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Genetic and Environmental Impact on Iron, Zinc, and Phytate in Food Sorghum Grown in Benin

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Seventy-six farmers' varieties of sorghum from Benin were distinguished by amplified fragment length polymorphism (AFLP) and clustered into 45 distinct genotypes. The genotype clusters were evaluated for their Fe, Zn, and phytate concentrations to assess the impact of genetic and environmental effects on the composition of the grains and to identify farmers' varieties with high potential Fe and Zn availability. The Fe concentration of the grains ranged from 30 to 113 mg/kg with an average of 58 mg/kg. The Zn concentration ranged from 11 to 44 mg/kg with an average of 25 mg/kg. The phytate concentration of the grain ranged from 0.4 to 3.5% with a mean of 1.2%. The grain-Fe and grain-Zn did not show consistent linkage to genetic variation, but varied significantly across field locations, suggesting a predominant environmental impact. The phytate concentration of the grains appeared to be environmentally as well as genetically determined. No varieties provide adequate Zn to meet nutritional requirements of sorghum consumers. The most promising varieties for Fe supply were tokogbessenou, mahi swan, biodahu, saï maï, mare dobi, sakarabokuru, and chabicouma, as they showed a [phytate]/[Fe] ratio of <14, which is the critical value above which Fe availability is strongly impaired. These varieties could therefore be recommended for the preparation of food products such as dibou, in which processing methods have only a slight diminishing effect on phytate levels. Further research is needed to test these varieties for the stability of [phytate]/[Fe] molar ratio across various environmental conditions.

KEYWORDS: AFLP; genotype; molar ratio

INTRODUCTION

Iron (Fe) and zinc (Zn) deficiencies constitute a major public health problem in many African countries. They mostly affect infants and pregnant women and may have serious consequences. Chronic micronutrient deficiencies, particularly of Fe, Zn, and vitamin A, cause child mortality, impaired mental and physical development, and decreased work output and contribute to morbidity from infections (*1*−*3*).

In semiarid tropics worldwide, sorghum [*Sorghum bicolor* (L.) Moench] is cultivated by farmers on a subsistence level for human consumption (*4*). The crop is processed into different local foods such as porridges and beverages, for adults and infants (*5*). The average yearly sorghum consumption amounts to 115 kg per capita in northern Benin (*6*) and 200 kg per capita in Burkina-Faso (*7*). Meat consumption is low in these regions as in most developing countries, and therefore cereals and vegetables are the main dietary sources of macro- and micronutrients (*8*). However, the minerals content of cereals such as sorghum is low, and their bioavailability is restricted by antinutritional factors such as phytate forming insoluble complexes with essential minerals such as calcium, iron, and zinc at physiological pH levels (*9*). Phytate may be partly responsible for the widespread mineral deficiencies observed in populations that subsist largely on sorghum and other cereals (*10*). The chelating properties of phytate depend on the levels of Fe, Zn, and phytate. Hence, the molar ratio of phytate to Fe or Zn was suggested as an index to estimate the availability of Fe and Zn in foods (*11*, *12*). A [phytate]/[Fe] molar ratio of $>10-14$ was reported to strongly impair Fe availability in rats fed wheat-based diets (*11*). Values of 10−15 for the [phytate]/[Zn] molar ratio are considered to be the critical values above which Zn availability decreases in humans (*13*, *14*). In the present study, we used the [phytate]/[Fe] and [phytate]/[Zn] molar ratios as indices for potential Fe and Zn availability from the local sorghum varieties.

Frossard et al. (*15*) postulated that improvement of bioavailability of Fe, Zn, and Ca in the edible part of staple crops, such as cereals, could be achieved by increasing the total Fe, Zn, and Ca levels combined with increasing the concentration of compounds that promote their uptake and/or by decreasing the

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concentration of compounds such as phytate or phenolic compounds, which inhibit their absorption. This could be supported by selection of varieties (for example, within local germplasm) containing enhanced levels of Fe and Zn concentration or by breeding for varieties with lower [phytate]/[Fe] and [phytate]/[Zn] ratios. Breeders need existing information on the genetic variation for a given trait as well as the determinants (for instance, genetic and environmental factors) in a collection of germplasm to justify selection for that trait (*3*). Genetic as well as environmental factors can significantly affect Fe and Zn levels in cereal grains as was shown in maize and wheat (*16*, *17*). There are limited data available on Fe, Zn, and phytate contents of sorghum grains.

Local sorghum landraces possess desirable characteristics; that is, they are well adapted to harsh environmental conditions, offer good food quality, are highly preferred by consumers, and thus play a significant role in local economies (*18*−*21*). This local germplasm may also have specific advantages in terms of mineral concentration and availability. A recent survey in northern Benin revealed the existence of a large number of farmers' varieties of sorghum that have been selected by farmers over the years and that fulfill relevant user criteria (*5*). Identification and promotion of local germplasm with high mineral availability can contribute to the improvement of the micronutrient status of the consumers, because they may have a relatively high chance of adoption by the users compared to newly bred varieties from elsewhere.

For the present study we collected sorghum samples (n = 76) from farmers' fields in northern Benin, grown under natural conditions and traditional farming systems to take the variability of these factors into account. Amplified fragment length polymorphism (AFLP; *22*), a genome fingerprinting technique, was applied to cluster farmers' varieties according to their genomic similarity. Clusters were evaluated for Fe, Zn, and phytate concentrations in relation to their growing environment and food quality. The farmers' varieties including environmental conditions with potential for adequate Fe and Zn supply to the consumers were identified.

MATERIALS AND METHODS

Plant Materials. Grains of 76 farmers' varieties of sorghum were provided by farmers in three communities in northern Benin, that is Banikoara (latitude °N, 11° 15′; longitude °E, 2° 23′); Toucountouna (latitude °N, 10° 27′; longitude °E, 1° 22′); and Djougou (latitude °N, 9° 43′; longitude °E, 1° 41′). The accessions studied represent farmers' varieties that are the product of development and/or maintainance of seeds by farmers. These farmers' varieties were previously surveyed by Kayodé et al. (*23*) and described as diverse in terms of their agromorphological traits and food properties. Of the varieties analyzed, 52% mature late in the season, 15% mature at an intermediate date, and 33% mature early. Most have long stems (87%), loose panicles (79%), large seeds (49%), and a pink or red seed color (52%). In addition, most of them are susceptible to drought (54%) and attacks by striga (79%), insects (99%), and birds (77%). The quality of the seeds for preparing porridges is high for 52−54% of the varieties, whereas 26% of the varieties are regarded as having a high quality for beverage making, according to the interviewed farmers and food processors.

The collected seeds were from crops grown in 2002 under the natural season of the Guinea Savannah climate of West Africa. The annual rainfall in the region varies from 1000 to 1300 mm, and the average yearly temperature is 26.5 °C (*24*). The soil is a tropical ferruginous type (*25*). After harvesting, the grains were dried to a moisture content of 11−13% (w/w). Parts of the seed samples were germinated to produce biomass for DNA extraction. The remainder was ground into flour using a Retsch mill fitted with a 0.5 mm screen and stored at −20 °C until analysis.

DNA Extraction and AFLP Protocol. DNA extraction and the AFLP protocol were performed as reported earlier by Kayodé et al. (*23*). A small piece (1 cm^2) of freeze-dried leaf tissue was ground with four glass pearls in a Retsch shaking mill, followed by DNA extraction according to the method of Fulton et al. (*26*). The AFLP method (*22*) was performed as described by Myburg et al. (*27*), with separation and detection on a LiCOR automated sequencer. Approximately 80 *Eco*RI/*Mse*I primer combinations were tested on four samples. Suitable combinations were selected on the basis of the number of unambiguously scorable polymorphic bands. Finally, two primer combinations were selected for analysis: *Eco*RI-AAC/*Mse*I-CCC and *Eco*R1-ACA/*Mse*I-CTG.

Iron and Zinc Determination. Approximately 0.4 g of sorghum flour was digested using hydrofluoric acid (40%) and concentrated nitric acid (65% w/w). Next, the concentrations of Fe and Zn were analyzed by using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Elan 6000, Perkin-Elmer, Norwalk, CT) (*28*). Analyses were performed in duplicate.

Phytate Determination. Approximately 10 mg of sorghum flour was extracted with 1 mL of 0.5 N HCl containing 50 mg/L *cis*-aconitate (internal standard). The mixture was boiled in a water bath at 100 °C for 15 min and then centrifuged at 14000*g* for 10 min. The supernatant was diluted 5 times in Millipore water and analyzed using HPLC (Dionex DX300, ICS2500 system, detector range of 10 μS) using the column AS11 (ATC column plus precolumn). Detection was with suppressed conductivity, and the suppression was done with water at the flow rate of 5 mL/min. The eluent and the elution times used were as follows: 0−5 min, 5 mM NaOH; 5−15 min, 5−100 mM NaOH; 15−20 min, 500 mM NaOH; and 20−35 min, 5 mM NaOH. A standard solution was prepared in Millipore water, containing 5.0 mg/L $NaNO_3$ (Merck p.a.), 5.0 mg/L Na_2SO_4 (Merck p.a.), 5.0 mg/L oxalic acid·$2H_2O$ (Merck p.a.), 10.0 mg/L Na_2HPO_4·$2H_2O$ (Merck 6346 p.a.), 10 mg/L citric acid·H_2O (Merck K23524044 719 p.a.), 5.0 mg/L *cis*-aconitate (Aldrich 27194-2), and 10 mg/L IP6·Na_{12} (Sigma P3168 lot 102K0053). Analyses were performed in triplicate.

1000 Kernel Weight (kw) Measurement. The 1000 kw was determined by weighing 100 grains of each sample on a 1/10000 precision balance and multiplying the obtained value by 10. The weight was expressed on a dry matter basis. Measurements were performed in duplicate.

Statistical Analysis. The data were analyzed using the statistical program SPSS 11.0. The one-way ANOVA model was used to compare means between groups by applying the LSD test. For the genetic data, the presence (1) or absence (0) of each polymorphic AFLP band was scored for all genotypes. The genetic similarity (GS_{ij}) was calculated according to method of Nei and Li (*29*): $GS_{ij} = 2N_{ij}/(N_i + N_j)$. N_{ij} is the number of bands present in genotypes i and j, N_i is the number of bands present in genotype i, and N_j is the number of bands present in genotype j. Tests for correlations between genetic variation and Fe, Zn, or phytate were performed using AMOVA 1.55 (*30*).

RESULTS AND DISCUSSION

Genetic Identification of Farmers' Varieties. The AFLP technique used in the present study has been reported as a powerful technique in the identification of many other crop species (*31*−*33*). On the basis of the presence or absence of the amplified fragments for each farmers' variety, all germplasm was identified by applying the genetic similarity index (GS). Farmers' varieties with a genetic similarity of >0.90 are considered to be related, whereas those with GS < 0.90 are regarded as distinct (*34*). The AFLP analysis revealed the genetic similarity of some varieties that were considered to be distinct by farmers (**Table 1**). Within the 76 samples analyzed, we grouped 42 farmers' varieties into 11 similarity clusters (**Table 1**). The remaining 34 farmers' varieties were distinct as revealed by AFLP. Within the clusters, the GS averaged 0.92 and varied from 0.90 between the farmers' varieties yacuba and fari to 0.98 between talemoula and toholamoula. Some farmers' varieties

Table 1. Fe, Zn, and Phytate (IP6) Contents and 1000 Kernel Weight (kw) of 45 Sorghum Genotype Clusters from Bénin

genotype	farmers' varieties included	Fe (mg/kg)	Zn (mg/kg)	IP6 (%)	1000 kw (g)
1	mahi swan, dobi monri, bio dahou, saï maï, yinan a, chamwoka a, chamwoka c, koussoubakou sinswan bodehem, tamabano, mare dobi a	56.3 ± 9.5 [44–77][a]	24.9 ± 3.0 [21–31]	0.92 ± 0.32 [0.6–1.45]	28.7 ± 5.5 [23.2–40.1]
2	dobi konouyirou, boussoukari, fari, yacouba, baniyani, yabakanoba	58.7 ± 12.0 [40–73]	27.2 ± 3.5 [22–32]	1.01 ± 0.37 [0.49–1.32]	27.2 ± 3.1 [23.2–31.4]
3	talemoula a, talemoula b, toholahamoula, agbaneri, somba hanni	47.8 ± 6.3 [38–53]	27.4 ± 5.6 [21–33]	1.56 ± 0.39 [1.02–2.01]	35.8 ± 3.3 [31.9–39.3]
4	chassissoya, soniya a, kaka, doniyoka	60.3 ± 18.6 [33–74]	23.0 ± 7.0 [19–34]	0.96 ± 0.56 [0.44–1.72]	26.9±4.1 [23.1–31.2]
5	natisoya a, natisoya b, tinoukpati	39.3 ± 1.5 [38–41]	23.0 ± 5.3 [19–29]	1.18 ± 0.19 [0.98–1.35]	31.0 ± 1.3 [30.1–32]
6	sakarabokuru b, dobi gnon faï, bonyinm	56.3 ± 19.6 [44–79]	31.7 ± 4.7 [28–37]	0.86 ± 02 [0.63–1.02]	29.1 ± 6.4 [25.1–36.6]
7	hanni kpare, zomora, zogua b	59.0 ± 12.5 [46–71]	20.3 ± 3.1 [17–23]	1.34 ± 0.37 [1.09–1.77]	25.3 ± 3.5 [21.7–28.7]
8	tokogbessenou b, mare dobi b	61.5 ± 24.7 [44–79]	20.0 ± 1.4 [19–21]	0.83 ± 0.12 [0.75–0.92]	nd[b]
9	soniya b, fissouka	63.5 ± 16.3 [52–75]	31.5 ± 3.5 [29–34]	1.28 ± 0.13 [1.19–1.37]	36.5 ± 1.4 [36–37]
10	kouwekifounan, koumborosoya	66.5 ± 9.2 [60–73]	21.3 ± 0.0 [21–21.5]	1.65 ± 0.12 [1.56–1.74]	32.4 ± 1.3 [31.5–33.4]
11	kpri hanni, zomora	68.0 ± 18.4 [55–81]	28.5 ± 6.4 [24–33]	2.27 ± 0.13 [1.48–3.07]	26.0 ± 6.1 [21.6–30.3]
12	gbango	42.5 [41–44]	26.0 [24–28]	1.03 ± 0.03	23.6 [23–24]
13	sakarabokuru a	64.1 [60–68]	21.4 [21–21.8]	0.72 ± 0.12	37 [37–37]
14	yerekou	45.0 [44–46]	23.0 [23–23]	0.92 ± 0.18	34.0 [33.5–34.6]
15	kobatia binyirou	92.5 [84–101]	29.0 [26–32]	0.97 ± 0.06	20.5 [20–21]
16	tokogbessenou a	68.0 [49–87]	20.5 [20–21]	0.75 ± 0.21	39.0 [39–39]
17	yibere kanyinse	51.0 [48–54]	23.5 [23–24]	0.86 ± 0.31	39.5 [39–40]
18	fara bonbo	46.0 [46–46]	14.0 [11–17]	0.58 ± 0.24	33.0 [32–34]
19	gourouma dobi	99.0 [96–102]	26.0 [24–28]	0.65 ± 0.07	27.0 [27–27]
20	yinan b	47.0 [44–50]	26.0 [26–26]	0.67 ± 0.21	nd
21	chamwoka b	nd	nd	0.87 ± 0.28	nd
22	chabikouman	40.5 [40–41]	19.0 [18–20]	0.47 ± 0.07	42.0 [41–43]
23	yowinka a	48.0 [46–50]	18.0 [17–19]	0.92 ± 0.09	28.1 [27.7–28.5]
24	yowinka b	77.0 [75–79]	24.0 [22–26]	0.84 ± 0.18	nd
25	sotakaman a	55.5 [51–60]	25.5 [25–26]	0.45 ± 0.17	31.6 [31.32]
26	sotakaman b	42.5 [41–44]	19.5 [19–20]	1.28 ± 0.18	nd
27	mousseman	56.5 [56–57]	16.5 [16–17]	1.40 ± 0.16	25.1 [25–25.2]
28	agbanni	47.5 [47–48]	24.5 [23–26]	1.35 ± 0.38	52.0 [51–53]
29	kassassahan	68.5 [68–69]	18.5 [17–20]	1.11 ± 0.11	29.0 [28–30]
30	zopira	63.0 [56–71]	24.0 [21–26]	1.17 ± 0.15	31.5 [31–32]
31	semoutche	53.5 [53–54]	37.5 [36–39]	1.57 ± 0.02	32.5 [32–33]
32	gaouri oleri	40.0 [39–41]	38.0 [32–44]	1.30 ± 0.06	40.5 [40–41]
33	zogua a	63.0 [60–66]	26.0 [25–27]	1.20 ± 0.24	48.5 [48–49]
34	moussi	63.0 [61–65]	28.5 [26–31]	0.95 ± 0.19	25.0 [24–26]
35	sobaki	86.0 [85–87]	16.5 [16–17]	1.62 ± 0.20	6.5 [5–8]
36	mahi a	38.5 [37–40]	20.0 [20–20]	1.14 ± 0.09	36.5 [36–37]
37	mahi b	79.5 [77–82]	20.5 [20–21]	1.68 ± 0.21	39.5 [39–40]
38	yabakawerou	67.0 [61–73]	31.0 [31–31]	1.45 ± 0.08	nd
39	gantim	74.5 [67–82]	33.0 [33–33]	1.15 ± 0.10	nd
40	sopoya	31.5 [30–33]	15.5 [14–17]	2.06 ± 0.37	25.5 [25–26]
41	sodaya	72.0 [70–74]	28.0 [27–29]	3.53 ± 0.41	34.5 [34–35]
42	saga pica	55.5 [52–59]	23.5 [23–24]	1.76 ± 0.19	23.0 [22–22]
43	hannitchre	40.0 [39–41]	18.5 [17–20]	2.22 ± 0.12	36.5 [36–37]
44	koussoubakou	38.6 [37–40]	26.5 [26–27]	1.59 ± 0.28	31.5 [31–32]
45	yebode	94.0 [75–113]	34.0 [28–40]	1.62 ± 0.40	nd
	average	58.8	24.4	1.2	31.7
	CV[c]	27.1	23.0	44.8	25.7

[a] Range is given in brackets. [b] Not determined. [c] Coefficient of variation.

considered to be similar and given the same name by the farmers were found to be genetically distinct. This is the case for the "varieties" doniyoka a and b, chamwoka a and b, sakarabokuru a and b, zogua a and b, tokogbessenou a and b, mare dobi a and b, yinan a and b, yowinka a and b, sotakaman a and b, and mahi a and b. Farmers use various criteria to identify their crops. The adaptation of a variety to particular agroecological conditions, its morphological aspects, and the taste of its products are key factors affecting crop identification by farmers, rather than purely genetic criteria. This leads to naming that is meaningful and significant. All varieties that meet the same criteria may get the same name, even though they may be genetically distinct. In some cases, the AFLP-based distinction agrees well with the farmers' assignment on the basis of similarity between varieties, as some varieties that are given the same name by farmers are also found to be similar by the AFLP analysis (for example, chamwoka a and c, talemoula a and b, and natisoya a and b). When similar farmers' varieties in clusters are examined, it appears in most cases that varieties in a specific cluster were obtained from the same region. All similar farmers' varieties in cluster 1 (except varieties chamwoka and tamabano) and in clusters 2, 6, and 8 are from Banikoara. Varieties in clusters 4, 5, and 10 are from Toucountouna, and those in clusters 3, 7, and 11 originated from Djougou. This regional resemblance in farmers' varieties may be explained as follows. First, it is plausible that during the seed collection in

Table 2. Analysis of Variance for the Effect of Locality on Fe, Zn, and Phytate (IP6)

genotype	region	village	DF[b]	*F* value[a] Fe	Zn	IP6
1	Banikoara, Toucountouna	Kokey, Gounbakou, Donparou, Tampégré	9	14**	4*	8.3**
2	Banikoara	Gounbakou, Kokey	5	47.6**	15.3*	3.5*
3	Djougou	Bareï, Banénou, Onklu	4	9.2*	9.4*	5.2*
4	Toucountouna	Tampatou, Tchaklakou, Tampégré	3	30.5*	243.9**	22.3**
5	Toucountouna	Toucountouna	2	0.1ns	14.1*	4.6ns
6	Banikoara	Kokey, Donparou	2	9.2ns	0.06ns	10.6*
7	Djougou	Bareï, Partogo	2	13.4*	16.9*	1.1ns

[a] *, *F* significant at the 0.05 level; **, *F* significant at the 0.01 level; ns, not significant. [b] Degrees of freedom.

a specific region, farmers mixed the varieties up, attributing different names to the same varieties. The second possible reason is linked to seed exchange between farmers and villages. In the adopting community the name of the traded farmers' variety often changed, taking either the name of the farmer who introduced it or the name of the village it came from.

Fe and Zn Concentrations of Grains. The Fe and Zn concentrations of the different farmers' sorghum varieties are presented in **Table 1**. The 45 sorghum genotype clusters vary significantly ($P < 0.01$) in their Fe and Zn concentrations. The Fe concentration ranged from 31.5 to 99.0 mg/kg with an average value of 58.8 mg/kg. Values for Zn ranged from 14.0 to 38.0 with an average of 24.4 mg/kg. In most genotypes the level of grain-Fe is higher than that of Zn, the difference being 1−5-fold. The level of Fe found in the present germplasm is in agreement with values reported in the literature. Jambunathan (*35*) reported an average Fe concentration of 59 mg/kg with a range of 26−96 mg/kg in samples of ∼100 varieties of sorghum.

Within the genotype clusters, samples grown in various locations show significantly different Fe and Zn levels, suggesting effects of the environment. The analysis of variance for the effect of locality on Fe, Zn, and phytate revealed a significant impact of the cultivation locations on the mineral concentrationof the grain (**Table 2**). The variation observed in grain-Fe and -Zn could not be explained by the observed genetic variation as the analysis of molecular variance failed to detect any significant correlation between the genetic distance of the varieties and grain-Fe and -Zn concentrations. Any genetic differences, if present, might have been masked by larger environmental differences. Even though the farmers' varieties were grown in the same agroecological zone on tropical ferruginous soils classified as Ferric lixisol by FAO (*36*), the soil fertility and microclimate may vary between regions/villages and induce differences in mineral accumulation by the plant (*37*). Banzinger and Long (*16*) found highly significant effects of "environment" and "genotype × environment" interaction on grain-Fe and -Zn concentrations among maize germplasm in Zimbabwe and Mexico. Further studies are needed to determine the contribution of different factors, that is genotype (G), environment (E), and G × E to the total grain-Fe and -Zn variation.

The 1000 kw also varies significantly ($P < 0.01$) among farmers' varieties and is inversely correlated with the grain-Fe: the larger the grain, the lower its Fe concentration (**Table 3**). In maize, Banzinger and Long (*16*) also reported the grain-Fe concentration to correlate inversely with grain size, corroborating the present finding. The authors ascribed this to the effect of dilution caused by enhanced grain-starch content. In wheat, there was no relationship between seed size and micronutrient density, probably because of the small amounts of microelements involved (*17*).

Farmers' varieties of perceived high and poor food quality showed no significant difference in terms of Fe and Zn concentrations. Likewise, the crop duration (late, intermediate, early) and the seed color (red, white, pink, yellow) showed no significant relationship with the grain-Fe and -Zn contents (**Table 4**). Implications of these findings for breeding are that selection for Fe or Zn does not automatically mean selection for good food quality properties and/or a certain plant maturity or seed color. Farmers' varieties are products of genetic makeup and cultivation conditions. To enable the establishment of a germplasm collection for high Fe and Zn concentrations, further experimentation will be required to eliminate effects of cultivation conditions. A collection with sufficient variability of mineral levels would allow selection for other important traits, such as good food quality, seed color, and crop duration.

Table 3. Pearson Correlation Matrix between Iron, Zinc, Phytate (IP6), Ash, and 1000 kw of Sorghum Grains[a]

	Fe	Zn	IP6	ash
Zn	0.168			
IP6	0.058	0.147		
ash	–0.008	0.047	0.374**	
1000 kw	–0.416**	0.091	–0.048	0.163

[a] *, significant at the 0.05 level; **, significant at the 0.01 level.

Table 4. Concentrations of Fe, Zn, and Phytate (IP6) of Farmers' Varieties of Sorghum Grouped by Agromorphological Traits

trait	Fe (mg/kg)	Zn (mg/kg)	IP6 (%)
maturity			
late ($n = 32$)	67.0 ± 18.4a[a]	29.0 ± 5.3a	1.14 ± 0.43a
early ($n = 20$)	68.0 ± 17.0a	27.1 ± 6.6a	1.41 ± 0.77a
intermediate ($n = 8$)	59.6 ± 14.1a	30.6 ± 8.6a	1.30 ± 0.50a
color			
red ($n = 11$)	70.0 ± 20.1a	29.6 ± 9.0a	1.45 ± 0.72a
white ($n = 29$)	64.9 ± 19.3a	28.9 ± 5.6a	1.12 ± 0.40a
pink ($n = 25$)	65.0 ± 14.5a	28.1 ± 5.7a	1.32 ± 0.62a
yellow ($n = 5$)	65.8 ± 15.0a	28.0 ± 4.5a	0.87 ± 0.25a
1000 kw[b]			
large ($n = 9$)	57.7 ± 12.4a	26.0 ± 3.3a	0.95 ± 0.43a
medium ($n = 24$)	63.3 ± 15.7ab	30.8 ± 7.2a	1.42 ± 0.72b
small ($n = 25$)	71.6 ± 19.0b	27.5 ± 6.1a	1.12 ± 0.42ab

[a] Means ± standard deviation. Means with the same letter are not significantly different according to the LSD at the 0.05 level. [b] One thousand kernel weight: large = 1000 kw > 40, medium = 30 < 1000 kw < 40, small = 1000 kw < 30.

Phytate Content and Fe and Zn Availability. The phytate concentration of the grain varied significantly ($P < 0.01$) among varieties (**Table 1**). Values ranged from 0.45 to 3.53% with a mean value of 1.2%. The highest phytate levels were found in the farmers' varieties sodaya (3.53%), hannitchre (2.22%), and sopoya (2.06%). Interestingly, these three varieties do not belong to the varieties that are highly preferred by farmers for foods. The phytate values found for these three farmers' varieties are higher than those earlier reported for sorghum. Frossard et al.

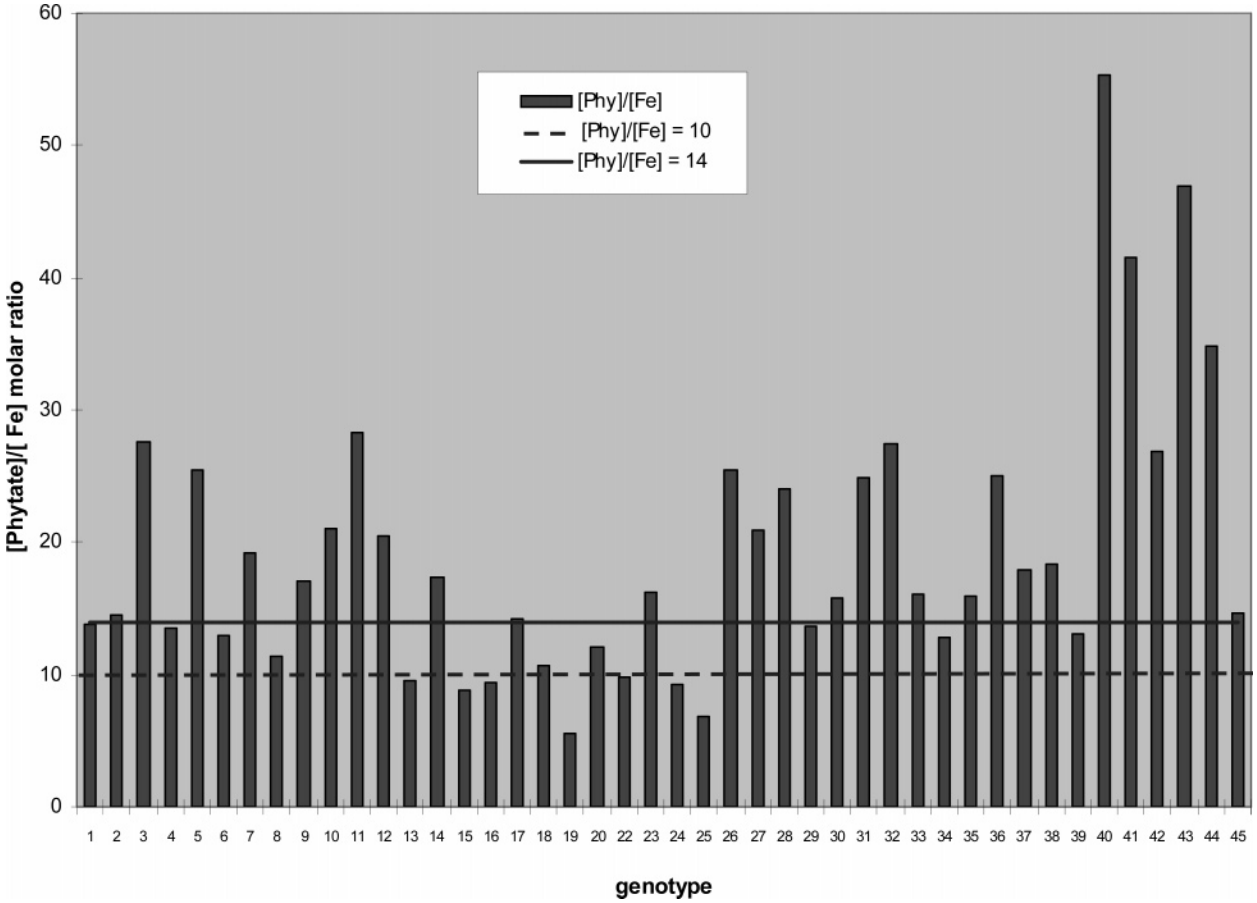

Figure 1. Sorghum varieties with [phytate]/[Fe] molar ratio and indication of range 10–14.

(*15*) reported phytate values ranging from 0.9 to 1.35% in sorghum, whereas Doherty et al. (*38*) reported values between 0.26 and 0.4% in 24 Indian sorghum varieties. The majority (56%) of the farmers' varieties (**Table 1**) have a phytate concentration of >1.0%. Farmers' varieties with a phytate concentration of <0.5% account only for 4%. The grain-phytate correlated with the ash content of the grain (**Table 3**), which could be expected as elements such as phosphorus, iron, and other minerals that account for the ash are also part of the phytate and phytate−mineral complex structure (*39*, *40*). The fact that the phytate concentrations of the grain vary significantly across villages (**Table 2**) and significantly correlate with the genetic structure among the varieties (data not shown) suggests that the grain-phytate concentration is environmentally, as well as genetically, determined.

We grouped the farmers' varieties according to their food quality traits as appreciated by farmers on a three-step scale, that is, high, medium, and low (*23*), and calculated the [phytate]/[Fe] and [phytate]/[Zn] molar ratios as an index for the potential mineral bioavailability (**Table 5**); that is, other food-processing beneficial effects were not taken into account. All varieties in each food quality group had [phytate]/[Zn] molar ratios >15, which suggests that Zn availability from the present varieties is very low and requires substantial improvement either by breeding, agronomy, or food processing. On the other hand, the average [phytate]/[Fe] molar ratios in the food groups ranged between 5 and 56. Therefore, farmers' varieties with a potentially adequate Fe availability are expected within the present germplasm. To identify varieties with adequate Fe availability, we determined the [phytate]/[Fe] molar ratio for each variety. Of the 45 farmers' varieties analyzed, 15% had [phytate]/[Fe] molar ratios below 10. Those with [phytate]/[Fe] molar ratios between 10 and 14 accounted for 20% (**Figure 1**). The varieties with [phytate]/[Fe] below 14 are unevenly distributed among the regions investigated. Most of them are located in Banikoara (25%) followed by Toucountouna (7%). Only one of these varieties originates from Djougou. Environmental effects (e.g., soil composition) may be a major contributing factor to the

Table 5. [Phytate]/[Fe] and [Phytate]/[Zn] Molar Ratios of Farmers' Varieties of Sorghum Grouped by Food Quality

food	varieties with quality perceived as	[phytate]/[Fe][a]	[phytate]/[Zn][b]
dibou	high ($n = 33$)	20.2 ± 10.1 [5.0–56.3][c]	51.6 ± 23.1 [22.3–127.6]
	medium ($n = 18$)	23.2 ± 4.7 [7.0–47.0]	58.5 ± 30.4 [18.7–124.9]
	low ($n = 9$)	12.7 ± 4.7 [5.6–20.3]	43.3 ± 24.0 [17.2–100.3]
sorou	high ($n = 32$)	20.8 ± 9.9 [7.8–56.3]	52.3 ± 22.5 [24.5–127.6]
	medium ($n = 17$)	20.6 ± 11.3 [5.0–47.0]	54.8 ± 29.0 [22.1–115.8]
	low ($n = 11$)	17.0 ± 9.3 [6.8–41.5]	45.6 ± 28.3 [17.1–124.9]
tchoukoutou	high ($n = 16$)	21.3 ± 9.6 [9.3–47.0]	57.2 ± 25.8 [18.7–115.8]
	medium ($n = 19$)	17.5 ± 8.8 [5.0–35]	44.8 ± 19.5 [22.1–87.3]
	low ($n = 25$)	20.1 ± 11.1 [6.8–56.3]	51.2 ± 29.0 [17.1–127.6]

[a] Phytate/Fe molar ratio = (mg of IP6/molecular weight of IP6)/(mg of Fe/molecular weight of Fe). [b] Phytate/Zn molar ratio = (mg of IP6/molecular weight of IP6)/(mg of Zn/molecular weight of Zn). [c] Range given in brackets.

uneven distribution. Interestingly, among these promising farmers' varieties for Fe availability, some—that is, tokogbessenou, mahi swan, biodahu, saï maï, mare dobi, chabicouman, and sakarabokuru—are regarded as having a high food quality by farmers (*23*). This group of top varieties in terms of Fe availability is recommended for the preparation of a food product such as dibou, for which the preparation technology mainly involves cooking, an operation that has only a minor diminishing effect on phytate. The top varieties could also be of interest to breeders as a basis for seed selection for high mineral availability. Further research would then be required to test these varieties under controlled conditions to assess the genetic impact on [phytate]/[Fe] molar ratios.

In conclusion, the AFLP technique allowed us to cluster the 76 collected farmers' varieties into 45 distinct genotypes. The grain-Fe and -Zn concentrations of the grains varied significantly among farmers' varieties, but this variation could not be related to genetic variance. Only the cultivation location affected the mineral concentration. Further studies are needed to assess the impact of genotype and environmental conditions on the grain-Fe and -Zn levels. The phytate concentration of the grain is governed by the growing environment, as well as the genetic makeup of the varieties, as revealed by the analysis of molecular variance. Within the farmers' germplasm we detected seven varieties with adequate Fe supply in food uses. Farmers' varieties with high [phytate]/[Fe] and [phytate]/[Zn] molar ratios may still be suitable to deliver Fe and Zn to consumers, provided that they are processed adequately. For instance, unit operations such as soaking, germination, and fermentation have been reported to induce a significant reduction of antinutritional factors and to improve the nutritional quality of cereals-based foods (*8*, *41*−*43*). Therefore, improved mineral availability could be expected from grains processed with such local methods. In further studies, attention will be focused on the impact of food-processing operations during the preparation of common sorghum-based foods of Benin.

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