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Enzyme diversity in pearl millet (*Pennisetum glaucum* L.)

3. Wild millet

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Summary. One hundred and eighty-eight accessions of wild millet, *Pennisetum glaucum* L. subsp. *monodii* [syn. *P. violaceum* (Lam.). L. Rich], representative of the species' geographic distribution, were studied by electrophoresis for polymorphism in eight enzymes: alcohol dehydrogenase (ADH), catalase (CAT), β-esterase (EST), glutamate oxalo acetate transaminase (GOT), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucoisomerase (PGI), and phosphoglucomutase (PGM). The frequencies of 46 alleles at 12 loci controlling the eight enzyme systems were analyzed by principal component analysis and confirmed by discriminant analysis. A pattern of enzymic variation corresponding to geographical zonation in five groups was discerned: Western Group (Senegal, Mauritania, western Mali), Central Group (eastern Mali and Niger), Aïr Group (Aïr Mountains of Niger), West Chad Group, and Darfur Group (encompassing eastern Chad and western Sudan). Nei's diversities varied from 0.161 ± 0.010 in the Aïr Group to 0.243 ± 0.004 in the Central Group. Comparison with Sahelian cultivated millet showed clear-cut divergence between populations of wild and cultivated millet in each region, even where they grow sympatrically. Cultivated and wild millet equal total Nei's diversity, but their locus by locus diversities are different. Wild millet, particularly populations growing far from the crop (allopatric wild accessions), is the most diverse for *Got A*, *Pgd A*, and *Cat A*, whereas cultivated millets are the most diverse for *Pgm A* and *Pgi A*. Allopatric wild millet populations are more divergent from cultivated populations than sympatric wild millets. The cultivated millets closest to wild millet were from western Mali, with the most remote in the Darfur Group. Based on these results, the process of pearl millet domestication is discussed.

Key words: Isoenzymes – Wild pearl millet – *Pennisetum glaucum* – Domestication – Genetic distances

Introduction

Lack of a representative collection of wild millet has up to now limited a thorough study of the relationship between cultivated millet and its wild relative. Nevertheless, there has been research in this area. Brunken (1977) and Brunken et al. (1977) greatly simplified the taxonomy of pearl millet by pooling, in the same biological species, the wild form [*P. violaceum* (Lam.) L. Rich] and the cultivated form (*P. americanum* subsp. *americanum*] on the basis of similarity in morphological characters. The wild form has a short involucral pedicel length, one small seed per spikelet, and low morphological diversity. It inhabits disturbed environments like temporary stream beds, roadsides, and around dwellings. After shedding, the seeds are probably dispersed by wind and water. Brunken (1977) suggests that the wild type has evolved little since the time of pearl millet domestication, but that its geographic distribution from Senegal to Ethiopia then extended much further north than today. He explains the high morphological diversity of cultivated millet by an adaptative post-domestication differentiation associated with the introgression of local wild millet germ plasm and the colonization of new environments. The history of pearl millet domestication can be traced only from observation of today's wild and cultivated millet, their geographic distributions, and genetic diversities, because archeological data are absent.

Grouzis (1979) gives ecological reasons to distinguish wild millet *sensu-stricto* growing far from the crop and

nitro-anthropophilous wild millet growing in the neighborhood of cultivation, although both are indistinguishable on the basis of domestication characters. According to his findings, wild millet is distributed from latitude 13°N to 21°N.

Clegg et al. (1984), studying a portion of chloroplastic DNA, found no polymorphism among several wild and cultivated millet samples. Gepts and Clegg (1989) observed greater diversity in wild millet than in cultivated for ribosomal DNA, and suggest a unique domestication event without being able to locate it. Laguda and Hanna (1989) noticed differences between wild and cultivated accessions for the distribution of *Est 1* allozymes, and a higher frequency of heterozygotes among cultivates accessions than among wild ones. On the basis of ADH variants, Gepts and Clegg (1989) split the Sahelian area into a western and an eastern region. In the enzymic study of West African millet by Tostain et al. (1987), a small sample of eight wild millet accessions appeared to form a cluster distinct from cultivated millet, but closer to early-maturing cultivars than to late-maturing types.

In order to verify these earlier results and study new sources of genetic diversity, wild millet was collected during 5 successive years across the Sahel and the accessions were described for their enzyme polymorphisms.

Materials and methods

A total of 240 accessions of wild millet was collected in the Sahelian zone of Africa from 1985 to 1989 (Tostain et al. 1986). Each accession studied comprised a bulk sample of seed from a large number of plants in the population. Some of the samples came from previous collecting trips made in 1975 in Mali by Grouzis and Marchais (Grouzis 1979), by Clément and Séquier in Cameroon in 1976, and by Clément and Leblanc in Mauritania in 1984. Two samples from Sudan (PS 65 and PS 66) were provided by Dr. Hanna of USDA, Tifton, USA.

Of this collection, 188 accessions were analyzed by electrophoresis. Their origins are shown in Fig. 1 and are as follows; Mauritania, 20; Senegal, 10; western Mali, 23; eastern Mali, 39, including 13 accessions from the Adrar des Iforas; Niger, 52, including 15 accessions from the Aïr Mountains; Burkina Faso, 6; western Chad, 11; eastern Chad, 10; Sudan, 12; Nigeria, 4; and Cameroon, 1. Ninety-one accessions came from the pastoral zone where there is no pearl millet cultivation and, following Grouzis (1979), who divides wild millets into two groups according to their association with the crop, can be called allopatric. Ninety-seven accessions were collected in the agricultural zone and are therefore termed sympatric. The collectors sampled plants that looked wild, but since the populations are open-pollinated, it is highly probable that the seeds from plants in the agricultural zone contain some alleles received from the neighboring crop, and that the genetic structure of the parental wild plants would therefore be slightly different. The comparison of these allopatric and sympatric wild millets should give an idea of the overall introgression of cultivated genes into wild populations. In addition, pairs of wild and cultivated accessions from the same village or from banks of the same temporary river in Mali (nine pairs) and in Chad (six pairs) were studied, in order to observe the degree of isolation between the subspecies.

A total of 123 accessions of cultivated millet was used for a comparison between wild millet and the crop. They include accessions from Sub-Saharan Africa already analyzed by Tostain et al. (1987) and Tostain and Marchais (1989), plus additional samples from northern Africa, Chad, and Mali. Their distribution is as follows. (a) Early-maturing cultivars from Western-Africa: Senegal, 7; Mauritania, 5; western Mali, 19; eastern Mali, 19, Niger, 16, including 6 accessions from Aïr oases; Togo, 3; Bénin, 1; Burkina Faso, 2; Ghana, 5; Guinée, 1. (b) East-central Africa: Chad, 23, including 17 early and 6 late-maturing cultivars; Sudan, 6, including 3 early- and 3 late-maturing cultivars; Cameroon, 2. (c) Northern Africa: oases in Algeria, 10; and Tunisia, 4.

Eight enzyme systems were studied: alcohol dehydrogenase (ADH), catalase (CAT), β-esterase (EST), glutamate oxalo acetate transaminase (GOT), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD) phosphoglucoisomerase (PGI), and phosphoglucomutase (PGM). The electrophoresis techniques and genetic control of these enzyme systems were explained in previous papers (Tostain et al. 1987; Tostain and Marchais 1989). ADH was separated on starch gels at pH 6.0 and pH 8.0 to reveal pH-sensitive allozymes. EST was separated on 7% polyacrylamide gels. CAT was extracted from non-pigmented younger leaves and separated on 11% starch gel at pH 8 and 100 V overnight, due to the large size and low charge of CAT tetramers. Other systems were analyzed on extracts from the young green leaves of 2- to 3-week-old plants. GOT and MDH were separated at pH 6.0, whereas PGI, PGM, and PGD were analyzed at pH 8.0. For each accession, 12 plants were used for ADH, CAT, GOT, MDH, PGI, and PGM, 24 plants for PGD, and 26 for EST. For each accession, 46 allelic frequencies corresponding to 12 loci were measured and analyzed, using principal component analysis on a variance-covariance matrix and discriminant analysis to identify groupings. In the second step, the groups were characterized by Nei's parameters (1975) calculated as follows: genetic diversity, Hx, of each sample X: $Hx = \Sigma_i\ (1-\Sigma_a\ X^2_{ai})/\ I$ with X_{ai} frequency to allele a from locus i and I the number of loci ($I = 12$); differentiation coefficient, Gst, between different samples: $G_{st} = (H_t - H_s)/H_t$ with H_t the group genetic diversity and H_s the average of the sample diversity; minimum distance, Dm, between two samples X, Y: $D_m(X, Y) = \Sigma_i\ \Sigma_a\ (X_{ai} - Y_{ai})^2/2I$.

Multivariate analyses were carried out using the software Stat-Itcf (ITCF 1987).

Results

Geographic distribution of wild millet

From the collecting expeditions, it appears that today, wild pearl millet is distributed from the Atlantic ocean to Sudan, a distance of about 5,000 km from longitude 17°W to 25°E, and from northern Nigeria to the Adrar des Iforas in Mali, latitude 12°N to 20°N (Fig. 1). In Senegal and western Mali, wild millet grows in disturbed habitats as a weed on roadsides, field borders, and around dwellings. Elsewhere, in Tagant in Mauritania, Gourma and Adrar des Iforas in Mali, Azawak Valley, Tiguidit Cliff, and Aïr in Niger, it grows as a wild grass mainly along the banks of temporary rivers, on flood plains with alluvial deposits, at the foot of rocky outcrops, or on the sandy borders of temporary ponds. In

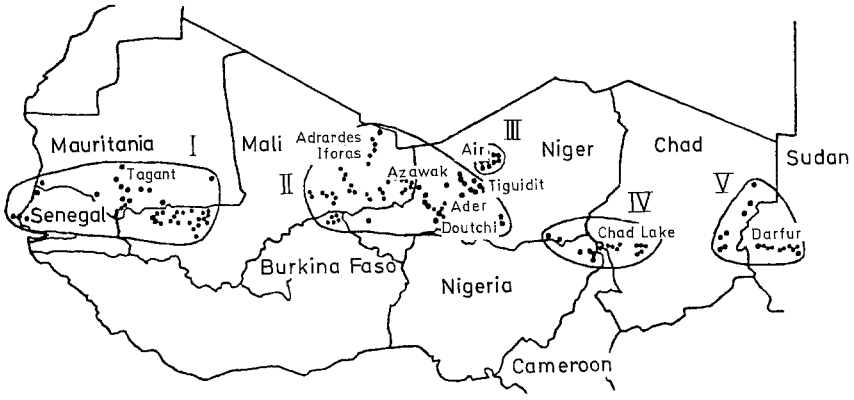

Fig. 1. Geographic distribution of the 177 wild millet accessions analyzed and their grouping by multivariate analysis. I = Western; II = Central; III = Aïr; IV = West Chad; V = Darfur

Ader Doutchi in Niger, western Chad, and Darfur in Sudan, it is found in typically wild habitats, but in contact with the crop. In the pastoral zone, allopatric wild millet populations grow in association with the annual grass *Cenchrus biflorus*, perennial grasses *Cymbopogon proximus*, *Panicum turgidum*, and the tree, *Acacia ehrenbergiana* (Grouzis 1979). Wild millet is often browsed by donkeys, but rarely by camels, goats, or cows. The populations are mainly distributed as scattered, dense patches. The biggest stand was observed in 1988 after abundant rains, south of Lake Chad, covering an area of about 700 km^2. Along the Tiguidit Cliff in Niger, wild millet forms a belt 100 km long of small dispersed patches.

Rainfall varies from 800 mm on the southern margin of the distribution zone to 50 mm in the Sahara (Adrar des Iforas, Aïr Mountains). In the latter case, because of erratic rains, populations vary greatly in size from year to year and can disappear. When climatic conditions are favorable, the population starts multiplying again from a small number of founder plants. Populations sympatric with the crop keep the botanical characteristics of allopatric wild millet, but have growth characteristics showing adaptation to a better environment (for example, taller size, better resistance to downy mildew).

Wild millet groups

Principal component analysis shows five groups: 1. Darfur and eastern Chad; 2. western Chad and Nigeria, called the West Chad Group; 3. Aïr; 4. eastern Mali and Niger, called the Central Group; 5. Senegal, Mauritania, and western Mali, called the Western group (Figs. 2 and 3). Axis 1 distinguishes the Aïr, West Chad, and Darfur Groups from the Western and Central Groups which are separated on axis 2. Axis 1 expresses variability for *Adh A2*, *Adh A4*, *Got A1*, *Cat A1*, *Mdh A3*, and *Pgm A2*, and axis 2 for *Adh A6* and *Adh A4*. Axis 3 separates the West Chad and Aïr Groups and expresses variability for *Est A4* and *Cat A2*. The validity of these five groups has been

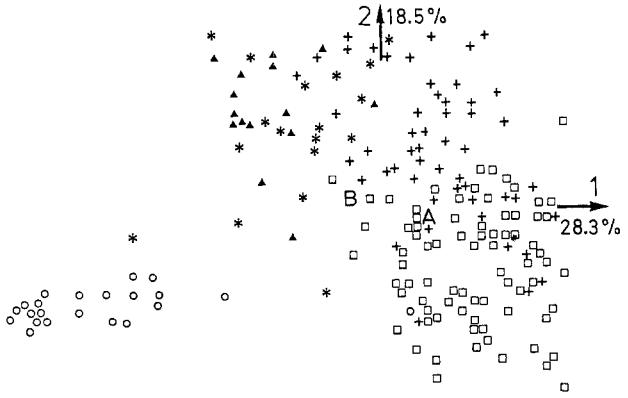

Fig. 2. Principal component analysis of 188 wild millet accessions. Projection on the plane (1, 2). + = Western; □ = Central; ▲ = Aïr; * = West Chad; o = Darfur. A: center of gravity of allopatric wild millets. B: center of gravity of sympatric wild millets

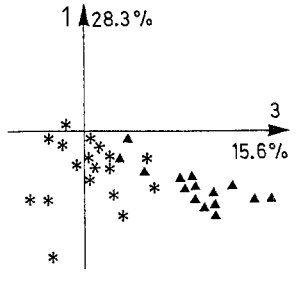

Fig. 3. Principal component analysis of 188 wild millet accessions. Projections on axes (1, 3) of accessions from West Chad (*) and Aïr (▲)

corroborated by a discriminant analysis, which shows 93% of the accessions well classified.

The groupings are distinguished by five alleles (Table 2), of which three are *Adh A* alleles. The most discriminating allele is *Adh A2*, which is only present in the Darfur Group with a high frequency. Several alleles are quasi-fixed in some groups: *Adh A4* and *Est A4* in the Adrar des Iforas millets; *Adh A6* and *Est A4* in the Aïr millets; *Adh A2* in the Darfur accessions.

Table 1. Nei's parameters for the five wild millet groups: gene diversity *Hx*, differentiation coefficient *Gst*, and minimum distances

Group	Accession number	*Hx*	*Gst*	Nei's minimum distance			
				Central	Aïr	West Chad	Darfur
Western	53	0.206±0.005	0.134	0.013±0.002	0.039±0.004	0.016±0.003	0.065±0.004
Central	80	0.243±0.004	0.149		0.044±0.004	0.026±0.003	0.066±0.004
Aïr	15	0.161±0.010	0.152			0.023±0.003	0.072±0.005
West Chad	18	0.233±0.009	0.119				0.046±0.005
Darfur	22	0.220±0.001	0.125				

Table 2. Mean frequencies of alleles discriminating the five groups

Allele	Enzymatic group				
	Western	Central	Aïr	West Chad	Darfur
Adh A2	0.01±0.00	0.00±0.00	0.00±0.00	0.10±0.15	0.83±0.16
Adh A4	0.35±0.21	0.62±0.19	0.21±0.16	0.20±0.12	0.10±0.15
Adh A6	0.62±0.21	0.34±0.17	0.78±0.16	0.65±0.24	0.05±0.06
Cat A1	0.64±0.18	0.54±0.19	0.11±0.15	0.31±0.16	0.25±0.18
Got A1	0.08±0.11	0.29±0.17	0.01±0.02	0.04±0.10	0.00±0.00

Table 3. Nei's diversities for individual loci and wild millet groups

Locus	Group					Wild millet
	Western	Central	Aïr	West Chad	Darfur	
Adh A	0.48±0.01	0.49±0.01	0.35±0.05	0.53±0.04	0.29±0.04	0.61±0.00
Cat A	0.46±0.01	0.49±0.00	0.19±0.05	0.43±0.03	0.38±0.03	0.50±0.00
Est A	0.79±0.01	0.77±0.01	0.48±0.03	0.80±0.01	0.80±0.01	0.78±0.02
Got A	0.15±0.02	0.41±0.02	0.04±0.02	0.09±0.03	0.00±0.00	0.26±0.01
Got B	0.03±0.01	0.09±0.02	0.07±0.03	0.02±0.06	0.05±0.02	0.06±0.01
Mdh A	0.02±0.01	0.01±0.01	0.18±0.05	0.03±0.02	0.28±0.04	0.06±0.01
Mdh B	0.01±0.01	0.01±0.00	0.19±0.05	0.00±0.00	0.01±0.01	0.02±0.01
Mdh C	0.01±0.00	0.02±0.01	0.00±0.00	0.01±0.01	0.01±0.01	0.02±0.00
Pgi A	0.22±0.03	0.12±0.02	0.06±0.03	0.22±0.05	0.18±0.04	0.16±0.01
Pgd A	0.21±0.03	0.36±0.02	0.37±0.04	0.39±0.04	0.20±0.04	0.32±0.01
Pgd B	0.00±0.02	0.01±0.00	0.00±0.00	0.00±0.00	0.03±0.02	0.01±0.00
Pgm A	0.07±0.02	0.11±0.02	0.01±0.01	0.26±0.04	0.40±0.03	0.15±0.01

Allopatric and sympatric wild millets are not differentiated by these analyses (Fig. 2).

Genetic parameters of groups

The Central Group, which comprises accessions from eastern Mali and Niger, has the highest diversity (Table 1), in particular millet from the Menaka region in Mali (0.257±0.007). It also has a high rate of differentiation (Gst), which reveals the existence of subgroups within this widely distributed group. Accessions from the Adrar des Iforas, for instance, form a separate subgroup, but it is less distinct than the Aïr Group. The Aïr Group is the least diverse but has the highest Gst, most probably due to the separate evolution of small populations in isolated valleys.

In the Western Group, the millets of Senegal and Mauritania have a lower diversity (0.190±0.011) than those of western Mali (0.219±0.007), which have also the lowest Gst in that group. The part played by an individual locus in the total diversity changes according to the group (Table 3). The *Adh* locus variability is higher in the total collection than for each of the groups. This can be explained by the high frequency of *Adh A2* in the Darfur accessions and absence of the allele elsewhere (Table 2). The same is true for *Cat A*, but due instead to an inversion of its frequencies between the west and east of the Sahelian zone. The low diversity of Aïr millets is due primarly to low diversity of *Est A*. The Central Group possesses a specific *Got A1* allele but with a frequency of about 0.30. The *Pgm A* diversity is highest in accessions from western Chad and Darfur in Sudan.

The Darfur Group is the most distant of all the groups and Western and Central Groups are the closest (Table 1). The separation between allopatric and sympatric millets is low, but sympatric millets have a slightly higher diversity (Table 6).

Comparison between wild and cultivated millets by groups

The Western Group. The 53 accessions of the Western Group were compared to 31 samples of cultivated millet from the same region. Wild and cultivated millets are clearly separated on axes 1 and 2 of the principal component analysis (Fig. 4). Among the cultivated accessions, those from western Mali appear distinct from samples from Senegal and Mauritania, as was shown in a previous paper (Tostain et al. 1987).

Wild millet accessions from western Mali, which are all sympatric with the crop, appear intermediate between allopatric wild millets and cultivated millets. The latter are more diverse than the wild millets, and their differentiation is high due to the divergence between the Senegal and western Mali accessions (Table 4).

Discriminant analysis corroborates the separation between wild and cultivated millets. The alleles discriminating between wild and cultivated accessions are *Pgm A1* (0.04 and 0.27, respectively), followed by *Adh A6* and *Adh A1*. Allopatric and sympatric wild millet accessions show 20% common grouping, which indicates their weak divergence, but *Adh A* diversity is higher in the allopatric samples.

The cultivated millets from western Mali are the closest to wild millet and consequently decrease the total variability of cultivated millets of that region from 0.234 to 0.221. The Western Group is exceptional in having closer wild and cultivated accessions than in other groups (Table 4). Within the group, the greatest distance between wild and cultivated accessions is found in those from Mauritania, and is probably due to the isolation of wild millet from the crop (Fig. 4).

This divergence between wild and cultivated millets holds true for the nine pairs of wild and cultivated samples from western Mali. They show the same separation as that of all the wild and cultivated millets from the region. The rare allele *Adh A1* was observed at a frequency of up to 0.27 in cultivated accessions and up to 0.04 in wild ones.

Central group. A total of 80 accessions of wild millet from the Central Group, 56 allopatric and 24 sympatric to the crop, were compared with 37 early-maturing cultivars from the same region. Here also, the distinction between wild and cultivated millet is strong and due to *Got A2*, *Pgm A1*, *Est A3*, *Pgi A5*, and *Adh A4* (Fig. 5). The discriminant analysis agrees with the initial classification in 98% of the cases. The groups have an equivalent diversity (Table 4), but the total diversity of the two groups together is higher (0.253) than that of each separate group. This and the high Gst indicate a greater divergence between wild and cultivated millets in this group compared to that of the Western group.

The distance between wild and cultivated accessions is twice as high in the Central Group than in the Western Group (Table 4), with the sympatric wild millets lying intermediate between allopatric wild and cultivated millets.

The variability of the wild millets is higher than that of cultivated millets for *Got A*, but lower for *Pgm A* and *Est A*.

West Chad Group. In the West Chad Group, the 4 allopatric and 12 sympatric wild millet accessions are well

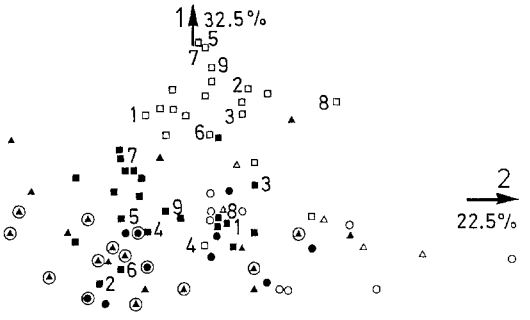

Fig. 4. Principal component analysis of the Western group. ○ = cultivated millet accessions from Senegal; □ = western Mali; △ = from Mauritania; ● = wild millet accessions from Senegal; ■ = western Mali; ▲ = Mauritania; ◭, ◉ = allopatric wild millet. Nos. 1–9: wild-cultivated pairs from the same village in western Mali

Table 4. Comparison of Nei's parameters, diversity (*Hx*), coefficient of differentiation (*Gst*), and minimum distance (*Dm*) between wild and cultivated accessions from different groups

Group	Wild		Cultivated		*Dm* between wild/cultivated
	Nei's diversity	*Gst*	Nei's diversity	*Gst*	
Western	0.206 ± 0.005	0.134	0.234 ± 0.058	0.146	0.013 ± 0.002
Central	0.243 ± 0.003	0.149	0.235 ± 0.005	0.131	0.030 ± 0.003
West Chad	0.239 ± 0.009	0.138	0.220 ± 0.006	0.126	0.055 ± 0.006
Darfur	0.219 ± 0.009	0.125	0.220 ± 0.006	0.126	0.090 ± 0.007

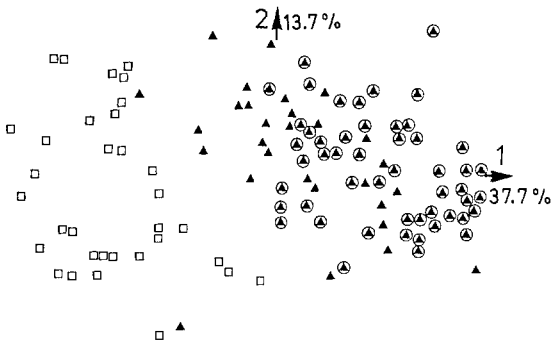

Fig. 5. Principal component analysis of the Central Group: □ = cultivated millets; ▲ = wild millets; ◬ = allopatric wild millets

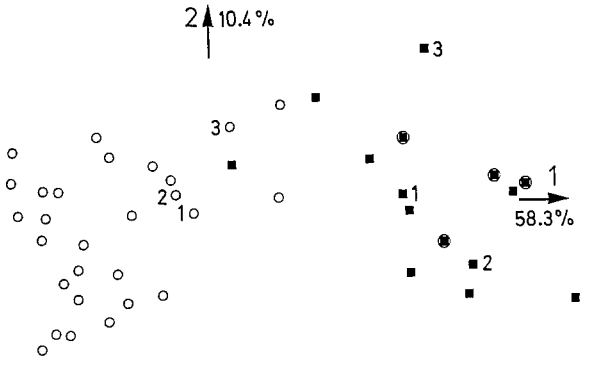

Fig. 6. Principal component analysis of the West Chad Group. ○ = Cultivated millets; ■ = wild millets; ◉ = allopatric wild millets. Nos. 1–3: wild-cultivated pairs from the same site

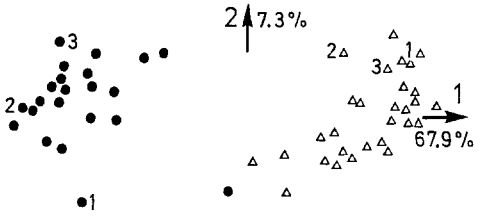

Fig. 7. Principal component analysis of the Darfur Group. ○ = Cultivated millets; ● = wild millets. Nos. 1–3: wild-cultivated pairs from the same wadi

separated from the cultivated millets of Chad and Sudan (Fig. 6). The discriminating loci are *Pgm A1*, *Pgd A2*, and *Cat A2*. The pooled wild and cultivated samples have a higher diversity (0.252) than each group separately (Table 4). Discriminant analysis gives 100% distinction between wild and cultivated accessions.

Allopatric and sympatric wild millets differ only for *Got A* (Table 5), the values of *Got A1* being higher for the sympatric wild millets. The distance between the wild and cultivated accessions is clearly greater than that in the Western and Central Groups. The three pairs of wild and cultivated samples from the same village perfectly fit this

Table 5. Nei's diversity *Hx*, of loci discriminating wild and cultivated accessions from different groups

Group	Locus	Wild		Cultivated
		Allopatric	Sympatric	
Western	*Pgm A*	0.062±0.033	0.076±0.022	0.387±0.028
	Adh A	0.389±0.043	0.051±0.014	0.630±0.019
	Total	0.187±0.010	0.211±0.055	0.234±0.005
Central	*Got A*	0.429±0.018	0.379±0.035	0.006±0.007
	Pgm A	0.063±0.017	0.228±0.040	0.467±0.015
	Est A	0.729±0.015	0.800±0.012	0.815±0.007
	Total	0.240±0.005	0.239±0.006	0.235±0.005
West Chad	*Pgm A*	0.230±0.093	0.272±0.055	0.384±0.030
	Cat A	0.432±0.071	0.470±0.027	0.252±0.035
	Pgd A	0.449±0.061	0.361±0.050	0.100±0.028
	Got A	0.279±0.096	0.028±0.025	0.008±0.009
	Total	0.242±0.018	0.234±0.010	0.219±0.005
Darfur	*Adh A*	–	0.291±0.045	0.657±0.024
	Cat A	–	0.377±0.035	0.252±0.035
	Pgm A	–	0.404±0.032	0.384±0.030

overall situation and indicate that gene flow between wild and cultivated populations is very limited.

Darfur Group. Twenty-two sympatric wild millet accessions in the Darfur Group were compared with the same 29 cultivated millets as used for the West Chad Group. Here the divergence between wild and cultivated millet reaches its maximum (Fig. 7). The diversity of the pool (0.264) and the coefficient Gst (0.272) are superior to that of each component, and the distance between wild and cultivated is high (Table 4). Discriminant analysis gives a 100% agreement with the classification. The discriminating alleles are *Adh A2*, *Adh A6*, *Pgm A1*, and *Cat A1*. *Adh A* locus is less diverse and *Cat A* more diverse in the wild millets than in the cultivated accessions, with the same locus variability in both groups.

The three pairs of wild and cultivated samples were collected from the banks of seasonal rivers where they were growing in competition. Their analysis corroborates the divergence of the two forms (Fig. 7). The *Adh A2* allele is a good marker to trace gene flow between these adjacent wild and cultivated populations. The allele was observed with a frequency of 0.79, 0.94, and 0.54 in the wild populations and 0.00, 0.25, and 0.00 in the cultivated populations. Nei's minimum distances in these pairs (0.077) are higher than those observed in the western Mali pairs (0.027).

General comparison between wild and cultivated millets

An analysis of all the 311 accessions together shows a divergence into two groupings for the wild and cultivated samples (Fig. 8), and confirms previous findings (Tostain et al. 1987). The additional accessions of cultivated millet

Table 6. Pooled comparison of wild and cultivated accessions

Locus	Genetic diversity			
	Wild		Cultivated	Cultivated + wild
	Allopatric	Sympatric		
Mean for 12 loci	0.239 ± 0.004	0.247 ± 0.004	0.247 ± 0.032	0.257 ± 0.002
Pgi A	0.129 ± 0.018	0.194 ± 0.020	0.324 ± 0.017	0.235 ± 0.011
Pgm A	0.073 ± 0.014	0.224 ± 0.019	0.497 ± 0.003	0.357 ± 0.010
Cat A	0.496 ± 0.003	0.501 ± 0.000	0.440 ± 0.011	0.494 ± 0.003
Got A	0.342 ± 0.019	0.172 ± 0.019	0.005 ± 0.003	0.171 ± 0.010
Pgd A	0.354 ± 0.021	0.277 ± 0.021	0.109 ± 0.014	0.240 ± 0.011
Adh A	0.530 ± 0.006	0.657 ± 0.008	0.633 ± 0.010	0.624 ± 0.006
Est A	0.723 ± 0.011	0.810 ± 0.005	0.822 ± 0.003	0.805 ± 0.003

Distance (*Dm*)	Sympatric wild	Cultivated
Allopatric wild	0.0073 ± 0.0009	0.0309 ± 0.0019
Sympatric wild		0.0162 ± 0.0015

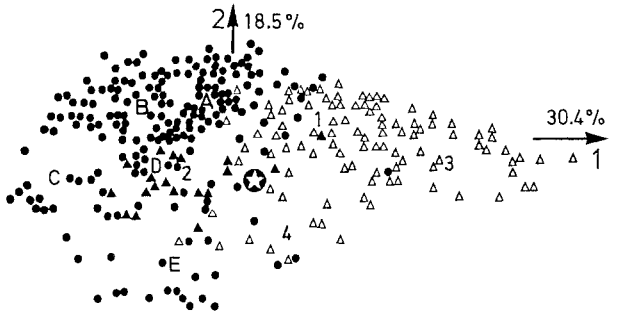

Fig. 8. Principal component analysis of 188 wild accessions and 123 early-maturing cultivars. ● = Wild accessions and their points of averaged diversity. A: Western, B: Central, C: Aïr, D: West Chad, E: Darfur. △ = Cultivated accessions and their points of averaged diversity. 1: Senegal, Mauritania, Niger, eastern Mali, Algeria, Tunisia. 2: western Mali. 3: Cameroon, Chad, Sudan. 4: Togo, Ghana, Bénin, Guinea. ▲ = Cultivated accessions from western Mali; ✪ = point of maximum diversity

from Mali, Algeria, Tunisia, and Chad clarify the previous picture by keeping western Mali accessions apart but grouping eastern Mali with Niger. Consequently, early-maturing cultivars from Sahelian Africa show a pattern of four clusters: 1. Senegal, Mauritania, eastern Mali, Niger, Algeria, Tunisia; 2. western Mali; 3. Togo, Ghana, Guinée, Bénin; 4. Cameroon, Chad, Sudan (Fig. 8).

The western Mali cultivated millets lie in the contact zone between the wild and cultivated clusters. They are the closest to wild millet, and the cultivated millets from Cameroon, Chad, and Sudan are the farthest. The same picture is given by the 15 wild and cultivated accession pairs from western Mali and Chad. Equal frequencies of allele *Got A1*, which is very rare in cultivated millet, were observed in only one pair. A hypothetical sample with maximum diversity for all loci (equifrequent alleles for each locus) would fall in the middle of the contact zone between the wild and cultivated groups (Fig. 8). This intermediate position indicates that each group possesses an equal but different part of the total diversity present in the species. This situation is shown in Table 6, where sympatric wild millets and cultivated millets have an equal diversity (0.247), only slightly inferior to that of the common pool (0.257). The composition of the total diversity differs between the groups: wild millets are more diverse than cultivated millets for *Got A*, *Pgd A* and *Cat A*, but less diverse for *Pgi A* and *Pgm A*. Differences are not significant for *Adh A* and *Est A*. The other loci are quasi-monomorphic in both groups (6% of the total diversity in wild millets and 4% in cultivated millets). The western Mali cultivated millets have frequencies close to that of wild millets for *Pgm A* and *Cat A*, but not for the other alleles.

A total of 83.6% of the 311 accessions was well classified by discriminant analysis; nine wild accessions and nine cultivated accessions were wrongly positioned.

Sympatric and allopatric wild millets are genetically very close, but sympatric accessions are closer to cultivated millet and their allelic diversities are generally intermediate (Table 6). The contrast between the allelic band patterns of the most widely separated allopatric wild millet and cultivated millet groups can be used to characterize an enzymic wild type and a cultivated type for most regions; moreover, it is unlikely that the alleles frequent in the past should have disappeared from all wild forms currently found. Thus, it is hypothesized that the allopatric wild millets taken together are the closest enzymatically to the wild species before domestication. This wild type, so defined, has a low diversity for loci *Pgi A*, *Pgm A*, *Adh A*, and *Est A* and a high diversity for *Got A*,

Pgd A, and *Cat A*. For loci *Pgi A*, *Pgm A*, and *Est A*, the diversity in cultivated millet is higher than that for the pooled wild and cultivated accessions (Table 6). This means that domestication has increased genetic diversity in these alleles. In contrast domestication has decreased the diversity at locus *Cat A* (towards fixation of *Cat A1* allele), locus *Got A* (suppression of *Got A1* allele), and *Pgd A* (fixation of *Pgd A2* allele).

Discriminant analysis confirmed the three groups, allopatric wild, sympatric wild, and cultivated, in 88.4% of accessions. Misclassifications occurred mostly between the allopatric and sympatric wild millet groups.

Discussion

The study has shown wild millet to have a large genetic diversity structured into five groups corresponding to geographical areas. A number of geographical barriers currently divide the distribution area of wild millet (Fig. 1): the Niger river delta and Taoudenit Desert in Mali (between Western and Central Groups), the Ténéré Desert in Niger (between Central and West Chad Groups), and the zone of heavy clay soils in central Chad (between West Chad and Darfur Groups). In these areas the soils, climate, or hydrological regime is unfavorable to the growth of wild millet. The geographical distribution observed in the extensive collections made between 1985 and 1989 has contracted in comparison to that observed in 1974, especially in Senegal and Mauritania (Grouzis 1979). The wild millets of central Sudan (Kordofan) and eastern Ethiopia, as described in floras and specimens (Brunken 1977), have not been sampled in the present study. The species has never been observed in eastern-southern Africa.

The optimum ecology for the species seems to be the Saharo-Sahelian pastoral zone. Anthropophilous habitats further southwards are perhaps the result of a later colonization of the agricultural zone. The present discontinuous distribution of wild millet could be the result of the disappearance of populations from regions previously uniformly covered thousands of years ago. Wild millet from the Aïr in Niger and Adrar des Iforas in Mali, which lie within the Sahara between 18°N and 20°N, survive today because of an altitudinal microclimate (Ingram 1990).

Wild millet genetic diversity measured on 188 accessions was 0.247 ± 0.002 and the coefficient of differentiation was 0.225. No fixed allele, specific to wild millet, was observed. In marginal areas where either the environmental (in Mauritania, the Adrar des Iforas, the Aïr) or man (in Senegal and the agricultural zone in Niger) imposes severe constraints to the expansion of wild millet, diversities appear low.

This study has shown that wild millet has a genetic diversity not larger than in cultivated millet. The divergence between wild and early-maturing pearl millet is clear. The wild enzymic type for wild millet is characterized by high frequencies of *Got A1*, *Pgd A3*, and *Cat A2*, and the cultivated type by high frequencies of *Pgi A5* and *Pgm A1*. Sympatric wild millet can be distinguished from allopatric, with loci *Adh A* and *Est A* more diverse in the former. Sympatric wild millet has a genetic diversity closest to that of cultivated millet.

Based on these facts, two hypotheses can be advanced for the domestication of pearl millet.

- Domestication occurred once or several times in different parts of the Harlan non-center (Harlan 1971), based on a few isolated plants with the "cultivated" enzyme type. This implies that the allozymes studied here are all on a small number of linked loci tied to the genes of domestication. This could explain that the same enzymic type may be selected from multiple domestications; however, the selection of founder plants with the same rare associations of several alleles remains improbable. The genetic distance between wild millets from Sudan and cultivated millet is too great for the former to be a source for domestication, contrary to a previous hypothesis put forward by Tostain et al. (1987).
- Domestication occurred as a unique event in the Western region (in Mauritania, Senegal, or western Mali), where today the greatest similarity is observed between wild and cultivated millet. For this it must be supposed that domestication has modified genetic diversities in the direction we observe today, either through genetic drift or by human and natural selection. The relative similarity between cultivated millet from western Mali and wild millet from Chad could result from a convergence by selection and not from common ancestry. Such a convergence has also been observed between teosinte and maize (Doebley 1990). Much also remains unknown about the people who domesticated pearl millet and the date and duration of the domestication.

Indirect clues such as the presence of potsherds, grinding tools, and sometimes agricultural tools (Amblard and Quéchon 1991) indicate the existence of agriculture as early as 9,500–9,000 BP in western Africa. It would have developed gradually during the 10th millenium when rains were abundant and regular. We could suppose that another grass, an indigenous species of rice (*Oryza glaberrima*), could have been used at that time without much effort to domesticate it (F. Paris, personal communication). As the drought increased, these first agriculturalists may have attempted to grow rice on the borders of lakes as they ebbed and on the surrounding sand dunes during the rainy season. These dunes probably would have been covered with dense stands of wild millet.

Teosinte, the ancestor of cultivated maize, is an allogamous species like wild millet. *Zea mays* subsp. *mexicana*, which grows as a weed sympatric to maize in the area of Mexico City, is enzymatically more distant from maize than *Z. mays* subsp. *parviglumis*, the teosinte allopatric to maize (Doebley et al. 1984). Weedy teosinte cannot be a genetic bridge between teosinte and maize and cannot be a natural hybrid (Doebley 1990). The domestication probably occurred after a migration away from its main distribution area and isolation over a century (Galinat 1988). In millet, allopatric wild millet populations are the most distant from cultivated millet, and the sympatric wild millet populations do not seem to be natural hybrids between cultivated and allopatric wild millet.

The pattern of geographically isolated, small wild populations probably resulted as periods of severe drought drastically decreased the size of wild millet stands. These isolated populations would have been subjected to domestication around 7,500–7,000 BP. Their differing ecologies would have given rise to plants with different phenotypes (Iltis 1983). These phenotypes would have had characters of interest to cultivators, and the associated specific isoenzymes would have then been quickly selected. Laredo and Pernès (1988), on the basis of a monogenic model for the domestication process, concluded that domestication can proceed quickly, especially if the flow of wild genes if reduced by geographic isolation.

Following the fixation of morphological and enzymic domesticated genotypes, cultivated millet must have coexisted for a long time with wild millet. Evidence of this is reported by Amblard and Pernès (1989), who observed impressions of wild spikelets mixed with cultivated seeds on pottery dated to 3,000 BP at Dhar Tichitt and Oualata in southeastern Mauritania.

Wild millet, like teosinte, has up to now not been used in the improvement of the crop (Goodman 1988). Nevertheless, the genetic diversity of wild populations is very different from that of cultivated and could be a source of useful genes. Our results corroborate, once more, that domestication produces new variation, but not speciation (Harlan et al. 1973).

Allopatric and sympatric wild millets are enzymatically difficult to distinguish, which shows that the wild form can adapt to modifications in the environment caused by agriculture. Introgression from wild populations to cultivated is said to influence their adaptability (Pernès 1986). Our observations show that gene exchange is rare, but it is necessary to study more precisely, *in situ*, the rate of hybridization between wild and cultivated millet.

Previous results from Tostain and Marchais (1989) permit a more rational utilization of cultivated millet collections. The current results could help in the exploitation and management of wild millet resources. Since wild millet diversity is structured into geographical groups, it is necessary to have populations from many different sites. It would also be interesting to follow the divergence in the years to come between samples conserved *ex situ* in a gene bank and the original populations evolving under drought conditions.

Cultivated millet retains its identity through the elimination by farmers of the natural hybrids with wild millet. However, blocks of wild genes can remain hidden in the backcrosses ($F_1 \times$ cultivated) and can be expressed in some environments (Galinat 1988). This would explain the occurrence of semi-wild genotypes (weedy forms) among cultivars growing far from wild populations. Use of enzymic markers would allow the detection and, therefore, the elimination of undesirable wild genes or, equally, the introgression of useful characters.

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References

Amblard S, Pernès J (1989) The identification of cultivated pearl millet (*Pennisetum*) amongst plant impressions on pottery from Qued Chebbi (Dhar Oualata, Mauritia). Afr Archeol Rev 7:117–126

Amblard S, Quéchon G (1991) L'agriculture néolithique au Sahara méridional: faits, théories et contradictions. In: Néolithisation en régions sahariennes. Critères, problèmes, perspectives. Colloque Montignac 1988 (in press)

Brunken JN (1977) A systematic study of *Pennisetum* sect. Pennisetum (Gramineae). Am J Bot 64(2):161–176

Brunken J, De Wet JMJ, Harlan JR (1977) The morphology and domestication of pearl millet. Econ Bot 31:163–174

Clegg MT, Rawson JRY, Thomas K (1984) Chloroplast DNA variation in pearl millet and related species. Genetics 106:449–461

Doebley JF, Goodman MM, Stuber CW (1984) Isoenzymatic variation in *Zea* (Gramineae). System Bot 9(2):203–218

Doebley J (1990) Molecular evidence and the evolution of maize. Econ Bot 44 [3 suppl]:6–27

Galinat WC (1988) The origin of corn. In: Corn and corn improvement, 3rd edn. pp 1–31 Agronomy Monograph No. 18, pp 1–31

Gepts P, Clegg MT (1989) Genetic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] at the DNA sequence level. Hered 80:203–208

Goodman MM (1988) The history and evolution of maize. CRC Critical Rev Plant Sci 7,3:197–220

Grouzis M (1979) Sur le *Pennisetum violaceum* sensu lato en Afrique de l'Ouest: formes, écologie et distribution géographique. Bull de l'I.F.A.N., T 41, Sér A, 2:300–316

Harlan JR (1971) Agricultural origins: centers and non-centers. Science 14:468–474

Harlan JR, De Wet JMJ, Price EG (1973) Comparative evolution of cereals. Evolution 27:311–325

Iltis HH (1983) From teosinte to maize: the catastrophic sexual transmutation. Science 222:886–894

Ingram GB (1990) Multi-gene-pool surveys in areas with rapid genetic erosion: an example from the Aïr mountains, northern Niger. Conserv Biol 4,1:78–90

ITCF (1987) Institut Technique des Céréales et des Fourrages. Boigneville, F 91720 France

Lagudah ES, Hanna WW (1989) Species relationship in the *Pennisetum* gene pool: enzyme polymorphism. Theor Appl Genet 78:801–808

Laredo C, Pernès J (1988) Models for pearl millet domestication as an example of cereal domestication. I. A one locus asymmetrical model. J Theor Biol 131:289–305

Nei M (1975) Molecular population genetics and evolution. In: Neuberger A, Tatum EL (eds) Frontiers of biology, Vol. 40. North Holland, Amsterdam-Oxford, 288 pp

Pernès J (1986) L'allogamie et la domestication des céréales: l'exemple du maïs (*Zea mays* L.) et du mil (*Pennisetum americanum* L. K. Schum.). Bull Soc Bot Fr Actual Bot 133(1):27–34

Tostain S, Marchais L (1989) Enzyme diversity in pearl millet (*Pennisetum glaucum*). 2. Africa and India. Theor Appl Genet 77:634–640

Tostain S, Hamon S, Bernus E, Marchais L, Ingram GB (1986) Collection of wild millets in Burkina Faso and Niger. FAO Plant Genet Res Newsl 68:11–15

Tostain S, Riandey MF, Marchais L (1987) Enzyme diversity in pearl millet (*Pennisetum glaucum*). 1. West Africa. Theor Appl Genet 74:188–193