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Assessment of genetic variability for micronutrient content and agro-morphological traits in rice (*Oryza sativa* L.)

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Abstract

Assessment of rice genetic diversity is critical step for trait specific varietal development program. In the present study, a collection of 281 Indian germplasm accessions were evaluated for genetic diversity study using 30 agro-morphological characters and grain iron and zinc contents in brown and polished rice. To identify the pattern of relatedness and associations, cluster analysis and principal component analysis coupled with correlation were used. Cluster analysis grouped 281 accessions into six main groups. Cluster 4 is the largest and had accessions with higher yield, zinc and iron content. Six components of principal component analysis indicated 76.4% of the total variation. The Principal Component (PC)1 showed 19.05%, while, PC2, PC3, PC4, PC5 and PC6 exhibited 14.23%, 13.61%, 11.58%, 7.59%, and 6.71% variability, respectively. Among the germplasm, three accessions IC145407, IC145357 and IC248034 have shown significant iron and zinc content in polished rice along with desirable grain yield. The information presented here will be useful in the development of rice varieties with high yield and micronutrient content.

Key words: Genetic diversity, germplasm, iron and zinc, polished rice, brown rice

Introduction

Malnutrition arising from dietary deficiency of one or more essential micronutrients affects two-third of world's population (White and Broadley 2009; Stein 2010). In developing countries, iron and zinc are the most commonly lacking mineral elements in the human diet with iron ranking the fifth and the zinc ranking the sixth among the top ten risk factors contributing to burden of disease (WHO 2017). Zinc plays a critical role in normal functioning of body and is integrated with several enzyme systems. Gene expression, cell division, immunity and reproduction are important biological functions of zinc (Brown et al. 2004). Zinc deficiency is commonly overlooked but several pathological effects and physiological disorders have been observed due to zinc deficiency in humans (Hagmeyer et al. 2015). The recommended dietary allowance (RDA) of iron and zinc for human population in the age group of 25-50 years are 10-15 and 12-15 mg respectively, (FAO/WHO, 2000). Iron is the central molecule of hemoglobin and it is an important component of human blood. It also regulates enzyme activity and plays an important role in the immune system (Abbaspour et al. 2014). Deviation from RDA levels of iron may cause deficiency symptoms like mental and psychomotor impairment in children, increased levels of both morbidity, mortality of mother and child during childbirth (Frossard et al. 2000).

Rice (*Oryza sativa* L.) has a unique position being a staple food crop for half of the world and Asia accounts for over 90% of the world's production of rice, mainly in China, India and Indonesia. Among all the Asian countries, India is the prominent rice growing country accounting for about 20% of all world rice production. In India, rice is grown in 44.7% of the total cropped land and accounts for 70.3% of the total food grain production in India (DAC&FW 2018). Rice has the largest collection of genetic diversity in the world and India is a home to diverse varieties of rice cultivars, landraces and many lesser known varieties those have been under cultivation since ages by farmers. The assessment of the genetic diversity is a basic principle in the breeding for the crop improvement, through the transfer of desirable traits to other genotypes (Sasaki

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2005; Varshney et al. 2008). Evaluation of locally adapted germplasm is the primary step for breeding towards a specific trait and land races are an ideal starting material for the breeder to raise crop varieties with beneficial characters (Shimelis and Laing 2012).

Majority of rice consumers prefer polished rice for the consumption but it is a poor source for iron and zinc (Bouis and Welch 2010). Most of the rice varieties have 12±14 μg g^{-1} of zinc and 1±2 μg g^{-1} iron in polished grains (Lu et al. 2013), thus consumption of polished rice is leading to malnutrition, especially for the people with low purchase power, for whom rice is only the affordable food item. Biofortification of rice with essential micronutrients is a sustainable and cost-effective approach to address micronutrient malnutrition, especially in the developing countries (Babu 2013). Understanding of the genetic mechanism of grain high iron and zinc in rice is essential for the systematic utilization of rice germplasm in rice biofortification programs (Swamy et al. 2016; Neeraja et al. 2017). Several researchers have characterized rice germplasm including the landraces, varieties and advance materials of diverse nature for morphological and physical-chemical quality parameters including zinc and iron (Roy and Sharma 2014); Wadbok et al. 2019; Haritha et al. 2020). The identification of promising landraces or traditional varieties with high zinc in polished rice and their use in the breeding programs appears to be the most beneficial strategy in rice improvement. The present investigation was therefore, intended to characterize the collection of rice germplasm based on the agro-morphological traits, yield and iron and zinc content in polished rice for assessing the existing genetic diversity and to identify promising donors with high zinc content in polished rice.

Materials and methods

Plant material and growth conditions

The experimental studies were undertaken in the farm of ICAR-Indian Institute of Rice Research (IIRR), Hyderabad in 2014.

The plant material comprised of 281 germplasm accessions was evaluated under normal agronomical conditions in an augmented block design with six blocks and four checks in each block. The seed material was sown in the raised nursery bed and transplanting of 26 days old seedlings was done in the main field. Germplasm accessions were planted in three rows with twenty plants in a space of 20 cm between rows and 15 cm between plants. The crop was grown under irrigated conditions, with recommended package of practices. Fertilizers were applied to the main field at the rate of 100 kg nitrogen, 60 kg phosphorus and 40 kg muriate of potash ha^{-1} at three different stages before transplanting, early tillering stage and booting stage.

Morphological observations

The germplasm accessions were evaluated to study genetic variability based on the important agro-morphological parameters along with grain iron and zinc content in brown and polished rice. The data on 30 agro-morphological traits were recorded from five randomly selected representative plants in all the genotypes. The traits were either measured or recorded through visual observation. The data was collected on 1) Basal leaf sheath color, 2) Leaf blade color, 3) Leaf pubescence, 4) Ligule shape, 5) Auricle colour, 6) Stigma color, 7) Stigma exsertion, 8) Apiculus color, 9) Panicle exsertion, 10) Panicle type, 11) Awning, 12) Flag leaf angle, 13) Sterile lemma color, 14) Culm strength, 15) Leaf senescence, 16) Threshability, 17) Hull color, 18) Seed coat color (kernel), 19) Aroma, 20) Days to 50% Flowering, 21) Leaf length, 22) Leaf width, 23) Plant height, 24) Total number of tillers per plant, 25) Effective tillers per plant, 26) Panicle length, 27) Grain yield per plant, 28) 1000 grain weight, 29) Grain length and 30) Grain width as per DUS (Distinctiveness, Uniformity and Stability) guidelines (Subba Rao et al. 2013).

Iron and zinc estimation

The seed samples of all the germplasm accessions were harvested separately from the pooled panicles of three middle row plants and divided into three parts to be analyzed as three replicates. The seeds were dehusked using JLGJ4.5 testing rice husker (Jingjian Huayuan International Trade Co. Ltd.) sponsored by Harvest Plus and polisher (Krishi International India Ltd.) with non ferrous and non zinc components. Each sample of brown and polished rice (5 g) was subjected to energy dispersive X-ray fluorescent spectrophotometer (ED-XRF) (OXFORD Instruments X-Supreme 8000) at ICAR-IIRR as per standardized protocols (Rao et al. 2014).

Data analysis

The data of fifteen quantitative traits *viz*., days to 50% flowering, leaf length, leaf width, plant height, total number of tillers per plant, no. of effective tillers per plant, panicle length, grain yield per plant, 1000 grain weight, grain length, grain width, iron and zinc contents in brown/polished grains from 281 rice germplasm accessions was analyzed using 'R' statistical software for correlation studies and principal component analysis. The genetic diversity was assessed using dendrogram generated by DARwin software, version 6.0.15 (http://darwin.cirad.fr/). Promising accessions were identified based on yield and mineral content using Venny2.1 online tool.

Results

Wide range of genetic variation was recorded across the gremplasm for the agro-morphological traits and grain in iron and zinc contents. The analysed data on variability of 19 qualitative traits along with mean differences iron and zinc contents in brown and polished rice are presented in Table 1. The descriptive statistics of eleven diverse quantitative traits along with grain iron and zinc contents in brown and polished rice of 281 germplasm accessions is shown in the Supplementary Table S1.

Pearson's correlation coefficient analyses showed high significant correlation between total number of tillers per plant with effective tillers per plant and zinc content in brown rice with zinc content in polished rice among the quantitative traits. Significant positive correlation was observed between test weight with grain width. Plant height has been positively correlated with leaf length, leaf width, grain yield, panicle length, iron and zinc content in brown and polished rice. Interesting significant positive correlation between iron and zinc content in brown and polished rice were recorded. Days to fifty percent flowering has shown negative correlation with test weight, plant height, panicle length, iron and zinc content in brown and polished rice (Table 2).

Promising accessions have been identified based on their grain yield (five plants yield is above 100g) along with zinc (>16µg g^{-1}) and iron (>4µg g^{-1}) content in polished rice (Fig. 1). A total of 169 accessions have shown high grain yield, 18 accessions showed high zinc and 18 accessions showed high iron in polished rice. Three accessions showed high yield, zinc and iron in polished rice (Table 3).

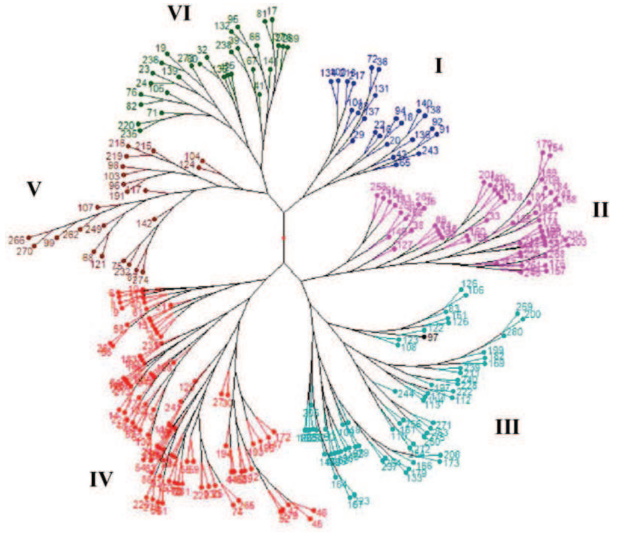

Fig. 1. Dendrogram of 281 accessions of Indian rice based on 15 quantitative traits

Dendrogram of 281 rice germplasm obtained for 15 quantitative characters based on UPGMA (Fig. 2, Supplementary Table S2) has shown six clusters with cluster 1 consisting of 24 accessions, cluster 2 with 52; cluster 3 with 63; cluster 4 with 88; cluster 5 with 23 and cluster 6 with 30 accessions. Accessions in cluster V were early to flower, with high leaf length, high grain yield, and high test weight but with short grain length. Likewise in cluster I, total number of tillers and effective tiller number were high. Cluster III had accessions which were high in iron content both in brown and polished rice whereas cluster IV had accessions with high zinc content both in brown and polished rice.

UPGMA dendrogram was generated using hierarchical cluster analysis using PC scores of different principal components, calculated on the basis of Eigen vectors of first six principal components for different qualitative and quantitative variables for each accession. Results obtained from UPGMA cluster analysis were corroborated by PCA. Six principal components accounted for approximately 72.80% variance. Discriminating power of principal components as inferred from PCA analysis was high for PC 1 (2.85) and low for PC 6 (1.0). First component (PC 1) explained 19.05 per cent of variation significantly positively correlated with DFF and had negative loadings for BZn, PZn, and BFe. Second component (PC 2) explained 14.23% variation positively correlated with TW, GL, GW and negatively correlated with TNT and ETN. Component 3 (PC 3) explained 13.61% variation correlated positively with TW, ENT, LW, TNT and GW whereas component 4 (PC 4) explained 11.58% variation correlated positively with TNT, ENT, GL. Component 5 explained 7.59% variation significantly positively correlated with DFF, LW and

Table 1. Agro-morphological traits with mean differences of grain iron and zinc content in brown and polished rice of 281 germplasm accessions

		Morphological trait	Frequency	Percentage	B_Fe μg g^{-1}	B_Zn μg g^{-1}	P_Fe μg g^{-1}	P_Zn μg g^{-1}
1	Basal leaf sheath color	Green-1	227	80.8	8.7	13.6	2.2	10.6
		Purple lines -2	44	15.7	8.6	12.5	1.8	9.3
		Light purple-3	3	1.1	9.1	9.2	2.6	6.3
		Purple-4	7	2.5	8.0	13.5	1.7	10.2
		Others-9	0	0.0	0.0	0.0	0.0	0.0
2	Leaf blade color	Light green-1	21	7.5	8.9	15.3	2.3	11.8
		Green-2	133	47.3	8.8	14.2	2.3	11.0
		Dark green-3	74	26.3	8.5	12.3	2.0	9.6
		Purple tips-4	0	0.0	0.0	0.0	0.0	0.0
		Purple margins-5	38	13.5	8.8	12.9	2.1	9.6
		Purple Blotch-6	13	4.6	8.2	10.6	1.7	7.8
		Purple-7	2	0.7	8.0	12.0	1.4	10.2
		Others-9	0	0.0	0.0	0.0	0.0	0.0
3	Leaf pubescence	Glabrous-1	65	23.1	9.0	14.5	2.6	11.3
		Intermediate-2	157	55.9	8.7	12.8	2.1	9.8
		Pubescent-3	59	21.0	8.3	13.8	1.9	10.6
4	Ligule shape	Truncate-1	0	0.0	0.0	0.0	0.0	0.0
		Acute-2	0	0.0	0.0	0.0	0.0	0.0
		Split-3	281	100.0	8.7	13.4	2.2	10.3
5	Auricle colour	Light green- 1	222	79.0	8.7	13.8	2.3	10.7
		Purple-2	59	21.0	8.5	12.1	1.8	8.9
6	Stigma color	White-1	188	66.9	8.7	13.3	2.2	10.3
		Light green-2	1	0.4	7.3	15.8	3.5	11.5
		Yellow-3	6	2.1	9.3	13.1	3.6	10.5
		Light purple-4	6	2.1	8.3	13.5	1.9	10.4
		Purple-5	80	28.5	8.7	13.5	2.0	10.4
		Others-9	0	0.0	0.0	0.0	0.0	0.0
7	Stigma exsertion	Well exs- 1	87	31.0	8.5	12.1	2.2	9.4
		Mod Exs- 3	99	35.2	8.9	14.3	2.2	11.0
		Just Exc- 5	95	33.8	8.6	13.7	2.1	10.5
8	Apiculus color	White-1	138	49.1	8.7	13.1	2.2	10.0
		Straw-2	57	20.3	8.8	13.5	2.3	10.7
		Brown-3	2	0.7	9.1	17.5	3.0	14.4
		Red-4	17	6.0	8.4	13.9	2.2	11.1
		Red apex-5	67	23.8	8.6	13.6	2.0	10.3
		Purple-6	0	0.0	0.0	0.0	0.0	0.0
		Purple apex-7	0	0.0	0.0	0.0	0.0	0.0
		Others-9	0	0.0	0.0	0.0	0.0	0.0
9	Panicle exsertion	Well exs-1	249	88.6	8.7	13.3	2.2	10.2
		Mod. Exs.-3	32	11.4	8.4	14.1	2.3	11.0
		Just exs.-5	0	0.0	0.0	0.0	0.0	0.0
		Partly exs-7	0	0.0	0.0	0.0	0.0	0.0
		Enclosed-9	0	0.0	0.0	0.0	0.0	0.0
10	Panicle type	Compact-1	0	0.0	0.0	0.0	0.0	0.0
		Intermediate-5	9	3.2	8.8	15.0	2.4	11.9
		Open-9	272	96.8	8.7	13.4	2.2	10.3
11	Awning	Absent-0	192	68.3	8.7	13.8	2.3	10.6
		Short and partly awned-1	24	8.5	8.3	14.3	2.2	10.8
		Short and fully awned-5	35	12.5	8.7	12.5	1.7	9.7

		Long and partly awned-7	0	0.0	0.0	0.0	0.0	0.0
		Long and fully awned-9	30	10.7	8.7	11.3	2.1	8.7
12	Flag leaf angle	Erect-1	149	53.0	8.6	13.4	2.1	10.3
		Semierect-2	128	45.6	8.7	13.4	2.2	10.2
		Horizontal-3	4	1.4	10.2	15.8	3.0	12.8
		Drooping -4	0	0.0	0.0	0.0	0.0	0.0
13	Sterile lemma color	Straw 1	257	91.5	8.7	13.6	2.2	10.5
		Gold 2	10	3.6	8.3	10.5	1.8	7.8
		Red 3	0	0.0	0.0	0.0	0.0	0.0
		Purple 4	12	4.3	8.6	12.7	1.9	9.7
14	Culm strength	Strong- 1	158	56.2	8.4	12.8	2.1	9.7
		Intermediate- 2	102	36.3	9.0	13.7	2.1	10.7
		Weak- 3	21	7.5	9.2	16.7	3.2	13.5
15	Leaf senescence	Early-1	24	8.5	8.4	12.8	2.1	9.9
		Medium -2	233	82.9	8.7	13.3	2.2	10.2
		Late-3	24	8.5	8.8	14.9	2.3	11.5
16	Threshability	Easy-1	270	96.1	8.6	13.3	2.1	10.2
		Intermediate-2	11	3.9	9.5	14.9	3.4	12.3
		Difficult-3	0	0.0	0.0	0.0	0.0	0.0
17	Hull color	Brown Line on Straw	66	23.5	8.4	12.3	2.0	9.5
		Brown	23	8.2	9.3	15.9	2.7	12.6
		Gold	17	6.0	8.6	14.0	2.0	11.2
		Purple lines on Straw	5	1.8	8.7	14.5	2.2	11.0
		Purple	10	3.6	8.6	13.0	2.2	10.7
		Straw	160	56.9	8.7	13.4	2.2	10.2
18	Seed coat color (kernel)	Brown line	4	1.4	8.8	12.2	1.6	10.0
		Brown	31	11.0	8.5	13.1	2.2	10.2
		Other green	3	1.1	8.7	15.0	2.9	12.0
		Red	85	30.2	8.9	13.8	2.2	10.6
		White	158	56.2	8.5	13.1	2.2	10.1
19	Aroma	Absent-0	268	95.4	8.7	13.4	2.1	10.3
		Present-1	13	4.6	9.0	13.9	2.7	10.9

B_Fe = Iron content in brown rice, B_Zn = Zinc content in brown rice, P_Fe = Iron content in polished rice and P_Zn = Zinc content in polished rice

negatively with PL, whereas component 6 correlated significantly with PL, GYP, BZn, PZn and negatively correlated with BFe and PFe.

Discussion

The plant genetic diversity is critical for the breeding programs as a resource for the genes of important agro-morphological and other traits. The conserved plant genetic resources are being utilized for crop improvement to meet global challenges in relation to food and nutritional security (Govindaraj et al. 2014). In the present study, a set of 281 germplasm accessions were characterized for qualitative and quantitative traits for identification of promising donors for zinc in polished rice and yield. Among the 19 qualitative traits stigma exsertion, apiculus color and leaf blade color have shown higher level of variation. Characters namely, basal leaf sheath color, leaf pubescence, auricle colour, stigma color, panicle exsertion, awning, flag leaf angle, culm strength, leaf senescence, hull color and seed coat color (kernel) displayed moderate level of variation. The remaining five traits include ligule shape, panicle type; sterile lemma color, threshability and aroma have shown lower level of variation. Among the 281 germplasm accessions, there is no significant difference in grain iron and zinc content in brown and polished rice as per the classification according to the 19 qualitative traits. Hence, identification of grain high iron and zinc genotypes appears to be difficult based on plant morphological observations in rice. Frequency distributions in the present study have also showed wide variability similar to the reports of variability for the 16 agro-morphological characters of 84 landraces (Roy and Sharma, 2014).

Table 2. Pearson's correlation coefficient among 15 quantitative traits of Indian rice germplasm

	Correlation coefficients, N = 281														
	DFF	LL	LW	PH	TNT	ETN	PL	GYP	TW	GL	GW	BFe	BZn	PFe	PZn
DFF	1.000														
LL	0.067	1.000													
LW	0.182	0.334***	1.000												
PH	-0.242***	0.386***	0.323***	1.000											
TNT	0.127*	0.067*	0.044*	0.001	1.000										
ETN	0.115*	0.127*	0.059*	0.019	0.930***	1.000									
PL	-0.137*	0.284***	0.071*	0.211**	-0.080*	-0.018	1.000								
GYP	-0.041*	0.188**	0.210**	0.235***	0.037	0.049*	0.148*	1.000							
TW	-0.190**	0.036	0.172**	0.255***	-0.081*	-0.071*	0.067*	0.118*	1.000						
GL	-0.057*	-0.107	0.023	-0.106	0.042**	0.031	-0.005	-0.032	0.474***	1.000					
GW	-0.032	-0.013	0.103*	0.083*	-0.038	-0.045*	-0.074*	0.079*	0.579***	0.389***	1.000				
BFe	-0.245***	0.057*	0.133*	0.327***	-0.031	-0.009	0.057*	0.027	0.165*	0.029	0.087*	1.000			
BZn	-0.218**	0.014	-0.02	0.259***	-0.021	-0.047*	-0.015	0.098*	0.048*	-0.045*	0.137*	0.398***	1.000		
PFe	-0.161*	0.038	0.050*	0.251***	0.081**	0.090**	0.009	0.054*	-0.096**	-0.210**	-0.190**	0.386***	0.229***	1.000	
PZn	-0.231***	-0.007	-0.015	0.242***	-0.02	-0.045*	-0.006	0.083*	0.032	-0.047*	0.112*	0.366***	0.946***	0.286***	1.000

* , ** and *** indicate significance at $P < 0.05$, < 0.01, and < 0.001 level respectively. DFF = Days to 50% flowering, LL = Leaf length, LW = Leaf width, PH = Plant height, TNT = Total number of tillers, ETN = Effective tiller number, PL = Panicle length, GYP = Grain yield, TW = Test weight, GL = Grain length, GW = Grain width. BFe = Iron in brown rice (µg g-1), BZn = Zinc in brown rice (µg g-1), PFe = Iron in polished rice (µg g-1) and PZn = Zinc in polished rice (µg g-1)

Efforts have been continuing for more than a decade to enhance iron levels in polished rice grains. The outer aleurone layer and embryo of the rice grains holds the majority of iron content and they are removed during the milling process, thus the edible endosperm contains very low amounts of iron. Therefore, most of the cultivated mega rice varieties hold only ~2 $\mu g\ g^{-1}$ of iron content in grains after polishing (Bouis et al. 2011). In the present study, 2 to 3.5 fold higher level of grain iron content (0.60-7.00) was shown by several accessions and these accessions have potential for rice biofortification breeding program. Breeding for higher endosperm iron content appears to be less feasible because of the low endosperm iron variation in rice germplasm (Meng et al. 2005). In order to achieve further increases in iron, the biofortification strategies need to consider approaches going beyond the uptake of iron by the roots (Paul et al. 2013). It has become evident that 2.54-fold increased amounts of iron can be obtained in the grains even after polishing as demonstrated in the transgene introgression a high yielding indica rice cultivar Swarna (Paul et al. 2014). Therefore, future strategies need to explore the identification of rice genotypes that can retain higher level of grain iron content even after polishing.

The relationship between grain mineral concentrations with grain yield is one of the most important aspects for the development of high nutrient rice. Analysis of Pearson's correlation studies in the present study indicated a positive correlation signifying that higher zinc and iron concentrations may occur

Table 3. Promising accessions for grain yield, zinc and iron content from the 281 germplasm accessions

	Genotype ID	GYP (gr)	P_Fe ($\mu g\ g^{-1}$)	P_Zn ($\mu g\ g^{-1}$)
	3 common elements in "GYP", "Iron" and "Zinc"			
1	IC145407	114.2	4.1	18
2	IC145357	103.4	4.5	23.1
3	IC248034	100.2	4.9	16.1
	12 common accessions in " GYP " and "Zinc":			
1	IC145440	287.8	2.2	18.1
2	IC145356	228.8	2.6	21.4
3	IC349669	173.8	1.5	17.4
4	IC264727	161.3	1.7	17.1
5	IC121884	156.1	2.5	16.1
6	IC262977	146.7	2	18
7	IC342904	130.1	2.6	16.4
8	IC340388	126.5	2.4	20.5
9	IC114186	125	2.5	24
10	IC258828	119.4	1.3	17.4
11	IC466773	105.8	2.4	18.1
12	IC203376	101.6	2.7	16.7
	11 common accessions in " GYP " and "Iron"			
1	IC351712	199.3	4.1	15.2
2	IC248028	135.6	4	12.9
3	IC248053	130.5	6.1	10.7
4	IC251465	126.5	5.2	14.7
5	IC145439	126.2	4.3	12.1
6	IC252289	125.5	4.5	12.2
7	IC145419	113.8	4.3	10.4
8	IC17042	113.5	4.7	15.8
9	IC248033	112.5	4.2	13.2
10	IC248024	102.5	4.6	12.8
11	IC248046	102.5	4.2	10.5
	1 common accession in "Iron" and "Zinc"			
1	IC203457	51.8	7	18.6

GYP = Grain yield per plant, P_Fe = Iron in polished rice ($\mu g\ g^{-1}$) and P_Zn = Zinc in polished rice ($\mu g\ g^{-1}$)

simultaneously in rice. Positive correlation between grain zinc and iron content has been previously reported in rice (Sperotto et al, 2009; Anuradha et al. 2012; Bollimedi et al. 2020) and in both brown and polished rice grains of three rice genotypes (Johnson et al. 2011; Madhubabu et al. 2017). A few studies have indicated the significant negative association between grain yield and zinc concentration (Gao et al. 2006; Jiang et al. 2008; Norton et al. 2010; Wissuwa et al. 2008), but a positive relationship was reported between grain yield with grain zinc content under zinc-deficient soil (Gregorio 2002). Non-significant correlations were also observed between yield and grain zinc in a panel of aromatic rice and land races under zinc sufficient conditions (Gangashetty et al. 2013; Sathisha 2013). Identification of high zinc donor accessions with high yield (Johnson et al. 2011) and development of transgenic accessions with grain high zinc content along with higher grain yield (Trijatmiko et al. 2016) suggests the possibility of combining high yield and high zinc. Recent release of high zinc accessions with high yield in India and Bangladesh also provide positive evidence for the possibility of combining high zinc and high yield potential in rice (Harvest Plus 2014; AICRIP 2015). Hence, using the donors identified in this study, high zinc rice varieties can be developed along with high grain yield.

Among 281 germplasm accessions, 169 accessions have been identified for high grain yield, 18 accessions showed high zinc in polished rice and 18 accessions have shown high iron. Three germplasm lines IC145407, IC145357 and IC248034 have shown higher grain yield with grain high zinc and iron content in polished rice. These accessions can be utilized as donors for the developing nutritional rich rice varieties. Till now, 28 accessions for grain iron and 38 accessions for grain zinc have been identified as donors through the evaluation of landraces and are being used in development of high zinc breeding accessions (Gregorio et al. 2000; Mahender et al. 2016). More recently, two promising accessions have been reported with high zinc content (20.6 $\mu g\ g^{-1}$, 24 $\mu g\ g^{-1}$) and moderate iron content (3.6 $\mu g\ g^{-1}$, 4.3 $\mu g\ g^{-1}$) in polished rice (Madhubabu et al. 2017). Screening large number of RILs developed through identified donors with differential grain mineral content also appears to be a reliable strategy to identify the promising genotypes for higher grain yield, zinc and iron content (Pulagam et al. 2015). Bollinedi et al. (2020) evaluated a large no. of germplasm lines for grain nutrients and physico-chemical traits and reported a wide range of variability.

The 281 rice genotypes were clustered into six groups, which implied a high level of genetic diversity in the rice genotypes. Cluster 4 is the largest cluster consisting of 88 accessions, followed by cluster 3 (63), 2 (52), 6 (30), 1 (24), 5 (23). Cluster 5, having 23 genotypes showed highest cluster means for grain yield, thousand grain weight and leaf length. Cluster 1, having 24 genotypes showed highest number of effective tillers, while other clusters had 9 to 10 tillers. Cluster 2 comprising of 52 genotypes had a unique accession IC203457 which had high iron and zinc content, while it is low yielder. To achieve a wide spectrum of variation among the segregates, genotypes having distant cluster, group 1 (accessions 6040, 6041, 6048, 6068, 6070, and 6067) could be hybridized with group 5 (accessions 7541, 7596, 3828, and 7545) and group 6 (accession 7508). Presence of considerable genetic diversity among the genotypes screened in the present study indicated that this material may serve as good source for selecting the diverse donor accessions for hybridization program aimed at developing high yielding varieties and also improving the micronutrient content of rice.

In general, Principal Component Analysis (PCA) measures the importance and contribution of each component to total variance. Principal Components (PCs) are orthogonal and independent of each other (Mohammadi and Prasanna, 2003). It can be used to quantity the independent impact of a particular trait to the total variance whereas each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. Higher the value of coefficients, regardless of the sign, the more effective they will be in discriminating between accessions (Nachimuthu et al. 2014). In this study, the total variability was explained by six PCs, which indicates the contribution of many traits with higher level of correlation to explain the genetic diversity. PCA has identified a few characters which plays important role in classifying the variation existing in the rice genotypes. The analysis identified days to 50% flowering, total number of tillers, effective tiller number, panicle length, thousand grain yield, grain length, grain width, Iron and zinc in brown and polished rice in different principal components are the most important traits contributing for classifying the variation. Gour et al. (2017) has reported similar results for panicle length, thousand grain weight, grain length, grain L/B ratio, decorticated grain length, decorticated grain L/B ratio, cooked grain length, 1000 grain weight, panicle length per plant, grain width, decorticated grain length, hulling %, milling %, head rice recovery and milling degree which were found to be the major factors contributing to the variation of 83 rice genotypes comprising traditional landraces and released varieties from India. Chakravorty et al. (2013) assessed the diversity of rice landraces in West Bengal state of India, in which PCA revealed six quantitative characters *viz*., grain length, culm diameter, culm number, number of grains per panicle, grain length/breadth ratio and leaf length significantly influenced the variation in cultivars studied. Rai et al. (2013) also reported similar results that grain characteristics along with plant height, panicle density and leaf length contributes for morphological diversity in a study involving Indian landraces of aromatic and non aromatic accessions. More recently, Bollinedi et al. (2020) studied a large collection of rice germplasm and identified promising donors for biofortification and grain quality improvement in rice.

Authors' contribution

Conceptualization of research (CNN, VRB); Designing of the experiments (CNN, VRB, MP); Contribution of experimental materials (LVSR); Execution of field/lab experiments and data collection (MP, RS, KS, MC, UC); Analysis of data and interpretation (MP, RAF, DSR, CNN); Preparation of manuscript (MP, CNN, VRB, RAF, LVSR).

Declaration

The authors declare no conflict of interest.

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Supplementary Table 1. Descriptive statistics of quantitative traits

	Name of the trait	Minimum	Maximum	Mean	Standard deviation	Standard error	Variance	*t* value	Coefficient of variation
1	Days to fifty percent flowering	87.00	141.00	105.90	8.77	0.52	76.87	202.49	8.28
2	Leaf length (cm)	19.80	82.05	46.90	9.76	0.58	95.17	80.60	20.80
3	Leaf width (cm)	0.65	2.25	1.31	0.26	0.02	0.07	86.44	19.39
4	Plant height (cm)	59.75	185.20	123.13	22.56	1.35	508.88	91.50	18.32
5	Total number of tillers per plant	5.00	23.00	11.11	3.11	0.19	9.68	59.84	28.01
6	Effective tillers per plant	3.00	22.00	9.40	3.13	0.19	9.80	50.35	33.29
7	Panicle length (cm)	14.20	41.50	24.75	3.70	0.22	13.71	112.02	14.96
8	Grain yield per five plant (g)	10.19	889.70	125.61	86.35	5.15	7456.73	24.38	68.75
9	1000 grain weight (g)	9.40	41.60	22.39	5.72	0.34	32.67	65.66	25.53
10	Grain length (mm)	1.68	10.86	8.16	1.32	0.08	1.75	103.37	16.22
11	Grain width (mm)	1.25	3.98	1.74	0.23	0.01	0.06	124.29	13.49
12	Iron in brown rice (μg g^{-1})	5.90	14.40	8.68	1.36	0.08	1.85	107.02	15.66
13	Zinc in brown rice (μg g^{-1})	4.70	28.80	13.41	4.25	0.25	18.05	52.89	31.69
14	Iron in polished rice (μg g^{-1})	0.60	7.00	2.17	0.99	0.06	0.99	36.63	45.77
15	Zinc in polished rice (μg g^{-1})	2.40	24.00	10.32	3.86	0.23	14.91	44.82	37.40

Supplementary Table 2. Clusters of Indian rice germplasm accessions according to cluster analysis

Clusters	Accession Numbers
Cluster-I	IC116100, IC125024, IC145356, IC145360, IC145440, IC203532, IC248020, IC256716, IC257219, IC282418, IC282430, IC282450, IC283218, IC299417, IC299457, IC300683, IC300875, IC300914, IC300945, IC300948, IC300991, IC337552, IC337560, IC362206
Cluster-II	IC145416, IC203448, IC203457, IC203506, IC211237, IC278770, IC280529, IC282425, IC300042, IC300477, IC300545, IC301090, IC301115, IC301143, IC301154, IC301172, IC301179, IC301180, IC301183, IC301424, IC301457, IC301473, IC301520, IC301543, IC310033, IC310435, IC310447, IC316276, IC316279, IC319353, IC321200, IC331148, IC332045, IC332052, IC332672, IC333018, IC334104, IC334202, IC334323, IC334349, IC335860, IC370818, IC449887, IC450022, IC462060, IC462074, IC462250, IC462277, IC462318, IC463011, IC464405, IC466403
Cluster-III	IC262967, IC262968, IC274369, IC285706, IC299544, IC299576, IC299705, IC299729, IC299766, IC299824, IC299924, IC299991, IC300159, IC300191, IC300202, IC300272, IC300369, IC300822, IC301093, IC301109, IC301116, IC301117, IC301119, IC301181, IC301185, IC301206, IC301346, IC301378, IC301442, IC301448, IC310017, IC313140, IC316315, IC319355, IC319488, IC330686, IC330793, IC331166, IC331645, IC334080, IC334234, IC335396, IC337604, IC337608, IC340559, IC349678, IC353826, IC360766, IC361321, IC363775, IC369301, IC462157, IC462256, IC462258, IC462939, IC462991, IC462992, IC463070, IC466426, IC466437, IC466773, IC463073, IC576936
Cluster-IV	IC17029, IC17037, IC17042, IC17054, IC17060, IC17084, IC70880, IC85745, IC86495, IC99441, IC114186, IC114652, IC115608, IC115845, IC145357, IC145407, IC145419, IC145439, IC182372, IC203376, IC247998, IC248021, IC248022, IC248024, IC248025, IC248026, IC248028, IC248029, IC248033, IC248034, IC248046, IC248053, IC251465, IC252053, IC252169, IC252243, IC252268, IC252289, IC252322, IC256616, IC256649, IC256803, IC256808, IC257234, IC258828, IC262964, IC262977, IC282423, IC283211, IC299465, IC299991, IC300166, IC300168, IC300566, IC300584, IC301107, IC301111, IC301114, IC301123, IC310012, IC310431, IC311005, IC319350, IC319472, IC319524, IC321833, IC326126, IC326439, IC328524, IC330135, IC336384, IC336650, IC337598, IC337601, IC339784, IC340388, IC340548, IC340690, IC340696, IC342904, IC350270, IC361569, IC362037, IC463175, IC540414, IC576898, IC576899, IC576968
Cluster-V	IC256789, IC260917, IC282408, IC283232, IC298338, IC298569, IC299479, IC299482, IC299573, IC300153, IC300186, IC300222, IC301064, IC324089, IC337148, IC337528, IC337593, IC349669, IC459573, IC463023, IC463182, IC466405, IC466769
Cluster-VI	IC115875, IC121884, IC132928, IC145400, IC145402, IC203386, IC211235, IC247991, IC248006, IC248014, IC252280, IC256749, IC256757, IC256821, IC264727, IC267428, IC282212, IC283230, IC299484, IC300812, IC300907, IC300989, IC300992, IC337598, IC351712, IC352990, IC356432, IC466404, IC466755, IC466791