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Genetic variation for micronutrients and study of genetic diversity in diverse germplasm of rice

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ABSTRACT

An experiment was carried out to explore high iron (Fe) and zinc (Zn) donors, and to study the extent of genetic divergence based on such micronutrients and agro-morphological traits including seed yield in a set of 92 diverse germplasm lines of rice. Grain Fe (8.3-52.15ppm) and Zn content (3.0-52.7ppm) revealed wide variation among the germplasm. P44 mutant selection-1, ORCZ 75-3-1, Basudha, Malliphulajhuli, Tikimahsuri and Nikipankhia were rich in both Fe and Zn. P44 mutant selection-1 and ORCZ 75-3-1 had high yield potential while above local land races were poor yielding. The total set of genotypes were grouped into 12 distinct clusters. Jabaphulla, Parijat and Sakaribanki emerged as most divergent genotypes, but moderate low in Fe and Zn content. Interestingly, most of the local land races and the breeding lines clubbed into two separate distinct clusters respectively. The Fe and Zn dense genotypes identified above belong to the same distinct single cluster that showed high Fe and Zn content. Hence, such donors may serve as valuable material for Fe and Zn biofortification breeding.

Keywords: Diversity, genetic variation, germplasm grain Fe and Zn content, Fe and Zn dense donors, rice.

Minerals play vital role in plant and human metabolism. In plants, Zn acts as a cofactor in more than 300 enzymes and plays a major role in gene expression. It stabilizes the structures of cellular membranes and it is needed for normal growth and resistance to biotic and abiotic stresses. While, iron is a constituent of several enzymes and some pigments, and assists in DNA synthesis, nitrate and sulphate reduction, and energy production within the plant. Besides, it has role in respiration and maintains chloroplast structure and function. In animals, Zn deficiency leads to loss of immunity to diseases, stunted growth, impaired learning ability, wound healing and reproduction; and increased risk of infection, DNA damage and cancer. While, Fe acts as an important component of haemoglobin and myoglobin (Sperotto *et al.*, 2010) in our body system and its deficiency causes metabolic imbalance resulting severe anaemic problems, osteoporosis, maternal mortality, preterm births, reduced immunity and stunted growth.

Rice is served as staple food for more than half of the world population which meet at least 50% of the daily calories. But, rice grains usually harbour very minimum amount of Zn (12-15mg kg^{-1}) and Fe (5-6mg kg^{-1}) as compared to the target fixed (Zn: 28-30 ppm and Fe: 40 ppm) to meet the recommended daily allowance (RDA) of 10-12mg $Zn.day^{-1}$ and 10-15mg $Fe.day^{-1}$ (FAO/WHO 2000 and Welch and Graham 2004). Therefore, there is a need for Zn and Fe-biofortified rice in the food chain and it can be achieved by reorienting the traditional breeding strategy. Grain Zn and Fe content are complex traits with appreciably high G x E interaction which hinders progress in development of stable biofortified rice. However, there is wide variation in iron (6.0-72.0 ppm) (Neelamraju *et al*. 2012) and zinc content (14.0-40.0 ppm) (Martinez *et al*. 2006) in brown rice suggesting tremendous scope for enrichment of these micronutrients in rice grains. Iron and zn content of brown rice ranged from 7.4-22.7 ppm and 16.5-33.0 ppm in North East Land Races (NELR) of rice using ED-XRF (Rao *et al.* 2014). A quest for truly stable nutrient dense donors and divergent genetic resources can pave the way for biofortification breeding. Therefore, an attempt was undertaken to assess genetic variation and extent of genetic diversity for micronutrients (Zn and Fe) along with agro-morphological traits in a set of diverse germplasm of rice.

MATERIALS AND METHODS

The experimental material includes 92 test genotypes including 53 local land races, 21 improved breeding lines and 18 released varieties of rice. These test entries were laid out in Randomized Block Design (RBD) with three replications to assess yield and ancillary traits. Before planting, average soil pH was 5.8 and the average iron and zinc content of soil were 450 ppm and 0.52 ppm respectively. Observations were recorded on seven agro-morphological traits along with seed yield and nine quality traits including grain Fe and Zn content. Dial micrometer was used to determine length and breadth of 10 grains and the respective kernels of each genotype.

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L/B ratios for grain and kernel were calculated taking respective mean values. Rice genotypes were classified into seven grain types *e.g.*, Short slender(Score 1), Short bold (Score 2), Medium slender (Score 3), Medium bold (Score 3.5), Long bold (4), long slender (Score 5) and extra long slender (Score 6) as per Govindaswamy (1985) with minor modification.

After harvest of the crop, the rice grains were oven dried at 50^{o}C for two hours to reduce the moisture content to 11-12% and the dried rice grains were manually dehulled. Fine ground samples of such brown rice of each of the genotypes in three replicates were digested by di-acid mixture of nitric acid (HNO3): and perchloric acid (HClO4) in 3:2 ratio following the standard procedure of Jahan *et al.* (2013) with minor modification (*i.e.* 3:2 instead of 1:2 diacid ratio). Fe and Zn content were estimated in the aliquot of seed extract by using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) at 238.2nm and 206.2nm wavelength respectively (Fig. 1.) at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The variation in replications for each sample did not exceed ± 1ppm. The mean of the three replicates were worked out to indicate Fe and Zn-content of each genotype.

Routine statistical procedures were followed for analysis of variance as per Singh and Choudhury (1985). Besides, the *inter se* varietal genetic distances between genotypes were determined following SPSS software (Version 16) and dendrogram was constructed based on morpho-agronomic and quality traits to assess genotypic divergence among the test genotypes.

RESULTS AND DISCUSSION

Genetic variation for grain Fe and Zn content

Modern high yielding rice varieties are deficient in Fe and Zn. Some land races (Roy and Sharma 2014, Dikshit *et al.*, 2016), basmati types (Brar *et al.*, 2011) and wild rice (Banerjee *et al.*, 2010) retain high grain Fe and Zn content but japonica rice harbour the least (Anuradha *et al.*, 2012a). Similarly, rice wild relatives, upland landraces and aromatic accessions, deep water rice and coloured rice are the best sources of high grain Zn and Fe (Mallikarjuna Swamy *et al.*, 2016). The grain iron content in the present investigation, was shown to be higher than Zn content in all test genotypes. This is ascribed to the fact that the crop was grown in iron toxic soil (Fe: 450ppm). In such condition, higher concentration of iron (Fe^{+2}) in the rhizosphere is reported to have antagonistic effect on uptake of many nutrients including zinc (Fageria *et al.*, 2008). Majority of grain iron content is present in aleurone layer of brown rice, while endosperm retains higher amount of zinc. There are between 1 and 5 aleurone layers in different rice accessions (del Rosario *et al.*, 1968); therefore, the high Fe levels in unpolished grains can be due to thickness of the bran layers. Paddy (rough rice) contains 38ppm of iron that is reduced to 8.8ppm in brown rice after processing (hulling) and finally 4.1ppm in milled (polished) rice (Majumder *et al.*, 2019). Besides, the loss of iron and Zn content due to milling and polishing is reported to ranged from 16.0-97.4 and 1.0- 45.0% respectively (Maganti *et al.*, 2019). Recently, the breeding target is approximately fixed at 40ppm for iron and 30ppm for zinc biofortification. In the present study, grain Fe and Zn content ranged from 8.3-52.15ppm and .3.0-52.7ppm respectively in brown rice. Liang *et al.* (2007) revealed variation in Fe content (9.45 to 25.2ppm) and Zn content (13.0 to 39.0ppm) in rice grain of 56 Chinese rice varieties. Considerable variation for grain Fe(6.9 to 22.3ppm) and Zn(14.5 to 35.3ppm) also exist in brown rice among local land races (Maganti *et al.*, 2019). Besides, Patil *et al.* (2015) reported highest variation of grain yield per plant followed by grain iron (Fe) content and number of productive tillers plant^{-1} but, moderate genetic variation in grain Zn content under aerobic condition. In the present study, most of the local land races showed rich source of above minerals as also reported by Anandan *et al.* (2011). The top Fe dense (≥40ppm) genotypes identified were Tikimahsuri (52.15ppm), Jabaphulla (52.15ppm), Kala Kusuna (52.1ppm), OR CZ 75-3-1(51.95ppm), P 44 mutant selection-1 (51.9 ppm), CR 2327-23(51.4ppm), Budhidhan (51.15 ppm), Kalamakhi (50.15ppm), Nikipankhia (47.2 ppm), ORM 405-8 (45.05ppm), Jadumani (42.75 ppm), Basudha (41.45ppm), Malliphulajhuli (41.35ppm) and Tulasibasa (40.35ppm) (Table 1). Interestingly, P44 mutant selection-1, ORCZ 75-3-1, Basudha, Malliphulajhuli, Tikimahsuri and Nikipankhia also revealed higher grain Zn content(>40.0ppm) in addition to iron. P44 mutant Sel.-1 and ORCZ 75-3-1 were derived from cv. P44 (popular in Haryana) and Pusa Basmati-1 (popular aromatic rice) respectively following mutagenesis with EMS at 0.5%. These had good yield potential (44q ha^{-1}) with better adaptability over diverse environments. This corroborates the findings of Jeng *et al.* (2012). They recovered two high yielding Fe-dense mutants “M-IR-75” and “M-IR- 58” from cv.IR64 which accumulated more Fe (28.10 and 27.26ppm, respectively) than the parent IR 64 (3.90ppm). A semi-dwarf high yielding IRRI rice variety IR 68144 derived from a cross IR 8/TN 1(Virmani and Ilyas-Ahmed, 2008) revealed 21 µg g^{-1} (21ppm) of iron concentration in brown rice and retains about 80% of its iron content even after polishing compared to other varieties (Sperotto *et al.*, 2012). The

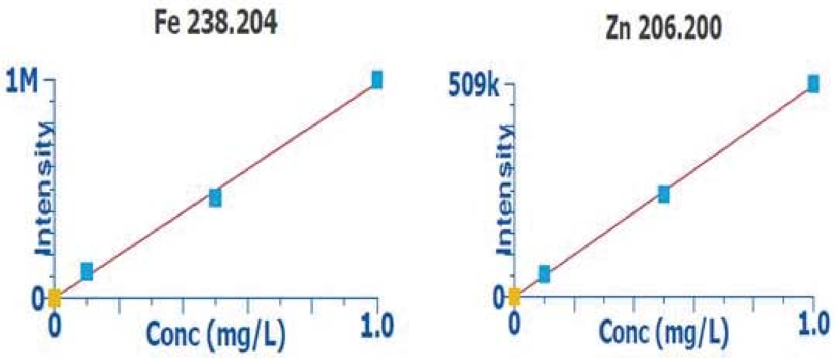

Fig. 1. Calibration of standard curve for grain Fe and Zn content using ICP-OES.

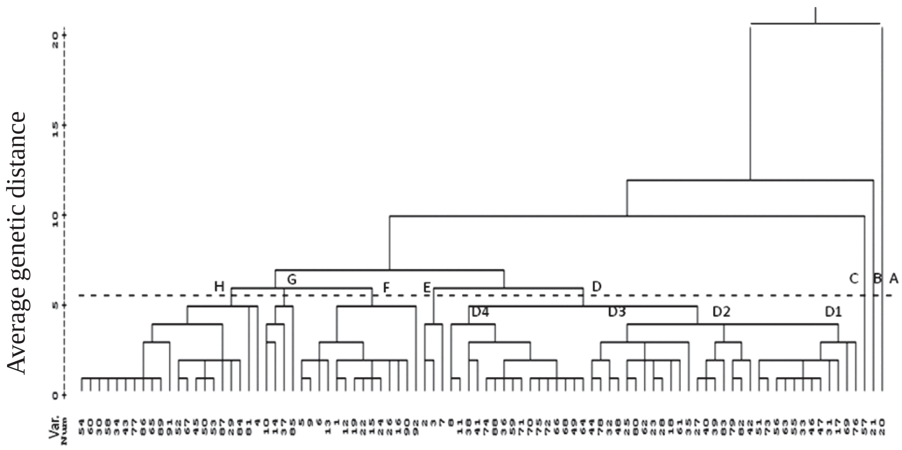

Fig. 2: Dendrogram showing hierarchical genetic relationship of test genotypes based on morpho-economic and quality traits.

erstwhile mentioned Zn and Fe dense donors *e.g*., Basudha, Malliphulajhulli and Tikimahsuri are lowland land races which recorded very low seed yield (20.2-27.9q ha^{-1}) except Nikipankhia which had shown moderate yield potential (34.0qtl ha^{-1}). The black pericarp rice genotypes are reported to harbour relatively higher iron content (15.4 to 162.4ppm) in rice grain (Zhang *et al*., 2000). Anuradha *et al*. (2012b) observed wide variability for Fe (0.2-224ppm) and Zn (0.4-104.0ppm) concentrations in unpolished rice of 168 RIL populations as compared Madhukar (Fe:17.3ppm, Zn:53.7ppm) and Swarna (Fe:22.5ppm, Zn:27.2ppm) used as parents. Several other researchers have also reported high grain Fe content in a few aromatic rice (Taraori Basmati and Palman 579: >180 ìg/g) (Brar *et al.*, 2011), rice hybrids (DRRH-29: 125.8ppm and Sahyadri-4:104.8ppm) (Ravindra Babu *et al*., 2013) and land race (cv. Swetonunia: 34.8 ìg.g^{-1}) (Roy and Sharma, 2014). Besides, a IRRI breeding line, IR68144-4B-2-2-3 is reported to have 80% more iron than IR64 (Gregorio *et al.*, 2000).

Wild species of rice such as *O. nivara*, *O. rufipogon*, *O. latifolia*, *O. officinalis*, and *O. granulata* retain high amounts of Zn, around 2-3 fold higher than in the cultivated rice. Besides, grain Zn content has also shown to be high in aromatic rice (Gregorio 2002) and local upland rice as high as 31ppm in 'Nam Roo' (Jaksomsak *et al.*, 2015). In this context, the rice genotypes identified in the present investigation, being rich in grain iron and zinc content, may serve as potential donors for biofortification breeding programme.

Genetic divergence

Genetic improvement mainly depends upon the amount of genetic variability present in the population. Therefore, assessment of genetic diversity in a set of breeding materials is a pre-requisite to distinguish the genotypes into genetically close and divergent types. The genotypes which are genetically distant enough are expected to generate wide range of genetic variation in recombination breeding and pave the way for greater scope for recovery of transgressive segregants (Zaman *et al.*, 2005 and Saxesena *et al.*, 2013). Therefore, an attempt has been made to assess the extent of genetic divergence in the present set rice genotypes.

Table 1: Grain yield and micronutrient content (of brown rice) of a set of 92 diverse germplasm.

Sl. No.	Genotype	Grain Zn (ppm)	Grain Fe (ppm)	Grain yield (q ha^{-1})	Sl. No.	Genotype	Grain Zn (ppm)	Grain Fe (ppm)	Grain yield (q ha^{-1})
1	Tikimahsuri	41.5+0.85[a]	52.1+1.00[b]	27.1	48	Kalkatti	11.1+0.43	13.4+0.46	18.6
2	Jayaphulla	18.0+0.95	52.1+0.91	15.9	49	Godikaveri	17.3+0.56	13.2+0.35	19.2
3	Kala Kusuna	10.1+0.89	52.1+1.00	10.8	50	Gajapati	20.8+0.67	13.2+0.50	42.8
4	OR CZ 75-3-1	45.2+1.00	51.9+0.80	43.8	51	Boudachampa	11.9+0.66	13.1+0.38	20.6
5	P 44 Sel.	52.7+1.00	51.9+0.90	44.2	52	Hiranmayee	20.8+0.83	12.8+0.48	48.2
6	CR 2327-23	31.7+1.00	51.4+0.95	41.4	53	OR CZ 83	13.1+0.50	12.6+0.40	44.4
7	Budhidhan	24.1+0.93	51.1+1.00	9.5	54	OR CZ 76-3	14.8+0.35	12.6+0.34	43.4
8	Kala makhi	23.8+0.63	50.1+0.78	12.8	55	Kantakarpur	13.0+0.56	12.4+0.38	17.0
9	Nikipankhia	42.8+0.88	47.2+0.88	34.2	56	Bhattadhana	14.0+0.60	12.3+0.36	17.0
10	ORM 405-8	30.1+0.89	45.0+0.79	40.5	57	ORCZ 80-1	34.8+0.60	9.0+0.28	40.0
11	Jadumani	32.1+0.52	42.7+0.80	18.6	58	OR CZ 76-5	10.6+0.28	12.1+0.40	42.0
12	Basudha	44.4+1.00	41.4+0.68	27.9	59	Raja hansa	9.5+0.40	12.1+0.39	18.0
13	Malliphulajhuli	43.8+1.00	41.3+0.90	20.2	60	OR CZ 76-15	16.0+0.39	12.0+0.43	45.4
14	Tulasibasa	15.1+0.86	40.3+0.69	25.6	61	Labangalata	30.6+0.60	11.8+0.30	30.8
15	Manika	42.7+1.00	39.4+0.71	38.3	62	Local Basumati	13.9+0.40	11.7+0.40	16.6
16	Swarna Sub-1	28.7+1.00	37.7+0.68	40.0	63	Kadalikandi	11.9+0.38	11.7+0.34	18.8
17	Jhaliamanju	24.7+0.90	36.4+0.66	16.5	64	Kalialendi	12.7+0.48	11.7+0.40	20.0
18	LalJagannath	18.2+0.82	33.1+0.78	29.4	65	OR CZ 76-16	14.5+0.34	11.6+0.29	42.4
19	Prachi	30.3+0.34	29.3+0.88	41.0	66	Majhalijhuli	13.4+0.43	11.6+0.40	18.6
20	Jaba phulla	19.0+0.58	28.1+0.82	15.8	67	OR CZ 48	20.0+0.68	11.3+0.38	46.6
21	Parijat	23.6+0.80	27.4+0.78	25.8	68	Sapurichudi	17.4+0.51	10.9+0.36	19.6
22	Mrunalini	32.0+0.78	27.3+0.69	45.2	69	Dinkisiali	28.0+0.72	10.8+0.34	16.4
23	Kalamugajai	23.7+0.94	25.8+0.66	22.5	70	Bitisapari	11.4+0.34	10.7+0.30	21.6
24	Ranjit	43.7+0.72	24.3+0.80	43.2	71	Puagi	17.2+0.40	10.5+0.39	21.6
25	Bhalusadi	21.2+0.24	23.6+0.82	24.0	72	Jhulpa	13.4+0.38	10.3+0.40	21.6
26	Upahaar	27.5+0.50	22.7+0.66	39.1	73	Turikanhei	12.1+0.28	10.3+0.34	21.6
27	Bhuvan	23.6+0.34	20.0+0.67	34.0	74	Mugudi	13.9+0.37	10.1+0.26	21.6
28	Padmavati	31.3+0.53	19.7+0.78	20.0	75	Karpuramati	17.3+0.60	10.1+0.29	21.6
29	OR(T)-31	19.8+0.56	19.4+0.45	44.0	76	Kalama	13.7+0.50	10.0+0.30	5.7
30	OR CZ 76-11	18.5+0.70	18.7+0.53	41.2	77	OR CZ 76-17	10.9+0.31	9.7+0.20	42.4
31	Jaygopal	14.5+0.86	18.4+0.60	22.0	78	Geleikathi	9.8+0.26	9.7+0.30	20.8
32	Raghuse	13.2+0.80	18.0+0.59	18.0	79	Budhamanda	23.2+0.56	9.5+0.39	30.6
33	Kathidhan	12.4+0.50	17.6+0.70	20.0	80	Thakurabhoga	12.2+0.30	9.5+0.32	21.6
34	OR CZ 76-4	14.8+0.48	17.3+0.56	40.0	81	Khandagiri -1	13.7+0.40	9.4+0.40	40.5
35	Basapatna	13.9+0.69	16.6+0.60	18.8	82	Kadalipenda	22.7+0.58	9.4+0.41	27.4
36	Chinamali	12.2+0.63	16.5+0.58	16.8	83	Jagannath	22.8+0.67	9.4+0.45	36.8
37	OR(T) 47	15.6+0.45	15.9+0.62	40.5	84	Tanmayee	21.7+0.56	9.3+0.29	39.0
38	Jhilli	11.6+0.70	15.7+0.80	26.8	85	Sakaribanki	29.9+0.70	12.2+0.36	17.0
39	Birupa	15.6+0.33	15.4+0.78	35.0	86	OR CZ 76-6	10.8+0.35	8.9+0.33	43.4
40	Bhanja	24.7+0.39	15.1+0.71	38.0	87	Pratikhya	14.3+0.45	8.9+0.40	53.3
41	Kharavela	21.5+0.49	15.0+0.66	32.5	88	Sambalpuri	3.3+0.09	8.8+0.42	20.6
42	Nilarpati	29.5+0.77	14.7+0.50	29.0	89	OR CZ 76-1	10.1+0.40	8.8+0.54	40.4
43	OR CZ 76-2	14.7+0.88	14.1+0.65	45.4	90	Dimapur	29.3+0.87	8.5+0.34	31.5
44	Buromal	11.2+0.42	13.8+0.70	22.0	91	OR CZ 76-13	8.7+0.30	8.3+0.31	40.4
45	ORCZ 84	14.9+0.49	13.7+0.68	42.4	92	Swarna(Check)	11.0+0.33	15.4+0.43	53.0
46	Raghusai	20.5+0.71	13.6+0.57	18.5		**Mean**	**20.4**	**20.2**	**29.5**
47	Ispit	5.0+0.25	13.4+0.48	16.2		**LSD(0.05)**	**4.2**	**8.2**	**12.8**

N.B.: [a] and [b] indicate mean estimates + SE of zinc and iron content of brown rice.

Table 2: Cluster composition of different clusters for 92 rice genotypes.

Cluster No.	No. of genotypes	Name of the genotypes
I	9	Tikimahsuri (1), OR CZ 75-3-1 (4), P 44 mutant Sel 1(5), CR 2327-23 (6), Nikipankhia (9), Basudha (12). Malliphulajhuli (13), Manika (15), Ranjit (24)
II	6	Jabaphulla (2), Kala kusuna (3), Budhidhan (7), Kala makhi (8), Jadumani (11), Jhaliamanju (17)
III	9	LalJagannath (18), Bhuvan (27), Jhilli (38), Birupa (39), Kharavela (41), Nilarpati (42), Labangalata (61), Kadalipenda (82), Tanmayee (84)
IV	2	OR(T) 47 (37), Khandagiri -1 (81)
V	1	ORCZ 80-1 (57)
VI	1	Parijat (21)
VII	1	Jaba phulla (20)
VIII	5	Padmavati (28), Jaygopal (31), Sakaribanki (85), Dinkisiali (69). Kalama (76)
IX	9	Swarna Sub-1 (16), Prachi (19), Mrunalini (22), Upahaar (26), OR(T)-31 (29), Bhanja (40), Jagannath (83), Dimapur (90), Swarna (Check) (92)
X	2	ORM 405-8 (10), Tulasibasa (14)
XI	30	Kalamugajai (23), Bhalusadi (25), Raghuse (32), Kathidhan (33), Basapatna (35), Chinamali (36), Buromal (44), Raghusai (46) , Ispit (47), Kalkatti (48), Godikaveri (49), Boudachampa (51), Kantakarpur (55), Bhattadhana (56), Raja hansa (59), Local Basumati (62), Kadalikandi (63), Kalialendi (64), Majhalijhuli (66), Sapurichudi (68), Bitisapari (70), Puagi (71), Jhulpa (72), Turikanhei (73), Mugudi (74), Karpuramati (75), Geleikathi (78), Budhamanda (79), Thakurabhoga (80), Sambalpuri (88)
XII	17	OR CZ 76-11 (30), OR CZ 76-4 (34), OR CZ 76-2 (43), ORCZ 84 (45), Gajapati (50), Hiranmayee (52), OR CZ 83 (53), OR CZ 76-3 (54), OR CZ 76-5 (58). OR CZ 76-15 (60). OR CZ 76-16 (65), OR CZ 48 (67), OR CZ 76-17 (77), OR CZ 76-6 (86), Pratikhya (87), OR CZ 76-1 (89), OR CZ 76-13 (91)

N.B.- Genotype serial number indicated in parenthesis.

Table 3: Inter-cluster distances among different clusters for 92 rice genotypes.

Clusters	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V	Cluster-VI	Cluster-VII	Cluster-VIII	Cluster-IX	Cluster-X	Cluster-XI
Cluster-II	58.5										
Cluster-III	39.8	49.5									
Cluster-IV	54.9	66.6	31.5								
Cluster-V	63.8	78.3	49.2	50.7							
Cluster-VI	78.1	60.7	68.2	82.6	113.0						
Cluster-VII	1261	1262	1259	1259	1253	1261					
Cluster-VIII	71.2	35.0	45.6	65.4	74.8	56.4	1260.8				
Cluster-IX	32.6	66.1	26.4	47.0	60.7	74.5	1260.7	65.7			
Cluster-X	44.9	46.8	39.6	43.4	43.4	91.4	1261.5	61.6	53.9		
Cluster-XI	62.7	37.0	30.1	48.8	61.6	63.1	1260.3	20.7	53.2	48.8	
Cluster-XII	45.5	72.4	26.2	28.3	50.4	85.4	1259.5	70.6	25.3	48.9	53.3

Table 4: Cluster means for different characters in a set of 92 rice genotypes.

Clusters	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V	Cluster-VI	Cluster-VII	Cluster-VIII	Cluster-IX	Cluster-X	Cluster-XI	Cluster-XII
Days to maturity	133.9	129.1	132.5	112.5	153.0	98.0	125.0	131.6	140.4	137.2	133.0	132.2
Plant height	99.23	124.5	111.2	125.2	146.0	82.0	135.8	123.3	90.5	138.6	123.6	105.8
Panicle length	23.20	23.65	22.77	24.45	27.60	20.0	27.60	21.28	22.49	26.20	21.77	23.76
No. of EBT hill^{-1}	5.80	3.95	5.83	5.85	6.40	8.50	4.00	5.10	6.92	5.55	3.50	6.28
Grains panicle^{-1}	131.0	88.6	119.5	135.2	140.0	70.40	90.00	78.56	128.6	127.6	96.19	140.3
1000-GW	21.1	19.35	22.56	23.20	30.00	21.60	12.8	20.40	22.09	20.35	20.88	23.26
Grain length	7.59	7.38	7.49	8.75	7.40	8.00	6.00	7.74	7.28	7.50	7.41	9.46
Grain breadth	2.42	2.32	2.69	2.55	2.90	2.80	2.80	2.40	2.58	2.55	2.61	2.26
Grain length/breadth	3.19	3.23	2.82	3.55	2.60	2.85	2.14	2.62	2.85	2.94	2.94	4.26
Grain type score	4.78	4.50	4.22	5.00	4.00	4.00	3.50	3.90	4.06	4.50	4.32	5.47
Kernel length	6.26	6.07	5.79	6.55	6.40	7.00	5.20	5.86	6.02	6.15	5.85	6.84
Kernel breadth	1.98	2.23	2.40	2.10	2.40	2.60	2.00	2.46	2.16	2.20	2.31	1.91
Kernel length/breadth	3.18	2.78	2.48	3.20	2.70	2.69	2.60	2.38	2.86	2.81	2.61	3.64
Zn-content	43.17	22.13	21.67	14.65	34.80	23.60	19.00	23.48	25.12	22.60	13.77	14.61
Fe-content	44.57	47.45	16.07	12.68	9.00	27.45	28.15	14.23	20.54	42.70	13.11	12.18
Seed yield.plant^{-1}	35.59	14.02	31.54	40.50	40.00	25.80	15.80	16.22	40.96	33.05	20.13	43.77

Non -hierarchical clustering pattern

Grouping of test genotypes into different clusters was made based on Euclidian genetic distance between all possible pairs of genotypes. In the present investigation, the total 92 test genotypes including standard checks (Swarna) were grouped into twelve non-heirarchical distinct genetic clusters (Table 2). Among these, Cluster-XI was the largest cluster which accommodated 30 genotypes followed by Cluster-XII (17 genotypes) and Cluster-I, III and IX (9 genotypes each) indicating genetic proximity of the test genotypes grouped in these clusters. Cluster IX included the mega variety "Swarna" and other high yielding genotypes e.g., Swarna Sub-1, Prachi, Mrunalini, Upahaar and OR(T)-31.

Cluster V, cluster VI and cluster VII were mono-genotypic which included Sakaribanki, Parijat and Jabaphulla while cluster IV and cluster X each contained two genotypes each such as OR(T)-47 and Khandagiri-1; ORM 405-8 and Tulasibasa respectively. The rest of the genotypes were distributed into cluster II and cluster VIII which included 6 and 5 genotypes respectively. It is interesting to note that most of the OUAT breeding lines have been clubbed into cluster XII, while a group of 30 landraces constitute the largest genotypic group (Cluster XI). This indicates that similar selection pressure might have been imposed while development of the OUAT breeding lines. Similarly grouping of as many as 30 local landraces into a single genotypic group may be ascertained to selection of local genotypes during the process of domestication in their area of native geographic location.

Inter cluster distance

Inter cluster distance among twelve genetic groups (Table 3) ranged from around 30.15(between Cluster III & Cluster XI) to as high as 1261.87 (between Cluster II & Cluster VII). Grouping of genotypes into different clusters is due to genetic variation that exists among the test genotypes. Genotypes having higher extent of inter se homology form the basis of grouping into different clusters. Genotypes with specific features not present in other genotypes would compel it to be separated into different genotypic group. Such a situation was revealed in the present investigation forming 3 mono-genotypic groups such as cluster V, Cluster VI and Cluster VII. In the present investigation, cluster VII emerged as the highest divergent genotypic group followed by cluster VI and cluster V as revealed from average genetic distance. In contrast rest of the clusters maintained almost equidistance between cluster pairs. Cluster VII merged as the single most divergent genotypic group with far genetic distance from rest of the genotypes and thus, it would have breeding implication. Genetic diversity studies for eight mineral concentrations of brown rice, using 653 accessions showed that there is greater average genetic diversity index for japonica accessions compared to indica accessions (Zeng *et al*., 2005).

The dendrogram constructed using SPSS software (version 16) showed clear picture of the hierarchical genetic relationship among 92 test genotypes based on seven morpho-economic traits including seed yield, seven physical quality traits and micronutrient (Fe and Zn) content in grain. The genotypes were distributed into seven broad clusters *e.g*., Cluster A, Cluster B, Cluster C, Cluster D, Cluster-E, Cluster F, Cluster G and Cluster H at average genetic distance approximately 5.8 (Fig. 2). Cluster-A , Cluster B and Cluster C were mono-genotypic and these were first separated as highly divergent from rest of the genotypes at average genetic distance 11.5 as also supported by earlier finding of Singh *et al.* (2018). Cluster A included the land race Jabaphulla, Cluster B contained Parijat while Cluster C is represents Sakaribanki. These clusters corresponds to the monogenic Cluster VII, Cluster VI and Cluster V. Rest of the genotypes were distributed sequentially and formed Cluster B, Cluster C, Cluster D, Cluster-E, Cluster F, Cluster G at average genetic distance 5.8. Cluster D was the largest genotypic group which contained most of the genotypes which were included in Cluster 12 of non-hierarchical clustering. Such group of genotypes formed four subgroups *e.g*, Cluster D1, Cluster D2, Cluster D3 and Cluster D4. Hierarchical clustering did not reveal much genetic difference among clusters beyond Cluster C as rest all clusters virtually configured at almost similar and lower average genetic distance. This was also evident from almost similar average inter cluster distances (151.4 to 166.2) of all cluster except Cluster V, Cluster VI and Cluster VII. In case of non-hierarchical clustering. Thus, hierarchical clustering (dendrogram) revealed almost similar clustering pattern to that of non- hierarchical clustering.

Characteristic features of clusters

In a set of test genotypes, some may have common features and therefore are clubbed into single cluster. Hence, common feature is the basis for clustering. Each of the cluster reflects specific morpho-economic and/ or quality features. In the present investigation, Cluster V (ORT 47 and Khandagiri 1) exhibited moderately tall plant stature with late maturity (Table 4). In contrast, Cluster VI (Parijat) revealed characteristic dwarf plant type with early maturity and the Cluster IX exhibited dwarf plant type wit late maturity. Tillers m^2 was maximum(8.50 per ill) in case of Cluster VI indicating profuse tillering ability, while genotypes included under Cluster II and Cluster XI exhibited bit shy tillering habit. Among the twelve genotypic groups, Cluster V and Cluster VII had shown highest panicle length (27.0cm) followed by Cluster X. Grain number panicle^{-1}, grain weight and fertility percentage are usually considered as major determinant of seed yield. Cluster V and Cluster XII revealed maximum fertile grain number panicle^{-1} (140) for which such clusters recorded higher mean seed yield (≥ 40g plant^{-1}). Similarly, Cluster IV with moderately higher number of grains panicle^{-1}, recorded high seed yield. Grain weight varied widely ranging from 12.8g in Cluster VII to as high as 30g in case of the mono genotypic group containing ORCZ 80-1.

Cluster XII exhibited long slender grain and grain length/breadth ratio, moderately long kernel type (Table 4). Such characteristic features associated with high yield potential (mentioned above) of this genotypic group may be the most preferable choice of the farmers as well as consumers. However, the said genetic cluster revealed low Fe and Zn content. Patil *et al*. (2019) revealed high genetic variation for grain yield plant^{-1} followed by grain iron (Fe) content and number of productive tillers plant^{-1} but, moderate genetic variation was shown by grain zinc (Zn) content. Rathod *et al*. (2017) studied genetic diversity of fifty six high iron and zinc genotypes of rice and revealed distinct genotypic groups for high micronutrient contents. In this context, Cluster I revealed high Fe and Zn content in grain (≥ 40ppm). Besides, Cluster II and Cluster X also recorded grain Fe content more than 40ppm along with moderate grain Zn content (22ppm). Such genotypic groups included erstwhile mentioned important Fe rich-genotypes *i.e*., Jabaphhulla, Kala Kusuma Budhidhan, Kalamaki, Jadumani, Jaliamanju, ORM 405-8 and Tulasibasa. These may serve as valuable materials for biofortification breeding.

Iron and zinc are important essential micronutrients required for normal metabolic function of animals and plants. Mineral deficiency of Fe and Zn is a world-wide problem affecting more than 40% of the human population. Since, rice is the staple food for more than half of the world population; it is being targeted for biofortification. Assessment of genetic variation and genetic diversity can detect heritable elite variants especially for complex traits like grain Fe and Zn content. In the present study, an exhaustive characterization of available germplasm revealed a wide array of variation in grain Fe and Zn content. Clustering pattern revealed grouping of the Fe and Zn rich genotypes together to form a distinct cluster. Elite Fe and Zn dense genotypes identified from such cluster can serve as donors for^{-1} biofortification breeding programme.

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