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Comparative Evaluation of Ground and Unground Pearl Millet and Sorghum Grain Samples for Determining Total Iron and Zinc

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Rapid and effective methods are needed to analyze large numbers of grain samples for iron (Fe) and zinc (Zn) to select cultivars that are denser in these minerals. This study was conducted for the comparative evaluation of ground and unground grain samples for determining total Fe and Zn in pearl millet and sorghum cultivars with a range in seed size. In general, the results of our study with 50 pearl millet and 49 sorghum cultivars showed that grain Fe and Zn, in these relatively small-seeded crops, can be routinely determined using unground samples. Highly significant positive correlations were found between the values of Fe and Zn in grains of these crops determined using ground and unground samples.

Keywords Biofortification of crops, iron and zinc malnutrition, grains denser in minerals, high volume routine method, seed size

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] and sorghum [*Sorghum bicolor* (L.) Moench] are important staple foods in the semi-arid tropics of Asia and Africa. These crops remain the principal sources of energy, protein, vitamins and minerals for millions of the poor people in these regions. Moreover, pearl millet and sorghum grow in harsh environment where other crops do not grow well. Improving productivity and nutritive value of these crops especially relative to micronutrients such as iron (Fe) and zinc (Zn) will significantly contribute to the household food security and nutrition of the population in the semi-arid tropical regions of Asia and Africa (Dwivedi et al. 2012; Welch and Graham 2004).

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and its partners have been working on pearl millet and sorghum biofortification for enhancing the grain Fe and Zn contents as biofortified crop cultivars; because wherever practical, biofortified cultivars of these crops offer a cost effective and sustainable means to tackle the micronutrient deficiencies (Velu et al. 2011; Ashok Kumar et al. 2012; Dwivedi et al. 2012). This requires rapid, accurate, and economic methods for analyzing grain samples of a large number of cultivars of sorghum and pearl millet to aid in the selection and breeding of cultivars that are dense in Fe and Zn.

It has been argued that if the analysis of grains is conducted without grinding them, this will not only make the method more rapid but will also avoid the possible contamination of the grain samples during grinding. Of course for the large-seeded crops, the grinding of grains is rather inevitable. But for the relatively small-seeded crops such as pearl millet and sorghum, the grinding step could probably be skipped; and the unground grain samples instead could be used for routine analysis in an analytical laboratory.

There is little information in the published literature on this aspect of providing analytical support through grain analysis. With this objective in view, a study was undertaken to compare

and evaluate the results of Fe and Zn analysis performed on unground and ground grain samples of pearl millet and sorghum cultivars with a range in seed size.

Materials and Methods

The study was conducted at the ICRISAT center analytical laboratory in Patancheru, Andhra Pradesh, India.

Fifty cultivars of pearl millet and 49 of sorghum were selected for the study from breeders' material so as to capture the diversity in seed size. Among the 50 pearl millet cultivars used, 35 were small-seeded (1000 seed wt. < 10g), and 24 were bold-seeded (1000 seed wt. > 10g). For sorghum, 25 cultivars were small-seeded (1000 seed wt. < 20g), and 24 were bold-seeded (1000 seed wt. > 20g). Before grinding and or use, all the grain samples were dried in the oven at 60°C for 48 h. Both ground and unground grain samples were used in the study.

The study consisted of three factors: two crops (pearl millet and sorghum), two treatments (ground and unground grain samples), and two grain sizes [pearl millet: small (1000 seed wt. < 10g), and bold (1000 seed wt. > 10g.), and sorghum: small (1000 seed wt. < 20g) and bold (1000 seed wt. > 20g)]. There were 8 treatment with 3 replications arranged in a 2 x 2 x 2 factorial complete randomized design.

Unground and ground grain samples in three replications were analyzed for Fe and Zn using the modified triacid (nitric acid, perchloric acid and sulfuric acid in the ratio of 10:2:0.5, v/v) digestion method (Sahrawat et al. 2002). Iron and Zn in the digests were determined using atomic absorption spectrophotometry (Mills and Jones 1996).

The data obtained were statistically analyzed using analysis of variance based on factorial complete randomized design. Also, the significance of the results was also tested. Correlations between the values of Fe and Zn obtained using ground and unground grain samples, were also determined.

Results and Discussion

The variability in 1000 seed wt. of the small-seeded pearl millet cultivars ($n = 35$) used in the study ranged from 4.7 to 9.4 g with a mean of 7.5g (Table 1); while in the case of bold-seeded pearl millet cultivars ($n = 15$), 1000 seed wt. ranged from 10.0 to 13.0 with a mean of 10.8g (Table 2).

The statistical analysis of the results showed that there was a significant difference in Fe content of small- and bold-seeded pearl millet cultivars between ground and unground treatments (Tables 1 and 2).

The Fe content in small-seeded pearl millet, ground cultivars varied from 26.6 to 77.9 mg kg^{-1} with a mean value of 51.3 mg kg^{-1} ; whereas for the unground samples, Fe content varied from 28.4 to 75.7 mg kg^{-1} with a mean value of 50.6 mg kg^{-1} (Table 1). For the bold-seeded grain samples, the Fe content varied from 27.7 to 81.5 mg kg^{-1} with a mean value of 49.1 mg kg^{-1} for the ground treatment; and for the unground treatment, it ranged from 26.5 to 81.7 mg kg^{-1} with a mean value of 47.8 mg kg^{-1} (Table 2).

The results for Zn content determined using ground and unground samples showed that there were no significant differences in the values of Zn in the grain samples of both bold- and small-seeded grains of pearl millet. The Zn content in small-seeded pearl millet varied from 18.7 to 73.7 mg kg^{-1} with a mean of 50.8 mg kg^{-1} for ground treatment, whereas for the unground treatment, Zn content varied from 19.5 to 72.1 mg kg^{-1} with a mean value of 49.9 mg kg^{-1} (Table 3). Similar results were obtained for the bold-seeded grain samples of pearl millet, which are summarized in Table 4.

The 1000 seed wt. of 25 small-seeded sorghum cultivars varied from 9.6 to 19.3g (Table 5); whereas those of 24 bold-seeded sorghum cultivars ranged from 26.9 to 46.4 g (Table 6).

The statistical analysis of the results on seed Fe content in sorghum grain samples showed that there were no significant differences in Fe content in ground and unground treatments both for small-seeded (Table 5) and bold-seeded (Table 6) sorghum cultivars.

As in the case of grain Fe, there were no significant differences in grain Zn in ground and unground treatments for both small-seeded (Table 7) and bold-seeded (Table 8) sorghum cultivars.

Further, simple correlation analysis between the values of grain Fe and Zn determined using ground and unground grain samples showed that there were highly significant positive correlations for both pearl millet and sorghum grains. In this analysis both small- and bold-seeded grains of the two crops were combined. The r^2 between the values for ground and unground treatments was relatively lower for Fe for both pearl millet ($r^2 = 0.91$, $n = 50$) and sorghum ($r^2 = 0.90$, $n = 49$) than for Zn contents (for pearl millet, $r^2 = 0.95$, $n = 50$; for sorghum, $r^2 = 0.94$, $n = 49$).

The results of our study on the comparative evaluation of determining Fe and Zn using ground and unground grain samples of selected pearl millet and sorghum cultivars showed that for routine analysis, the use of unground grain samples seem satisfactory. Obviously, the use of unground grain samples of these crops for Fe and Zn, and probably other minerals will make the method relatively rapid. The use of unground grain samples will also potentially eliminate the contamination of samples. Our results merit further evaluation using large numbers of cultivars of sorghum and pearl millet, and other relatively small-seeded crops such as wheat and rice.

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Table 1. Comparison of Fe content in pearl millet grains using ground and unground samples of pearl millet cultivars with small grain size (n = 35)

Cultivar	1000 seed weight (g)	Fe (mg kg ⁻¹)	
		Ground	Un-ground
FeZn –sta-T Rp 3A-125	4.7	68.4	62.7
ICMB 89111	5.2	66.5	64.7
ICMB 91444	5.2	71.4	70.6
81B	5.2	32.2	31.4
FeZn –sta-T Rp 3A-208	5.9	39.4	37.8
FeZn –sta-T Rp 3A-223	6.3	39.7	39.0
ICMB 92111	6.3	43.4	48.9
ICMB 91777	6.6	56.8	56.1
ICMB 04555	6.7	39.8	40.2
ICMB 00999	6.8	44.8	44.1
FeZn –sta-T Rp 3A-112	6.9	66.5	62.2
841B	7.0	45.5	48.5
ICMB 02666	7.0	26.6	28.4
ICMB 00666	7.2	59.2	58.3
FeZn –sta-T Rp 3A-110	7.2	31.1	30.7
ICMB 91666	7.2	56.6	59.0
ICMB 04222	7.3	41.4	40.9
FeZn –sta-T Rp 3A-214	7.6	37.0	37.0
ICMB 02222	7.7	56.9	56.0
FeZn –sta-T Rp 3A-306	8.0	45.7	44.8
FeZn –sta-T Rp 3A- 122	8.1	77.9	75.7
ICMB 03111	8.1	60.3	58.6
FeZn –sta-T Rp 3A-216	8.3	31.4	31.5
FeZn –sta-T Rp 3A- 324	8.3	75.7	72.1
ICMB 04777	8.4	58.5	57.2
ICMB 04999	8.5	42.7	41.3
FeZn –sta-T Rp 3A-305	8.5	52.3	58.6
ICMB 04888	8.6	48.6	48.2
ICMB 00777	8.6	40.3	40.1
ICMB 88006	8.8	32.6	30.9
ICMB 04666	8.9	40.1	39.9
ICMB 03999	9.0	55.9	56.8
843B	9.0	72.5	71.6
ICMB 93222	9.1	74.2	73.3
FeZn –sta-T Rp 3A-116	9.1	63.2	54.2

Mean 7.5 51.3 50.6
SEm± 0.00025
LCD (P = 0.05) 0.0004
CV (%) 0.57

Table 2. Comparison of Fe content in pearl millet grains using ground and unground samples in pearl millet cultivars with bold grain size (n =15)

Cultivar	1000 seed weight (g)	Fe (mg kg ⁻¹)	
		Ground	Unground
FeZn -sta-T Rp 3A-130	10.0	45.8	48.7
FeZn -sta-T Rp 3A-303	10.0	61.1	58.7
842B	10.1	34.3	33.9
ICMB 97222	10.1	53.8	47.0
FeZn -sta-T Rp 3A-112	10.1	34.6	33.5
FeZn -sta-T Rp 3A-125	10.2	52.1	46.6
ICMB 88004	10.2	50.8	42.8
ICMB 02444	10.6	59.4	59.4
ICMB 02111	10.9	39.7	36.8
FeZn -sta-T Rp 3A-218	10.9	27.7	26.5
FeZn -sta-T Rp 3A-330	11.0	47.6	47.2
ICMB 03333	11.2	53.2	52.0
ICMB 04444	11.7	81.5	81.7
FeZn -sta-T Rp 3A-305	12.0	39.1	42.6
ICMB 00888	13.0	56.2	58.9
Mean	10.8	49.1	47.8
SEm±		0.00025	
LCD (P = 0.05)		0.0004	
CV (%)		0.57	

Table 3. Comparison of Zn content in pearl millet grains using ground and un-ground samples of pearl millet cultivars with small grain size (n =35).

Cultivar	1000 seed weight (g)	Zn (mg kg ⁻¹)	
		Ground	Un-ground
FeZn -sta-T Rp 3A-125	4.7	67.0	59.9
ICMB 89111	5.2	72.1	67.7
ICMB 91444	5.2	69.1	71.1
81B	5.2	30.0	29.8
FeZn -sta-T Rp 3A-208	5.9	50.4	46.3
FeZn -sta-T Rp 3A-223	6.3	59.5	59.9
ICMB 92111	6.3	50.6	55.7
ICMB 91777	6.6	73.7	72.1
ICMB 04555	6.7	18.7	19.5
ICMB 00999	6.8	49.1	45.8
FeZn -sta-T Rp 3A-112	6.9	55.2	62.2
841B	7.0	44.0	48.3
ICMB 02666	7.0	23.8	25.0
ICMB 00666	7.2	43.7	43.2
FeZn -sta-T Rp 3A-110	7.2	40.5	37.4
ICMB 91666	7.2	60.2	59.6
ICMB 04222	7.3	38.9	37.5
FeZn -sta-T Rp 3A-214	7.6	48.7	53.7
ICMB 02222	7.7	48.5	48.9
FeZn -sta-T Rp 3A-306	8.0	54.2	57.3
FeZn -sta-T Rp 3A- 122	8.1	59.7	65.9
ICMB 03111	8.1	46.9	45.3
FeZn -sta-T Rp 3A-216	8.3	50.7	57.3
FeZn -sta-T Rp 3A- 324	8.3	60.5	55.5
ICMB 04777	8.4	47.2	45.4
ICMB 04999	8.5	43.3	40.5
FeZn -sta-T Rp 3A-305	8.5	69.0	65.1
ICMB 04888	8.6	45.8	39.1
ICMB 00777	8.6	33.0	26.8
ICMB 88006	8.8	32.5	31.1
ICMB 04666	8.9	51.4	50.4
ICMB 03999	9.0	41.9	42.8
843B	9.0	62.0	59.1
ICMB 93222	9.1	68.5	71.7
FeZn -sta-T Rp 3A-116	9.1	67.0	61.9
Mean	7.5	50.8	50.3
SEm±			0.0003
LCD (P = 0.05)			NS (Not significant)
CV (%)			0.6

Table 4. Comparison of Zn in pearl millet grains using ground and unground samples in pearl millet cultivars with bold grain size (n =15).

Cultivar Name	1000 seed weight (g)	Zn (mg kg ⁻¹)	
		Ground	Unground
FeZn -sta-T Rp 3A-130	10.0	60.9	59.9
FeZn -sta-T Rp 3A-303	10.0	61.5	59.3
842B	10.1	36.1	35.5
ICMB 97222	10.1	56.0	53.1
FeZn -sta-T Rp 3A-112	10.1	45.8	45.7
FeZn -sta-T Rp 3A-125	10.2	56.8	53.8
ICMB 88004	10.2	42.8	42.0
ICMB 02444	10.6	52.8	55.7
ICMB 02111	10.9	32.3	32.0
FeZn -sta-T Rp 3A-218	10.9	47.7	45.0
FeZn -sta-T Rp 3A-330	11.0	53.8	49.3
ICMB 03333	11.2	45.0	43.7
ICMB 04444	11.7	62.3	53.2
FeZn -sta-T Rp 3A-305	12.0	45.4	49.2
ICMB 00888	13.0	49.3	51.4
Mean	10.8	49.9	48.6
SEm±		0.0003	
LCD (P = 0.05)		NS	
CV (%)		0.6	

Table 5. Comparison of Fe in sorghum grains using ground and unground samples for sorghum cultivars with small grain size (n = 25).

Cultivar	1000 seed weight (g)	Fe (mg kg ⁻¹)	
		Ground	Un-ground
III MNNTT 1030	9.6	42.2	41.5
II MNTT 1001	9.6	26.9	24.9
II MNTT 1095	10.4	35.8	39.0
III MNNTT 1001	10.8	53.3	53.6
III MNNTT 1044	14.5	46.5	42.7
III MNNTT 1032	15.0	43.3	42.9
III MNNTT 1019	16.8	50.8	49.0
III MNNTT 1026	17.5	39.9	38.2
II MNTT 1107	17.6	32.8	24.6
III MNTT 1003	17.7	65.0	53.2
II MNTT 1087	17.8	20.8	18.8
III MNNTT 1047	18.2	54.6	50.7
II MNTT 1080	18.4	27.0	25.2
II MNTT 1053	18.5	24.9	25.2
II MNTT 1086	18.5	21.8	25.9
II MNTT 1040	18.6	21.1	19.7
II MNTT 1076	18.6	47.8	42.6
II MNTT 1116	18.6	25.5	23.5
II MNTT 1068	18.7	29.0	28.6
II MNTT 1089	18.7	32.5	28.2
I MNTT 1117	18.8	38.8	31.2
II MNTT 1071	18.9	50.9	65.1
II MNTT 1019	19.0	19.0	18.2
II MNTT 1066	19.0	24.8	21.8
I MNTT 1008	19.3	26.8	26.0
Mean	6.8	36.1	34.4
SEm±			0.00019
LCD (P = 0.05)		NS	
CV (%)		2.08	

Table 6. Comparison of Fe in sorghum grains using ground and unground samples of sorghum cultivars with bold grain size (n = 24).

Cultivar	1000 seed weight (g)	Fe (mg kg ⁻¹)	
		Ground	Unground
I MNTT 1065	26.9	27.9	24.4
I MNTT 1018	27.0	28.4	26.3
I MNTT 1010	27.7	28.8	27.8
I MNTT 1118	27.7	26.2	24.2
I MNTT 1061	27.8	27.9	37.6
II MNTT 1006	28.9	37.1	30.5
II MNTT 1067	30.3	34.3	34.3
I MNTT 1109	31.4	41.5	35.6
IRAT 204 Bc23/R04	32.0	38.9	38.8
II MNTT 1060	32.5	33.3	27.1
I MNTT 1025	33.0	37.6	26.4
I MNTT 1032	33.3	62.2	62.4
I MNTT 1111	33.4	48.7	47.9
III MNTT 1042	33.4	36.6	35.1
I MNTT 1011	34.5	36.8	33.5
I MNTT 1020	34.9	22.5	25.0
II MNTT 1007	36.6	29.0	37.6
I MNTT 1114	38.5	31.4	30.5
I MNTT 1033	38.7	23.0	23.7
I MNTT 1112	39.2	54.8	54.4
I MNTT 1045	39.6	52.7	49.9
I MNTT 1043	41.7	31.9	27.8
I MNTT 1027	42.0	33.4	26.1
I MNTT 1062	46.4	34.2	37.4
Mean	34.1	35.8	34.4
SEm±		0.00019	
LCD (P = 0.05)		NS	
CV (%)		2.08	

Table 7. Comparison of Zn in sorghum grains using ground and unground samples for sorghum cultivars with small grain size (n = 25).

Cultivar	1000 seed weight (g)	Zn (mg kg ⁻¹)	
		Ground	Un-ground
III MNNTT 1030	9.6	34.5	32.5
II MNTT 1001	9.6	30.7	31.4
II MNTT 1095	10.4	47.5	47.3
III MNNTT 1001	10.8	38.4	36.2
III MNNTT 1044	14.5	44.7	34.3
III MNNTT 1032	15.0	31.1	32.4
III MNNTT 1019	16.8	31.2	30.1
III MNNTT 1026	17.5	34.1	32.9
II MNTT 1107	17.6	38.7	34.6
III MNTT 1003	17.7	61.0	59.8
II MNTT 1087	17.8	29.2	27.9
III MNNTT 1047	18.2	51.6	48.4
II MNTT 1080	18.4	16.9	17.5
II MNTT 1053	18.5	35.9	30.8
II MNTT 1086	18.5	21.6	16.5
II MNTT 1040	18.6	34.4	29.2
II MNTT 1076	18.6	44.7	34.0
II MNTT 1116	18.6	37.7	33.2
II MNTT 1068	18.7	18.9	17.1
II MNTT 1089	18.7	40.3	34.4
I MNTT 1117	18.8	33.1	30.1
II MNTT 1071	18.9	37.7	32.8
II MNTT 1019	19.0	26.4	25.9
II MNTT 1066	19.0	22.3	18.4
I MNTT 1008	19.3	25.3	25.5
Mean	16.8	34.7	31.7
SEm±		0.00015	
LCD (P = 0.05)		NS	
CV (%)		1.58	

Table 8. Comparison of Zn in sorghum grains using ground and unground samples of sorghum cultivars with bold grain size (n = 24).

Cultivar	1000 seed weight (g)	Zn (mg kg ⁻¹)	
		Ground	Unground
I MNTT 1065	26.9	23.0	21.3
I MNTT 1018	27.0	24.8	23.3
I MNTT 1010	27.7	26.5	25.8
I MNTT 1118	27.7	23.9	20.5
I MNTT 1061	27.8	30.3	21.8
II MNTT 1006	28.9	57.5	47.3
II MNTT 1067	30.3	24.1	22.2
I MNTT 1109	31.4	45.3	45.0
IRAT 204 Bc23/R04	32.0	32.8	29.9
II MNTT 1060	32.5	26.9	24.0
I MNTT 1025	33.0	41.0	37.5
I MNTT 1032	33.3	29.3	25.8
I MNTT 1111	33.4	44.0	42.0
III MNTT 1042	33.4	25.8	25.8
I MNTT 1011	34.5	33.0	30.0
I MNTT 1020	34.9	26.4	25.3
II MNTT 1007	36.6	39.2	33.5
I MNTT 1114	38.5	25.5	22.9
I MNTT 1033	38.7	18.9	18.1
I MNTT 1112	39.2	60.6	61.0
I MNTT 1045	39.6	31.0	27.5
I MNTT 1043	41.7	38.4	35.2
I MNTT 1027	42.0	36.8	37.3
I MNTT 1062	46.4	32.3	31.3
Mean	34.1	33.2	30.6
SEm±			0.00015
LCD (P = 0.05)			NS
CV (%)			1.58

