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Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions

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Abstract
The lack of micronutrients such as Fe and Zn in staple food crops is a widespread nutrition and health problem in developing countries. Biofortification is one of the sustainable approaches, for improving the Fe and Zn content and their bioavailability in rice grain. Screening germplasm for Fe and Zn content is the initial step of biofortification. We analyzed brown rice of 126 accessions of rice genotypes for Fe and Zn concentration. Iron concentration ranged from 6.2 ppm to 71.6 ppm and zinc from 26.2 ppm to 67.3 ppm. Zn concentration and grain elongation (-0.25) was significantly correlated. The wild accessions had the highest Fe and Zn. Thus, wild species are a good source for biofortification of popular rice cultivars using conventional, acceptable, non transgenic methods.

Keywords: Iron, Zinc, germplasm screening, biofortification, brown rice

INTRODUCTION

Rice is a major food staple and energy source of more than half the world population, being the major source of carbohydrate and even protein. However, rice is a poor source of essential micronutrients such as Fe and Zn [1]. Micronutrient malnutrition, and particularly Fe and Zn deficiency affect over three billion people worldwide, mostly in developing countries [2, 3].

Production of varieties containing high amounts of bioavailable Fe would improve Fe nutrition in regions where iron deficiency is prevalent [4]. It is necessary to improve both the net Fe & Zn concentration and their bioavailability in rice grain for improving the Fe & Zn intake in populations dependent on rice as a staple food. Food fortification has been recommended as one of the preferred approaches for preventing and eradicating iron and zinc deficiency [5]. Scientists have coined the term “biofortified” for genotypes that deliver increased levels of essential minerals or vitamins. Biofortification, when applied to staple crops, such as rice, is a sustainable approach, provided that access to the technology in the form of seeds is unrestricted.

In addition to agronomical management, selecting genotypes with high efficiency of Fe & Zn accumulation in the endosperm and their bioavailability from existing germplasm collection may be an efficient and reliable way to deliver Fe nutrition benefits to farmers and local population [6]. Germplasm has been screened for high Fe and Zn in many crops including rice. Cheng et al. [7] screened 113 rice landraces from 12 provinces of China. They reported that japonica rice had higher Fe than that of indica rice variety. 11,400 rice samples of brown and milled rice were evaluated for Fe and Zn during 2006-2008 by Martinez et al. [8]. They found that brown rice had 10-11 ppm Fe and 20-25 ppm Zn while milled rice had 2-3 ppm Fe and 16-17 ppm Zn. Banerjee et al. [9] screened 46 rice lines including cultivated and wild accessions and showed that wild rice accessions have higher grain Fe and Zn concentration.

Micronutrient-dense cultivars can be selected from within existing germplasm, or can be generated de novo through genetic modification. Plant breeders involved in breeding staple food crops with more Fe, Zn need to identify donor parents carrying the target traits. Perl’s Prussian Blue and DTZ staining method are standardized for Fe and Zn estimation respectively to conduct the initial screening of genotypes. Although these methods are simple and inexpensive but qualitative instead of quantitative [10]. Accurate estimation of Fe and Zn concentration is normally achieved through inductively coupled plasma-optical emission spectrophotometry (ICP-OES) or atomic absorption spectroscopy (AAS) [11]. Around 75% of total grain Zn was reported to be present in the endosperm of brown rice [12], while Takahashi et al. [13] revealed that Zn is most abundant in the embryo and in the aleurone layer using X-ray fluorescence imaging. Fe has been localized in the aleurone layer and in the embryo using histochemical techniques [14,15,16] and in the endosperm by X-ray fluorescence imaging [13].

A distinction has to be made between content and concentration. The content of iron and zinc in rice depends on the grain size. Aromatic long grain basmati lines are known to be high in iron content. The high or low content of mineral elements in grain largely determine the nutrient value of rice. Zhang et al. [17] showed that single grain selection of narrow grains tends to increase the content of Zn, Mn and P; long grains tends to increase the content of Fe and Mn; short grains tend to increase content of Zn and P while selection of single plants with bigger grain weight tends to increase the content of P.

The objectives of the present study were to (i) screen rice germplasm for iron and zinc concentration in brown rice (ii) analyze the correlation between Fe and Zn concentration and seed dimensions, if any and (iii) identify lines with high Fe and Zn

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concentration.

MATERIALS AND METHODS

Samples

126 accessions including 7 accessions of two wild species used for the present study are given in Table1. All accessions are maintained at Directorate of Rice Research (DRR), Hyderabad, India. All accessions were grown in DRR field during kharif 2006 and seed harvested from these lines was used. Some accessions were listed twice or more as their seeds were collected in different lots to study the extent of variation.

Image analysis for seed morphological traits

65 accessions were selected and used for image analysis. 11 parameters were analyzed for the measurement of seed (with husk) using Biovis PSM image analysis system for plant science and agriculture. The parameters analyzed were length, width, area, perimeter, density, roundness, compactness, elongation, roughness, perimeter/length, perimeter/width.

Fe and Zn concentration analysis

Fe and Zn concentration in all the accessions was analyzed using Atomic Absorption spectrophotometer at National Institute of Nutrition, Hyderabad, India (AAS, Varian). Seeds from all varieties were dehusked gently using a palm dehusker. Concentration was expressed in parts per million (ppm). A minimum of two replications from each of the cultivars and wild accessions were analyzed for the two micronutrients. The triacid method of digestion was followed. The variation in replications for each sample did not exceed ± 4ppm for Fe and ± 2 ppm for Zn. The mean of the two replicates is presented in results (Table 1).

RESULTS

126 accessions including cultivated indica and japonica rice cultivars, germplasm accessions and wild rice genotypes were analyzed for Fe and Zn concentration in brown rice. Fe concentration ranged from 6.2 ppm to 71.6 ppm and Zn concentration ranged from 26.2 ppm to 67.3 ppm. Both Fe and Zn were high in wild rice genotypes and least in japonica. The mean value of Fe concentration in all wild varieties was 27.5 ppm ranging from 11.9 ppm in SL-12 to 71.5 ppm in SL-32. The mean value of Zn concentration in all wild accessions was 50.7 ppm ranging from 31.7 ppm in SL-12 to 67.2 ppm in SL-48. Five wild accessions had both Fe and Zn in high concentration among which SL-32 had 71.5 ppm Fe and 61.1 ppm Zn **(**Table 1).

Table 1. Iron and Zinc concentration of brown rice in 126 rice accessions

S.No	Accession	Fe (ppm)	Zn (ppm)	S.No	Accession	Fe (ppm)	Zn (ppm)	S.No	Accession	Fe (ppm)	Zn (ppm)
1	ADT-36	8.3	37.2	43	Jalmagna	12.4	51	85	Pusa Sugandha	9.5	33.1
2	ADT-41	10.5	42	44	Jaya	7.3	30.9	86	Pusa Sugandha	10.6	35
3	Aghoni bora	8.2	51.6	45	Jaya	10	25.2	87	PY-3	14.9	40
4	Amulya	8.2	35	46	Jyoti	40.3	27.1	88	Rasi	16	36.8
5	Annada	48.4	36.1	47	Kalanamak	19.9	31.1	89	Sabita	14.1	36.1
6	ASD-16	41.3	45.8	48	Kanehna	13	48.7	90	Salivahna	14	42.8
7	Athira	6.6	34.8	49	Kasturi	15	45.7	91	Sasyasri	14.3	53.4
8	Badsha Bhog	11.9	36.1	50	Kavitha	23.2	36.9	92	SLC 11	15.1	34.5
9	Basmati-370	12.7	44.7	51	KJT-5	12	33	93	SLC-2	6.8	29.4
10	Benibhog	16.6	51.3	52	KJT-jaya	10.3	36	94	SLC-5	13.1	34.3
11	Bhararie	12.7	44.2	53	KRH-2	9.5	38.4	95	Sugandhamati	9.1	39.2
12	BPT-5204	13.4	47.8	54	Krishnahamsa	14.3	46.6	96	Sukaradhan	11.7	32.9
13	BSI-115	8.8	31.8	55	Krishnahamsa	13.1	40.7	97	Sarjoo-52	13.9	30.4
14	CH-45	40.9	41.1	56	Lalat	16.1	48.6	98	Suraksha	12.7	45.8
15	Chittimuthyalu	12.3	47.4	57	Leimaphou	14	38.8	99	Swarna	32.1	58.2
16	Co-47	8.5	39.7	58	Madhukar	11.5	42.2	100	Swarnadhan	24.8	46.9
17	Cohondoresolu	6.9	26.2	59	Mahsuri	11.1	29.8	101	Swarnaprabha	8.3	37.9
18	Dhanrasi	9.6	45.7	60	Mahsuri	13.3	42.3	102	Taraori Basmati	12.2	37.9
19	Dinesh	10	38.8	61	Mandya vijaya	8.4	29.4	103	Taraori Basmati	26.8	38.4
20	DRR-H2	14.7	42.9	62	Mansarovar	10.9	35.5	104	Thapanthi	9.1	36.2
21	Dular	13.7	33.9	63	MTU-1010	24.6	34.3	105	Tulasi	11.9	32.1
22	Govinda	11.3	38.2	64	N-22	30.3	43.2	106	TKL-9	26.8	38.6
23	GR-101	11.6	41.4	65	NDR-6278	23.7	37.9	107	TL J-1	17.7	47.4

24	GR-4	12.2	45.5	66	NDR-80	28	32.4	108	TN 1	13.6	40.7
25	GR-4	8.3	33	67	NDR97	10.1	36.6	109	Triguna	9	31.4
26	GR-5	10.2	40.2	68	Nidhi	14.7	38.4	110	Aishvarya	13.3	40.4
27	HKR120	12.3	36.7	69	Nootripathu	10.1	49.3	111	TRY-5	11.9	44.3
28	HKR-126	33.9	38.8	70	Norungan	8.6	62.7	112	Vajrama	15.6	40.9
29	HKR-46	8.4	32.2	71	Pusa Basmati	14	30.7	113	Varallu	13.1	32.6
30	HPR 2143	12.7	31.6	72	Pusa Basmati	18	46.7	114	Vibhava	7.2	31.7
31	HPR 2036	11.6	39.7	73	PA-6201	7.2	41.9	115	Vijetha 1001	14	35.7
32	HR-12	29.4	47.2	74	Phalguna	10.7	27.9	116	Vikramarya	12.1	46.6
33	IET-10750	12.8	38.8	75	Punshi	13.8	37.8	117	Vivekdhan 82	16.3	35.4
34	INRC-10192	9.7	34.8	76	Phovidhi	14.3	33.9	118	Vivekdhan-62	9.5	36.8
35	IR-29	20.3	58.9	77	PR-106	25.4	35.1	119	W 10 B	27.1	29.9
36	IR-36	18.7	47.6	78	PR-113	11.7	44.5	120	SL 17 (IRGC-105318)	27.6	51
37	IR-36	9.8	39.4	79	PRBYO 02266	12.8	45.8	121	SL-29 (IRGC-106099)	12.3	41.2
38	IR-50	8.9	39.5	80	PSD-3	11.7	30.3	122	SL-32(IRGC-106187)	71.6	61.2
39	IR-64	15.5	38.7	81	Puja	12.5	35.7	123	SL-18(IRGC-105320)	27.9	57.8
40	IR-64	11.1	38.9	82	Purnendu	17.5	34.3	124	SL-12(IRGC-81848)	12	31.7
41	IR-72	12.1	39.6	83	Pusa Sugandha-1	23.1	53.5	125	SL-48(IRGC-86476)	21.2	67.3
42	Jalmagna	10.2	33.5	84	Pusa Sugandha	12.8	35.6	126	SL-55(IRGC-106086)	20.6	45.3

The highest concentration in cultivars was 14.8 ppm for Fe and 39.4 ppm for Zn. The Fe concentration in cultivated varieties ranged from 6.6 ppm in Athira to 48.3 ppm in Annada and Zn concentration ranged from 25.1 ppm in Jaya to 62.7 ppm in Norungan. Among cultivated varieties Annada, ASD-16, CH-45, Jyoti, HKR-126, Swarna, N-22 had high Fe (>30 ppm) and Norungan, IR-29, Swarna, Jalmagna, Sasyasri, Pusa-Sugandha-1, Aghonibora, Beni bhog had high Zn (>50ppm). Out of 126 accessions only 8 lines had >30 ppm Fe and 12 lines had >50 ppm Zn. When 8 high Fe lines were analysed for Zn, it was found that these lines also had high Zn (>30 ppm). On the other hand when 8 high Zn lines were analysed for Fe, only three lines Swarna, SL-32 and SL-18 had high Fe (>25ppm) (Fig. 1).

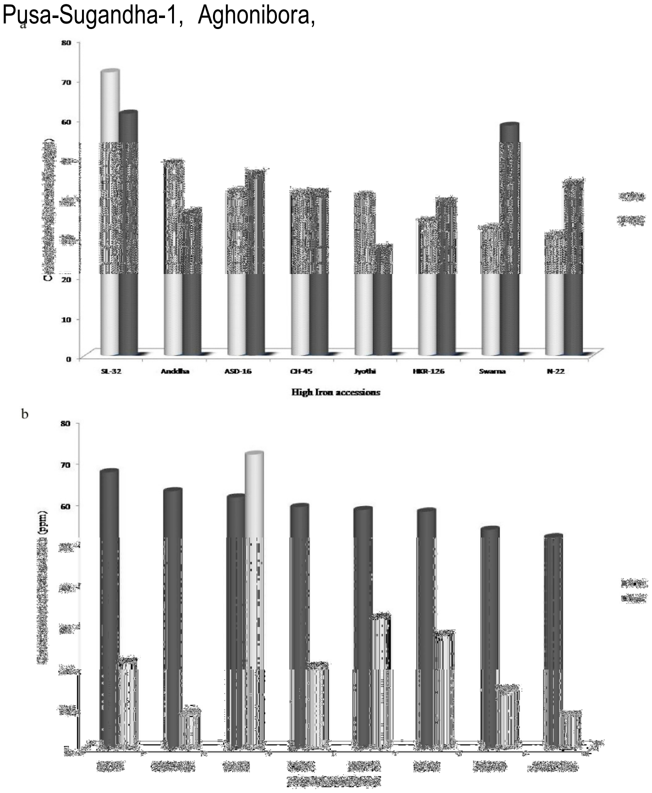

Fig 1. Concentration of Zn in high Fe lines (a) and Fe in high Zn lines (b)

9 accessions were screened more than once for Fe and Zn concentration, as their seeds were collected in different lots to determine the extent of variation. Fe concentration varied greatly from 8.3 to 12.2 ppm in GR-4, 11.1 to 13.3 ppm in Mahsuri, 13.1 to 14.3 ppm in Krishnahamsa, 14 to 18 ppm in Pusa Basmati, 12.2 – 26.8 ppm in Taraori basmati, 9.8 – 18.7 ppm in IR-36, 11.1 -15.5 ppm in IR-64, 7.3 -10 in Jaya and 9.3-23.1 ppm in Pusa Sugandha. But on the other hand Zn concentration was quite consistent in the duplicate samples of all the 9 accessions.

Seeds of 65 rice varieties out of 126 accessions were also analysed for seed dimensions using image analysis (Table 2). Seed length varies from 7.8 mm in Vivekdhan-62 & Beni bhog to 10.7 mm in Sugandhamati and Pusa Basmati and seed width varies from 3 mm in GR-4 to 4.8 mm in Nidhi. Pearson correlation coefficient was determined for 13 grain parameters used in the study and Fe and Zn concentration of 65 germplasm accessions (Table 3). Only one correlations between Zn and elongation (-0.25) was significant.

Table 2. Morphological observation of seeds from 65 accessions

S. No.	Name	Area (mm)	Length (mm)	Width (mm)	Peri-meter (mm)	Density	Round -ness	Compa-ctness	Elong -ation	Rough-ness	Peri/ Length	Peri/ Width
1	Dinesh	22.9	7.9	4.0	21.5	222.7	0.6	20.3	2.0	1.1	2.7	5.4
2	Annada	22.6	7.9	3.8	20.3	213.9	0.7	18.4	2.1	1.1	2.6	5.4
3	HKR -120	25.3	9.7	3.6	24.4	217.9	0.5	23.7	2.7	9.5	2.5	6.9
4	Krishnahamsa	22.3	9.3	3.3	23.3	204.0	0.5	24.6	2.9	5.1	2.5	7.2
5	Badshahbhog	14.5	6.3	3.2	16.8	206.3	0.6	19.6	2.0	1.1	2.7	5.3
6	Mahsuri	22.3	9.2	3.3	22.9	218.2	0.5	23.5	2.8	11.5	2.5	6.9
7	PY - 3	22.7	8.7	3.7	22.3	216.5	0.6	22.0	2.4	1.1	2.5	6.0
8	Sarjoo -52	21.5	8.6	3.3	22.1	210.4	0.6	23.0	2.6	1.1	2.6	6.7
9	ASD - 16	20.7	7.9	3.6	20.8	217.9	0.6	22.3	2.2	1.1	2.7	5.9
10	PR - 106	26.2	9.4	3.7	25.2	204.6	0.5	24.7	2.6	3.9	2.7	6.8
11	HPR - 2143	24.4	10.0	3.5	24.2	216.7	0.5	24.1	2.9	7.8	2.4	7.0
12	Vijetha	29.4	9.7	4.2	26.2	204.1	0.5	23.5	2.3	3.3	2.7	6.3
13	Jyoti	23.7	8.4	3.8	21.4	207.6	0.6	19.4	2.2	6.6	2.6	5.6
14	Purundu	21.9	8.3	3.5	21.7	217.9	0.6	21.8	2.4	4.2	2.6	6.2
15	IR - 29	24.7	9.3	3.6	23.4	220.6	0.6	22.3	2.6	5.4	2.5	6.6
16	Nidhi	26.7	10.1	4.8	27.6	209.6	0.5	28.7	2.4	45.9	2.7	6.3
17	Varallu	19.3	9.0	3.1	22.2	197.0	0.5	26.0	3.0	7.2	2.5	7.5
18	Vajram	25.3	9.5	3.6	25.5	205.3	0.5	27.6	2.8	9.6	2.7	7.4
19	Kasturi	26.9	10.0	3.9	25.9	193.0	0.5	25.3	2.7	1.1	2.6	6.9
20	Vikramarya	32.4	10.0	4.7	26.8	201.8	0.6	22.5	2.2	17.3	2.7	5.8
21	HPR - 2036	22.2	9.0	3.4	23.0	218.0	0.5	24.3	2.7	4.5	2.5	6.9
22	Phalguna	34.5	10.2	4.7	28.0	207.8	0.6	23.5	2.3	4.5	2.7	6.2
23	TLJ-1	26.3	9.0	3.9	23.4	206.1	0.6	21.0	2.3	1.1	2.6	6.0
24	GR - 4	16.4	7.3	3.0	18.3	212.5	0.6	20.4	2.5	4.5	2.5	6.2
25	Govindha	23.8	9.7	3.3	24.0	210.4	0.5	24.6	2.9	1.1	2.5	7.3
26	Manasarovar	21.4	7.9	3.6	20.3	212.3	0.7	19.5	2.2	4.0	2.6	5.6
27	Sasyasri	26.2	9.3	3.9	24.2	207.6	0.6	22.7	2.4	4.8	2.6	6.2
28	Kanehna	22.8	7.9	3.9	20.3	218.6	0.7	18.1	2.0	1.1	2.6	5.2
29	Suraksha	23.1	8.7	3.7	23.9	205.3	0.5	25.4	2.4	12.0	2.7	6.5
30	PR- 113	28.2	9.1	4.4	25.9	205.3	0.5	24.2	2.1	1.2	2.9	6.0
31	NDR -80	21.3	8.3	3.5	21.7	210.1	0.6	22.5	2.4	1.1	2.6	6.2
32	IR-36	21.9	8.0	3.7	21.4	202.9	0.6	21.0	2.2	4.1	2.7	5.7
33	Sukradhan	22.8	9.3	3.4	26.1	209.4	0.4	30.9	2.8	7.9	2.8	7.6

34	Lalat	25.3	9.6	3.6	25.4	209.6	0.5	26.0	2.7	5.5	2.6	7.1
35	HKR - 126	22.3	9.6	3.2	24.2	208.5	0.5	26.7	3.1	9.6	2.5	7.8
36	CH - 45	22.9	8.5	3.6	22.1	212.9	0.6	21.8	2.4	7.8	2.6	6.1
37	IR - 72	22.1	8.8	3.3	22.1	217.7	0.6	22.4	2.6	1.1	2.5	6.6
38	Triguna	22.7	9.5	3.2	23.7	215.7	0.5	25.1	3.0	3.6	2.5	7.5
39	Athira	25.0	9.2	3.7	22.9	198.5	0.6	21.2	2.5	5.0	2.5	6.3
40	KJT-5	27.1	10.0	3.8	26.1	212.6	0.5	25.3	2.7	4.8	2.6	7.0
41	Leimaphou	30.1	9.6	4.4	26.7	205.1	0.5	24.0	2.3	7.6	2.8	6.3
42	GR - 101	26.2	9.2	3.8	23.8	219.7	0.6	21.6	2.5	3.1	2.6	6.3
43	Shalivahana	25.2	8.1	4.3	22.9	208.7	0.6	21.0	1.9	8.1	2.8	5.4
44	Beni bhog	20.6	7.8	3.6	20.5	216.9	0.6	20.5	2.2	9.6	2.6	5.7
45	TN1	25.3	8.6	3.9	22.5	206.6	0.6	20.0	2.2	4.1	2.6	5.8
46	IET - 10750	28.3	9.6	4.0	25.1	197.3	0.6	22.5	2.4	1.1	2.6	6.3
47	PSD -3	27.5	10.4	3.6	25.8	223.1	0.5	24.4	2.9	10.7	2.5	7.2
48	Phovidi	24.6	9.5	3.6	23.3	220.7	0.6	22.1	2.7	4.1	2.5	6.6
49	DRRH-2	25.7	9.7	3.7	25.0	212.7	0.5	24.8	2.7	1.1	2.6	7.0
50	KRH - 2	22.6	9.2	3.3	22.6	220.5	0.6	22.7	2.8	7.2	2.5	6.9
51	PA 6201	20.6	9.0	3.0	22.2	221.2	0.5	24.1	3.0	4.1	2.5	7.3
52	IR - 50	22.2	8.7	3.4	21.3	217.8	0.6	20.6	2.6	1.1	2.4	6.3
53	Sugandhmati	26.4	10.7	3.6	27.8	209.2	0.4	29.9	3.1	20.9	2.6	7.9
54	GR - 5	22.5	8.9	3.4	22.0	216.5	0.6	21.8	2.7	1.1	2.5	6.5
55	Vivekdhan 62	21.2	7.8	3.7	21.0	215.0	0.6	21.7	2.1	5.5	2.7	5.7
56	Amulya	23.9	9.0	3.6	22.5	209.7	0.6	21.4	2.5	10.1	2.5	6.3
57	ADT - 36	21.9	8.7	3.5	22.1	200.8	0.6	22.5	2.5	4.6	2.5	6.4
58	Aishwarya	24.2	9.1	4.0	25.6	198.2	0.5	28.4	2.3	6.9	2.8	6.5
59	HKR - 46	20.6	8.0	3.5	20.1	214.3	0.6	19.8	2.3	3.9	2.5	5.8
60	Vibhava	19.3	8.5	3.1	21.1	210.0	0.5	23.2	2.8	15.4	2.5	6.9
61	Co - 47	23.1	8.1	3.8	20.7	218.1	0.7	18.7	2.1	4.4	2.5	5.5
62	HR-12	25.0	9.6	3.6	24.2	196.5	0.5	23.8	2.7	4.1	2.5	6.7
63	Pusa Basmati	26.9	10.7	3.5	26.6	207.1	0.5	26.6	3.1	9.3	2.5	7.7
64	Dhanrasi	21.8	8.1	3.6	21.3	219.7	0.6	21.2	2.3	5.0	2.6	6.0
65	Tulasi	21.5	8.4	3.5	20.9	215.3	0.6	20.5	2.4	4.1	2.5	6.0

Table 3. Pearson correlation coefficient among Fe & Zn concentration, grain area, length, width, perimeter, density, roundness, compactness, elongation, roughness,

	Fe (ppm)	Zn (ppm)	Area (mm)	Length (mm)	Width (mm)	Perimeter (mm)	Density (mean)	Roundness	Compactness	Elongation	Roughness	Peri/ Length	Peri/ Width
Fe (ppm)	1.00												
Zn (ppm)	0.08	1.00											
Area(mm)	-0.06	0.02	1.00										
Length(mm)	-0.15	-0.13	0.74**	1.00									
Width(mm)	-0.12	0.15	0.78**	0.27	1.00								
Perimeter(mm)	-0.12	-0.06	0.85**	0.91**	0.53**	1.00							
Density(mean)	-0.07	-0.04	-0.27*	-0.20	-0.27*	-0.33**	1.00						
Roundness	0.19	0.14	-0.30*	-0.76**	0.06	-0.75**	0.28*	1.00					
Compactness	-0.12	-0.12	0.29*	0.69**	0.01	0.74**	-0.29*	-0.97**	1.00				
Elongation	-0.16	-0.25*	-0.01	0.64**	-0.54**	0.37**	0.03	-0.74**	0.64**	1.00			
Roughness	-0.09	-0.07	0.19	0.35**	0.31*	0.39**	-0.07	-0.40**	0.46**	0.16	1.00		
Peri/Length	0.08	0.17	0.33*	-0.11	0.65**	0.30*	-0.34**	-0.06	0.20	-0.59**	0.11	1.00	
Peri/Width	-0.16	-0.23	0.10	0.71**	-0.42**	0.53**	-0.09	-0.88**	0.82**	0.96**	0.20	-0.34**	1.00

peri/length, peri/width of 65 different rice germplasm accession.

DISCUSSION

Plant breeding programs in biofortification of staple food crops such as rice and wheat require screening of germplasm, varieties and elite lines having Fe and Zn-dense grains to be used as donor parents [18]. An increase in concentration of Fe and Zn in grain is a high-priority research area. Exploitation of large genetic variation for Fe and Zn existing in cereal germplasm is an important approach to minimize the extent of Fe and Zn deficiencies in developing world. Maximum micronutrients are frequently present in some landraces and /or genetically distant wild varieties [19].

Among the germplasm screened for Fe & Zn concentration, the highest values were obtained in the wild accessions. Among wild accessions SL-32 (*O. nivara*) was found to be high for both iron and zinc. It is interesting to note that all the wild accessions had high zinc but only one had high iron. Some cultivars such as Annada had high Fe and Norungan had high zinc. Our results are consistent with study by Banerjee et al. [9] who estimated Fe and Zn concentration in 46 rice accessions including 3 wild genotypes *O. nivara*, *O. latifolia* and *O. officinalis*. They showed that wild accessions had high iron and zinc. In addition they also got 3 homozygous breeding *indica* genotypes having high grain Fe and Zn concentration. Four lines MTU1010, IR64, Nagina22 and Swarna were common between their and our study. When compared, these lines had different Fe and Zn concentration. In our study, Fe concentration in these four lines MTU1010, IR64, Nagina22 and Swarna was 24.6, 13.4, 12.6 and 8.4 ppm and Zn concentration was 34.3, 38.8, 26.8 and 13.9 ppm respectively while Fe was 8, 8, 9 and 13 ppm and Zn was 18, 20, 13 and 26 ppm in their study.

In another study 220 rice genotypes were analysed for Fe and Zn and indica and aromatic rice varieties with high Fe and Zn content were identified [19]. Out of these 220 rice genotypes 13 accessions were common with our study. Indica and aromatic varieties had high Fe but not Zn [19] but our results show that these accessions had high Zn but not Fe. Anandan et al., [20] reported that the content of Fe and Zn in traditional genotypes were significantly higher than that of improved cultivars. These result show that there is a significant genetic diversity or variation in the existing rice germplasm. We observed that the high Fe lines (>30ppm) also had high Zn but the high Zn lines (>40ppm) did not have high Fe. This interesting observation is also supported by our results from 128 Backcross Introgression Lines (BILs) from the cross of BPT5204 x *O. nivara* (unpublished data). The top 5 high Fe BILs also had high Zn but the top 5 high zinc BILs did not have high Fe. The same was also observed in 126 BILs derived from the cross Swarna x *O. nivara* [21].

It is clear from previous work and our results that there are no fixed values of Fe and Zn for an accession. These can vary depending on sample lots even from one accession as seen in the eight lines for which we had more than one sample. The position of grain on the panicle may also influence its Fe and Zn levels but there are no such detailed studies on Fe Zn distribution within the panicle. Variations in Fe and Zn values in different samples of the same accession can also arise due to presence or absence of embryo in grains, time of harvest or different digestion or analytical methods. This variation in Iron and Zinc values is also due to homeostasis regulating their translocation, absorption, and transport within the plant system [3]. Another factor contributing to difference in Iron and Zinc values is the phloem sap loading and unloading rates within the reproductive organs [22]. Different seed lots of the same accession had different Fe Zn concentration even though they were harvested from the same plot. Thus there is a range of Fe and Zn concentration and no fixed values quite akin to the trait yield.

Secondly, soil properties also influence the grain Fe and Zn concentration. The pH, organic matter content and Fe/Zn levels of native soil showed significant effects on grain Fe and Zn content [23]. Iron and zinc when applied to soil singly significantly increased the seed weight per plant in soybean [24]. Mishra et al., [25] studied the effect of biofertilizers on nutrient content of cultivated variety of fenugreek. They showed non significant changes in Fe and Zn content on application of biofertilzers. Fe concentration is known to vary with location but Zn values appear to be more consistent [23]. Also, the range of variation is much more for Fe concentration than for Zn. Environment, genotype and genotype × environment interaction significantly affected Fe concentration in rice grains [26]. While grain Fe content showed significant genotype × environment interaction effect, Zn content of brown rice was significantly influenced by native soil properties [9, 23, 26]. Thus, in general grain zinc appears to be more consistent than grain Fe content.

Sellappan et al. [15] suggested that the number of aleurone layers, size of the embryo and size of the caryopsis determines the quantity of important micronutrients such as iron, zinc in the grains. The high genetic correlation between grain characteristics and some mineral element contents can be used to conduct indirect selection of a grain characteristic for mineral element content in a breeding program [17]. In our study, seed dimensions were not significantly correlated with high iron and zinc. In wheat, it was shown that smaller seeds of *Aegilops longissima* had upto twice higher iron and zinc content than durum wheat cultivar with larger seeds [27]. The F2 seeds and amphidiploids despite being large showed 42%–70% higher Fe and 60%–80% higher Zn indicating better genetic systems for uptake, translocation and sequestration into seeds influence Fe and Zn rather than seed size.

CONCLUSION

In conclusion, it was found that the wild accessions *O. nivara* and *O. rufipogon* had the highest Fe and Zn in brown rice. Some cultivars such as Annada, ASD16, CH45, HKR126, Nagina22 also had more than 30ppm of both Fe and Zn. Lines with high Fe invariably had high Zinc but not vice versa. Zn concentration was significantly correlated to grain elongation. That the wild species are a good source of high Fe and high Zn is supported by our subsequent unpublished work on BILs derived from Swarna x *O.nivara* and BPT5204 x *O.rufipogon* [20] and high iron and zinc lines have been developed.

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