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EXPLORING GENETIC VARIABILITY FOR GRAIN IRON AND ZINC CONTENT IN A DIVERSE SET OF RICE [*Oryza sativa* (L.)] GERMPLASM

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ABSTRACT

Characterization of genetic variability in existing germplasm for any trait of economic importance is a basic plant breeding strategy enabling identification and deployment of suitable donors. Present study aimed at estimating Fe and Zn content in a set of 206 rice germplasm lines, which includes 63 recombinant inbred lines (RILs) derived from hybridizing parents with low and high Zn content. The material was planted along with three standard checks; PR114, PR121 and PR 126 in Augmented Design. Sufficient variability was recorded in the brown rice of the rice germplasm lines for grain Fe (4.6-37.5, 6.7-16.1 ppm) and Zn (16.3-41.0, 15.0-58.2 ppm) contents. First two principal components (PCs) in rice germplasm lines (60.78%) and first three PCs in RIL population (75%) showed maximum variability. The entire set of germplasm was delineated into three diversity groups. Fe and Zn dense genotypes identified in present study could be used as donors for improving Fe and Zn content in popular cultivars.

Keywords: Cluster, Iron, PCA, Rice germplasm, RILs, Zinc

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Rice (*Oryza sativa* L.), a major staple food and a source of energy, contribute up to 70% of daily calories for nearly 50% of the world's population. However, rice grain lacks essential micronutrients such as Iron (Fe) and Zinc (Zn). Across the globe about two billion people endure Fe and Zn deficiency associated health problems (Rampa *et al.*, 2020). Zinc is required for several functions in human body including gene expression, enzymatic reactions, immune function, protein synthesis, DNA synthesis, wound healing, growth and development and its deficiency causes stunted growth, loss of appetite, abnormal neurosensory changes, male hypogonadism, and impaired immune response (Murata *et al.*, 2006). Similarly, Fe is a fundamental element of hemoglobin and myoglobin and its insufficiency give rise to serious health problems, such-as anemia, weak immune system, mental retardation, increased mortality rates. Preventing micronutrient deficiencies is one of the most serious global challenges for human health today (Muthayya *et al.*, 2013).

Because of its high consumption, rice is considered as an ideal vehicle for large scale delivery of micronutrients such as Fe and Zn. The biofortification of rice with enhanced levels of Fe and Zn may be a cost-effective and sustainable solution to assist in combating Fe and Zn malnutrition. Any breeding program directed to enrich rice grain with higher Fe and Zn content requires screening and identification of donors with high Fe and Zn in the germplasm. At International Rice Research Institute (IRRI), Philippines, studies on the genetic variability for grain Fe and Zn content in rice was initiated in 1992 and 1995, respectively. Since then, numerous researchers have characterized rice germplasm sets to elucidate variability for grain Fe and Zn content in cultivated and wild accessions (Wadbok *et al.*, 2019). A wide variability has been reported from different studies for grain Zn content ranging from 7.3 to 58.4 ppm in brown rice, and from 4.8 to 40.9 ppm in polished rice with polishing losses from 11.1 to 28.0% (Swamy *et al.*, 2016; Sanjeeva Rao *et al.*, 2020). Likewise, genetic variation for Fe ranged from 2 to 147 ppm in brown rice (Zeng *et al.*, 2010). Identification of promising donors with high Fe and Zn content in rice grains and their use in the breeding programs seems to be the most viable strategy in improving rice nutritional composition. Present research was, therefore, planned to evaluate the genetic variability in the set of rice germplasm for their grain Fe and Zn content in brown rice. To identify the relatedness among genotypes, cluster analysis and principal component analysis were used to delineate them into different diversity groups.

MATERIALS AND METHODS

Plant materials

The experimental material consists of 143 germplasm lines consisting of landraces, released cultivars, and advanced breeding lines and 63 recombinant inbred lines (RILs) derived from the cross of low zinc parent, Samba Mahsuri with high zinc parent, Kaliboro was sown during *kharif* 2019 and *kharif* 2020. The material was planted along with three standard

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checks; PR114, PR121 and PR 126 in Augmented Design. The designation of germplasm lines used has been given in Table 1.

Morphological analysis and Iron-zinc content estimation

Quantification of Fe and Zn was done by inductively coupled plasma-optical emission spectrophotometer (ICP-OES). Around 500 mg of each seed sample of brown rice were digested with 6 ml HNO_3 at 180°C for 30 minutes using a microwave digestion system (Microwave 300, Anton PAAR, Graz, Austria). The digested sample was diluted to a final volume of 25ml with double distilled water prior to analysis. Quantification of Fe and Zn was done by inductively coupled plasma-optical emission spectrophotometer (ICP-OES) procedure at School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. Each sample was analyzed in two replications and concentrations of Fe and Zn were determined and presented as ppm. Data for some of the morpho-agronomic traits *viz*; days to 50% flowering (DTF), Ear bearing tillers per meter square (EBT/m^2) and Plant height (PH-cm) was recorded from five random plants of each entry per genotype.

Statistical analysis

The analysis was carried out using the software SPAD (Rahore *et al*., 2004). PCA was done by R-software. Cluster analysis was done using Minitab software (21.1).

RESULTS AND DISCUSSION

The analysis of variances (Table 2) was carried out using data recorded for Fe, Zn, PH, EBT, and DTF on rice germplasm lines, RIL population and three check varieties sown in augmented block design during *kharif* 2019 and *kharif* 2020. The mean square for rice germplasm lines and RIL population were found significant for indicating that the highly significant differences were present between the rice germplasm lines and between RILs at *p* value <*0.01*. The mean square for blocks were non-significant for rice germplasm and RIL population indicating the low heterogeneity among the blocks (*p* value <*0.01*). Significant differences were present between rice germplasm lines and checks and between RILs and checks for all traits except for Fe content between RILs and checks. Germplasm lines and RILs with high EBT/m^2 along with less DTF and small PH are desirable in rice breeding programs.

Average Fe content of germplasm lines (Fig. 1a) ranged from 4.6 ppm (CSR 30) to 37.5 ppm (R-RHZ-R 56). In RIL population, Fe content varied from 6.7 ppm (RIL No. 21) to 16.1 ppm (RIL No. 11) (Fig. 1b). Average Fe content of two parents of RIL population was 8 ppm (Samba Mahsuri) and 15.4 ppm (Kaliboro). Average Fe contents of standard checks were 11.6, 12.2 and 12.7 ppm for PR114, PR121 and PR126, respectively. Similarly, average Zn content of germplasm lines ranged between 16.3 ppm (CR 2829-PLN 109) to 41ppm (RP 5177-435-8-5-3) (Fig. 1a). The Zinc content in RIL population ranged from 15 ppm (RIL No. 63) to 58.2 ppm (RIL No. 51) (Fig. 1b). Average Zn content of two parents of RIL population was 42.8 ppm (Kaliboro) and 20.1 ppm (Samba Mahsuri). Average Zinc contents of standard checks were 23.2, 24.4 and 24.3 ppm for PR114, PR121 and PR126, respectively.

For developing varieties with high Fe/Zn in polished/brown rice via conventional breeding, variability for grain Fe/Zn should be present in the germplasm. We observed a wide variation in Fe and Zn contents in brown rice grain of RIL population, which could be attributed due to the diverse combination of alleles from the two contrasting parents for Zn content (Kumar *et al.*, 2014). In present study, a total of 15 germplasm lines and 34 RILs have high Zn content (≥35 ppm) and the promising genotypes with Zn content ≥35 ppm in brown rice could be used as donors to meet the target Zn (Sanjeeva-Rao *et al.*, 2020). Similarly, 73 germplasm lines and 6 RILs have high Fe content i.e. ≥15 ppm. So, these lines with high Fe as well as high Zn could be used as promising donors for the development of mapping populations. We identified 12 RILs which had significantly variable and remarkably higher Zn contents in rice grains (up to 58.2 ppm) as compared to the superior parent. The variability created for grain Fe and Zn content can be exploited for nutritional enhancement and crop improvement.

In some of previous studies, Fe content varied from 6.2 to 71.6, 1.55 to 16.58, 0.25 to 34.8, 3.38 to 36.99, 7.45 to 29.85, 6.9 to 22.3, 13.23 to 45.83, 8.3-52.15, 8.3-52.15, 1.6 to 15.2 and Zn content varied from 26.2 to 67.3, 15.72 to 65.01, 0.85 to 195.3, 3.32 to 42.49, 14.68 to 59.65, 14.5 to 35.3, 18.67 to 38.01, 3.0 to 52.7, 6.2 and 33.2 ppm (Anuradha *et al*., 2012; Pinson *et al*., 2014; Roy and Sharma, 2014; Patil *et al.*, 2015; Umadevi *et al*., 2016; Maganti *et al.*, 2019; Raza *et al*., 2019; Tripathy, 2020; Chandu *et al.*, 2020, respectively for Fe and Zn). Grain Fe and Zn content of several mapping populations including RILs, DHs, F_2, BILs have been analyzed in brown rice grain and varied between 4.7 to 15.8 ppm and 11.1 to 27.3 ppm (Suman *et al.*, 2021), 14.0 to 26.5 ppm and 12.5 to 28.0 ppm (Lee *et al.*, 2020), 21.9 to 41.3 ppm (Zn) (Mohiuddin *et al.*, 2020), 18.0-55.4 ppm and 16.2-47.2 ppm (Liu *et al.*, 2020), respectively.

Principal component analysis is one of the important multivariate analyses and it identifies the traits that

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Table 1. List of rice germplasm lines used in the present study

Sr. No.	Designation	Sr. No.	Designation	Sr. No.	Designation
1	RP 4920-Bio-51 (B)	49	2K6-2012-4-3-1-1-2-1-1-1-1	96	PR 118
2	RP 5898-182-22-4-3-2-1	50	RP 5910-69-11-4-2-2-2	97	PR 120
3	RP 5912-17-8-4-3-2-2	51	RP 5706-112-4-5-3-2	98	PR 121
4	RP 5898-38-7-2-1-1	52	IR58025B	99	PR 122
5	RP 5898-54-21-9-4-2-2	53	CSR-58	100	PR 123
6	RP 5898-138-14-9-8-4-2	54	CR 2826-1-1-1-2B-1-2B-1	101	PR 124
7	NP-9685	55	ARB-10	102	PR 126
8	CR 2829-PLN-32	56	NPQ-23	103	PR 127
9	CR 2829-PLN-100	57	RP 4993-183-40-5-2-1-1	104	PR 128
10	Gontra Bidhan	58	RP Bio 4918-236-K	105	PR 129
11	RP Bio 5477-NH 787-1	59	RTN RR-4	106	PAU 201
12	RP 5898-101-3-2-1-1	60	RP 5177-435-8-5-3	107	Pusa 44
13	R-RHZ-L1-23	61	R-RHZ-LI-22	108	Punjab Mehak 1
14	RP 5898-19-8-6-1-1-1	62	NPQ-50	109	PB-3
15	R-RHZ-SM-14	63	CR 2829-PLN 23	110	Pusa 1121
16	Dadaji HMT	64	ARB-11	111	Bas 386
17	CR 2819-1-5-3-B-8-2B-1	65	RP 5958-IR 94052-117-13-4-11-1-1	112	Pusa 1509
18	IR 82475-110-2-2-1-2			113	Bas 370
19	CB 14740	66	NPQ 49	114	PB-2
20	HPR 2795	67	RP Bio 4918-233-k	115	PB 5
21	RP 5115-111-24-3-1-1	68	CR 2915-1-1-3-1-B-6-2B-1	116	PB 1718
22	PAU 2K10-23-54-14-52-20-0-4	69	R-RHZ-MI-30	117	CSR 30
23	RP 5948-36-21-11-5-2-1	70	ARB 13	118	Swarna
24	HPR 2720	71	2K6-2012-9-1-2-2-1-1-1-1-1-1	119	Zhang 123
25	RP 5898-22-4-3-2-1	72	ARB 12	120	ASD 18
26	RP 5948-59-19-8-6-1-1	73	CR 2007	121	Yu-Xiiangzan
27	RP 5977-MS-M-37-3-2-3-4-3	74	R-RHZ-MI-32	122	Doddi
28	CR 2830-PLS-156	75	RP 5898-101-9-3-1-1	123	Om1723
29	R-GM-AS43	76	RP 5163-102-3-5-2-2	124	OM 997
30	KaliKamod	77	R-RHZ-R 56	125	93072
31	R-RHZH-7	78	RP 5948-26-11-3-1-1-1	126	BG 94-1
32	RP Bio 5477-NH 686	79	PAU 6886-6346-1-1-B	127	TGMS 29
33	R-RHZ-2	80	R-RHZ-1B-13	128	PSBRC 28
34	RP 5886-HP3-IR 80463-B39-3	81	CN-1987-4-2	129	Govind
35	CR 2829-PLN-37	82	RHZ-5M-14	130	UPR 191-66
36	KMP-200	83	Jaya	131	R644
37	RP 5898-182-22-4-3-2-1	84	IR8	132	Nan 29-2
38	NLR 3238	85	IR 50	133	Rasi
39	RP 5917-HP-8-IR94064	86	IR 64	134	Cisedani
40	RP 5889-117-46-9-3-3-2	87	IR 72	135	IR58025B
41	PAU 2769-20-2-3-3-2-1-2-M-9	88	PR 103	136	JP5
42	RP 5911-52-13-3-2-2-1	89	PR 106	137	C71
43	CR 2829-PLN-36	90	PR 108	138	Amol 3 (Sona)
44	CR 2829-PLN 109	91	PR 111	139	IR 66897B
45	2K6-2012-4-2-2-1-2-1-1-1-1	92	PR 113	140	Zhong 413
46	OR 2545-5	93	PR 114	141	Hua-Gen-Xian 74
47	MINGUI 63	94	PR 115	142	Ajaya
48	RP 5892-32-9-5-4-3-2	95	PR 116	143	Khole marshi

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Table 2. ANOVA for Fe, Zn, PH, and EBT in diverse rice germplasm lines

Trait	Source	Df	SS	MS	*F*	*P-value*	CV
Fe (Germplasm lines)	Genotypes	145	2877.6	19.8	*22.4*	*0.0*	6.0
	Block	10	0.8	0.1	*0.1*	*1.0*	
	Genotypes-vs-Control	1	435.7	435.7	*492.8*	*0.0*	
Zn (Germplasm lines)	Genotypes	145	2734.9	18.9	*14.6*	*0.0*	4.3
	Block	10	32.8	3.3	*2.5*	*0.0*	
	Genotypes-vs-Control	1	20.7	20.7	*16.1*	*0.0*	
PH (Germplasm lines)	Genotypes	145	29848.9	205.9	*43.7*	*0.0*	2.2
	Block	10	70.1	7.0	*1.5*	*0.2*	
	Genotypes-vs-Control	1	6058.9	6058.9	*1285.0*	*0.0*	
EBT (Germplasm lines)	Genotypes	145	503921.7	3475.3	*36.1*	*0.0*	3.0
	Block	10	926.7	92.7	*1.0*	*0.5*	
	Genotypes-vs-Control	1	1139.4	1139.4	*11.8*	*0.0*	
Fe (RILs)	Genotypes	67	324.8	4.8	*5.6*	*0.0*	7.0
	Block	4	0.3	0.1	*0.1*	*0.9*	
	Genotypes-vs-Control	1	1.7	1.7	*2.0*	*0.2*	
Zn (RILs)	Genotypes	67	4579.5	68.4	*58.5*	*0.0*	3.0
	Block	4	10.3	2.6	*2.2*	*0.2*	
	Genotypes-vs-Control	1	1322.8	1322.8	*1132.4*	*0.0*	
PH (RILs)	Genotypes	67	70545.8	1052.9	*186.4*	*0.0*	1.8
	Block	4	49.6	12.4	*2.2*	*0.2*	
	Genotypes-vs-Control	1	33426.5	33426.5	*5916.2*	*0.0*	
EBT (RILs)	Genotypes	67	242494.5	3619.3	*66.2*	*0.0*	2.2
	Block	4	547.6	136.9	*2.5*	*0.1*	
	Genotypes-vs-Control	1	1905.1	1905.1	*34.8*	*0.0*	
DTF (RILs)	Genotypes	67	3518.3	52.5	*22.7*	*0.0*	1.5
	Block	4	1.1	0.3	*0.1*	*0.9*	
	Genotypes-vs-Control	1	213.6	213.6	*92.2*	*0.0*	

Days to 50% flowering (DTF), Ear bearing tillers per meter square (EBT/m^2) and Plant height (PH-cm), Iron (Fe), Zinc (Zn)
**P≤0.05; **P≤0.01*

Supplementary Table S1. Principal component, eigenvalue, percentage of total variation, cumulative percentage and factor loading with respect to four principal components in rice germplasm lines and RIL population

Component	Eigen Value	Variation %	Cumulative variation %	Zn (ppm)	Fe (ppm)	PH	EBT/m^2	DTF
Rice germplasm lines								
PC1	1.35	33.8	33.8	0.65	0.70	0.27	-0.08	
PC2	1.07	26.7	60.6	-0.21	0.02	0.67	0.71	
PC3	0.90	22.6	83.1	-0.36	0.01	0.63	-0.69	
PC4	0.68	16.9	100.0	0.64	-0.71	0.29	-0.07	
RIL population								
PC1	1.57	31.4%	31.4%	-0.614	-0.525	-0.352	0.010	0.472
PC2	1.18	23.6%	55.1%	0.227	-0.490	0.196	0.809	-0.122
PC3	1.00	20.0%	75.0%	-0.004	0.320	-0.833	0.355	-0.277
PC4	0.83	16.6%	91.6%	-0.510	-0.219	0.127	-0.142	-0.810
PC5	0.42	8.4%	100.0%	0.558	-0.577	-0.357	-0.446	-0.173

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contribute most for the observed variation within a group of genotypes and has a practical application in the selection of best genotypes for breeding purpose. PCA was performed for four traits of rice germplasm lines and five traits in a RIL population (Table S2). In rice germplasm lines, first two principal components (PCs) exhibited more than 1.00 eigenvalue and showed maximum variability about 60.6% among the traits studied (Fig 2a,b). The PC1 had the highest variability (33.9%) followed by PC2 (26.7%), PC3 (22.5%), and PC4 (16.9%). As per the PCA-biplot (Fig. 2b) and factor loading of different characters (Table S1), the PC1 was mostly associated with trait such as, Fe and Zn. These traits had the major role in divergence and responsible for major portion of its variability. PC2 was consisting of mainly PH and EBT/m^2. For RIL population, first three PCs with eigen value greater than or equals to one showed 75.0% cumulative variation (Table S1, Fig 2c). Highest variability was exhibited by PC1 (31.4%) followed by PC2 (23.6%) and PC3 (20.0%). Factor loading (Table S1) and PCA-biplot (Fig. 2d) revealed that PC1 was associated with Fe and Zn, PC2 with EBT/m^2, PC3 with PH and PC4 with DTF.

It can be concluded from the above results that Fe and Zn were having the highest variation in PC1 in germplasm lines and RIL population. Selection of trait via the traits that contribute to maximum morphological diversity through these major PCs would be beneficial. Calayugan *et al*. (2020) reported first two PCs explained 55.94% of the total variation observed in the DH population. Madhubabu *et al*. (2020) reported six PCs showed 76.4% of the total variation. The PC1 indicated 19.05%, while, PC2, PC3, PC4, PC5 and PC6 revealed 14.23%, 13.61%, 11.58%, 7.59%, and 6.71% variability, respectively. In another study by Singh *et al*. (2020), 5 PCs were reported with eigen value more than 1.0 contributing 73.84% of cumulative variability. Sheela *et al*. (2020) revealed 4 PCs with eigen value more than 1.0 and accounting 27.73, 19.12, 15.27 and 10.12 % of variability. Suman *et al*. (2021) revealed four PCs with eigen values more than or equal to one and together explaining around 68% of variability in a RIL population. Wang *et al*. (2021) analyzed multi-element content of rice and reported six PCs with eigen value more than one contributing cumulative variation of 87.878%. Our results are consistent with these previous findings.

Cluster analysis aims to group genotypes in form of clusters and the genotypes present within same cluster are more identical as compared to genotypes present in other clusters. The dendrogram was constructed using Minitab statistical software (version 20) based on squared eucledian distances to identify genetic relationship among 143 rice germplasm lines based on four traits including Fe, Zn, PH and EBT/m^2 (Fig. 3, Table S2). The complete linkage technique of hierarchical clustering on the basis of Squared

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Supplementary Table S2. Cluster composition for 143 rice genotypes

Cluster	Number of genotypes	Name of genotypes
I	76	RP 4920-Bio-51 (B),RP 5912-17-8-4-3-2-2,RP 5898-38-7-2-1-1,RP 5898-138-14-9-8-4-2,NP-9685,GontraBidhan,RP Bio 5477-NH 787-1,RP 5898-101-3-2-1-1,RP 5898-19-8-6-1-1-1,R-RHZ-SM-14,CR 2819-1-5-3-B-8-2B-1,CB 14740,RP 5115-111-24-3-1-1,RP 5898-22-4-3-2-1,RP 5977-MS-M-37-3-2-3-4-3,R-GM-AS43,R-RHZH-7,RP Bio 5477-NH 686,R-RHZ-2,RP 5886-HP3-IR 80463-B39-3,RP 5898-182-22-4-3-2-1,NLR 3238,RP 5917-HP-8-IR94064,RP 5889-117-46-9-3-3-2,PAU 2769-20-2-3-3-2-1-2-M-9,RP 5911-52-13-3-2-2-1,2K6-2012-4-2-2-1-2-1-1-1-1,RP 5892-32-9-5-4-3-2,RP 5910-69-11-4-2-2-2,IR58025B,CSR-58,RP 5177-435-8-5-3, NPQ-50,CR 2829-PLN 23,ARB-11,NPQ 49,RP Bio 4918-233-k, ARB 13,2K6-2012-9-1-2-2-1-1-1-1-1-1,ARB 12,R-RHZ-MI-32,RP 5898-101-9-3-1-1,RP 5163-102-3-5-2-2,R-RHZ-1B-13,RHZ-5M-14,IR 64,PR 103,PR 113,PR 114,PR 115,PR 116,PR 120,PR 122,PR 126,PR 127,PAU 201,Pusa 44,Pusa 1509,Zhang 123,Yu-Xiiangzan,Doddi,Om1723,OM 997,93072,TGMS 29,Govind,UPR 191-66,R644,Nan 29-2,Rasi,JP5,Amol 3 (Sona),IR 66897B,Hua-Gen-Xian 74,Ajaya,Khole marshi
II	21	RP 5898-182-22-4-3-2-1,CR 2829-PLN-32,Dadaji HMT,IR 82475-110-2-2-1-2,HPR 2795,RP 5948-36-21-11-5-2-1,HPR 2720,RP 5948-59-19-8-6-1-1,CR 2830-PLS-156,KaliKamod,ARB-10,RP 4993-183-40-5-2-1-1,RP Bio 4918-236-K,CR 2915-1-1-3-1-B-6-2B-1,R-RHZ-MI-30,R-RHZ-R 56,IR 72,Bas 370,Swarna,PSBRC 28,C71
III	46	RP 5898-54-21-9-4-2-2,CR 2829-PLN-100,R-RHZ-L1-23,PAU 2K10-23-54-14-52-20-0-4,CR 2829-PLN-37,KMP-200,CR 2829-PLN-36,CR 2829-PLN 109,OR 2545-5,MINGUI 63,2K6-2012-4-3-1-1-2-1-1-1-1,RP 5706-112-4-5-3-2,CR 2826-1-1-1-2B-1-2B-1,NPQ-23,RTN RR-4,R-RHZ-LI-22,RP 5958-IR 94052-117-13-4-11-1-1,CR 2007,RP 5948-26-11-3-1-1-1,PAU 6886-6346-1-1-B,CN-1987-4-2,Jaya,IR8,IR 50,PR 106,PR 108,PR 111,PR 118,PR 121,PR 123,PR 124,PR 128,PR 129,Punjab Mehak 1,PB-3,Pusa 1121,Bas 386,PB-2,PB 5,PB 1718,CSR 30,ASD 18,BG 94-1,Cisedani,IR58025B,Zhong 413

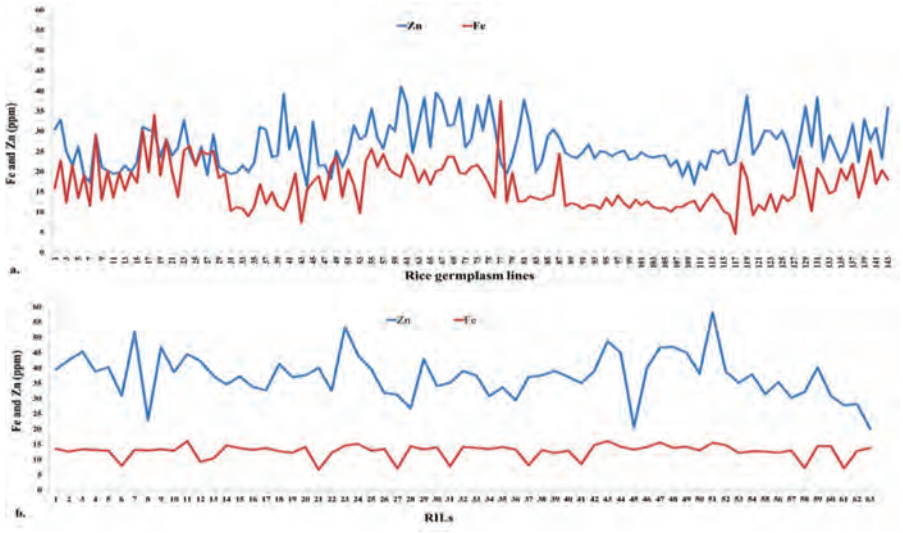


Fig. 1. Iron and Zinc contents in (a) 143 genotypes and (b) RIL population

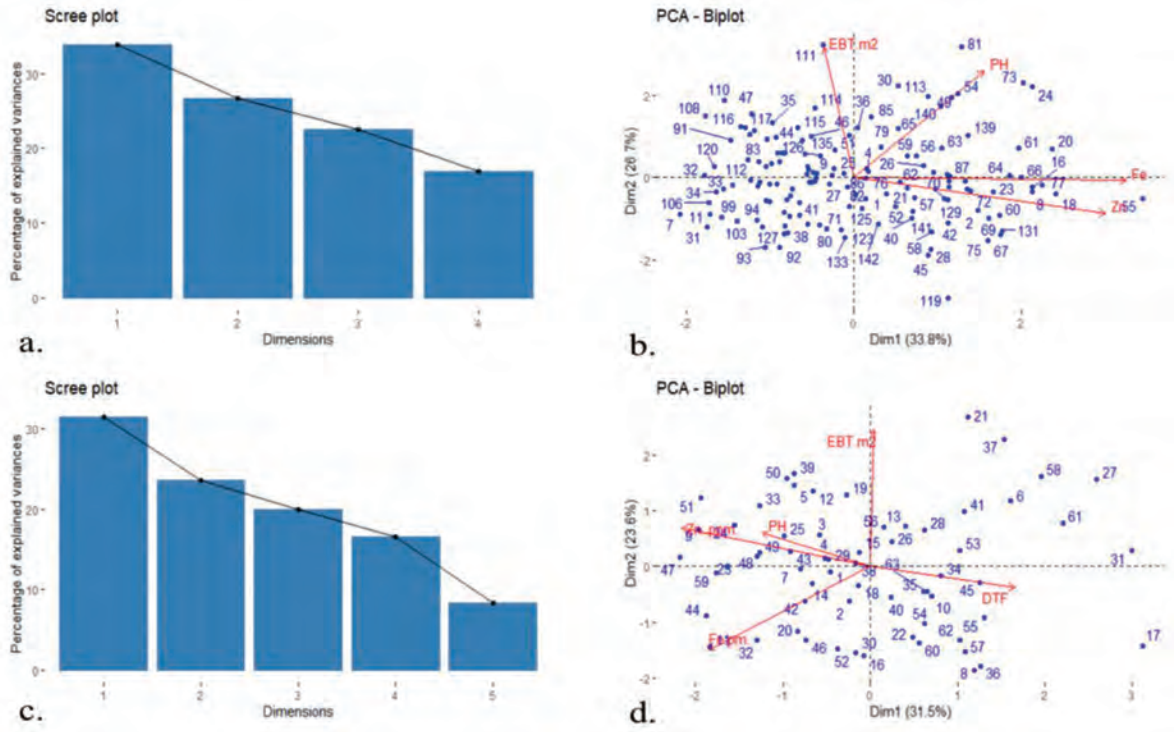


Fig. 2. Scree plot and PCA-biplot rice germplasm (a,b) and RIL population (c,d)

Euclidean Distance, grouped 143 rice genotypes into three clusters. Besides grouping of accessions into diverse clusters, inter cluster distance was also determined (Table S3). The minimum similarity and maximum genetic distance (0.0% and 54.4) were found between RP 4920-Bio-51(B) and RP 5898-54-21-9-4-2-2 followed by RP 4920-Bio-51 (B) and RP 5898-182-22-4-3-2-1 (18.6% and 44.3) and RP 5898-182-22-4-3-2-1 and HPR 2795 (40.8% and 32.2).

Among these, RP 4920-Bio-51 (B) has high Zn (30.5ppm) and Fe (15.8ppm) in brown rice as compared to RP 5898-54-21-9-4-2-2 (26.2 and 13.5 ppm, respectively). Similarly, RP 5898-182-22-4-3-2-1 had high Fe (22.8 ppm) and Zn (32.8 ppm) concentrations than that of RP 4920-Bio-51 (B). These are the most diverse combinations which can be used in Fe and Zn hybridization programs in rice. Therefore, superior recombinants could be found by crossing between genotypes across the clusters. Genotypes lied in same cluster signifies that these are similar and closely linked and had a lesser amount of genetic variation among them. Hybridization programs employing diverse

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Supplementary Table S3. Distances between cluster centroids

	Cluster1	Cluster2	Cluster3
Cluster1	0	1.94257	1.66077
Cluster2		0	2.41742
Cluster3			0

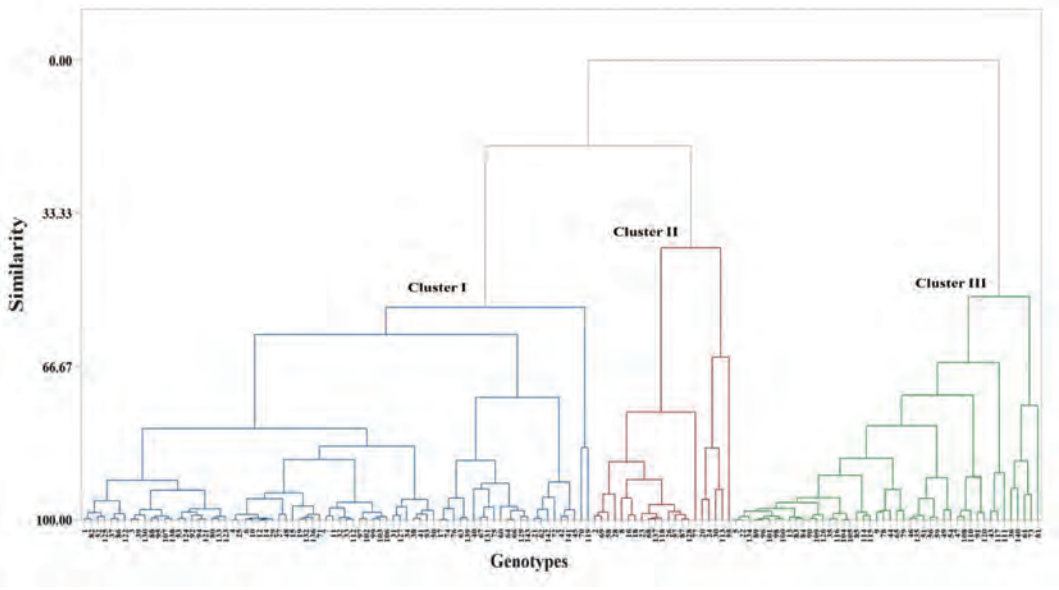


Fig. 3. Dendrogram showing genetic relationship of rice genotypes based on Fe, Zn, PH and EBT/m^2

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clusters will be beneficial for rice improvement.

Conclusively, Fe and Zn dense genotypes identified in present study could be used as donors in crosses with high yielding varieties of Punjab to enrich their grain with high concentrations of Fe and Zn. Further those derived lines can be employed for the identification of QTLs/ genomic regions responsible for Zn and Fe uptake, translocation and remobilization into grains. As Punjab produced over 11 million metric tons of rice in 2020 and is a main contributor of rice to the Indian economy (contributing 40 percent of rice), delivering nutritional benefits through biofortified rice varieties would be beneficial to human health worldwide. Additionally, the value of rice to producers and consumers would be significantly increased.

Authors' contribution

Conceptualization of research work (RK); Data collection (RK); Statistical analysis (NK, RK, GS); Analysis of data and interpretation (NK, RK, GS); Review of manuscript (RK); Preparation of manuscript (RK).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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