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Genetic diversity analysis of rice (*Oryza sativa* L.) genotypes with high grain zinc content for yield and yield traits

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Abstract

Assessment of genetic diversity was made based on the data recorded for sixteen yield and yield contributing characters in twenty one rice genotypes using Mahalanobis's D^2 statistics. By use of Tocher method twenty one genotypes were grouped into six clusters. Out of which Cluster I had eleven genotypes followed by Cluster II with five genotypes, Cluster VI with two genotypes and single genotype was included in cluster V, cluster III and Cluster IV respectively. The maximum intra-cluster distance was found in cluster VI (82.07) and the minimum was recorded for cluster I (35.37), while it was zero value for cluster III, IV and V as they had only single genotype each. The minimum inter-cluster D^2 values was recorded between cluster III and V (83.05) while the maximum D^2 value was found between cluster I and VI (485.23) followed by cluster IV and VI (379.12), cluster V and VI (349.78) and cluster III and cluster VI (338.24). The cluster I recorded high mean values significant for grain zinc content and 1000 grain weight whereas cluster II for days to first flowering, days to 50% flowering, days to maturity and spikelet per panicle. The cluster III recorded high mean values significant for spikelet per panicle, no of grains per panicles, spikelet fertility percent and grain weight per panicle. While cluster IV recorded high values significant for plant height, panicle length, spikelets per panicle, number of grain per panicle and grain weight per panicle. Whereas cluster V recorded high mean values significant for 1000 grain weight, grain weight per panicles, plant height and L/B ratio. The cluster VI had high mean value significant for days to first flowering, days to 50% flowering, days to maturity, no of filled grains per panicle, spikelet fertility percent, grain yield per plant, grain yield per plot, yield per ha., the crosses between BRRIdhan 62 x Swarna, BRRIdhan 62 x Sambamahsuri (cluster I x cluster VI) and BRRIdhan 64 x Swarna, BRRIdhan 64 x Sambamahsuri (cluster IV x cluster VI), IR 97443-11-2-1-1-1-1 –B x Swarna, IR 97443-11-2-1-1-1-1 –B x Sambamahsuri (cluster III x cluster VI), could be suggested for the exploitation of transgressive segregants for both yield and zinc biofortification.

Keywords: genetic divergence, D^2 statistic, inra-cluster distance, inter-cluster distance transgressive segregants and biofortification

Introduction

Rice (*Oryza sativa* L.) is a self-pollinated short day monocotyledonous angiosperm has a place with the genus *Oryza* of family Poaceae. Rice is mainly grown in hot humid tropical climate and its distribution varies from humid tropical to subtropical climate. South-East Asia is the probable primary centre of origin of *Oryza sativa* (Huang *et al.*, 2012). It is the staple nourishment for 33% of the total population and involves very nearly one-fifth of the aggregate land territory occupied under cereals (Ren *et al.*, 2006) [8]. Rice is produced in 114 countries across the globe estimating production of 753mt (499mt milled rice, 2016) and forecasting 758mt (503.6mt milled rice, 2017) with world rice acerage of 161.1 mha (FAO, 2017). But 90 percent of world's rice is grown in Asia i.e 686.1mt (455.3mt milled rice i.e 0.7% more from the 2016 production) (FAO, 2017). Rice production, consumption and trade are mostly confined in Asia. One third of Asia's rice production is consumed in China and one fifth in India. Among the rice growing countries in the world, India occupied the largest area under rice crop (about 45 million ha.) having the second position in production next to China, (IRRI 2016, standard evaluation system for rice.). Essentially, the Indian rice generation is relied upon to be around 109 mt during the year 2016-17 which is the most astounding ever creation of rice till date (AICRIP yearly gathering report 2017). India having rice production of 165.5mt (110.4mt milled rice 2017) i.e 1% increment from the previous production of 163.7mt (109.2mt milled rice, 2016) (FAO, 2017). The micronutrient insufficiencies, in individuals between 25 and 50 years of age require 10 to 15 mg Fe and 12 to 15 mg of zinc (Welch and Graham, 2004) [16] leads to most important health hazards in children like diarrhoea, pneumonia, stunting, child mortality, prompts anorexia,

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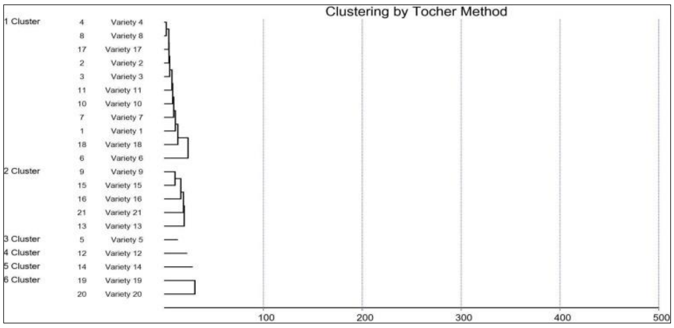
sadness and psychosis, weakened development and improvement, modified conceptive science, gastrointestinal issues and impaired immunity (Solomons 2003) [12]. To overcome this situation we should go for biofortification. Biofortification is a genetic approach which aims at biological and genetic enrichment of food stuffs with vital nutrients like vitamins, minerals and proteins (Bouis, 2002). For biofortification purpose all the experimental rice genotypes having high grain zinc content in mg/kg were taken. The accumulation and assessment of viable germplasm lines are essential goal for any breeding program. Genetic diversity plays a key role in plant breeding since progeny obtaining from divergent parents exhibit greater heterosis and provide wide spectrum of variability in segregating generations. Diversity not only bring about genetic variation but also brings new recombination of genes in gene pool. Hybridization among different genotypes and handling of their segregants is the most common and effective means of generating variability. Information on the genetic distance among parents plays a vital role in hybridization programme.

Material and Methods

This experiment was conducted to study the genetic divergence for yield and yield traits among twenty-one diverse rice genotypes with high grain zinc content over five environmentsduring Kharif 2017.Net Plot size was 2.4 m×2.4m for each location under study. Inter and intra row spacing was 20cm and 15cm, respectively in each location. In each plot twelve rows were grown. They were grown in a randomized block design with three replications and observations were recorded on randomly selected five plants for the traits; days to first flowering, days to 50% flowering, days to maturity, number of effective tillers per plant, plant height (cm), panicle length (cm), number of spikelets per panicle, number of filled grains per panicle, spikelets fertility percent, grain weight per panicle (g), grain yield per plant (g), 1000-grain weight (g), Grain yield per plot (kg), Grain yield per ha (kg), L/B ratio, and grain zinc content(mg/kg) were considered. Zinc content of rice grains was estimated in the aliquot of seed extract by using Atomic Absorption Spectrophotometer (AAS) at 213.86 nm for Zinc. The data thus generated from the twenty-one genotypes were subjected to D^2 statistics and Tocher method and statistical analysis with the help of indostat service for the objectives under study.

Results and Discussions

The analysis of variance for dispersion of 21 genotypes is presented in Table 2. The significance of genotypes clearly indicated the significant pooled effect of all the characters between different genotypes. By analyzing Mahalanobis's D^2 statistics and Tocher method, the twenty-one genotypes were grouped into six clusters. The distribution of genotypes into six clusters were presented in Table 3 and illustrated in Fig 1. Cluster I had eleven genotypes. Cluster II had five genotypes. Cluster VI had two genotypes. Single genotype was included in cluster V, cluster III and Cluster IV.As earlier similar results were obtained by Singh and Choudhary (1977), Vivekanandan and Subramanian (1993) [15] and Tuwar *et al*. (2014). The genetic diversity among the genotypes may be due to factors like history of selection, heterogeneity, selection under diverse environments and genetic drift. Therefore, for hybridization, the selection of parents should be based on genetic diversity besides *per se* performance and realistic knowledge of the breeder.



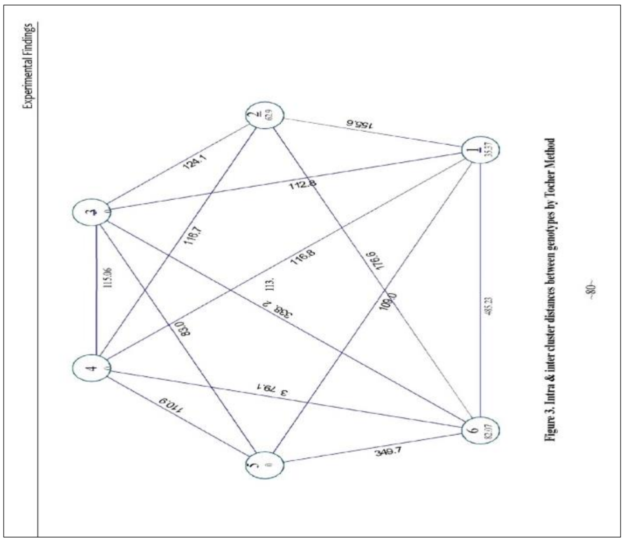


Fig 1: (Clustering of genotypes by Tocher Method)

Table 1: List of 21 genotypes and their sources

S. No.	Name of Genotype	Source	Grain Zinc Content (ppm)
1	IR 95044:8-B-5-22-19-GBS	IRRI South Asia Hub, Hyderabad.	20.6
2	IR 84847-RIL 195-1-1-1-1	IRRI South Asia Hub, Hyderabad.	21.8
3	IR 99704-24-2-1	IRRI South Asia Hub, Hyderabad.	14.67
4	IR 99647-109-1-1	IRRI South Asia Hub, Hyderabad.	23.7
5	IR 97443-11-2-1-1-1-1 -B	IRRI South Asia Hub, Hyderabad.	14.45
6	IR 97443-11-2-1-1-1-3 -B	IRRI South Asia Hub, Hyderabad.	23.47
7	IR 82475-110-2-2-1-2	IRRI South Asia Hub, Hyderabad.	24.73
8	IR 96248-16-3-3-2-B	IRRI South Asia Hub, Hyderabad.	27.18
9	R-RHZ-7	IRRI South Asia Hub, Hyderabad.	26.61
10	CGZR-1	IRRI South Asia Hub, Hyderabad.	24.43
11	BRRIdhan 62	IRRI South Asia Hub, Hyderabad.	23.33
12	BRRIdhan 64	IRRI South Asia Hub, Hyderabad.	24.97
13	BRRIdhan 72	IRRI South Asia Hub, Hyderabad.	20.7
14	DRR Dhan 45	IRRI South Asia Hub, Hyderabad.	18.13
15	DRR Dhan 48	IRRI South Asia Hub, Hyderabad.	19.2

16	DRR Dhan 49	IRRI South Asia Hub, Hyderabad.	17.63
17	IR 64	IRRI South Asia Hub, Hyderabad.	23.57
18	MTU1010	IRRI South Asia Hub, Hyderabad.	21.70
19	Sambamahsuri	IRRI South Asia Hub, Hyderabad.	24.47
20	Swarna	IRRI South Asia Hub, Hyderabad.	18.89
21	Local check	IRRI South Asia Hub, Hyderabad	16.9

Table 2: Analysis of variance for dispersion in 21 genotypes of rice by pooled data of five different environments

Source of Variations	Df	Sum of Squares	Mean Squares	F Ratio	Probability
Varieties	20	1.8036E-08	9.0178E-10	1.950E00	0.03664 *
Error	39	- 1.8036E-08	- 4.6245E-10		
Total	59	0.0000E00	0.0000E00		

Among the clusters, cluster VI had the maximum intra-cluster distance (82.07) and the minimum was recorded for cluster I (35.37), while it was zero value for cluster III, IV and V as they had only single genotype each. The minimum inter-cluster D^2 values was recorded between cluster III and V (83.05) while the maximum D^2 value was found between cluster I and VI (485.23) followed by cluster IV and VI (379.12), cluster V and VI (349.78) and cluster III and cluster VI (338.24) (Table 4). The inter-cluster distance was minimum between cluster III and V indicating close relationship and similarity for most of the characters of rice genotypes falling in these clusters while the maximum inter-cluster distance was recorded between cluster I and VI suggesting highest genetic divergence existing between the genotypes of these clusters.

Table 3: Cluster composition of 21 rice genotypes (Tocher's method) under five different environments

Cluster	Number of genotypes	Name of the genotypes
I	11	IR 99647-109-1-1, IR 96248-16-3-3-2-B, IR 64, IR 84847-RIL 195-1-1-1-1, IR 99704-24-2-1, BRRIdhan 62, CGZR-1, IR 82475-110-2-2-1-2, IR 95044:8-B-5-22-19-GBS, MTU1010, IR 97443-11-2-1-1-1-3 –B
II	5	R-RHZ-7, DRR Dhan 48, DRR Dhan 49, Local check, BRRIdhan 72
III	1	IR 97443-11-2-1-1-1-1 –B
IV	1	BRRIdhan 64
V	1	DRR Dhan 45
VI	2	Sambamahsuri, Swarna

Hence, the crosses between BRRIdhan 62 x Swarna, BRRIdhan 62 x Sambamahsuri (cluster I x cluster VI) and BRRIdhan 64 x Swarna, BRRIdhan 64 x Sambamahsuri (cluster IV x cluster VI), IR 97443-11-2-1-1-1-1 –B x Swarna, IR 97443-11-2-1-1-1-1 –B x Sambamahsuri (cluster III x cluster VI), could be suggested for the exploitation of transgressive segregants for both yield as well as quality. Hence, hybridization among these genotypes drawn from widely divergent clusters with high yield potential is likely to produce more heterotic effect. The inter-cluster distances were higher than the intra-cluster distances which indicate the existence of substantial diversity among the parents. Similar results of inter and intra cluster distances in rice were reported by Kandhola and Panwar (1999) [3], Senapati and Sarkar (2005) [9], Singh *et al.* (2006) [11], Kuchanur *et al.* (2009) [5], Shahidullah *et al.* (2009) [10] and Vennela *et al.* (2015).

Table 4: Inter-cluster and Intra-cluster (diagonal) average of D2and D values (parenthesis) of 21 rice genotypes (Tocher's method) over five different environments.

	Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster
Cluster 1	35.37	155.61	112.81	116.83	109.07	485.23
	(5.947)	(12.474)	(10.621)	(10.808)	(10.443)	(22.027)
Cluster 2		62.99	124.11	116.73	113.00	176.64
		(7.936)	(11.14)	(10.804)	(10.63)	(13.29)
Cluster3			0.00	115.06	83.05	338.24
			(0.000)	(10.726)	(9.113)	(18.391)
Cluster 4				0.00	110.97	379.12
				(0.000)	(10.534)	(19.471)
Cluster 5					0.000	349.78
					(0.000)	(18.702)
Cluster 6						82.07 (9.059)

Table 5: Cluster means for sixteen characters in 21 genotypes of rice (Tocher's method) under five different environments

	Days to first flowering	Days to 50 % Flowe ring	Day s to Matu rity	Tillers Per Plant	Plant Height (cm)	Panicle Length (cm)	SpikeletsPer Panicle	Filled grains Per Panicle	Spikelets Fertilit y %	Grain Weight Per Panicle(g)	Grain Yield Per Plant (g)	1000-grain Weight (g)	Grain Yield Per Plot (kg)	Yield/ha(kg)	L / B Ratio	Grain Zinc Contet (ppm)
1 Cluster	77.36	82.15	108.79	8.3	107.81	27.61	93.12	71.18	77.08	1.43	11.73	20.46	0.95	3957.57	4.16	25.48
2 Cluster	96.13	100.13	128.87	8.13	112.27	26.74	134	100.13	76.23	1.71	13.53	17.40	1.1	4564.99	4.03	24.03
3	80.33	84	111	6	108.37	27.42	157.33	131.67	84.53	2.3	13.86	17.09	1.12	4677.77	3.92	17.2

Cluster																
4 Cluster	83.00	87.33	114.33	5.33	133.39	28.51	156	110.33	71.46	2.36	12.31	21.56	1	4152.77	3.02	25.73
5 Cluster	86.33	90.67	118.67	7	126.47	27.34	123	84.33	69.55	1.86	12.73	23.37	1.03	4294.44	4.40	23.63
6 Cluster	111.17	115.33	144.33	8.83	111.33	27.57	123.67	107.83	86.84	1.68	14.83	16.10	1.2	5004.85	4.12	19.07

The cluster means for different characters indicated differences between the clusters for all the characters (Table 5). The cluster means for each of 16 characters are furnished in Table 4. The cluster means for days to first flowering ranged from 77.36 (Cluster I) to 111.11 (Cluster VI).For 50 percent flowering cluster means ranged from 82.15(cluster I) to 115.33(cluster VI). Days to maturity ranged from 108.79 (Cluster I) to 144.33 (Cluster VI). For plant height, cluster means ranged from 107.81 (Cluster I) to 133.39 (Cluster IV). Number of effective tillers per plant ranged from 5.33 (Cluster IV) to 8.83(Cluster VI). Similarly, panicle length ranged from 26.74 (Cluster II) to 28.51 (Cluster IV), whereas the number of spikelets per panicle ranged from 93.12 (Cluster I) to 157.33 (Cluster III) and number of filled grains per panicle ranged from 71.18 (Cluster I) to 131.67 (Cluster III). The cluster mean for spikelets fertility percent ranged from 69.55 (Cluster V) to 86.84 (Cluster VI). The cluster mean for grain weight per panicle ranged from 1.43 (Cluster I) to 2.36 (Cluster IV) and for grain yield per plant ranged from 11.73 (Cluster I) to 14.83 (Cluster VI). The 1000-grain weight ranged from 16.1 (Cluster VI) to 23.37 (Cluster V). Similarly, cluster mean for grain yield per plot ranged from 0.95 (Cluster I) to 1.2 (cluster VI). Likewise the cluster mean for yield hectare ranged from 3957.57 (Cluster I) to 5004.85 (Cluster VI).The cluster mean for LB ratio ranged from 3.02 (Cluster IV) to 4.4(Cluster V) and the cluster mean for Grain zinc content from 17.2 (Cluster III) to 25.48 (Cluster I).The cluster I recorded high mean values significant for grain zinc content and 1000 grain weight. The cluster II higher mean value significant for days to first flowering, days to 50% flowering, days to maturity and spikelet per panicle. The cluster III recorded high mean values significant for spikelet per panicle, no of grains per panicles, spikelet fertility percent and grain weight per panicle. While cluster IV recorded high values significant for plant height, panicle length, spikelets per panicle, number of grain per panicle and grain weight per panicle. Whereas cluster V recorded high mean values significant for 1000 grain weight, grain weight per panicles, plant height. The cluster VI had high mean value significant for days to first flowering, days to 50% flowering, days to maturity, no of filled grains per panicle, spikelet fertility percent, grain yield per plant, grain yield per plot, yield per ha. The genotype belonging to clusters V, VI and IV could be used in hybridization programme to obtain better recombinants. Apart from the divergence, the performance of genotypes and the character with maximum contribution towards divergence should also be given due consideration which appear as desirable for improvement of rice.The characters appeared in first rank more it contributed towards diversity. Among all the characters, days to first flowering contributed the maximum (29.63%) to the diversity by taking first rank in 90 times, followed by grain zinc content (29.05% with 61 times ranked first), LB ratio (13.33% with 28 times ranked first) and grain yield per ha (7.62% with 16 times ranked first). The characters viz., grain yield per plot, panicle length and 1000-grain weight contributed 1.43%, 1.43% and 1.9%, respectively. Other characters *viz.*, Days to maturity, Number of filled grains per panicle, no of effective tiller, contributed 0.95, 0.48 and 0.48 percent, respectively to the genetic divergence. On contrary days to 50 per cent flowering, number of spike lets per panicles, spikelet fertility percent and grain yield per plant had no contribution towards genetic divergence. Hence, days to first flowering followed by grain zinc content, Grain LB ratio, grain yield per ha, 1000-grain weight, grain yield per plot and panicle length were the important traits contributing maximum towards divergence in rice over five different environments (Table 6). Similar results in rice were also reported by Kaw (1995) for plant height and days to 50% flowering; Kuchanur *et al.* (2009) [5], Bhati *et al.* (2015) [1] for days to 50% flowering and Meena *et al.* (2017) [7] for number of effective tillers and grain yield per plot.

Assessment of genetic diversity was made based on the data recorded for sixteen yield and yield contributing characters in twenty one rice genotypes using Mahalanobis' D^2 analysis. The significance of Wilk's 'V' (statistic) value indicated that the genotypes differed significantly among themselves when all the characters were considered simultaneously. This indicated that there is considerable diversity in the material. Twenty one genotypes were grouped into six clusters and each has the genotypes from different geographical regions indicating that there was no association between geographical distribution and genetic diversity. The cluster I recorded high mean values significant for grain zinc content and 1000 grain weight whereas cluster II for days to first flowering, days to 50% flowering, days to maturity and spikelet per panicle. The cluster III recorded high mean values significant for spikelet per panicle, no of grains per panicles, spikelet fertility percent and grain weight per panicle. While cluster IV recorded high values significant for plant height, panicle length, spikelets per panicle, number of grain per panicle and grain weight per panicle. Whereas cluster V recorded high mean values significant for 1000 grain weight, grain weight per panicles, plant height and L/B ratio. The cluster VI had high mean value significant for days to first flowering, days to 50% flowering, days to maturity, no of filled grains per panicle, spikelet fertility percent, grain yield per plant, grain yield per plot, yield per ha. The genotypes belonging to clusters V, VI and IV could be used in hybridization programme to obtain better recombinants. Hence, the crosses between BRRIdhan 62 x Swarna, BRRIdhan 62 x Sambamahsuri (cluster I x cluster VI) and BRRIdhan 64 x Swarna, BRRIdhan 64 x Sambamahsuri (cluster IV x cluster VI), IR 97443-11-2-1-1-1-1 –B x Swarna, IR 97443-11-2-1-1-1-1 –B x Sambamahsuri (cluster III x cluster VI), could be suggested for the exploitation of transgressive segregants for both yield and quality. Hybridization among these genotypes drawn from widely divergent clusters with high yield potential is likely to produce more heterotic effect also.

Table 6: Relative contribution of 16 characters to genetic diversity in 21 genotypes of rice over five different environments

Character	Times Ranked 1st	Contribution (%)
Days to first flowering	90	41.86 %
Days to 50 per cent flowering	0	0.01 %
Days to maturity	2	.95 %
Total number of effective tillers per plant	1	.48 %
Plant height (cm)	1	.48 %
Panicle length (cm)	3	1.43 %
Number of spikelets per panicle	0	0.01%
Number of filled grains per panicle	1	.48%
Spikelets fertility percentage	0	0.01%
Grain weight per panicle(g)	2	.95 %
Grain yield per plant (g)	0	0.01 %
1000-grain weight (g)	4	1.9 %
Grain yield per plot (kg)	3	1.43 %
Grain yield per ha (kg)	16	7.62 %
L/B Ratio	28	13.33 %
Grain Zinc content (ppm)	61	29.05 %

Conclusion

The crosses between BRRIdhan 62 x Swarna, BRRIdhan 62 x Sambamahsuri (cluster I x cluster VI) and BRRIdhan 64 x Swarna, BRRIdhan 64 x Sambamahsuri (cluster IV x cluster VI), IR 97443-11-2-1-1-1-1 –B x Swarna, IR 97443-11-2-1-1-1-1 –B x Sambamahsuri (cluster III x cluster VI), could be suggested for the exploitation of transgressive segregants for both yield as well as quality like bifortification for zinc in rice genotypes.

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