niger	U	C	I	
		Central and eastern Africa				
IS 2262	1	Sudan	K-C	C	III	
IS 2787	1	Kenya	C	C	III	
IS 3073	1	Sudan	C	C	II	
IS 3511	1	Sudan	K-C	C	III	
IS 10234	1	Central African R.	G-C	C		
IS 11435	1	Ethiopia	C	C		
IS 15857	1	Cameroon	C	C	III	
215	2	Ethiopia	U	C	III	
		Southern Africa				
IS 2807	1	Zimbabwe	C	C	III	
IS 2814	1	Zimbabwe	C	C	III	
IS 9331	1	South Africa R.	K	C	II	
		Western Africa				
546	2	Niger	G-C	D	III	
973	2	Senegal	U	D	III	
1040	2	Senegal	D	D		
		Central and eastern Africa				
IS 929	1	Sudan	D	D	III	
IS 1398	1	Sudan	D	D	III	
IS 2263	1	Sudan	D	D	III	
IS 8542	1	Ethiopia	D	D	II	
IS 11575	1	Ethiopia	G-C	D		
		Asia				
IS 1037	1	India	D	D		
IS 1259	1	Burma	B	D	n. c.	
IS 5239	1	India	D	D	n. c.	
IS 8330	1	India	C	D	n. c.	
IS 8336	1	Pakistan	D	D		
IS 8339	1	Pakistan	D	D		
IS 17818	1	India	G-C	D		
		Western Africa				
205	2	Senegal	U	G	II	1
232	2	Burkina Faso	G	G	I	1

Table 1 (continued)

Accession number	Seed Source[a]	Geographic origin	Racial classification[b]		Isozyme classification[c]	
			(A)	(B)	(C)	(D)
249	2	Burkina Faso	G	G	I	1
261	2	Senegal	G	G	I	1
379	2	Mali	G-K	G	II	n.c.
557	2	Niger	U	G	III	n.c.
1057	2	Senegal	G	Gma	II	3
		Southern Africa				
IS 14317	1	Swaziland	G	G		2
IS 14331	1	South Africa R.	G	G		2
IS 14351	1	Malawi	C	G		2
IS 14414	1	Malawi	G	G	II	2
IS 14417	1	Malawi	G	G	II	2
IS 23100	1	Tanzania	G	G		2
IS 24072	1	Tanzania	G	G		2
		Asia				
IS 3958	1	Nepal	G	G		2
IS 4963	1	India	G	G		2
IS 12931	1	China	G	G		2
IS 19467	1	Sri Lanka	G	Gma		3
		Southern Africa				
IS 3151	1	South Africa R.	C	K	II	
IS 9303	1	South Africa R.	K	K	II	
IS 9337	1	South Africa R.	K	K	II	
IS 9450	1	South Africa R.	K	K		
IS 9468	1	South Africa R.	K	K	II	
IS 9473	1	South Africa R.	K	K	II	
IS 9508	1	South Africa R.	K	K	II	
IS 9527	1	South Africa R.	K	K	II	
IS 9549	1	South Africa R.	C	K	II	
IS 14282	1	Botswana	C	K	II	
		Western Africa				
IS 19437	1	Burkina Faso	G-C	G-C		
		Southern Africa				
IS 13452	1	Zimbabwe	G-C	G-C		
IS 21391	1	Lesotho	G-C	K-C		
IS 13481	1	Zimbabwe	G-C	K-D		
IS 14443	1	Lesotho	C-B	K-D		
		Asia				
IS 10302	1	Thailand	C	C-D	n. c.	
IS 303	1	China	K-B	K-B	n. c.	
IS 311	1	China	K-B	K-B	n. c.	
IS 313	1	China	K-B	K-B	n. c.	
IS 22584	1	Indonesia	G-D	K-D		
		Western Africa				
367	2	Mali	U	U	II	
535	2	Niger	U	U	III	
547	2	Niger	C	U	II	
1046	2	Senegal	U	U	III	
		Central and eastern Africa				
IS 10736	1	Chad	G-C	U		
IS 19187	1	Sudan	G-C	U		
		Southern Africa				
IS 23092	1	Tanzania	G-C	U		
1293	2	Rwanda	U	U		
1294	2	Rwanda	U	U		
		Asia				
655	2	Pakistan	U	U		
1474	2	China	C	U		

[a] Seed source: 1 ICRISAT 2 CIRAD – GERVEX

[b] A: classification as in the ICRISAT or CIRAD – GERVEX catalog
B: classification based on the discriminant traits identified by Chantereau et al. (1989); B, bicolor; C, caudatum, D, durra; G, guin-

ea, Gma, G margaritiferum; K, kafir; X-Y, intermediate between X and Y; U, unspecified
[c] C: classification in the scheme of Ollitrault et al. (1989); groups I, II, III and not classified (n.c.)
D: classification in the within-guinea scheme of Dégremont (1992); groups 1, 2, 3 and not classified (n.c.)

ple of accessions carefully selected to take advantage of earlier studies conducted with other types of markers. This allows testing association among traits and parameters of different nature. This first step will serve to construct a wider sample in order to approach a core collection (Frankel and Brown 1984; Brown 1989). The congruence with results obtained earlier with other types of markers is tested.

Materials and methods

Plant material

A sample of 94 sorghum cultivars (Table 1) was chosen on the basis of their geographical origin and where possible on the basis of biochemical and morphological criteria obtained previously (Chantereau et al. 1989; Ollitrault et al. 1989 and Dégremont 1992). Seeds were obtained from ICRISAT (Hyderabad, India) or from the CIRAD Seed Unit (Montpellier, France). Two representative inbred lines of maize (Mo 17 and B 73) were included in this study to test proper maize-probe hybridization.

RFLP analysis

Taking advantage of the homology between maize and sorghum genomes (Hulbert et al. 1990; Aldrich and Doebley 1992; Binelli et al. 1992; Whitkus et al. 1992; Bennetzen and Melake-Berhan 1993), heterologous maize probes were used, and their location on maize chromosomes served as a basis to cover the genome of sorghum.

The maize probes used were kindly provided by D. Hoisington (CIMMYT). They are low-copy-number genomic fragments produced at the University of Missouri, Columbia (designated UMC) or at the Brookhaven National Laboratory (designated BNL). They were chosen to provide a fairly uniform coverage of the genome with four probes per chromosome (see Table 2).

In order to have access to possible heterogeneity within the accessions, DNA was isolated from a mixture of leaves of three 35-day-old seedlings according to the protocol of Dellaporta et al. (1983) modified by Cordesse et al. (1990). About 2 μg of DNA per sample were digested separately with the restriction enzymes *Hin*dIII and *Xba*I according to the supplier's instructions (Bethesda Research Laboratory) and in about three-fold excess. Restricted DNAs and, for each sample, two additional internal fragments, 1.5 and 24.8 kb in size, of digested lambda DNA, were electrophoresed (in 0.7% agarose gels) and transferred to nylon membranes (Hybond N, Amersham) applying the Southern procedure.

There were four combs and 30 lanes per comb for each gel. A molecular-weight marker (Raoul I, Appligene) was loaded at every seventh and 24th lane. The maize DNA probes were isolated by electrophoresis in low-melting-point agarose gels and labelled with ^{32}P-dCTP applying the random primer method (Feinberg and Vogelstein 1983). Prehybridization, hybridization and washes were performed under standard conditions used at CIMMYT (Hoisington 1992). Autoradiographs were exposed at −80 °C for 5 days using Fuji RX films and two intensifying screens.

Statistical analyses

Fragment lengths were estimated with a computer program (Hoisington and Gonzalez-de-Leon personal communication) by interpolation from a standard curve obtained from the molecular-weight marker and the two internal lambda fragments. The factor analysis of correspondences (FAC) method (Benzecri 1973) was used to obtain a synthetic picture of the organization of molecular variation. This method treats qualitative data. For all of the probe/enzyme combinations, each band was considered as a character and each accession was characterized by all the bands it displayed. The presence or absence of a band in a lane was coded as 1 or 0, respectively. The FAC identifies several independent axes (eigenvectors) that account for the largest part of the variation. These axes are linear combinations of the characters, and each accession can be located along the axes. The loadings of the axes on the characters measure the discriminative power of these characters. When a few accessions are strongly differentiated from the others due to specific bands, the main axes of the FAC will be largely determined by these markers and may not resolve the rest of the accessions. It may be worthwhile repeating the analysis after taking these bands as inactive variables. The specific bands of groups identified by the previous FAC were successively taken as inactive variables. "Rare" bands, that is to say, those in only one accession, were treated in the same way. Thus, three FACs were performed iteratively.

A hierarchical classification analysis was performed to determine the aggregation of the accessions into various clusters. For doing so, Dice's (1945) index of distance d(j, k)=1−(2x No. shared fragments)/(No. fragment j+No. fragment k) was calculated for all pairwise comparisons. Only probe-enzyme combinations which generated polymorphisms were used for the calculation of the distance index. Then, a dendrogram was constructed via the unweighted pair-group method with average squared distance minimization. All computations were performed by using appropriate procedures of ADDAD (1983) software.

Results

Genetic variation for RFLPs

A set of 41 maize probes, well scattered over the maize genome, was tested on the 94 accessions. Under the stringency conditions employed, 33 of the probes (80%) provided a sufficient quality of hybridization to sorghum DNA digested with both enzymes and two did so with only one enzyme. Most probes revealed only one band for most accessions. The exceptions were probe BNL 7.49, BNL 15.20 and BNL 16.06, which revealed two strong bands per accession for both enzymes. The most likely explanation is that these probes hybridize to sequences that are duplicated in sorghum. Five other cases of possible duplication were observed with UMC 38, UMC 42, UMC 64, UMC 113 and UMC 124, which generally revealed one strong band and one faint band for both enzymes; here the stringency used allowed strong hybridization at only one locus.

Four probes did not detect an RFLP regardless of the restriction enzyme used and 11 detected polymorphism with only one enzyme. Finally, 50 polymorphic probe-enzyme combinations yielded 158 polymorphic RFLP bands which were taken into account for the statistical analyses. The polymorphism (% polymorphic probes) detected with *Hin*dIII and *Xba*I was 74.2% and 72.7%, respectively (Table 2). The maximum number of RFLPs detected by a single probe-enzyme combination was seven. The average

Table 2 Probes tested and number of bands revealed in our sorghum sample

Maize chromo-some	Probe	*Hin*dIII		*Xba*I	
		1 band per accession	2 bands per accession	1 band per accession	2 bands per accession
2	UMC 4	2		2	
2	UMC 6	(1)		(1)	
3	UMC 10	3		(1)	
4	UMC 15	3		5	
5	UMC 27	4		3	
8	UMC 30				
3	UMC 32	(1)		3	
6	UMC 38	2	*	3	*
3	UMC 39	(1)		(1)	
4	UMC 42	2	*	3	*
5	UMC 43				
10	UMC 44	2		3	
6	UMC 46	(1)			(2)
2	UMC 53	3		4	
10	UMC 64	3		6	*
5	UMC 68	(1)		3	
1	UMC 83	(1)		2	
6	UMC 85	4		2	
8	UMC 89				
8	UMC 93	2		5	
9	UMC 94	3		(1)	
1	UMC 107	3	*	3	
7	UMC 110				
9	UMC 113	2		4	*
9	UMC 114	(1)		(1)	
7	UMC 116				
8	UMC 124	5	*	4	*
5	UMC 126	2			
2	UMC 135	4		3	
5	UMC 147	(1)		2	
1	UMC 157	3		(1)	
1	UMC 167	4		3	
7	UMC 168	3		(1)	
10	BNL 3.04		(1) + 2	2	
9	BNL 5.09	4			4
5	BNL 6.25				
10	BNL 7.49		(1) + 2		(2)
8	BNL 9.11		(2)	2	
7	BNL 14.07	3		(1)	
3	BNL 15.20		6		7
7	BNL 16.06		(1) + 2		(1) + 2

Whether the probe reveals generally one or two bands per accession is indicated except for UMC 32 for which 1 or 2 bands per accession are equally frequent

* Identifies cases where there were additional weak bands not taken into account in the analysis

Parentheses identify monomorphic bands

No information means that no distinct band could be scored

number of variants detected by probes that detect one or more polymorphic locus was 3.1 and 3.4 for *Hin*dIII and *Xba*I, respectively. A large fraction of these variants were very rare; in particular, 14% of them occurred in only a single accession.

Multilocus structure

Considering all loci simultaneously, the level of variation detected enabled differentiation of 90 types among the 94 accessions surveyed. The number of differences between two accessions ranged between 0 and 60%, and averaged 33% of the 158 polymorphic bands. Relationships among the accessions were investigated using multivariate analysis. The first FAC identified two small groups, one composed of two bicolor accessions from Ethiopia and the other of two guinea accessions belonging to sub-race margaritiferum. A second FAC identified five guinea accessions from western Africa. The third FAC identified three main axes which accounted for 26.4%, 15.2%, and 10% of the whole variation, respectively. The distributions of the accessions in plane (1.2) are shown in Fig. 1. A geographic differentiation in Africa is noted along axis 1 (Fig. 1 a), the left part of the plane (negative coordinates) being essentially populated with southern African accessions whereas the right part is populated with accessions from the rest of Africa. Considering the morphological classification previously established, a pattern related to the race can be noted (Fig. 1 b), with kafir accessions in the upper left portion of the plane, most durra varieties in the upper right portion, and most caudatum varieties in the lower right portion. Within race guinea, the accessions from southern Africa and Asia cluster in the lower left portion, whereas the accessions from western Africa (which were already isolated in the previous FAC) cluster in the center right portion of the plane. The bicolor accessions had a widespread distribution

The dendrogram obtained from Dice's distances (Fig. 2) confirms the results of the FAC. Besides two distant bicolor accessions, six major clusters can be distinguished, by decreasing magnitude of divergence:

- a small group including the two margaritiferum accessions, two atypical accessions and one bicolor accession;
- a group of western African accessions mostly belonging to race guinea;
- a group of accessions from various geographic origins with a majority of durra accessions from Asia and central and eastern Africa;
- a group of accessions from diverse geographic origins, mostly belonging to race caudatum;
- two groups of accessions essentially from southern Africa, one corresponding to race kafir, the other corresponding to race guinea.

Discriminative markers

The most discriminative markers were identified using their loadings on the main axes of the three FACs. Eighteen probes allow a good discrimination between the clusters of the hierarchical classification. In three cases (BNL 15.20, UMC 4 and UMC 107), the two enzymes (*Hin*dIII and *Xba*I) convey the same information, while in six cases (UMC 27, UMC 42, UMC 53, UMC 93 and UMC 113) the information of *Xba*I is complementary to *Hin*dIII. Five probes (BNL 14.07, UMC 10, UMC 15, UMC 44 and UMC 126) are informative in combination with the enzyme *Hin*dIII, and four probes (BNL 5.09, UMC 32, UMC 68 and UMC 124) in combination with *Xba*I.

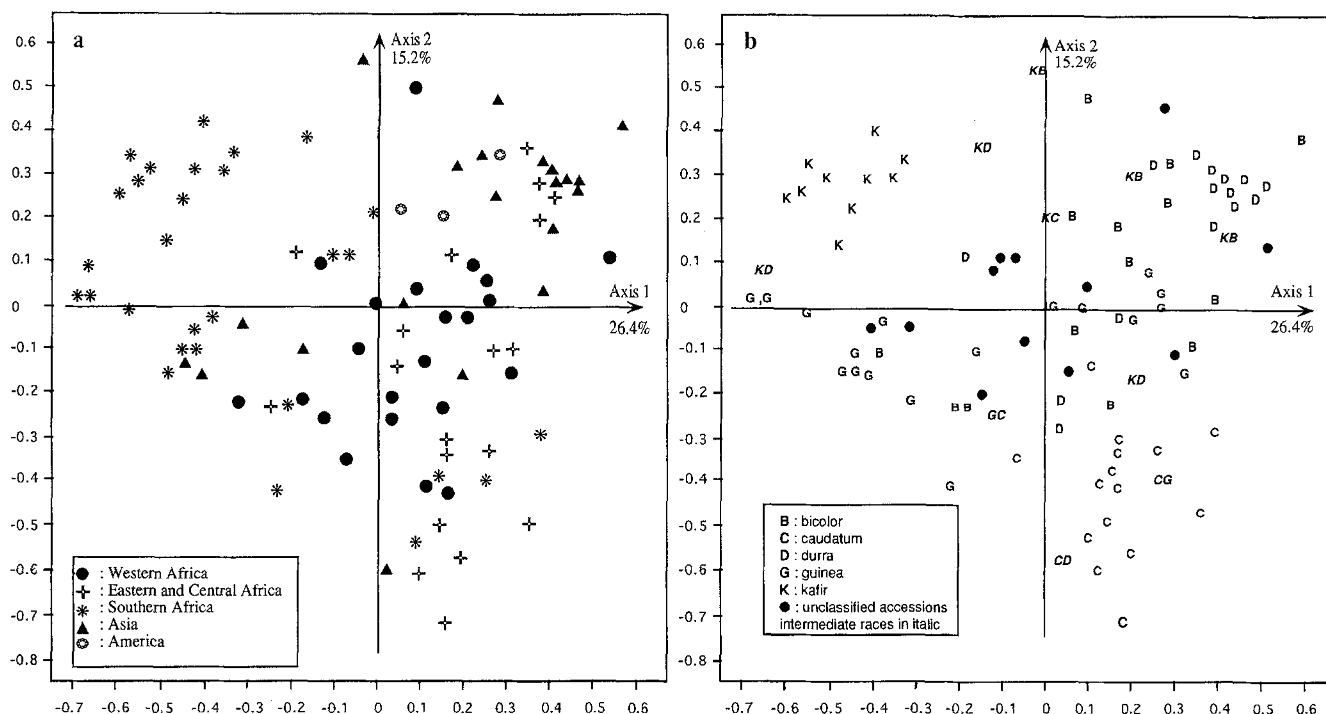

Fig. 1 a, b Distribution of 94 accessions of sorghum in plane (1, 2) of the factor analysis of correspondences among the RFLP data for 50 probe-enzyme combinations, using 110 of 158 observed bands as active variables (see text); the accessions are symbolized according to **a** their geographic origin, **b** their morphological race

Given the present state of knowledge of the molecular map of *Sorghum*, it is not possible to precisely analyze the distribution of the markers which discriminate the morphological races. The regions involved in racial differentiation, as defined by the markers used, appear to be scattered over the whole sorghum genome as compared to the maize map, which is indicative of the sorghum map to a significant extent (data not shown).

Discussion

Our study revealed an important degree of RFLP polymorphism in cultivated sorghum as did the earlier study of Aldrich and Doebley (1992).

The large amount of molecular variation allows a thorough analysis of the organization of genetic diversity within cultivated germplasm. Earlier studies using morphological traits distinguished races bicolor, caudatum, durra, guinea and kafir, although the differentiation was weaker between bicolor and guinea and between caudatum and kafir. Biochemical markers did not distinguish well between these morphological races. Instead, they showed the importance of geographic origins, for the varieties tended to cluster into a western African group, a southern African group and a central-eastern African group rather than into racial clusters. They also showed that race guinea can be split into three groups corresponding to sub-race margaritiferum, whatever the geographic origin, to western African forms and to southern African forms (Ollitrault et al. 1989; Dégremont 1992). Other studies also highlighted this relation to the geographic origins (Morden et al. 1989).

RFLPs offer a slightly different perception of the species organization and interracial relationships. Race bicolor is the most variable race. Its representatives do not form a specific group but they are distributed among the various clusters; some of them are strongly differentiated from the rest of the species, like the two accessions from Ethiopia. Race guinea appears with RFLPs as it appeared with isozymes, i.e., organized into three groups, corresponding to the margaritiferum sub-race, to the western African forms and to the southern African forms. Caudatum accessions cluster into one main group, despite their widespread geographic origin. Durra accessions, which were sampled mainly from central-eastern Africa and Asia, cluster into a single group. Kafir accessions, all of which come from southern Africa, form a separate group.

As compared to studies with isozymes, our analysis highlights the relationships between molecular variation and racial differentiation. The new information resides in the molecular differentiation between races kafir and guinea in southern Africa and between caudatum and durra in central-eastern Africa. This corresponds to axis 2 of the final FAC (Fig. 1). This observation remains compatible with the domestication scheme proposed by various authors (Vavilov 1950; Doggett 1970; De Wet and Harlan 1971; Harlan and Stemler 1976) and discussed by Ollitrault et al. (1989). Future studies will also have to take into account cytoplasmic DNA diversity and will need to give more emphasis to races guinea and bicolor, which appear much more variable than other races. Wild-types can then

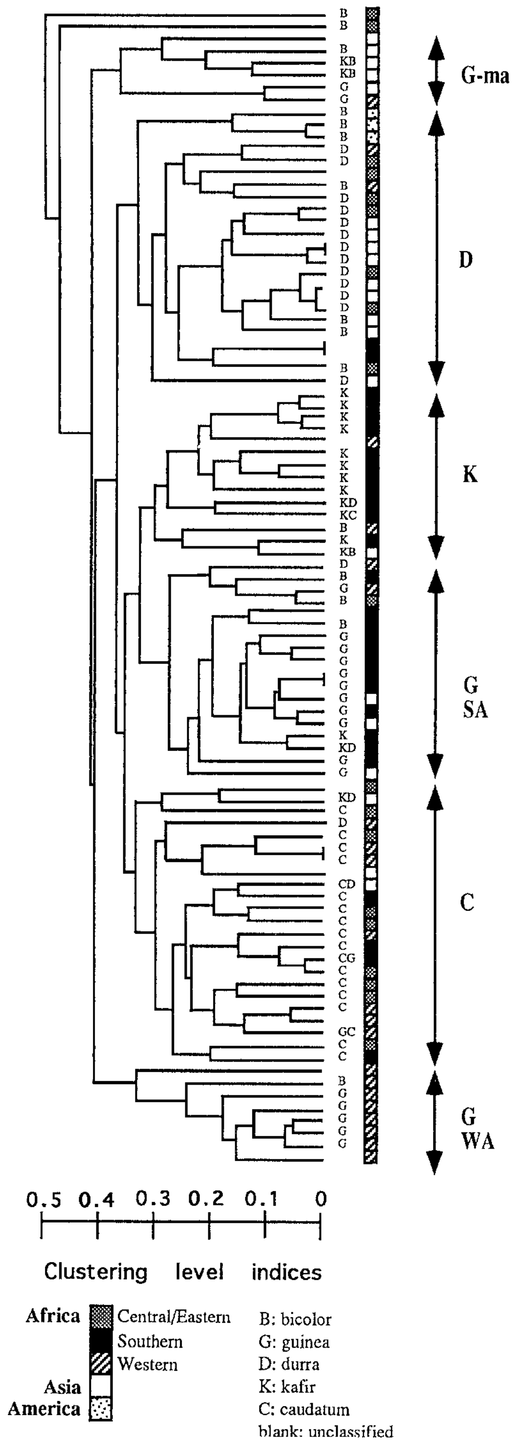

Fig. 2 Cluster analysis based on RFLP data among 94 cultivated sorghum accessions using the squared distance of Dice and the average distance minimization criterion

be studied in relation to the genetic structure within cultivated sorghum.

The congruence between the morphological classification and the classification based on molecular markers raises the question of the pressures which affect the two types of characters. Morphological traits are obviously affected by human selection whereas molecular markers are most likely neutral markers. Initially, these correlations probably arose from multitrait associations resulting from the founder effect associated with domestication. The persistance of such correlations requires a restricted recombination between the genetic factors underlying these traits. This restriction can be due to a limited or only recent contact between the various races, a limited cross pollination or reproductive barriers. It is known that the rate of allogamy can be significant in cultivated sorghum, ranging from 5 to 7% in race durra (Doggett 1988) and from 10 to 30% for race guinea (Ollitrault 1987). It is also known that reproductive barriers, if any, are at the most very weak and that gene segregation in interracial hybrids is normal (Dégremont 1992). The gene exchanges between the races may, however, be limited by asynchronous flowering, by ecological specialization, and by a contrasting microgeographic pattern related to ethnical distributions of farmers in Africa (Harlan and Stemler 1976; De Wet 1978). Lastly, restricted recombination can be the consequence of genetic linkage. If some of the molecular markers are closely linked to genetic factors controlling the traits responsible for race differentiation, then these markers will be affected by linkage drag during selection by humans which maintains the phenotypic differentiation between the races. Although this hypothesis is rather unlikely, it deserves testing for further interpretation of phenotypic diversity in sorghum. We are now engaged in a program aiming at mapping the genes responsible for grain and panicle traits in the genome of sorghum; this will allow both for testing the validity of the above hypothesis and assessing the possibility for marker-assisted selection of grain quality factors in sorghum.

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