



Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

GENETIC EVALUATION OF RICE (*Oryza sativa*. L) GENOTYPES FOR YIELD AND NUTRITIONAL QUALITY TRAITS

M. S. Umar Farooq^{1*}, J. R. Diwan¹, K. Mahantashivayogayya², Vikas V. Kulkarni³,
N. M. Shakuntala⁴

¹Department of Genetics and Plant Breeding, University of Agricultural Sciences, Raichur-584104, Karnataka, India.

²AICRP on Rice Breeding, ARS, Gangavathi-583227, Karnataka, India.

³MARS, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Raichur-584104, Karnataka, India.

⁴Department of Seed Science and Technology, University of Agricultural Sciences, Raichur- 584104, Karnataka, India.

Received – January 04, 2019; Revision – February 20, 2019; Accepted – March 28, 2019

Available Online – April 10, 2019

DOI: [http://dx.doi.org/10.18006/2019.7\(2\).117.127](http://dx.doi.org/10.18006/2019.7(2).117.127)

KEYWORDS

Iron
Zinc
Rice
Yield
Biofortification
XRF
Amylose content

ABSTRACT

The increase in food production is greatly contributed by green revolution, thereby leading to reduction in people's starvation. However, this caused greater depletion of micronutrient reserve in soil and thereby accentuated wide spread deficiencies of micronutrients in crop species. Hence, biofortification program has been initiated to identify varieties having high iron and zinc along with high yield. In the present study 46 rice genotypes along with 4 checks were received from Indian Institute of Rice Research (IIRR), Hyderabad, India and were evaluated for yield and yield attributing traits, grain quality parameters and estimation of micronutrients. For yield and yield attributing characters most of the traits showed high heritability associated with high genetic advance indicating fixation of genes and presence of additive gene action in these traits. The results obtained by micronutrient estimation revealed that iron and zinc contents of dehusked grains differed significantly between the genotypes. Among various studied genotypes the promising genotypes with highest grain iron and zinc content along with higher yields and with intermediate amylose content were identified. These promising genotypes identified can be released to farmers after testing in multilocation trials for their stable performance or they can be used as parents in hybridization programme.

* Corresponding author

E-mail: umar.gpb@gmail.com (M. S. Umar Farooq)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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1 Introduction

Rice (*Oryza sativa* L.) is a “Global Grain” cultivated widely across the world and feeds millions of mankind. It is the staple food for over half the world’s population. The countries like China and India account for 50% of the rice grown and consumed. It provides upto 50% of the dietary caloric supply for millions living in poverty in Asia and is, therefore, critical for food security (Muthayya et al., 2014). Rice constitutes starch as chief component along with lipids, minerals, proteins and negligible levels of micronutrients, hence rice supplies very less micronutrients leading to micronutrient deficiency known as “hidden hunger” (Khush & Virk, 2005).

Iron (Fe) is an essential element for blood production, about 70 percent of the body’s iron is found in the red blood cells called hemoglobin. The hemoglobin is involved in transfer of oxygen from the lungs to the tissues (Roohani et al., 2013). Iron deficiency is one of the most prevalent micronutrient deficiencies, affecting around two billion people globally (WHO, 2016). Micronutrient deficiencies or “hidden hunger” affect about 38% of pregnant women and 43% of preschool children worldwide and are most prevalent in developing countries. More than 30% of the world’s population is anaemic. Global studies estimate that approximately half of this is due to Iron-Deficiency Anaemia (IDA). IDA can affect productivity and cause serious health consequences, including impaired cognitive development in children, a weakened immune system, and increased risk of morbidity. (Trijatmiko et al., 2016)

Zinc (Zn) helps in protein synthesis and regulates immune system of the human body. It also has role at the time of pregnancy, hair care, night blindness, eye care, appetite loss, and many other conditions (Roohani et al., 2013). Zinc deficiency is a major cause of stunting among children. About 165 million children with stunted growth run a risk of compromised cognitive development and physical capability (Trijatmiko et al., 2016).

The deficiency of zinc and iron not only affects the human health but also leads to crop yield losses. The WHO (2009) report says half of the human population affected by iron and zinc deficiency indicating its importance in human nutrition. This is more pronounced in the developing countries of Asia and Africa (Gómez-Galera et al., 2010). The other health related issues due to deficiency of Fe and Zn are reduced immunity, fatigue, poor growth, irritability, weakness, hair loss, wasting of muscles, morbidity, sterility and even death in acute cases (Stein, 2010).

To alleviate iron and zinc deficiency biofortification program has been suggested to be a sustainable, targeted, food-based and cost effective approach in alleviating Fe and Zn deficiency. There are popular projects which aim at Fe biofortification of rice, cassava,

beans, and sweet potato. Similarly Zn biofortification of wheat, rice, beans, sweet potato and maize led to release of varieties contributing to reduction in malnutrition (Jena et al., 2018; Jagadish et al., 2018). Further, seeds with increased micronutrients also perform better in micronutrient deficient soils by producing longer roots leading to better absorption and yield (Graham et al., 2001) and improved disease resistance and stress tolerance (Bouis & Welch, 2010). The present study was carried out with the aim of evaluating rice genotypes possessing combination of both high yield and nutritional quality.

2 Materials and Methods

The material used for the present research consisted of 50 rice genotypes including 4 checks. All the collected genotypes were provided by Indian Institute of Rice Research (IIRR), Hyderabad. These genotypes were grown during *kharif* 2017 at Agricultural Research Station, Gangavathi under University of Agricultural Sciences (UAS), Raichur in Randomized Block Design (RBD) with three replications. The seeds of the selected genotypes were sown on the seed bed on 2nd August 2017 and seedlings were transplanted to the main field on 25th August 2017 with a spacing of 20×15 cm. Five representative plants for each genotype in each replication were randomly selected to record observations on various yield and yield attributing traits. The estimation of micronutrients by XRF (X-Ray Fluorescence Spectrometry) was carried out at IIRR, Hyderabad.

2.1 Procedure of XRF method of micronutrient estimation:

After harvesting, all the samples were processed using non-iron and zinc husker to avoid iron and zinc contamination. The dehusked brown rice sample was cleaned by tissue paper. Then arrange ten dustless cleaned rice samples sachets in a row. Weigh nearly 5 grams of each samples and pour into pre-identified aluminium cup. Carefully tap the sample tray along with the aluminium cups by holding the ends of tray with both hands in order to spread the grain uniformly in all the aluminium cups and place each aluminium cup into a sample sleeve of XRF machine without disturbing the grain starting from 1 to 10. Insert x-ray lamp key to the machine and turn it on. Iron and Zinc values appear in a new window on the screen, note down the values and click on to accept the results and repeat for each sample. The estimation of amylose content in each rice sample was carried out by following modified method of Juliano (1971). Amylose content was determined by the following formula and expressed on dry weight basis.

Amylose content=Optical density×Slope of the curve×Dilution factor.

Based on the amylose percentage varieties can be grouped as proposed by Juliano (1971) and mentioned as in table 1.

Table 1 Classification based on amylose content.

Category	Amylose content (%)
Waxy	1-2
Very low amylose	3-9
Low	10-19
Intermediate	20-24
High	25-30

Table 2 Analysis of Variance (ANOVA) for different yield and yield attributing characters in rice genotypes

Source of variation	Mean sum squares (MSS)			CV (%)
	Replications	Treatments	Error	
(d.f)	2	49	98	
DFF	24.09	130.93**	5.91	2.45
TN	0.28	6.14**	1.42	7.85
PN	0.10	4.26**	1.57	9.16
PL	4.89	5.14**	2.33	9.43
PB	0.21	12.41**	0.46	6.90
SB	198.94	354.39**	8.45	10.05
PH	43.25	371.21**	29.86	5.44
%FS	173.27	227.22**	12.06	4.40
GN	331.04	2340.36**	38.29	5.12
TW	0.24	36.47**	2.17	5.68
GY	4.92	52.19**	3.24	5.19

Where, *df*-Degrees of freedom (*d.f*); *PH*- Plant height; *DFF*- Days to 50% flowering; % *FS*- Percentage of fertile spikelets; *TN*- Tiller number per plant; *GN*- Grain number per panicle; *PN*- Panicle number per plant; *TW*- Test weight; *PL*- Panicle length; *GY*- Grain yield per plant; *PB*- Primary branches per panicle; *SB*- Secondary branches per panicle; ** - Significance at 1% level

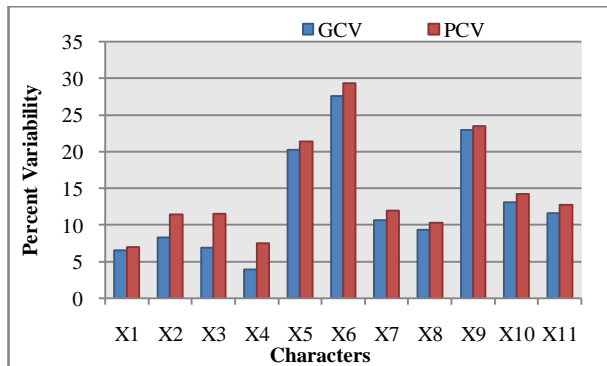


Figure 1: Phenotypic (PCV) and genotypic (GCV) coefficient of variation of various yield and yield attributing traits in 50 rice genotypes

2.2 Statistical analysis

The data recorded for yield and yield attributing traits were processed with statistical parameters *viz.* range, mean, standard error and coefficient of variation for all traits. The data was subjected to randomized block design analysis. The data were analyzed using software, Windostat version 8.5 and frequency distribution curves obtained using SPSS version 16.0 software.

3 Results

3.1 Yield and yield attributing characters

The grain yield per plant ranged from 22.10 to 40.14 g with a mean value of 34.74 g. The highest grain yield was recorded by genotype IR 93354:19-B-12-21-9-IRGA-2RGA-1-B (40.14 g) while lowest grain yield per plant was recorded in IR14M117 (22.10 g).

The analysis of variance for different characters along with coefficient of variation is presented in Table 2. The significant differences for all the characters under study at 1% level of significance was observed, indicating presence of sufficient amount of variability for all the traits among the genotypes studied.

As per the results of mean performance, a wide range of variation was found for most of the characters. Through this study an endeavor was made to assess the extent of variability in rice genotypes which portray the mean performance of fifty genotypes, for eleven quantitative traits. Genetic variability parameters for different yield and yield attributing traits are presented in Table 3 while the PCV, GCV, h^2 (bs), and genetic advance for different yield and yield attributing traits are presented graphically in Figure 1 and 2 and 2a.

3.2 Grain iron and zinc content

The mean performance of rice genotypes for grain Fe and Zn is given in Table 4. The top three genotypes were selected on the

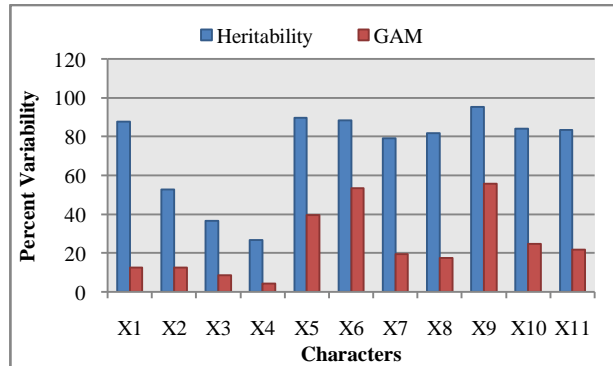


Figure 2: Heritability and genetic advance as percent of mean for various yield and yield attributing traits in 50 rice genotypes

Table 3 Estimates of mean and other genetic parameters for different yield and yield attributing characters in rice genotypes

Sl. No.	Characters	Mean	Min	Max	Variances		Coefficient of variation %		h ² (bs) (%)	Genetic Advance	GAM (%)
					Genotypic (Vg)	Phenotypic (Vg)	GCV	PCV			
1	Days to 50% flowering	98.99	89.33	110.33	41.67	47.58	6.52	6.96	87.58	12.44	12.57
2	Tiller number per plant	15.15	13.00	19.47	1.57	2.99	8.28	11.41	52.62	1.87	12.37
3	Panicle number per plant	13.67	11.67	17.00	0.89	2.46	6.93	11.49	36.43	1.17	8.62
4	Panicle length	23.74	21.07	26.67	0.85	3.18	3.89	7.51	26.80	0.98	4.15
5	Primary branches per panicle	9.83	7.10	13.43	3.98	4.44	20.26	21.41	89.60	3.89	39.52
6	Secondary branches per panicle	28.91	15.5	39.5	63.49	71.94	27.56	29.34	88.25	15.42	53.34
7	Plant height	100	78	125	113.78	143.64	10.62	11.93	79.21	19.55	19.47
8	Percentage of fertile spikelets	78.77	52.70	91.27	53.73	65.79	9.30	10.29	81.67	13.64	17.32
9	Grain number per panicle	120.65	64.00	181.33	767.35	805.64	22.95	23.52	95.24	46.15	55.69
10	Test weight	25.88	15.31	32.40	11.43	13.59	13.06	14.24	84.07	6.38	24.67
11	Grain yield per plant	34.74	22.10	40.14	16.31	19.57	11.64	12.75	83.43	7.60	21.91

GCV: Genotypic Coefficient of variation, PCV: Phenotypic Coefficient of variation, h² bs: Broad sense heritability, GAM: Genetic Advance as Percent of Mean

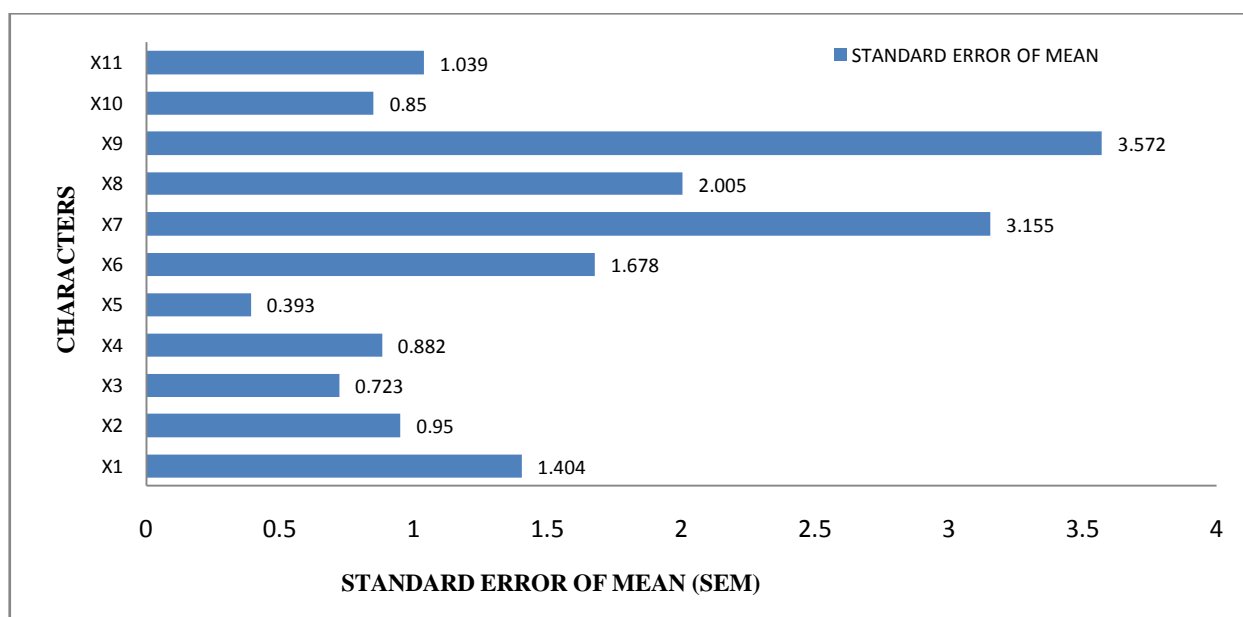


Figure 2 a: Standard Error of mean for various yield and yield attributing traits in 50 rice genotypes

Where, X1-Days to 50% flowering, X2-Tiller number per plant, X3-Panicle number per plant, X4-Panicle length, X5-Primary branches per panicle, X6-Secondary branches per panicle, X7-Plant height, X8- Percentage of fertile spikelets, X9-Grain number per panicle, X10- Test weight, X11-Yield per plant

Table 4 Mean performance of rice genotypes for grain Fe and Zn content

Sl. No.	Genotype name	Fe (ppm)	Zn (ppm)
1	IR15M1293	9.70	19.30
2	IR15M1298	8.20	15.90
3	IR15M1319	10.65	20.75
4	IR15M1328	9.35	16.50
5	IR15M1341	8.95	18.50
6	IR10M210	9.80	16.95
7	IR 95044:8-B-5-22-19-GBS	9.70	17.85
8	IR95048:1-B-11-20-10-GBS	11.40	16.70
9	IR 95097:4-B-20-18-8-GBS	12.05	19.60
10	IR 95133:1-B-16-14-10-GBS	9.15	16.30
11	IR 95133:18-B-2-22-5-GBS	9.90	16.10
12	IR 95040:12-B-3-10-2-GBS	10.30	15.70
13	IR15M1178	10.60	16.65
14	IR 99637-123-1-3-B	11.40	17.35
15	IR 93354:19-B-12-21-9-1RGA-2RGA-1-B	9.90	17.80
16	IR 93342:14-B-23-18-5-1RGA-2RGA-1-B	10.95	18.55
17	IR 93346:1-B-13-7-6-1RGA-2RGA-1-B	10.65	14.80
18	IR15M1053	11.00	14.40
19	IR15M1054	10.30	14.30
20	IR15M1266	8.45	14.85
21	IR15M1274	8.60	13.75
22	IR15M1329	8.60	16.35
23	IR1M1330	8.30	15.55
24	IR 92195-398-1-1-1-1-1	9.00	13.00
25	IR 92195-411-1-1-1-1-1	8.95	16.20
26	IR15M1315	8.30	15.50
27	IR 95097:3-B-16-11-4-GBS	13.00	19.65
28	IR 95041:9-B-7-5-17-GBS	8.40	15.30
29	IR14M117	9.55	16.05
30	IR14M118	9.60	16.45
31	IR14M121	9.40	18.55
32	IR14M123	10.35	18.05
33	IR14M124	8.85	16.15
34	IR14M126	10.20	23
35	IR14M102	8.35	19.60

Table 4 Mean performance of rice genotypes for grain Fe and Zn content

Sl. No.	Genotype name	Fe (ppm)	Zn (ppm)
36	IR15M1003	9.35	16.35
37	IR15M1284	12.25	20.45
38	IR15M1302	8.15	13.15
39	IR15M1322	8.90	14.40
40	IR15M1337	7.50	12.70
41	IR14M211	8.60	15.00
42	IR95080:1-B-9-12-17-3	9.55	16.05
43	IR95052:12-B-3-6-13-B	8.50	15.00
44	IR93337:50-B-20-19-13-IRGA-2RGA-1-B	10.50	18.00
45	IR93337:37-B-15-15-22-IRGA-2RGA-1-B	9.95	20.00
46	IR 93337:37-B-15-15-22-IRGA-2RGA-1-B	9.40	16.50
47	Chittmuthyalu (C)	10.00	20.35
48	DRRDhan-45 (C)	9.70	17.90
49	BPT-5204 (C)	8.65	14.95
50	GangavathiSona (C)	11.90	15.90
	MEAN	9.70	16.76
	Minimum	7.5	12.7
	Maximum	13	23
	CD @ 5%	1.50	3.50
	CD @ 1%	2.00	4.60

C in the parenthesis indicates Check

Table 4a Top three genotypes selected based on high grain micronutrients

SL. No	Fe	Zn
1	IR 95097:3-B-16-11-4-GBS (13 ppm)	IR14M126 (23 ppm)
2	IR15M1284 (12.25 ppm)	IR15M1319 (20.75 ppm)
3	IR 95097:4-B-20-18-8-GBS (12.05 ppm)	IR15M1284 (20.45 ppm)

basis of high grain micronutrients is presented in Table 4 (a). The grain iron content ranged from 7.5 ppm to 13 ppm with a mean value of 9.70 ppm. The highest grain Fe content was recorded in the genotype IR 95097:3-B-16-11-4-GBS (13 ppm) while the lowest was reported from the genotype IR15M1337 (7.5 ppm). The frequency for the trait is shown graphically in Figure 3 (a). The grain Zn content in test entries was recorded with a mean value of 16.77 ppm and range from 13 to 23 ppm. The lowest grain Zn content was recorded in the genotype IR 95097:3-B-16-11-4-GBS (13 ppm) and highest in the genotype IR14M126 (23 ppm) this frequency for the trait is shown graphically in Figure 3 (b).

3.3 Amylose Content

Highest amylose content of 25.2 per cent was observed in genotype Chittmuthyalu and majority of the genotypes falls under low (10-20 %) amylose content compared to check BPT-5204 (24.10%). The lowest amylose content observed was 11.28 per cent in the genotype IR 95133:18-B-2-22-5-GBS. Overall mean of the genotypes for amylose content was 18.30 percent and Frequency distribution for the trait is shown in Figure 4(a) and variation in amylose content is shown in Figure 4(b). Forty six rice genotypes was compared with the check BPT-5204 and found that most of the genotypes that are statistically at par with it for traits like grain length, grain breadth, grain L/B ratio, amylose content, grain Zn, Fe content and grain yield are listed in Table 5.

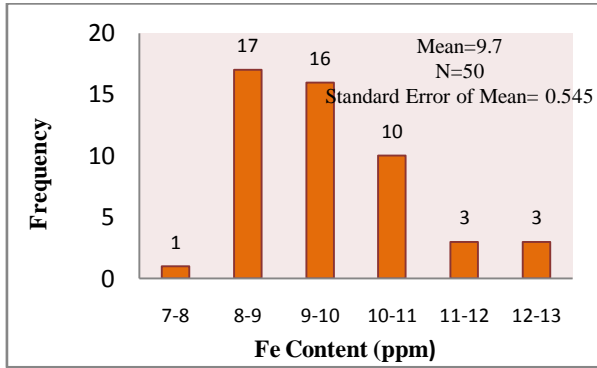


Figure 3 a: Frequency distribution for grain iron content

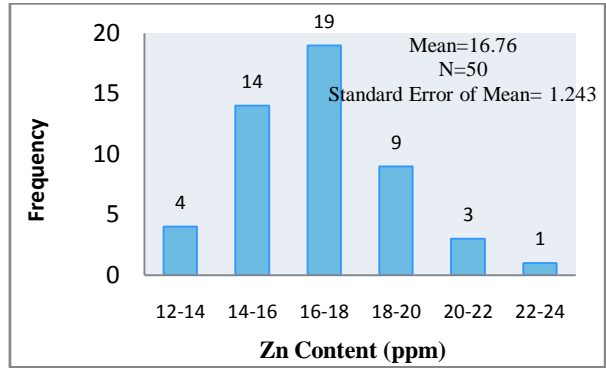


Figure 3(b): Frequency distribution for grain zinc content

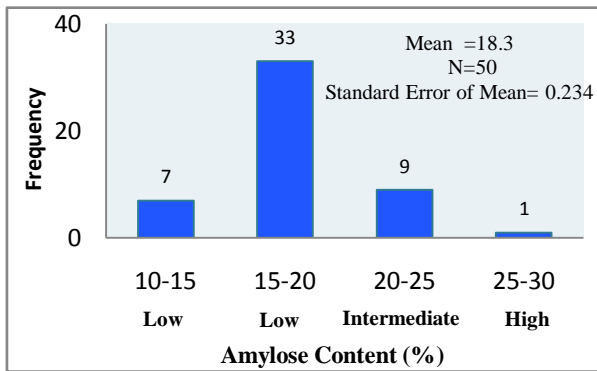


Figure 4 a: Frequency distribution for amylose content



Figure 4 b: Variation in amylose content

Table 5 Grouping of genotypes statistically on par with check BPT-5204

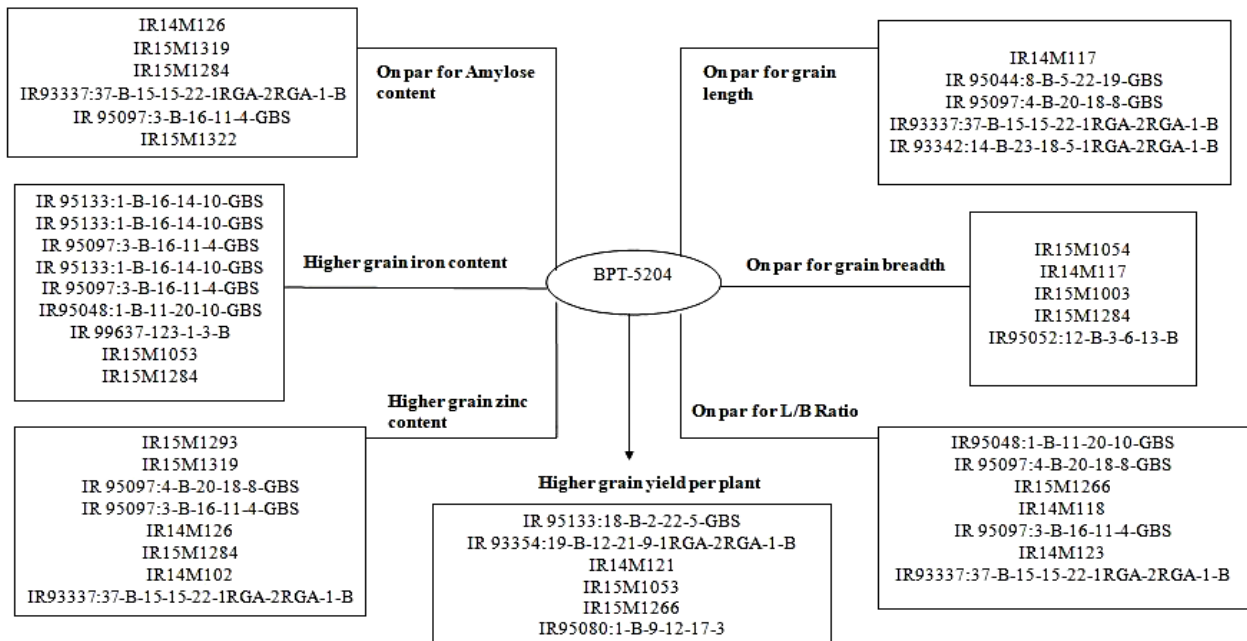


Table 6 List of genotypes with highest grain micronutrients along with highest grain yield with intermediate amylose content on comparison with checks

Genotypes	Grain Zinc content (ppm)	Grain Iron content (ppm)	Grain yield/plant (g)	Intermediate amylose content (%)	Grain type
IR14M126	23.00	10.20	35.11	21.17	Long Slender
IR15M1319	20.75	10.65	37.43	21.40	Long Bold
IR15M1284	20.05	12.25	37.93	20.50	Long Slender
IR93337:37-B-15-15-22-IRGA-2RGA-1-B	20.00	10.95	35.86	20.13	Medium Slender
IR 95097:3-B-16-11-4-GBS	19.65	13.00	35.66	20.30	Medium Slender
BPT-5204 (Check)	14.95	8.65	31.22	24.12	Medium Slender
GangavathiSona (Check)	15.90	11.90	37.53	20.43	Medium Slender
DRRDhan-45 (Check)	17.90	9.70	33.30	20.70	Medium Slender
Chittmuthyalu (Check)	20.35	10.00	35.27	25.20	Short Bold
CD @ 5%	3.50	1.50	2.73	0.61	
CD @ 1%	4.60	2.00	2.06	0.46	
Standard Error of Mean	1.24	0.54	1.03	0.23	

3.4 Selection of top performed genotypes

Among all the evaluated rice genotypes, the top five genotypes (IR14M126, IR15M1319, IR15M1284, IR93337:37-B-15-15-22-IRGA-2RGA-1-B and IR 95097:3-B-16-11-4-GBS) are selected based on presence of high grain micronutrients along with high grain yield with moderate amylose content on comparison with checks. Details of these are presented in Table 6.

4 Discussion

Iron and zinc are important elements for plant development, out of the 16 essential elements needed for plant growth, iron is used for the synthesis of chlorophyll and is essential for the function of chloroplasts (Rout & Sahoo, 2016). Zinc is involved in membrane integrity, enzyme activation and gene expression. In addition to being essential to plants, these are also an essential mineral nutrient for human beings (Yang et al., 2017). Micronutrient malnutrition is a serious health problem worldwide, affecting more than three billion people. There is evidence that the number of people and the proportion of global population suffering from micronutrient malnutrition have increased over the last four decades (Haider & Bhutta, 2017). Rice is a staple food eaten in large quantities everyday by majority of Asian population. Therefore, addition of even small quantities of micronutrients is beneficial. Once rice is biofortified with vital nutrients, the farmer can grow indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way. This is the only feasible way of reaching the malnourished population.

Furthermore, the use of Zn and Fe dense grains results in greater seedling vigour and increased crop yields when the seeds are sown in micronutrient poor soils. To address this problem, a genetic approach called biofortification which aims at enrichment of food grains with micronutrients has been evolved and used (Hussain et al., 2018).

The phenotypic variance (V_p) and phenotypic coefficient of variation (PCV) were higher than their corresponding genotypic variance (V_g) and genotypic coefficient of variation (GCV) for number of tillers per plant, panicle number per plant, panicle length and grain yield per plant indicating the more role of environment compared to genotype on expression of these traits. In this study the difference between values of phenotypic and genotypic variance along with their corresponding PCV and GCV for days to 50% flowering, primary branches per panicle, secondary branches per panicle, plant height, percentage of fertile spikelets, grain number per panicle and test weight was low indicating sensitivity to environment, consequently higher role of genetic factors influencing the characters expression. The characters with almost equal value of V_p and V_g coupled with their corresponding PCV and GCV can be considered as stable. Similar findings were illustrated by Roy et al. (2014) and Kishore et al. (2015). The genotypic estimates of variability (V_g) and GCV are the most consequential parameters and avails in the quantification of the contribution of the genotype to the expression of a particular character and gives clue to compare the genetic variability for different characters. This gives scope for selection of plants which contribute for better yield attributing

traits. Results of the present study are also supported by the results obtained by Yadav et al., (2010), Ullah et al., (2011), Singh et al., (2011), Dutta et al., (2013) and Hussain et al., (2014). Heritability is a measure of the extent of phenotypic variation caused by the action of genes. High heritability associated with high genetic advance was found in number of primary branches per panicle, number of secondary branches per panicle, number of grains per panicle, test weight and grain yield, while days to fifty percent flowering and percentage of fertile spikelets exhibited high heritability but moderate genetic advance. High heritability estimates indicated a high response to selection for these traits; the high heritability and genetic advance values have explained the predominance of additive gene action and selection for these traits can be carried in early generations. Therefore, selection of genotypes based on these characters would be more effective to be successful for target plant selection and these findings are in accordance with the findings of Rajendra Prasad et al. (2017).

Plant breeding programmes based on biofortification needs screening of germplasm having Zn and Fe-dense grains to be used as donor parents. The large genetic variations present in cereals must be exploited and it is important approach to eradicate Fe and Zn deficiencies (Brar et al., 2011). The significance of such an intervention leading to higher acquisition of nutrients by land races of rice would reflect in cutting costs worth billions presently being used for malnutrition related diseases (Chandel et al., 2010).

Yet rice feeds the largest number of people in the world, the demand for superior grain quality represents a major issue in rice breeding. Now a days, breeders and scientists are more and more focused on improving the quality of rice for different purposes and markets. The preference of different rice consuming people varies with geographical location and culture. The people in the far east prefer sticky and soft rice, but in India, a non-sticky type is preferred. Consumers from developed countries look for grain with good cooking quality and victualing characteristics, but in many developing regions, alimantal value is crucial as rice is the most consumed staple victuals. Grain quality is a concept which tells about many characteristic features ranging from physical to physiological and biochemical properties. Amending grain quality is a paramount goal in rice breeding program. The grain quality traits such as amylose content play a crucial role in determining grain quality of rice (Suzuki et al., 2006). The rice starch is made up of amylose and amylopectin and assessed by properties such as amylose content (AC), amylopectin structure, viscosity, gelatinization temperature (GT), and gel consistency. All the starch-related properties have varied degrees of impact on the eating and cooking quality of rice. Among this, amylose content is widely recognized as the most important factor affecting the eating and cooking quality of rice grain. The

cooked rice kernels property differs with amylose content. The higher value (>25%), the kernels are dry, separate, less tender and become hard upon cooling. The Intermediate amylose content (20–25%) rice is most preferred in the world which is soft and flaky whereas those with low amylose (12–20%) are glossy, soft, and sticky. This starch property also decides about glycemic index which is important for diabetic people. Amylose synthesis in grains is primarily regulated by the *Waxy* (*Wx*) gene and its expression is regulated organ specifically. The differential expression of *Wx* gene is observed, as it found in the endosperm and pollen but not in other organs. In rice genotypes, the *Wx* gene encodes the Granule-Bound Starch Synthase I (GBSSI) enzyme and the level of grain amylose is determined by the amount of GBSSI enzyme in the endosperm. The *Waxy* gene locus is present on chromosome 6 which has 13 exons and 12 introns. The *Wx^a* found in *indica* subspecies and *Wx^b*, found in *japonica* subspecies. These determine high and low amylose content in rice (Pang et al., 2016).

Based on the above studies five promising genotypes were selected from forty six genotypes comparing with checks based on the presence of high grain iron and zinc content along with highest yields and with intermediate amylose content. The genotypes viz., IR14M126, IR15M1319, IR15M1284, IR93337:37-B-15-15-22-IRGA-2RGA-1-B and IR 95097:3-B-16-11-4-GBS (Table 6) are the promising genotypes selected. These identified genotypes may be used in future hybridization program to achieve desired segregants for varieties with higher grain micronutrients, higher yields and intermediate amylose content and the selected genotypes can be tested and released.

Conclusions

In the present investigation, useful variation among fifty rice genotypes were observed for yield and yield attributing traits, grain micronutrients and amylose content. The five promising rice genotypes identified in the study are the best source to supplement the iron and zinc and eradicate the problem of hidden hunger and all the micronutrient deficiency related diseases furthermore these genotypes exhibited higher yields compared with the checks, also these genotypes possess intermediate amylose content which results in soft and flaky rice when cooked. Hence these genotypes can be released to farmers after testing in multilocation trials for their stable performance or they can be used as parents in hybridization programme.

Acknowledgements

This research was supported by Indian Institute of Rice Research, Hyderabad, India.

Conflict of Interest: Nil

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