Title

Sorghum germplasm profiling to assist breeding and gene identification for biofortification of grain mineral and protein concentrations

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Introduction

Sorghum (*Sorghum bicolor*) is the world's fifth most important grain crop. Sorghum is a heat and drought tolerant C4 plant and is a widely consumed cereal staple in subtropical semi-arid regions of Africa and Asia (Kresovich et al., 2005). Mineral concentrations and bioavailability are limited in cooked sorghum grain (Kayode et al., 2006), and populations relying on this food have high rates of iron and zinc deficiencies. Thus, biofortification of sorghum by increasing mineral micronutrient (especially iron and zinc) and protein concentration is of widespread interest (Pfeiffer and McClafferty, 2007; Zhao, 2008).

Genetic and genomic resources for sorghum are rapidly developing. The sorghum genome was recently sequenced (Paterson et al., 2009), and a genotyped association mapping panel of nearly 400 accessions has been established (Casa et al., 2008). Utilization of these resources, in combination with natural variation in sorghum phenotypic traits, will allow identification of the underlying genetic factors. Knowledge of markers or specific genes that affect traits such as grain iron and zinc concentration can be used for biofortification. Additionally, identifying accessions with extreme differences in traits could provide contrasting subjects to better understand the physiology and molecular biology of nutrient uptake and translocation to the grain. Thus, our objective was to profile a panel of sorghum accessions for grain mineral concentrations to determine the range of diversity in sorghum germplasm, and to compare these results with previously determined digestibility and crude protein values (Hooks et al., 2006). Here, we report results for concentration of eight minerals (Cu, Fe, K, Mg, Mn, P, S, and Zn), crude protein, and digestibility in 95 sorghum accessions.

Materials and methods

Accessions were chosen from a prior large-scale screen of 2882 accessions for grain protein concentration by NIR spectroscopy (Hooks et al., 2006). Digestibility of 2881 accessions was determined by the 12 h in vitro dry matter digestibility method (Richards et al., 1995). Within the pool of 2882 accessions, 66 accessions are also included in the association mapping panel (Casa et al., 2008), and were selected for mineral analysis. In addition, the 10 highest protein accessions and 19 lowest protein accessions were selected. The sorghum grain was grown as described previously (Hooks et al., 2006) and kept in cold storage until mineral analysis was performed. One hundred seeds were selected, dried in a drying oven at 65 C for 24 h and weighed. This sample was ground in a coffee mill. Subsamples of 0.35 g were acid digested and mineral concentrations were determined by ICP.

Results

Except for protein concentration, which was non-randomly selected, the traits observed in the 95 accessions exhibited a wide range of values. We observed an apparent normal distribution of grain size (Fig. 1), digestibility (Fig. 2), and mineral concentrations (Fe and Zn shown, Fig 3.). The range of values are shown in Table 1, and in most cases exceeded 2-fold.

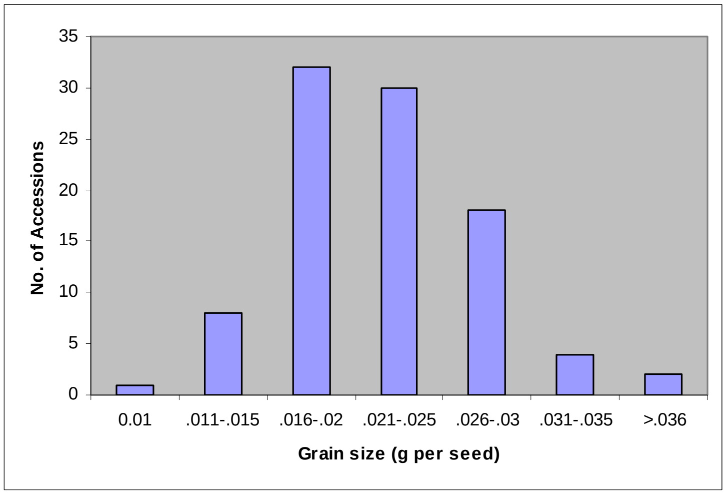

Figure 1. Grain size (g per individual grain) in the 95 accession panel.

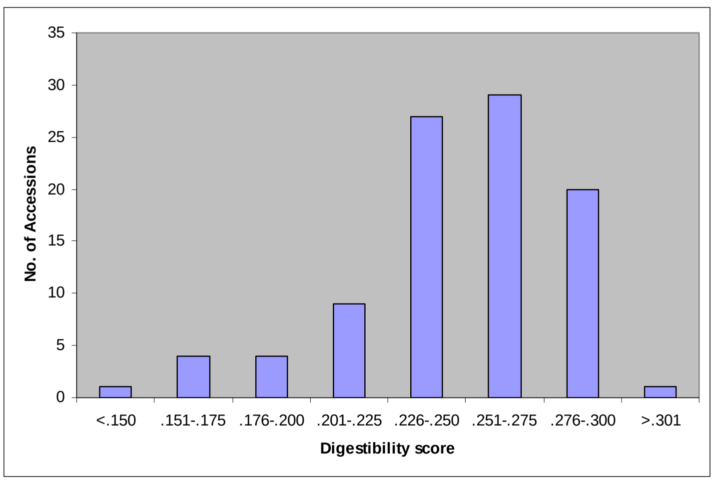

Figure 2. Digestibility scores in the 95 accession panel.

Table 1. Phenotypic diverstiy within the 95 accessions.

Trait	Low value	High value	Range	Mean value
Protein (%)	9.2	18.2	2.0	12.5 ± 2.3
Digestibility	0.149	0.301	2.0	0.25 ± 0.03
Cu (ppm)	1	11	11.0	4.4 ± 2.0
Fe (ppm)	24	73	3.0	41.1 ± 9.6
K (%)	0.3	0.54	1.8	0.4 ± 0.05
Mg (%)	0.15	0.3	2.0	0.21 ± 0.03
Mn (ppm)	10	34	3.4	20.7 ± 4.8
P (%)	0.28	0.59	2.1	0.42 ± 0.07
S (%)	0.1	0.21	2.1	0.14 ± 0.03
Zn (ppm)	15	59	3.9	32.4 ± 9.4

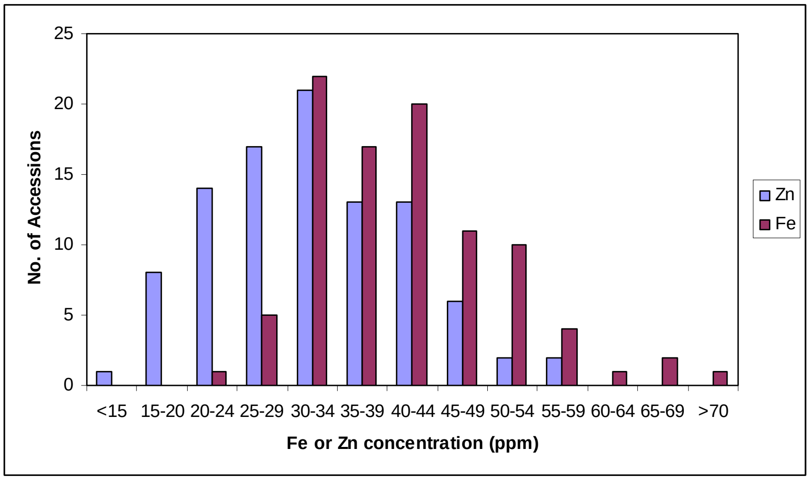

Figure 3. Frequency distribution of grain Fe and Zn concentrations in the 95 accessions.

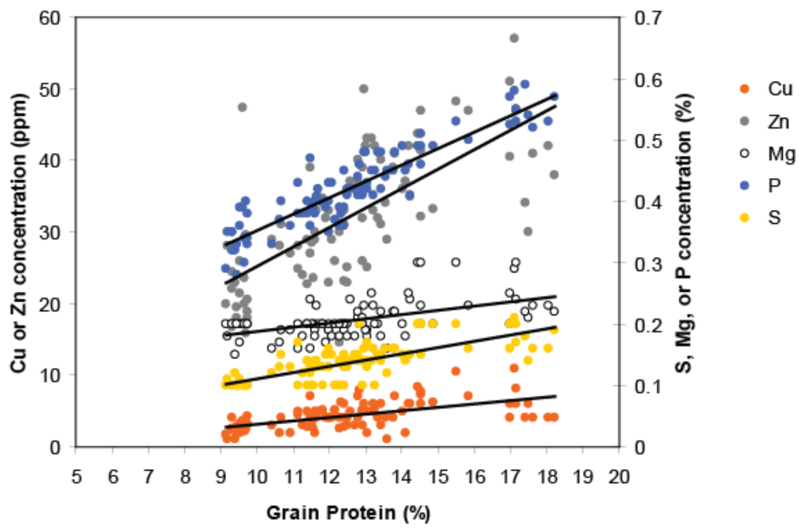

Figure 4. Correlation between grain protein and Cu, Zn, Mg, P, and S concentrations. Linear regression equation for Cu, y=0.4734x-1.5826; S, y=0.0102x+0.0093; Mg, y=0.0068x+0.1202; Zn, y=2.7201x-1.9577; P, y=0.0268x+0.0846.

Table 2. Correlation coefficients between sorghum grain characteristics in the 95 accession panel.

	Protein	seed weight	Cu	Fe	K	Mg	Mn	P	S	Zn
Protein	*									
Seed weight	0.099	*								
Cu	0.327	0.08	*							
Fe	0.101	0.056	0.311	*						
K	0	0.038	0.022	0.004	*					
Mg	0.275	0.047	0.437	0.355	0.003	*				
Mn	0.019	0.043	0.184	0.242	0.005	0.168	*			
P	0.833	0.114	0.406	0.113	0.002	0.296	0.033	*		
S	0.567	0.044	0.457	0.128	0	0.224	0.055	0.594	*	
Zn	0.4945	0.047	0.481	0.242	0.02	0.31	0.047	0.611	0.471	*
Digestibility	0.008	0.125	0.004	0.0126	0.027	0.0049	0.0107	0	0.005	0.005

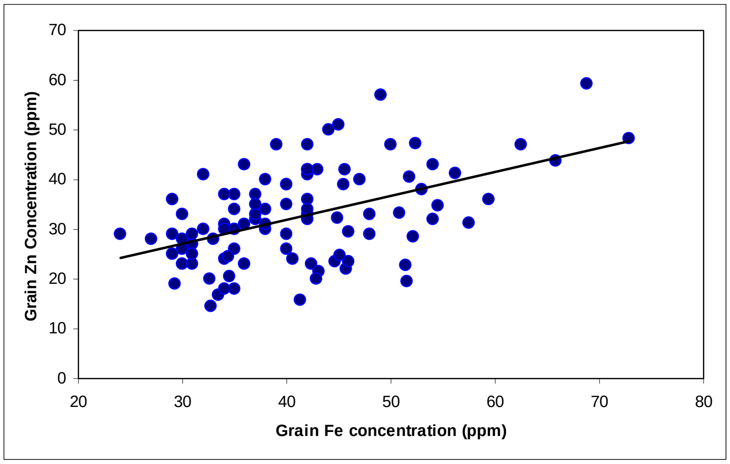

Figure 5. Correlation between grain Fe and Zn concentrations in the 95 accession panel. Equation for linear regression; y=0.4816x+12.624.

Table 3. Grain chacteristics for the 5 highest and 5 lowest Fe and Zn accessions in the 95 accession panel.

Accession	Seed Wt. (g)	Digestibility	Protein (%)	Cu (p)pm	Fe (p)pm	K (%)	Mg (%)	Mn (ppm)	P (%)	S (%)	Zinc (ppm)
PI 533991	0.025	0.28	13.6	1	24	0.39	0.16	14	0.45	0.12	29
PI 308484	0.028	0.26	9.2	1	27	0.43	0.18	13	0.35	0.11	28
PI 533949	0.021	0.26	12.5	3	29	0.43	0.18	17	0.41	0.14	25
PI 533957	0.016	0.22	12.1	4	29	0.43	0.18	25	0.43	0.15	29
PI 563593	0.030	0.23	9.7	4	29	0.40	0.20	27	0.33	0.10	19
PI 534096	0.016	0.27	12.9	8	59	0.40	0.20	29	0.46	0.20	36
PI 562744	0.014	0.18	17.2	8	63	0.40	0.30	34	0.55	0.20	47
PI 534088	0.031	0.30	14.5	8	66	0.40	0.30	24	0.49	0.20	44
PI 534127	0.015	0.21	15.5	10	73	0.40	0.30	30	0.53	0.20	48
TX430	0.035	nd	nd	8	69	0.30	0.20	32	0.40	0.20	59

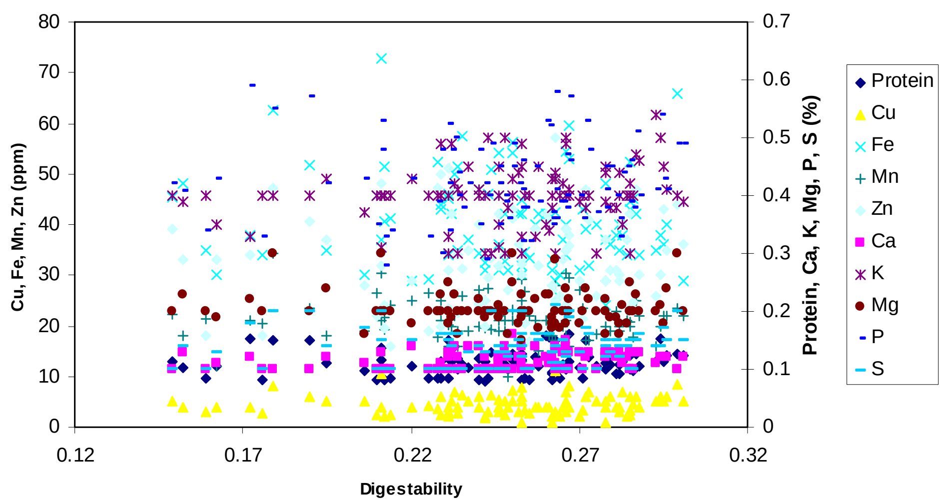

Figure 6. Correlation between grain digestibility and grain nutrient concentrations.

Several minerals showed strong positive correlations with protein concentration (Fig. 4, Table 2), suggesting that mineral and protein density in grain can be improved together. Several minerals were positively correlated, (i.e. Fe and Zn, Fig. 5, Table 2), suggesting that improving accumulation of one of these minerals might also result in increases in others. Full results of the 5 highest and 5 lowest Fe and Zn lines are presented in Table 3. None of the mineral or protein concentrations were correlated with digestibility (Fig. 6).

Discussion

Diversity in mineral concentrations for biofortification has been studied in a number of species (White and Broadley, 2009). Markers associated with seed mineral variation have been identified by QTL mapping in Arabidopsis (Vreugdenhil et al., 2004; Waters and Grusak, 2008), bean (Guzman-Maldonado et al., 2003; Gelin et al., 2007), and rice (Stangoulis et al., 2007). Sorghum varieties collected from farmers in Benin exhibited an approximately 4-fold range in grain Fe and Zn concentration (Kayode et al., 2006). The authors cautioned that environmental variability could strongly affect concentration values. The samples tested here were grown over two years in a single location, precluding testing for environmental interactions. Inference of relationships between grain mineral and protein differences to the underlying genetic differences are therefore limited to the parameters of this study. However, based on the variation within the 95 accessions tested here, sufficient diversity is present in existing sorghum germplasm to warrant further investigations into genes or markers for use in biofortification or sorghum breeding programs.

QTL mapping for grain nutritional traits could be carried out with existing mapping populations, or specific populations could be constructed based on wide variation in mineral, protein, or digestibility profiles in screens such as the one presented here. The QTL information could be used for marker assisted selection breeding programs, or for positional cloning studies to identify specific genes that affect grain mineral traits. The association mapping panel (Casa et al., 2008) offers the opportunity for identifying QTL without the expensive and time consuming process of developing mapping populations. Traditional breeding methods that integrate data on grain mineral concentrations into the selection process for parental lines (Ortiz-Monasterio et al., 2007) could result in significant improvement in nutritional quality of newly released varieties of sorghum. Individual genes identified by genetic mapping could be used in transgenic approaches to develop lines with higher accumulation of nutrients in grain. Likewise, comparative studies using transcriptomic or proteomic approaches between extreme high and low lines could allow identification of biofortification target genes.

One outcome of the profiling approach taken here is the indication that grain protein concentration is positively correlated with concentrations of several minerals, most notably Zn. This is similar to results in control and transgenic wheat lines, in that protein and Zn (and Fe) concentrations were correlated (Uauy et al., 2006). It may be possible that breeding for increased protein concentration will also increase Fe and Zn (and certain other minerals) in grain, either because these minerals are associated with storage proteins, or because these minerals use common source-to-sink translocation mechanisms. Another important result is that grain digestibility was not correlated to protein or any of the mineral concentrations, suggesting that sorghum breeders can select for improved digestibility independently of mineral or crude protein concentration. Thus, the potential exists for increasing bioavailability of the quantity of mineral already in the seed, while simultaneously increasing the absolute mineral content, with a synergistic impact on total nutritional value.

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