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GENETIC DIVERSITY IN TRADITIONAL GENOTYPES FOR GRAIN IRON, ZINC AND β -CAROTENE CONTENTS REVEAL POTENTIAL FOR BREEDING MICRONUTRIENT DENSE RICE

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ABSTRACT

Rice is the staple food for half of the world's population, however, its edible grain part is deficient in essential micronutrients, especially Fe and Zn. Breeding for micronutrient dense rice demands exploration of available genetic diversity for grain Fe, Zn and β -carotene contents. In this study, we analysed brown and polished grain samples of 26 traditional rice genotypes for Fe and Zn concentration. Fe concentration varied from 13.23 ppm to 45.83 ppm and 1.10 ppm to 36.45 ppm in brown and polished rice, respectively. Whereas, Zn concentration in brown and polished rice ranged from 18.67 ppm to 38.01 ppm and 5.86 ppm to 23.88 ppm, respectively. The landraces had higher Fe and Zn contents than improved varieties and advance uniform lines. Significant positive correlation was present between Fe and Zn concentration in brown ($r = 0.694$, $P < 0.01$) and polished rice ($r = 0.533$, $P < 0.01$). Fe concentration was greatly reduced by the polishing process than Zn concentration and approved varieties lost less Fe and Zn contents than advance uniform lines. Minimal levels of beta-carotene were detected in rice genotypes indicating need for genetic modifications to enhance pro-vitamin A contents in rice endosperm.

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1 Introduction

Malnutrition or 'Hidden hunger' affects at least 2 billion peoples worldwide and 45% of deaths among children under 5 years of age are linked to under nutrition (McGuire et al., 2015; WHO, 2015). In developing countries, micronutrient malnutrition, particularly iron (Fe) and zinc (Zn) deficiency, is a continuing public health issue. Global studies estimated that more than 30% of the world's population is anemic due to iron-deficiency anemia (IDA) (Brotanek et al., 2005; Stevens et al., 2013). IDA affects productivity of women's along with other severe health problems, including mental retardation, weakened immune system and increased morbidity and mortality rates (Black, 2003). Zinc deficiency is a major cause of stunted growth, male hypogonadism, abnormal neurosensory changes and impaired immune function (Maret & Sandstead, 2006). Vitamin A deficiency (VAD) is also a major health concern and causes xerophthalmia and night blindness (WHO, 2009).

In past, supplementation and fortification of food items have been practiced, however, these interventions have not been able to eradicate the problem, mainly due to the fact that these are not economically sustainable (Meenakshi et al., 2010). For instance, vitamin A supplementation programs in 103 priority countries has stagnated at 58% success rate in ten years, with high year to year fluctuation (Dalmiya & Palmer, 2007). Among different approaches to mitigate malnutrition, biofortification is the most cost effective and sustainable intervention. It is a process of bolstering the nutritional value of edible parts of crop plants through agronomic, breeding and transgenic techniques.

Rice (*Oryza sativa* L.) is one of the widely grown and most consumed crops worldwide. Half of the world's population depend on it to meet their daily calorie requirements (Meng et al., 2005). However, its edible grain part (endosperm) contains insufficient levels of Fe, Zn and β -carotene (pro-vitamin A) to meet the dietary requirements. Therefore, biofortified rice with enriched levels of essential nutrients can significantly improve global human health. In order to provide 30%, 40% and 50% of the dietary estimated average requirements (EAR), the polished rice should contain 15 ppm of Fe, 28 ppm of Zn and 17 ppm of β -carotene (Bouis et al., 2011). Exploration of available genetic diversity for Fe, Zn and β -carotene in economically important parts of the crop plants is a first step towards developing the nutrient dense crops (Bouis & Saltzman, 2017).

Germplasm has been screened for grain Fe, Zn and β -carotene contents in many crops, including rice. Cai et al. (2009) evaluated 113 rice landraces, including japonica and indica accessions. The variation in Fe ranged from 8.1 ppm to 19.9 ppm and japonica rice contained significantly higher Fe contents than indica rice. Banerjee et al. (2010) assessed genetic diversity in cultivated and wild accessions of rice and found wide genetic variation in grain

Fe, Zn and protein levels. Fe contents varied from 4.82 - 22.69 ppm and Zn contents varied from 13.95 - 41.73 ppm, respectively. They also found high micronutrient densities in wild accessions of rice. Anandan et al. (2011) found wide genetic variability in traditional and improved rice genotypes and reported that Fe and Zn contents were higher in traditional genotypes than improved cultivars. Brar et al. (2011) assessed genetic diversity in a collection of 220 rice genotypes. The Fe and Zn contents in brown rice varied significantly from 5.1 - 441.5 ppm and 2.12 - 39.4 ppm, respectively. Anuradha et al. (2012) analysed Fe and Zn concentration in brown rice of 126 accessions and reported that wild accession had highest concentration levels. In wheat, genetic variability for grain Fe, Zn and β -carotene contents had also been assessed by Badakhshan et al. (2013). Based on two year's data, concentration of Fe, Zn and β -carotene ranged from 41.36 to 67.67, 36.37 to 73.80 and 0.96 to 1.69 ppm, respectively. Roy & Sharma (2014) evaluated 84 rice accessions and concentration of Fe ranged from 0.25 - 34.8 ppm and Zn ranged from 0.85 - 195.3 ppm. Patil et al. (2015) screened 60 rice genotypes and found that Fe & Zn contents in brown rice ranged from 3.38 - 36.99 ppm and 3.32 - 42.49 ppm, respectively. Umadevi et al. (2016) assessed genetic variability in 57 rice genotypes for Fe and Zn contents in brown rice. The Fe and Zn concentration varied from 7.45 - 29.85 ppm and 14.68 - 59.65 ppm, respectively.

Genetic diversity in local rice germplasm of Pakistan for micronutrients and vitamins has not been assessed previously. Therefore, we decided to explore it in approved varieties and advance uniform lines for fast track development of nutrient dense rice cultivars. The objectives of this study were to explore genetic diversity in traditional rice genotypes for Fe and Zn contents in brown and polished rice, as well as β -carotene levels in polished rice; analyse association between Fe and Zn concentration in brown and polished rice; examine losses in Fe and Zn contents during polishing process and identify Fe and Zn donor parents for their exploitation in nutrition breeding program.

2 Material & Methods

2.1 Experimental site, plant material and growth conditions

Present study was conducted at the Rice Research Institute, Kala Shah Kaku (RRI, KSK), Pakistan. Twenty-six (26) rice genotypes including twelve (12) approved varieties and fourteen (14) advance uniform lines were used in this study and are listed in Table 1. During Kharif season 2017, all genotypes were grown in the experimental field of RRI, KSK under normal field conditions and seed harvested from this trial was used for further analysis. NPK and Zn fertilizers were applied at rates of 116:87:62 and 12 kg/ha, respectively. Standard agronomic and plant protection practices were followed. Soil and water samples were also collected and analysed for determination of physiochemical characteristics (Table 2).

Table 1 List of genotypes*

Sr. No.	Genotype	Status with release year	Type
1	Basmati 370	Approved Variety-1933	Aromatic
2	Basmati 198	Approved Variety-1972	Aromatic
3	Basmati Pak	Approved Variety-1968	Aromatic
4	Basmati 385	Approved Variety-1985	Aromatic
5	Super Basmati	Approved Variety-1996	Aromatic
6	Basmati 2000	Approved Variety-2000	Aromatic
7	Basmati 515	Approved Variety-2011	Aromatic
8	PK 386	Approved Variety-2013	Non- Aromatic
9	PK 1121 Aromatic	Approved Variety-2013	Aromatic
10	Kissan Basmati	Approved Variety-2016	Aromatic
11	Chenab Basmati	Approved Variety-2016	Aromatic
12	Punjab Basmati	Approved Variety-2016	Aromatic
13	PK 8892	Advance uniform line	Aromatic
14	PK 9194	Advance uniform line	Aromatic
15	RRI 3	Advance uniform line	Aromatic
16	PKBB 15-1	Advance uniform line	Aromatic
17	PKBB 15-6	Advance uniform line	Aromatic
18	PKPB 8	Advance uniform line	Aromatic
19	PK 10683	Advance uniform line	Aromatic
20	PK 10355	Advance uniform line	Aromatic
21	PKBB 15-116	Advance uniform line	Aromatic
22	PK 10029	Advance uniform line	Aromatic
23	PK 9966	Advance uniform line	Aromatic
24	PK 9533	Advance uniform line	Aromatic
25	PK 10161	Advance uniform line	Aromatic
26	PK 10967	Advance uniform line	Aromatic

* These genotypes are maintained at RRI, KSK.

Table 2 Physiochemical characteristics of soil and water samples

Soil characteristics		Water characteristics	
Sampling Depth (inch)	0-6	Electrical Conductivity (mScm ⁻¹)	1.3
Electrical Conductivity (mScm ⁻¹)	1.5	Calcium+Magnesium(Ca ²⁺ &Mg ²⁺)	5.0
pH	8.1	Sodium (Na ⁺)	6.0
Organic matter (%)	0.8	Carbonate (CO ³⁻)	-
Available Phosphorus (mg/kg)	7.0	Bi-Carbonate (HCO ³⁻)	5.2
Available Potassium (mg/kg)	146	Chloride (Cl ⁻)	2.6
Saturation (%)	42	Residual Sodium Carbonate	3.2
Texture	Loam	Sodium Absorption Ratio	4.1
Fe (ppm)	12.2	Fe (ppm)	1.12
Zn (ppm)	0.87	Zn (ppm)	0.96

2.2 Sample preparation

Rice panicles were harvested and hand threshed to avoid any metal contamination. For preparation of brown rice, the husk was removed by using a paddy dehusker (Satake, Japan). Brown rice samples were polished with a laboratory polishing machine (Grainman 60M-250-50-DT Sheller, USA) for the same intervals (40 s). The dehusker and polishing machine were thoroughly cleaned after preparation of each sample by brushing the bran and broken rice kernels. For estimation of Fe and Zn, whole grains were collected, washed with double distilled deionized water (d_3H_2O) to remove any metal contamination and oven dried at $70^\circ C$ to bring the moisture level at 10-12 percent.

2.3 Estimation of Fe and Zn

Standard procedure was followed for estimation of Fe and Zn contents (George et al., 2013). One gram of sample weighed separately for each genotype, transferred quantitatively into 100 mL pyrex digestion tubes. Ten mL of di-acid mixture (HNO_3+HClO_4 , 2:1) was added to each tube and allowed to stand overnight until the vigorous reaction phase was over. After preliminary digestion, digestion tubes were placed in a cold-block digester and temperature was raised to $150^\circ C$ for 60 minutes until all traces of HNO_3 disappeared. Then, temperature was slowly raised to $235^\circ C$. When white dense fumes of $HClO_4$ appeared, digestion was continued for 30 more minutes, until white precipitates settled down at the bottom of digestion tubes. d_3H_2O was added in small increments for dissolving the crystals and washing of the tube walls. The contents were filtered through Whatman No. 41/42 filter paper and 50 mL dilutions were made with d_3H_2O . Finally, extract was fed to atomic absorption spectrophotometer (200 Series AA, Agilent Technologies, USA) available at Soil and Water Testing Laboratory, Thokar Niaz Baig, Lahore for determination of Fe and Zn concentration. Each digestion batch contained 11 samples and one blank (no plant material). The analysis was repeated thrice for each genotype and mean value is presented in results. Fe and Zn concentrations were expressed in parts per million (ppm).

2.4 Estimation of β -carotene

Standard procedure with little modification was followed for estimation of β -carotene contents (Nagata, 2009). Polished rice grains were grounded using a pestle and mortar. For each genotype, accurately weighed 0.225 g powder was taken into 15 mL falcon tubes and 3 mL of 80% acetone solution was added to it. After vortexing the mixture for 5 minutes, it was allowed to stand overnight. Next day, the homogenous mixture was centrifuged at 3000 RPM for 5 minutes and supernatant was taken into newly labelled falcon tubes. Finally, the absorbance of extract was measured at 479 nm, 645 nm and 663 nm wavelengths by

using the spectrophotometer (T80⁺ UV/VIS spectrophotometer, PG instruments Ltd., UK). β -carotene concentration was calculated by putting the absorbance values in following formulae.

$$\beta \text{ carotene (ppm)} = 0.854(479 \text{ nm}) - 0.312(645 \text{ nm}) + 0.039(663 \text{ nm}) - 0.005 \text{ (r = 0.992, SE = 0.0096 ppm)}.$$

Where,

r = correlation coefficient

SE = standard error

2.5 Data analysis

The one-way analysis of variance and Tukey HSD all pair wise comparison tests were used to compare the mean values of Fe and Zn contents in both brown and polished rice at probability level of $\alpha \leq 0.05$. Statistical analysis was carried out using the SPSS software (SPSS 16.0, Chicago, USA).

3 Results

Fe and Zn concentration was analysed in brown and polished grain samples of 26 rice genotypes and significant genetic variation was observed (Table 3). Wide genetic variation in Fe concentration of brown and polished rice was found as compared to Zn concentration. Fe concentration in brown rice ranged from 13.23 ppm (PK 10355) to 45.83 ppm (Basmati 370), while in polished rice it ranged from 1.10 ppm (PK 10355) to 36.45 ppm (Basmati 370). Whereas, Zn concentration in brown rice ranged from 18.67 (PK 10967) ppm to 38.01 ppm (Basmati 370) and in polished rice it ranged from 5.86 ppm (PK 9533) to 23.88 ppm (Kissan Basmati). The mean value for Fe concentration in brown rice was 25.21 ppm, whereas in polished rice it was 11.81 ppm. Similarly, the mean value for Zn concentration in brown rice was 23.95 ppm, whereas in polished rice it was 12.69 ppm (Table 3). Additionally, large variation in grain Fe contents was due to high availability of this mineral to rice genotypes from the experimental soil (Table 2).

Overall, all approved varieties had high Fe and Zn concentration in both brown and polished rice when compared with advance uniform lines. In approved varieties, Fe concentration in brown rice ranged from 16.93 ppm (Chenab Basmati) to 45.83 ppm (Basmati 370), with an average of 27.77 ppm. Whereas in advance uniform lines, Fe concentration in brown rice ranged from 13.23 ppm (PK 10355) to 39.32 ppm (PK 8892), with an average of 23.01 ppm (Table 3). Similarly, Zn concentration in brown rice of approved varieties ranged from 19 ppm (Punjab Basmati) to 38.01 ppm (Basmati 370) with an average of 26.76 ppm, however, in advance uniform lines it ranged from 18.67 ppm

Table 3 Fe and Zn concentration in 26 rice genotypes

Genotype	Brown Rice		Polished Rice	
	Fe (ppm)	Zn (ppm)	Fe (ppm)	Zn (ppm)
Approved Varieties				
Basmati 370	45.83±0.0212 ^a	38.01±0.0778 ^a	36.45±0.0354 ^a	11.69±0.0242 ^j
Basmati 198	36.37±0.0354 ^d	33.03±0.0495 ^b	18.58±0.0566 ^f	18.87±0.0210 ^e
Basmati Pak	27.80±0.0221 ^f	26.71±0.2051 ^c	10.80±0.0145 ^k	10.39±0.0230 ⁿ
Basmati 385	41.65±0.0358 ^b	27.09±0.0283 ^c	21.08±0.0152 ^d	22.27±0.0212 ^c
Super Basmati	20.13±0.0212 ^m	24.65±0.0232 ^h	5.20±0.0141 ^s	7.87±0.0424 ^s
Basmati 2000	25.10±0.0360 ^h	26.29±0.0424 ^d	14.35±0.0210 ⁱ	12.53±0.0778 ⁱ
Basmati 515	21.95±0.0372 ^j	23.36±0.0707 ⁱ	16.70±0.0140 ^h	18.46±0.0265 ^f
PK 386	25.17±0.0283 ^h	24.98±0.0141 ^g	17.73±0.0228 ^g	19.25±0.0354 ^d
PK 1121 Aromatic	20.45±0.0216 ^{lm}	26.78±0.0283 ^c	11.65±0.0360 ^j	9.52±0.0141 ^o
Kissan Basmati	30.75±0.0358 ^e	26.51±0.0512 ^d	24.75±0.0212 ^c	23.88±0.0157 ^a
Chenab Basmati	16.93±0.0141 ^p	24.72±0.0566 ^{gh}	4.55±0.0200 ^t	12.54±0.0283 ⁱ
Punjab Basmati	21.15±0.0849 ^k	19.00±0.0861 ^o	7.55±0.0145 ^o	8.94±0.0354 ^q
Mean	27.77	26.76	15.78	14.68
Minimum	16.93	19.00	4.55	7.87
Maximum	45.83	38.01	36.45	23.88
Advance Uniform Lines				
PK 8892	39.32±0.4808 ^c	26.37±0.0217 ^d	26.40±0.0150 ^b	23.24±0.0424 ^b
PK 9194	28.07±0.0495 ^f	19.55±0.0390 ⁿ	5.68±0.0141 ^q	11.31±0.0293 ^k
RRI 3	22.98±0.0566 ⁱ	19.18±0.0636 ^o	10.63±0.0210 ^l	10.82±0.0181 ^m
PKBB 15-1	25.23±0.0212 ^h	25.27±0.0778 ^f	7.95±0.0919 ⁿ	8.68±0.0768 ^r
PKBB 15-6	27.15±0.0354 ^g	21.92±0.0560 ^k	5.50±0.0225 ^r	10.44±0.0432 ⁿ
PKPB 8	25.33±0.0919 ^h	21.77±0.0200 ^k	6.70±0.0636 ^p	10.35±0.0438 ⁿ
PK 10683	20.85±0.0360 ^{kl}	19.96±0.0283 ^m	10.30±0.0424 ^m	9.37±0.0490 ^p
PK 10355	13.23±0.0210 ^f	20.50±0.1556 ^l	1.10±0.0653 ^w	13.56±0.0220 ^h
PKBB 15-116	19.67±0.1202 ⁿ	25.90±0.0707 ^e	3.00±0.0168 ^u	16.54±0.0238 ^g
PK 10029	17.97±0.0283 ^o	22.59±0.0350 ^j	7.90±0.0707 ⁿ	10.88±0.0849 ^l
PK 9966	15.02±0.0570 ^q	19.98±0.0428 ^m	4.50±0.0140 ^t	7.81±0.0636 ^s
PK 9533	22.93±0.0424 ⁱ	20.03±0.0420 ^m	20.80±0.1414 ^e	5.86±0.0280 ^u
PK 10161	22.63±0.0241 ⁱ	20.01±0.0454 ^m	1.71±0.0156 ^v	6.06±0.0283 ^t
PK 10967	21.73±0.0374 ^j	18.67±0.0283 ^p	5.45±0.0354 ^f	8.95±0.0345 ^q
Mean	23.01	21.55	8.40	10.99
Minimum	13.23	18.67	1.10	5.86
Maximum	39.32	26.37	26.40	23.24
Coefficient of variation (%)	31.65	18.88	73.95	41.29
Overall Mean	25.21	23.95	11.81	12.69
Overall Minimum	13.23	18.67	1.10	5.86
Overall Maximum	45.83	38.01	36.45	23.88
Standard Deviation	7.97	4.52	8.73	5.24

Mean ± standard deviation. Values sharing same alphabets in columns differ non-significantly (P>0.05).

(PK 10967) to 26.37 ppm (PK 8892) with an average of 21.55 ppm. Similar Fe and Zn acquisition patterns were also observed in polished rice, where concentration of Fe in approved varieties ranged from 4.55 ppm (Chenab Basmati) to 36.45 ppm (Basmati 370) with an average of 15.78 ppm, however, in advance uniform lines it ranged from 1.10 ppm (PK 10355) to 26.40 ppm (PK 8892) with an average of 8.40 ppm. Likewise, concentration of Zn in approved varieties ranged from 7.87 ppm (Super Basmati) to 23.88 ppm (Kissan Basmati) with an average of 14.68 ppm, whereas in advance uniform lines it ranged from 5.86 ppm (PK 9533) to 23.24 ppm (PK 8892) with an average of 10.99 ppm (Table 3).

Fe and Zn contents were decreasing in rice grains with the passage of time. Among all approved varieties, 7 varieties had high Fe density (>25 ppm) in brown rice (Basmati 370, Basmati 198, Basmati Pak, Basmati 385, Basmati 2000, PK 386 and Kissan Basmati) and almost all of these varieties also contained high Zn density (>25 ppm) (Figure 1). However, only 5 advance uniform lines showed high Fe density in brown rice (PK 8892, PK 9194, PKBB 15-1, PKBB 15-6 and PKPB 8) and just 2 out of total 5 high Fe density advance uniform lines also contained high Zn density. Furthermore, 3 approved varieties (Basmati 370, Basmati 385 and Kissan Basmati) and only 2 advance uniform lines (PK 8892 and PK 9533) contained high Fe density in white rice (>20 ppm). Similarly, 2 approved varieties (Basmati 385 and Kissan Basmati) and only one advance uniform line (PK 8892) contained high Zn density in white rice (>20 ppm) (Table 3).

Significant positive correlations were observed between these two

mineral elements and possibility for simultaneous improvement. Grain Fe and Zn were strongly correlated in brown rice ($r = 0.694$, $P < 0.01$) and polished rice ($r = 0.533$, $P < 0.01$). High Fe genotypes (>25ppm) also contained high Zn (>25ppm) and vice versa (Figure 1). Among 12 high Fe genotypes, 9 genotypes contained high Zn contents in brown rice. Similarly, 9 genotypes, out of total 10 high Zn genotypes also contained high Fe in brown rice.

Polishing process greatly reduced the Fe and Zn concentration in polished rice. Loss in Fe concentration was greater than Zn concentration. In approved varieties, less reduction in Fe and Zn concentration was observed as compared with advance uniform lines (Figure 2). Among approved varieties, maximum Fe was lost in Super Basmati (74.17%) and minimum in Kissan Basmati (19.51%), whereas maximum Zn was lost in Basmati 370 (69.26%) and minimum in Kissan Basmati (9.92%). Among advance uniform lines, maximum Fe was lost in PK 10161 (92.43%) and minimum in PK 9533 (9.30%), whereas maximum Zn was lost in PK 9533 (70.72%) and minimum in PK 8892 (11.86%). Overall, four genotypes (Kissan Basmati, Basmati 515, PK 386 and PK 8892) were found to be least affected by the polishing process and lost minimum Fe and Zn contents (Figure 2). We also found significant positive correlations in Fe ($r = 0.800$, $P < 0.01$) and Zn concentration ($r = 0.440$, $P < 0.05$) between polished rice and brown rice.

Narrow genetic variability was present among studied rice genotypes for β -carotene concentration that ranged from 0.000 (Basmati 198, PK 10029) to 0.055 ppm (PK 8892), with an average of 0.012 ppm (Figure 3). Only 4 genotypes, including 2

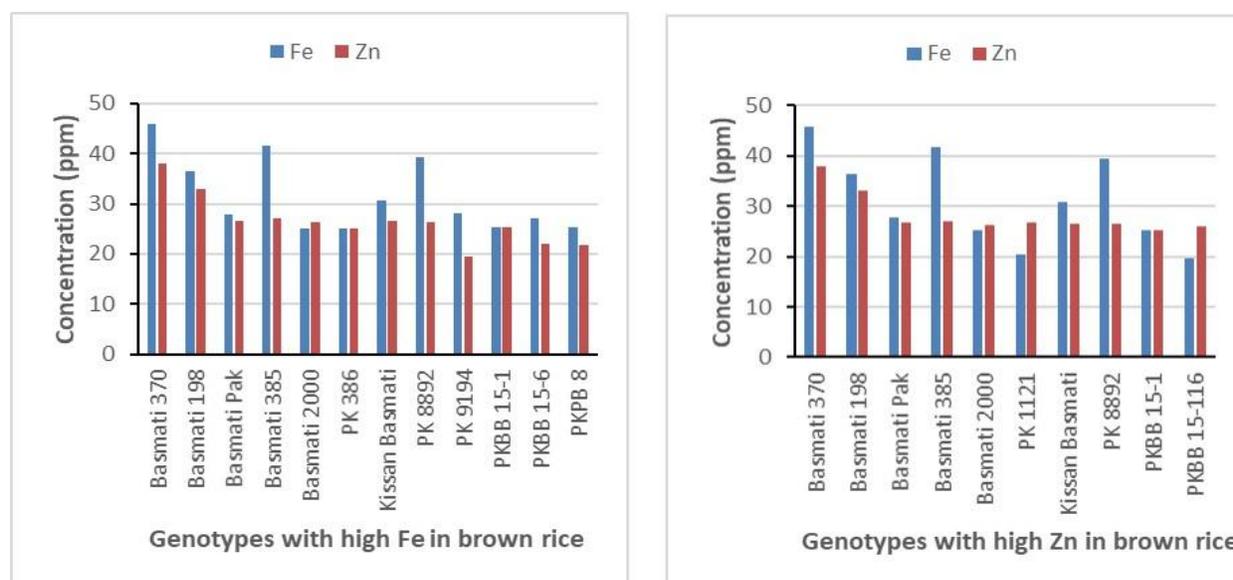


Figure 1 Concentration of Fe and Zn in genotypes containing ≥ 25 ppm Fe or Zn in brown rice.

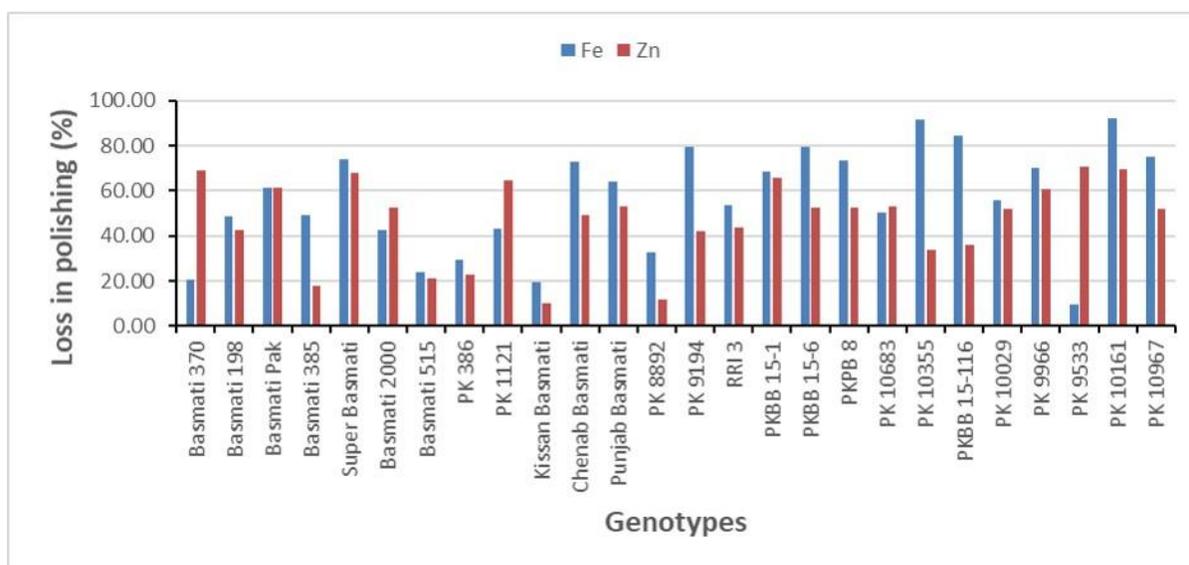


Figure 2 Effect of polishing on Fe and Zn concentration of all traditional rice genotypes

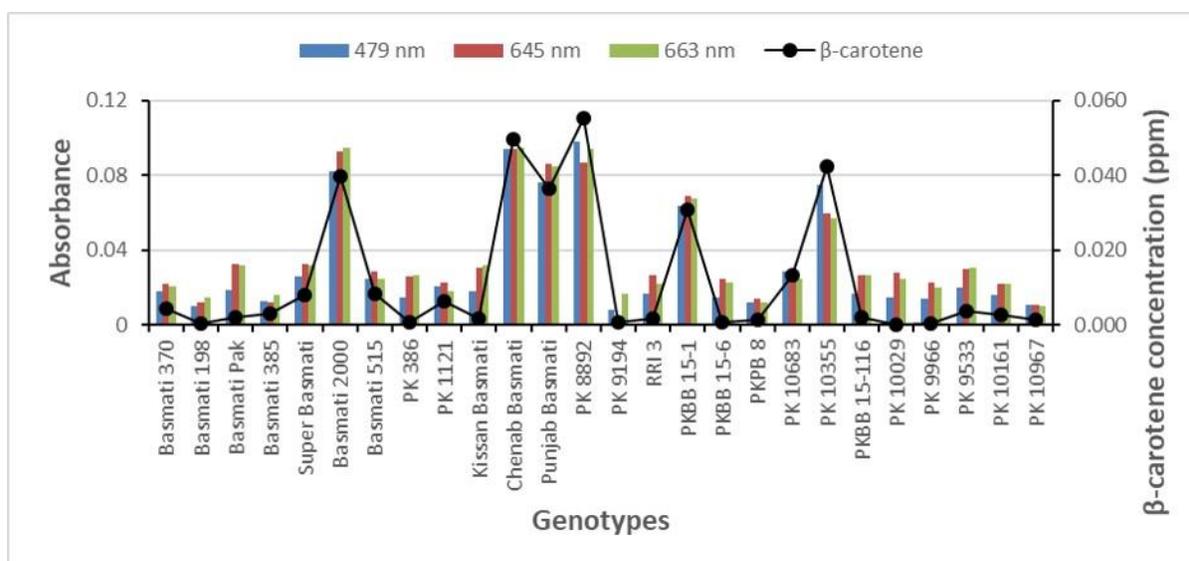


Figure 3 beta-carotene concentration levels detected in all traditional rice genotypes

approved varieties (Basmati 2000, Chenab Basmati) and 2 advance uniform lines (PK 8892, PK 10355), contained ≥ 0.040 ppm beta-carotene in polished rice indicating need for genetic modification of the rice plant so that the grain accumulates higher concentration of beta-carotene.

4 Discussions and Conclusion

Biofortification is a process of increasing the concentration of essential micronutrients and vitamins in a crop plant through

agronomic practices, plant breeding or transgenic techniques. Exploring the available genetic diversity for Fe, Zn and pro-vitamin A in edible parts of the crop plants is a first step towards developing the nutrient dense biofortified crops (Bouis & Saltzman, 2017). The objectives of this screening are to identify the donor parents for initiating the breeding programs and existing varieties or pre-varieties in the release pipeline for "fast-tracking." Fast-tracking refers to commercializing the genotypes that combine the desired micronutrient concentration without compromising farmer preferred agronomic traits so they can be

released without delay. Increasing Fe, Zn and pro-vitamin A density in cereal grains is a high priority research area. Exploration and exploitation of available genetic diversity in cereal germplasm for Fe, Zn and pro-vitamin A is a basic approach for alleviating the malnutrition of billions worldwide.

In rice, large genetic variation has been reported for grain Fe and Zn concentration (Cai et al., 2009; Banerjee et al., 2010; Anandan et al., 2011; Brar et al., 2011; Anuradha et al., 2012; Roy & Sharma, 2014; Patil et al., 2015; Umadevi et al., 2016). In this study, 26 rice genotypes including approved varieties and advance uniform lines were screened for Fe, Zn and β -carotene contents in rice grains. Significant genetic variation was present in brown rice, as well as in white rice, for Fe and Zn concentration (Table 3), thus holding promise for further trait improvement. Among screened genotypes, those which were released until 2000 (the landraces), contained high Fe and Zn densities (>25 ppm) in brown rice, except Super Basmati. However, those genotypes which were released after 2000 or are in release pipeline, contained lower Fe and Zn densities in brown rice (<25 ppm), except Kissan Basmti, PK 8892 and PKBB 15-1 (Table 3). These data indicate that landraces contains higher Fe and Zn densities in their grains and may be used as donor parents in future rice breeding programs. Previously, maximum Fe and Zn densities have been reported in some landraces and /or distantly related wild species (Anandan et al., 2011; Brar et al., 2011; Anuradha et al., 2012; Roy & Sharma, 2014; Patil et al., 2015; Umadevi et al., 2016), which support the consistency and accuracy of our data.

In previous studies, strong positive association between grain Fe and Zn concentration has been reported (Baxter et al., 2013; Moreno-Moyano et al., 2016). In this study, we also found strong positive association between grain Fe and Zn concentration in brown rice ($r = 0.694$, $P < 0.01$), as well as, in polished rice ($r = 0.533$, $P < 0.01$). Furthermore, high Fe varieties (> 25 ppm) also had high Zn (> 25 ppm) and vice versa (Figure 1). This inherent association between these two mineral elements suggest presence of linkage between micronutrient accumulation genes or pleiotropic effects of these genes. Majority of the genes encode metal transporter proteins and some of which transport multiple metals (Qin et al., 2012). Thus, grain Fe and Zn can be improved simultaneously.

Rice is mainly consumed worldwide as polished rice. The polishing process is performed to improve the physical and sensory attributes, as well as to increase the storage stability of rice (Monks et al., 2013). However, polishing process diminishes the overall nutritional value by removing the bran layers and germ which are rich source of minerals, proteins, vitamins, fibers and fats (Paiva et al., 2016). In this study, Fe and Zn concentration was greatly reduced by the polishing process. However, loss in Fe concentration was greater than Zn concentration. Moreover,

advance uniform lines lost more Fe and Zn contents as compared to approved varieties (Figure 2). These unequal losses in Fe and Zn might arise due to high concentration of Fe, but not Zn, in experimental soil (Table 2). We also found significant positive correlations in Fe ($r = 0.800$, $P < 0.01$) and Zn concentration ($r = 0.440$, $P < 0.05$) between polished rice and brown rice. Wei et al. (2012) investigated the effects of foliar iron applications on Fe and Zn concentration in polished rice and demonstrated that concentration of Fe, but not Zn, was greatly reduced in polished rice. They also found significant positive correlation in Fe concentration between polished rice and brown rice. Their findings are consistent with our results and indicate predominant deposition of Fe and Zn in bran layers and endosperm, respectively.

In Asia, vitamin A deficiency is associated with the poverty related predominant consumption of rice, which lacks pro-vitamin A in endosperm (Paine et al., 2005). Minimal levels of β -carotene are reported in rice endosperm (Beyer, 2010) and wheat (Badakhshan et al., 2013). In this study, we also found narrow genetic diversity among rice genotypes for β -carotene contents in endosperm (Figure 3), suggesting use of transgenic techniques rather than conventional breeding efforts for increasing the pro-vitamin A concentration in rice endosperm.

In conclusion, wide genetic diversity exists in rice for grain Fe and Zn concentration and narrow genetic diversity for β -carotene concentration. Landraces contain higher Fe and Zn concentration in their grains and are less affected by the polishing process, thus, can be good sources of high Fe and Zn in future nutrition breeding programs. Strong inherent associations between Fe and Zn in brown rice and polished rice provide opportunity for simultaneous improvement of both microelements.

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Conflict of interest

All the authors declare that there is no conflict of interest.

References

- Anandan A, Rajiv G, Eswaran R, Prakash M (2011) Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. *Journal of food science*, 76: H122-H130.
- Anuradha K, Agarwal S, Batchu AK, Babu AP, Swamy BM, Longvah T, Sarla N (2012) Evaluating rice germplasm for iron

- and zinc concentration in brown rice and seed dimensions. *Journal of Phytology* 4: 19-25.
- Badakhshan H, Moradi N, Mohammadzadeh H, Zakeri MR (2013) Genetic variability analysis of grains Fe, Zn and beta-carotene concentration of prevalent wheat varieties in Iran. *International Journal of Agriculture and Crop Sciences* 6: 57-62.
- Banerjee S, Sharma D, Verulkar S, Chandel G (2010) Use of in silico and semiquantitative RT-PCR approaches to develop nutrient rich rice (*Oryza sativa* L.). *Indian Journal of Biotechnology* 9: 203-212.
- Baxter IR, Gustin JL, Settles AM, Hoekenga OA (2013) Ionomic characterization of maize kernels in the intermated B73× Mo17 population. *Crop Science* 53: 208-220.
- Beyer P (2010) Golden Rice and 'Golden' crops for human nutrition. *New Biotechnology* 27: 478-481.
- Black R (2003) Micronutrient deficiency: an underlying cause of morbidity and mortality. *SciELO Public Health*.
- Bouis HE, Hotz C, McClafferty B, Meenakshi J, Pfeiffer WH (2011) Biofortification: a new tool to reduce micronutrient malnutrition. *Food and nutrition bulletin* 32: S31-S40.
- Bouis HE, Saltzman A (2017) Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security* 12: 49-58.
- Brar B, Jain S, Singh R, Jain R (2011) Genetic diversity for iron and zinc contents in a collection of 220 rice (*Oryza sativa* L.) genotypes. *Indian Journal of Genetics and Plant Breeding* 71: 67-73.
- Brotanek JM, Halterman JS, Auinger P, Flores G, Weitzman M (2005) Iron deficiency, prolonged bottle-feeding, and racial/ethnic disparities in young children. *Archives of Pediatrics & Adolescent Medicine* 159: 1038-1042.
- Cai JC, Cao JI, Shu AP, Jin ZQ, Han LZ (2009) Difference of iron contents in rice landraces. *Journal of Plant Genetic Resources* 1: 0-12.
- Dalmiya N, Palmer A (2007) Vitamin A supplementation: A decade of progress. UNICEF.
- George E, Rolf S, John R (2013) *Methods of Soil, Plant, and Water Analysis. A Manual for the West Asia and North Africa region*. International Center for Agricultural Research in the Dry Areas (ICARDA).
- Maret W, Sandstead HH (2006) Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements in Medicine and Biology* 20: 3-18.
- Meenakshi JV, Johnson N, Manyong V, Groote D, Javelosa H, Yanggen JD, Naher F (2010) How cost-effective is biofortification in combating micronutrient malnutrition? An ex-ante assessment. *World Development* 38: 64-75.
- Meng F, Wei Y, Yang X (2005) Iron content and bioavailability in rice. *Journal of Trace Elements in Medicine and Biology* 18: 333-338.
- McGuire S, FAO, IFAD, WFP (2015) *The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress*. Rome: FAO, Oxford University Press.
- Monks JLF, Vanier NL, Casaril J, Berto RM, de Oliveira M, Gomes CB, de Carvalho MP, Dias ARG, Elias MC (2013) Effects of milling on proximate composition, folic acid, fatty acids and technological properties of rice. *Journal of Food Composition and Analysis* 30: 73-79.
- Moreno-Moyano LT, Bonneau JP, Sánchez-Palacios JT, Tohme J, Johnson AA (2016) Association of increased grain iron and zinc concentrations with agro-morphological traits of biofortified rice. *Frontiers in plant science* 30: 73-79.
- Nagata MA (2009) Simple spectrophotometric method for the estimation of β -carotene content in spinach acetone extracts. *Bulletin of the National Institute of Vegetable and Tea Science* 8: 1-5.
- Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology* 23: 482-487.
- Paiva FF, Vanier NL, Berrios JDJ, Pinto VZ, Wood D, Williams T, Pan J, Elias MC (2016) Polishing and parboiling effect on the nutritional and technological properties of pigmented rice. *Food chemistry* 191: 105-112.
- Patil R, Diwan J, Nidagundi J, Lokesh R, Ravi M, Boranayak M, Dikshith S (2015) Genetic diversity of brown rice for iron and zinc content. *Electronic Journal of Plant Breeding* 6: 196-203.
- Qin H, Cai Y, Liu Z, Wang G, Wang J, Guo Y, Wang H (2012) Identification of QTL for zinc and iron concentration in maize kernel and cob. *Euphytica* 187: 345-358.
- Roy SC, Sharma B (2014) Assessment of genetic diversity in rice [*Oryza sativa* L.] germplasm based on agro-morphology traits and zinc-iron content for crop improvement. *Physiology and Molecular Biology of Plants* 20: 209-224.
- Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, Peña-Rosas JP, Bhutta ZA, Ezzati M, Group NIMS (2013) Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in

children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. *The Lancet Global Health* 1: e16-e25.

Umadevi M, Saraswathi R, Shanthi P, Suresh R, Rao D, Rajendran R (2016) Assessment of genetic variability in traditional rice varieties based on agro-morphological traits and iron zinc content for crop improvement. *International Journal of Science and Nature* 7: 244-250.

Wei Y, Shohag MJ, Yang X, Yibin Z (2012) Effects of Foliar Iron Application on Iron Concentration in Polished Rice Grain

and Its Bioavailability. *Journal of Agricultural and Food Chemistry* 60:11433-11439.

WHO (2009) Global prevalence of vitamin A deficiency in populations at risk 1995–2005. Available at http://apps.who.int/iris/bitstream/10665/44110/1/9789241598019_eng.pdf?ua=1 accessed on 15 January 2019.

WHO (2015) Malnutrition. Available at: <https://www.who.int/news-room/factsheets/detail/malnutrition> accessed on 15 January 2019.