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GRAIN QUALITY ASSESSMENT OF SELECTED RICE (*Oryza sativa* L.) GENOTYPES

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Abstract

Grain quality improvement has now become the primary consideration in rice breeding programs. In the present research, grain physical properties as well as nutritional quality [iron (Fe) and zinc (Zn) content] of eleven advanced rice lines with two check varieties were investigated for the estimation of the variation during the Aman season 2021. The lines EFSD-01, EFSD-66, EFSD-59, IZSD-10, IZSD-30, IZSD-44 and IZSD-67 were categorized as to produce long slender grain. The highest milling percentage and head rice recovery percentage were recorded in IZSD-67 (71.67%) and IZSD-30 (67.33%), respectively. Grain physical properties of the genotypes which showed higher heritability in broad sense and moderate genetic advance rendering them more amenable to improvement through selection than the other traits, and could be suitable for plant hybridization. First two principal components contributed up to 72.5% of the total variance cumulatively. The genotypes were grouped into five clusters and the maximum intra-cluster and inter-cluster distances were found in cluster IV and between cluster I and V, respectively. Besides, the minimum inter-cluster distance was found between cluster I and cluster V but there was no intra-cluster distance in Binadhan-20 and EFSD-01, because they were separated into a single genotype. In micronutrient estimation study, Fe content varied from 6 to 13 mg kg^{-1} and 0 to 7.33 mg kg^{-1} whereas Zn content ranged from 33.33 to 44.33 mg kg^{-1} and 20.33 to 27.67 mg kg^{-1} in unpolished and polished rice, respectively. Considering physical grain quality and Fe, Zn content, the advanced rice lines EFSD-66, IZSD-44, EFSD-59, EFSD-58, IZSD-26, IZSD-67 and IZSD-10 could be utilized in plant breeding to develop premium quality and nutrient enriched rice varieties, which in turn will ensure nutritional security in Bangladesh.

Keywords: Rice (*Oryza sativa* L.), Grain quality, Iron (Fe), Zinc (Zn)

Introduction

The primary food source for more than fifty percentage of the world's population is rice, which is also a suitable food for people of all ages (Al-Daej, 2022). Rice has a more complex grain character than other cereals and it is a staple food in Bangladesh where it is consumed as whole grain. Milling percentage, head rice recovery percentage, physical attractiveness, and nutritional content are significant market price criteria and these characteristics are essential for figuring out the quality and preference of rice among different customer groups (Prom-u-thai and Rerkasem, 2020; Sellappan *et al.*, 2009; Dela Cruz and Khush, 2000).

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For assessing grain quality, the average kernel length-breadth ratio and kernel shape are important factors (Rita and Sarawgi, 2008). Plant breeders therefore prioritize grain size and shape when developing new rice varieties to be released for commercial cultivation. Additionally, different factors had an impact on quality. For example, rice millers preferred varieties with superior milling potential, whereas consumers placed more emphasis on physiochemical properties (Merca and Juliano, 1981). The milled rice recovery (milling percentage) and head rice recovery (heading percentage) of a variety of rice are significant traits that enhance its economic performance (Butardo and Sreenivasulu, 2019).

Micronutrient deficiency is one of the major risks to food and nutrition security, especially in developing countries, and there is growing recognition of a food-based strategy to tackling it (Maganti *et al.,* 2019). Over three billion people globally, mostly in developing nations, suffer from micronutrient malnutrition, which is made worse by deficits in Fe and Zn (Sperotto *et al.,* 2010; Welch and Graham, 2004). For those between the ages of 25 and 50, the recommended daily allowances (RDA) for Fe and Zn are 10-15 mg and 12-15 mg, respectively (Madhubabu *et al.,* 2020). The use of polished grains, such as rice, wheat, and maize, was the major cause of these deficiencies (Pfeiffer and McClafferty, 2007). Modern high yielding rice varieties have weak sources for the essential micronutrient Fe and Zn (Zimmermann and Hurrel, 2002). Because it contains less Fe (2.14 times less iron, from 8.8 to 4.1 ppm) and Zn (1.83 times less Zn, from 33 to 18 ppm) content than brown rice (Majumder *et al.,* 2019). Zn is necessary for a strong immune system and normal development in humans. Zn deficiency, which affects 45% of primary school going students also 57% of non-pregnant and non-lactating women in Bangladesh, is the most prevalent nutritional shortfall there (Islam *et al.,* 2016).

Over the last ten years, there has been an increase in interest for developing varieties of staple grain crops with greater concentrations of Zn and Fe, to improve the nutritional value of grain for human consumption (Cakmak, 2008; Wissuwa *et al.,* 2008; White and Broadley, 2005). Plant breeders are always attempting to develop new varieties with improved agronomic traits in order to deliver higher grain yields. But currently, while developing new varieties to release, it has been taken into account the assessment of advanced lines for grain quality and nutritional characteristics. In order to collect essential information for both consumers and forthcoming rice breeding programs, this research set out to determine the nutritional content and rice grain quality of some promising lines from the advanced breeding generation of rice.

The current study's major goals are to determine the grain quality and nutritional content (Fe and Zn) of selected rice genotypes. Also, study the genetic advance through genotypic and phenotypic as well as heritability and principal component analysis for all traits under study.

Materials and Methods

The experiment was carried out at the experimental farm and laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), BAU Campus, Mymensingh-2202, Bangladesh during the Aman season of 2021. Randomized complete block design (RCBD) was adopted with three replications. Eleven promising rice lines were developed from BINA and two high yielding check varieties BRRI dhan84 (released from the Bangladesh Rice Research Institute, Gazipur-1701, Dhaka, Bangladesh) and Binadhan-20 (BINA released variety) were used for this study. The methods used in the current inquiry are outlined below, along with the observations that were made (Dela Cruz and Khush, 2000).

Milling percentage: 100-gram of rice sample was washed, dried (14% moisture content), and dehusked. After dehusking, the rice was polished for 45 seconds using a milling machine and the polished rice was then weighed. The milling percentage was determined to be as follows:

$$\text{Milling percentage} = \frac{\text{Weight of polished rice sample}}{\text{Weight of paddy sample}} \times 100$$

Head rice recovery: Whole grain rice was separated and weighed using an electronic balance after the bran was removed during polishing, and the percentage of recovered head rice was estimated as follows:

$$\text{Head rice recovery percentage} = \frac{\text{Weight of head rice}}{\text{Weight of paddy sample}} \times 100$$

Whole grain length (mm): Length of five seeds were taken with the help of a slide caliper and its average length was taken.

Kernel length (mm): Ten randomly selected dehusked whole kernels were measured for length with the help of slide calipers and its average length was taken to find out kernel length. The length of kernel was expressed in millimeter (mm).

Kernel breadth (mm): Five dehusked whole kernels were measured for breadth with the help of a slide caliper and its average breadth was taken to find out kernel breadth. The breadth of kernel was expressed in millimeter (mm).

Kernel length-breadth ratio (KL/KB): KL/KB of various genotypes were determined on the basis of average length-breadth ratio of kernel (Bhattacharjee and Kulkarni, 2000).

$$\text{KL/KB} = \frac{\text{Average kernel length of rice}}{\text{Average kernel breadth of rice}} \times 100$$

The scores are recorded for brown rice to evaluate the traits as genetic characteristics avoiding the effect of milling on size and shape. The rice grains were classified by standard evaluation system (SES) scoring (IRRI, 2013).

Fe and Zn content: Fe and Zn content in brown rice samples was estimated using non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000; Oxford Instruments plc, Abingdon, UK) from International Rice Research Institute (IRRI), Bangladesh office. A non-metallic de-husker was used to de-husk about 10 g of well-dried paddy sample from each genotype (Krishi international 810 de-husker) having roller made of polymer to avoid Fe and Zn contamination. De-husked rice was cleaned by removing broken grains and debris before weighing and transferring 5 g of each sample to sample containers. The sample containers were gently shaken for uniform distribution of samples and kept for analysis. Content of Fe and Zn was expressed in micrograms per gram ($\mu g\ g^{-1}$) or parts per million (ppm) and converted into milligrams per kilogram ($mg\ kg^{-1}$).

Statistical tools: RStudio with required packages, Minitab 18 and Microsoft Excel were used to analyze the data and their graphical visualization.

Results and Discussion

Physical grain quality

Analysis of variance (ANOVA)

Analysis of variance revealed significant ($p < 0.001$) differences among the rice genotypes for grain physical parameters except milling yield percentage (Table 1).

Table 1. Analysis of variance (ANOVA) of physical grain properties of the rice genotypes

Sources of variation	df	Milling yield (%)	Head rice recovery (%)	Whole grain length (mm)	Kernel length (mm)	Kernel breadth (mm)	Kernel length breadth ratio
Replications	2	5.564	4.410	0.005	0.009	0.001	0.003
Genotypes	12	4.325^{ns}	41.637^{***}	1.442^{***}	1.179^{***}	0.013^{***}	0.252^{***}
Error	24	2.620	1.938	0.005	0.005	0.001	0.002

*** indicates significant at 0.1% level of probability, ns = Statistically not significant and df = Degrees of freedom

Least significant difference (LSD) on grain physical properties

The LSD test was done to find out the mean performance among the studied genotypes (Table 2). The whole grain length of the rice genotypes varied from 8.06 mm to 10.15 mm with an average of 9.28 mm. The shortest grain was presented by IZSD-42, and the longest grain was shown by EFSD-59. The kernel length of various rice genotypes ranged from 5.84 mm (IZSD-42) to 8.29 mm (Binadhan-20) with an average value of 6.67 mm. The kernel breadth of various rice genotypes ranged from 2.02 mm (IZSD-42) to 2.20 mm (IZSD-44) with an average value of 6.67 mm and their length breadth ratio ranged from 2.87-4.02. Quality assessment was done based on kernel length and kernel length-breadth ratio. The kernel shape and size of Binadhan-20 and BRRI dhan84 are extra-long (>7.5 mm) and slender (KL/KB > 3.0) (Table 3). The lines EFSD-01, EFSD-59, EFSD-66, IZSD-10, IZSD-30, IZSD-44 and IZSD-67 had the long grain (6.6 to 7.5 mm) and the higher kernel length-breadth ratio (>3.0) indicating that the lines produced long slender grain (Table 3). Other lines produced medium grain length and shape (IZSD-26 and IZSD-42) also medium slender (EFSD-58 and EFSD-21) grain (Rani *et al.*, 2022; Khatoon and Islam, 2020; Maganti *et al.*, 2019). The maximum milling percentage was recorded in IZSD-67 (71.67%) and the minimum was recorded in EFSD-01 (67.67%) with an average 69.95% (Table 2). Significant differences among the genotypes based on head rice recovery percentage showed that the highest value was found in BRRI dhan84 (68.67%) and the lowest value was in EFSD-01 (57.33%) with an average value 63.64% (Pokhrel *et al.*, 2020; Babu *et al.*, 2013).

Table 2. Mean performance of the rice genotypes for determining physical grain properties

Genotypes	MY (%)	HRR (%)	WGL (mm)	KL (mm)	KB (mm)	KL/KB
IZSD-10	70.00 ab	65.33 c	10.12 a	7.05 c	2.19 ab	3.22 d-f
IZSD-26	70.00 ab	66.67 a-c	8.36 g	5.87 h	2.04 fg	2.87 h
IZSD-30	67.67 b	68.33 ab	9.56 c	6.93 d	2.08 de	3.32 bc
IZSD-42	70.67 a	67.00 a-c	8.06 h	5.84 h	2.02 g	2.89 h
IZSD-44	71.00 a	60.33 ef	9.70 b	7.16 b	2.20 a	3.25 de
IZSD-67	71.67 a	64.67 cd	9.70 b	6.88 de	2.16 bc	3.19 ef
EFSD-01	67.67 b	57.33 g	9.15 de	6.62 f	2.05 d-g	3.22 d-f
EFSD-21	70.33 ab	60.33 ef	8.44 g	6.26 g	2.13 c	2.93 h
EFSD-58	70.67 a	59.67 fg	8.97 f	6.54 f	2.07 d-f	3.17 fg
EFSD-59	70.67 a	60.33 ef	10.15 a	7.07 bc	2.09 d	3.38 b
EFSD-66	69.00 ab	66.00 bc	9.10 e	6.64 f	2.03 fg	3.26 cd
Binadhan-20	69.67 ab	62.67 de	10.10 a	8.29 a	2.05 e-g	4.02 a
BRRI dhan84	70.33 ab	68.67 a	9.24 d	6.77 e	2.19 ab	3.10 g
CV(%)	2.31	2.19	0.76	0.99	1.09	1.28
$LSD_{(0.05)}$	2.73	2.35	0.12	0.11	0.04	0.07
Mean	69.95	63.64	9.28	6.76	2.10	3.22
SD (±)	1.20	3.73	0.69	0.63	0.07	0.29
SE (±)	0.33	1.03	0.19	0.17	0.02	0.08
Maximum	71.67	68.67	10.15	8.29	2.20	4.02
Minimum	67.67	57.33	8.06	5.84	2.02	2.87

MY = Milling yield, HRR = Head rice recovery, WGL = Whole grain length, KL = kernel length, KB = kernel breadth, KL/KB = Kernel length-breadth ratio

Table 3. Classification of the rice genotypes based on kernel length and shape (IRRI, 2013)

Kernel length	Kernel shape (length-breadth ratio)	Genotypes
Medium (5.51 to 6.6 mm)	Medium (2.1 to 3.0)	IZSD-26, IZSD-42
	Slender (over 3.0)	EFSD-58, EFSD-21
Extra long (more than 7.5 mm)	Slender (over 3.0)	Binadhan-20, BRRI dhan84
Long (6.6 to 7.5 mm)	Slender (over 3.0)	EFSD-59, EFSD-01, EFSD-66, IZSD-10, IZSD-44, IZSD-30, IZSD-67

Genetic variability, heritability and genetic advance

The genetic variability parameters for all the grain physical properties are presented in Table 4. The results indicated that the genotypes showed a wide range of genetic variability for the grain physical parameters. For almost all the parameters, the phenotypic variances (σ^2p) were higher than the genotypic variances (σ^2g). The maximum genotypic and phenotypic variances were obtained for head rice recovery percentage (13.23 and 15.17, respectively), followed by those for milling yield percentage, whole grain length, kernel length, kernel breadth, kernel length-breadth ratio. Similarly, the estimated phenotypic co-efficient of variation (PCV) of the grain physical parameters were slightly higher than the genotypic co-efficient of variation (GCV) on the expression of the traits studied (Anis *et al.*, 2016). Phenotypic coefficient of variation (PCV) was found to be the highest in kernel length (9.30%) while milling percentage had the least PCV value (2.55%). Similarly, GCV was found to be the highest in kernel length (9.25%), but milling percentage had the least PCV value (1.08%). Similar results were obtained by Paikhomba *et al.* (2014) and Verma *et al.* (2013). The highest heritability was exhibited by whole grain length (98.97%), followed by kernel length (98.86%), kernel length-breadth ratio (98.00%), grain breadth per kernel (88.89%), head rice recovery (87.23%) and milling yield percentage (17.83%). In the current study, the high heritability shown by all the parameters implied that a simple selection approach based on phenotypic characters can be followed in the breeding program to improve the characters of interest of the genotypes (Roy & Shil, 2020; Rathi *et al.*, 2010).

All the studied grain physical traits exhibited a moderate estimate of GA (%) whereas milling yield percentage and grain breadth per kernel showed lower GA (%). The highest GA (%) was exhibited by kernel length (18.95), followed by the kernel length-breadth ratio (18.32) (Table 4). The high estimates of heritability coupled with medium estimates of genetic advance were observed for whole grain length (98.97% and 15.28%), kernel length (98.86% and 18.95%) and kernel length-breadth ratio (98.00% and 18.32%) (Anis *et al.*, 2016; Nayak and Reddy, 2005). Thus, it is interpreted that the traits i.e. kernel length, kernel breadth showed high heritability and moderate genetic advance as percentage of mean rendering them more amenable to improvement through selection than the other characters (Singh *et al.*, 2016).

Table 4. Estimation of genetic variability and parameters of grain quality traits of the rice genotypes.

Traits	σ^2g	σ^2p	GCV (%)	PCV (%)	H^2 (%)	GA	GA (%)
MY (%)	0.57	3.19	1.08	2.55	17.83	0.66	0.94
HRR (%)	13.23	15.17	5.72	6.12	87.23	7.00	11.00
WGL (mm)	0.48	0.48	7.46	7.50	98.97	1.42	15.28
KL (mm)	0.39	0.40	9.25	9.30	98.86	1.28	18.95
KB (mm)	0.00	0.00	3.01	3.19	88.89	0.12	5.85
KL/KB	0.08	0.09	8.98	9.08	98.00	0.59	18.32

MY = Milling yield, HRR = Head rice recovery, WGL = Whole grain length, KL = Kernel length, KB = Kernel breadth, KL/KB = Kernel length-breadth ratio, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, H^2 = Heritability in broad sense, GCV = genotypic co-efficient of variation, PCV = phenotypic co-efficient of variation, GA = genetic advance and GA (%) = Genetic advance as percentage of mean

Relative contribution of different grain physical parameters

Six principal components were obtained through principal component analysis (PCA) and among the six components, two were found with an eigenvalue of more than 1 (Figure 1a). These two components contributed 72.5% of the cumulative variance. PC1 accounted for the highest variance (47.2%), followed by PC2, which accounted for 25.3% of the variation (Figure 1b).

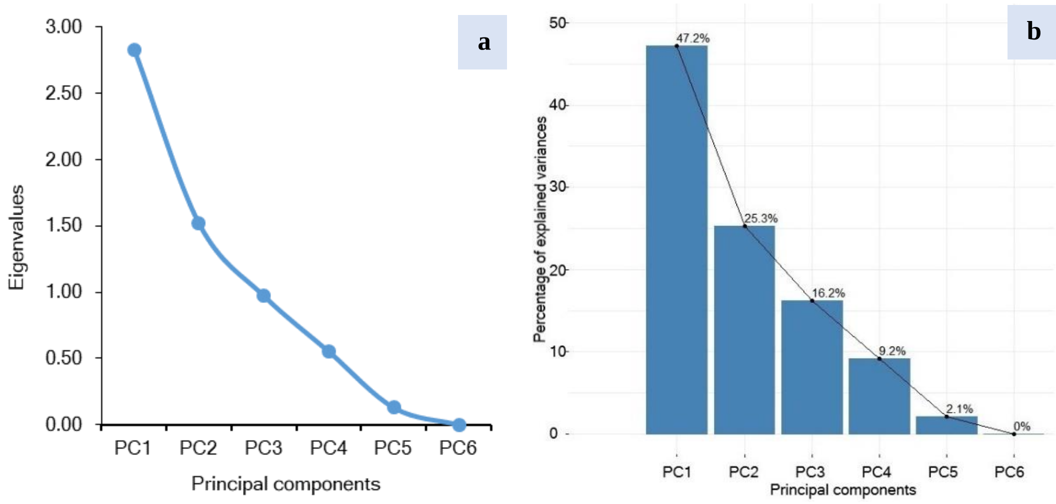

Figure 1. a) Eigenvalues of different principal components (PCs) b) contribution of each principal component to the total explained variance based on the phenotypic traits of the rice genotypes.

The characters that together contribute to form a principal component should be emphasized and taken into consideration in breeding programs because they tend to be exploited together (Chakravorty *et al.*, 2013). Kernel length-breadth ratio contributed highly to creating the variability in the first two principal components, which were responsible for 72.5% of the variation present in the study population. These two characters could be considered during parental selection to start a breeding program for improving the grain physical properties of rice genotypes.

The results of PCA and vector loading in Table 5 showed that kernel length (0.58) had the highest contribution to the first principal component. Whole grain length (0.55), kernel length-breadth ratio (0.54) and kernel breadth (0.18) also positively contributed to PC1 whereas milling yield percentage (-0.03) and head rice recovery percentage (-0.16) contributed negatively. PC2 was positively contributed by the kernel breadth (0.68), milling yield percentage (0.67), whole grain length (0.12) and head rice recovery percentage (0.05), whereas kernel length (-0.04) and kernel length breadth ratio (-0.26) contributed negatively. The contribution of different traits to the genotypes in the first two principal components are shown in Figure 2.

Table 5. Principal component values for six grain physical traits of the rice genotypes

Traits	PC1	PC2	PC3	PC4	PC5	PC6
MY (%)	-0.03	0.67	-0.19	-0.71	0.09	-0.0055
HRR (%)	-0.16	0.05	0.96	-0.21	0.01	0.0004
WGL (mm)	0.55	0.12	0.11	0.16	0.80	-0.0001
KL (mm)	0.58	-0.04	0.07	-0.13	-0.38	-0.6997
KB (mm)	0.18	0.68	0.12	0.56	-0.36	0.2264
KL/KB	0.54	-0.26	0.03	-0.32	-0.28	0.6776

MY = Milling yield, HRR = Head rice recovery, WGL = Whole grain length, KL = kernel length, KB = kernel breadth, KL/KB = Kernel length-breadth ratio, PC = Principal component

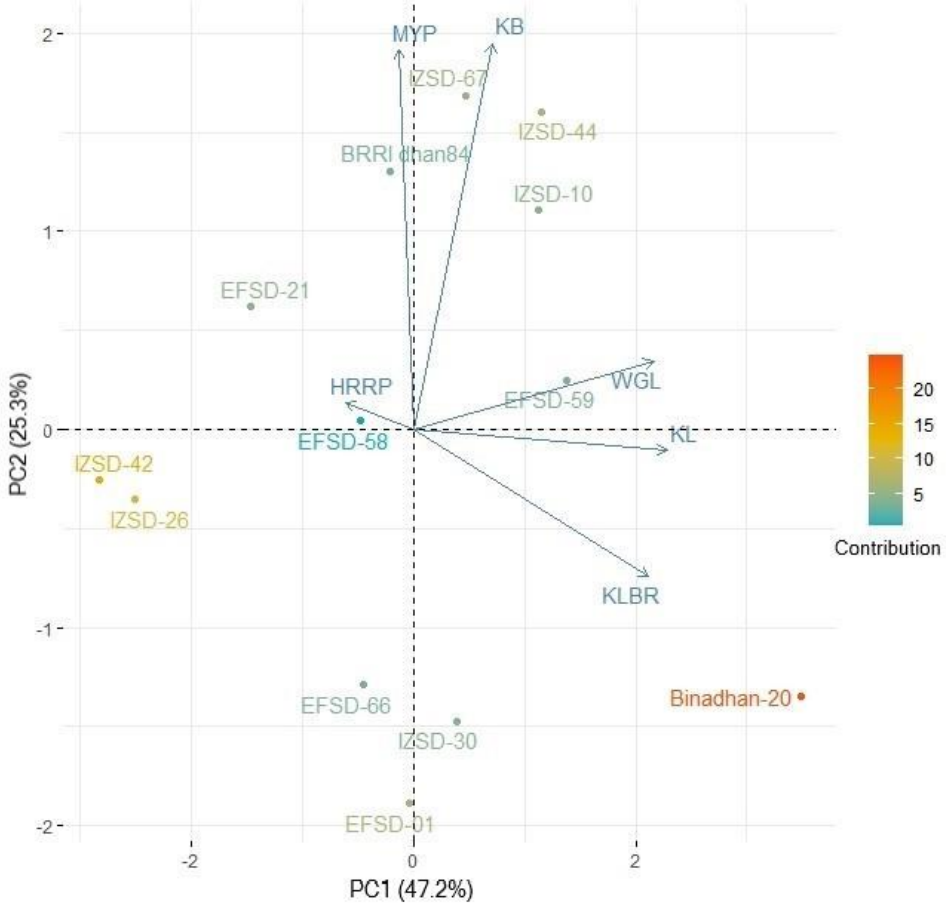

Figure 2. Biplot on main two dimensions based on the grain quality traits of the rice genotypes and the contribution of the variables.

MYP= Milling yield percentage, HRRP= Head rice recovery percentage, WGL= Whole grain length, KL= Kernel length, KB= Kernel breadth, KLBR= Kernel length-breadth ratio, PC= Principal component. Right sided legend means contributions of the variables to the genotypes.

Clustering of the genotypes

Mahalanobis distance analyzed by D^2 statistics were applied to elucidate the genetic divergence among the genotypes. Modified Tocher's method was used to group the genotypes. The genotypes were grouped into five clusters on the basis of the six grain physical traits (Table 6). Cluster I and IV were the larger and included four genotypes. Cluster II, which included three landraces, was the next-larger cluster. Cluster III and V included one genotypes. The maximum intra-cluster distance (D) was found in cluster IV (D = 3.00) followed by that in cluster I (D = 2.88) (Table 7). The intra-cluster distance was 0.00 in cluster III and V because both of the clusters included only one genotype. The maximum inter-cluster distance was found between clusters I and V (D = 6.44), followed by that between clusters IV and V (D = 4.99). The minimum inter-cluster distance was obtained between clusters II and IV (D = 3.98).

Mean values of the clusters of six characters indicated a wide range of variation among the characters (Table 6). The maximum mean value of the kernel length-breadth ratio (4.02), whole grain length (10.10) and kernel length (8.29) were shown by cluster V. Cluster III showed the minimum mean values for rest of the three traits.

Table 6. Clusters' mean with Tukey's honest significant ($p < 0.05$) test based on the traits of the rice genotypes.

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
MY (%)	70.42 ab	71.11 a	67.67 b	69.25 ab	69.67 ab
HRR (%)	63.42 a	61.78 a	57.33 a	67.08 a	62.67 a
WGL (mm)	8.45 b	9.85 a	9.15 ab	9.51 a	10.10 a
KL (mm)	6.13 c	7.04 b	6.62 bc	6.85 b	8.29 a
KB (mm)	2.07 a	2.15 a	2.05 a	2.12 a	2.05 a
KL/KB	2.96 c	3.27 b	3.22 bc	3.23 bc	4.02 a

MY = Milling yield, HRR = Head rice recovery, WGL = Whole grain length, KL = kernel length, KB = kernel breadth, KL/KB = Kernel length-breadth ratio, PC = Principal component

The maximum mean values of kernel breadth (2.15 mm) and milling yield percentage (71.11%) were found for cluster II. However, the minimum whole grain length (8.45 mm), kernel length (6.13 mm), kernel length-breadth ratio (2.96) were shown by the cluster I. The maximum head rice recovery percentage (67.08%) was found in cluster IV. A short inter-cluster distance implies that the cluster members are closely related (Dhakal *et al.,* 2020). In this study, Cluster II and IV had the minimum distance indicating genotypes of these clusters were closely related. Cluster II included EFSD-59, IZSD-44 and IZSD-67 (Figure 3). These three genotypes have good physical characteristics viz. long slender grain, milling yield percentage and head rice recovery >60% etc. whereas cluster IV included the genotypes BRRI dhan84, IZSD-10, IZSD-30 and EFSD-66 those genotypes have similar traits. So, these lines could be selected for releasing a new variety. Widely distant clusters represent genotypes with a wide genetic distance. So, hybridization should be conducted between the genotypes from widely distant clusters (clusters I and V) to develop a desirable genotype (Figure 3). Parental selection in a hybridization program should be done based on the highest genetic divergence to obtain a wide range of variability and transgressive segregations with the highest heterotic effect (Chandra *et al.*, 2007).

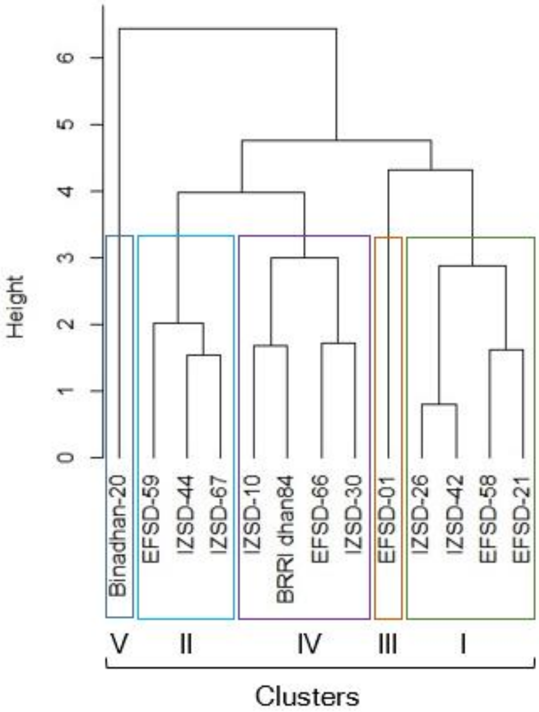

Figure 3. Euclidean distance of the genotypes shown in a dendrogram.

Table 7. Average intra-cluster (diagonal bold values) and inter-cluster distances (D= $\sqrt{D^2}$ alues) of the rice genotypes.

Clusters	I	II	III	IV	V
I	**2.88**	4.76	4.31	4.58	6.44
II		**2.02**	4.28	3.98	4.41
III			**0.00**	4.35	4.63
IV				**3.00**	4.99
V					**0.00**

Fe and Zn determination:

Analysis of variance revealed significant ($p < 0.001$) differences among the rice genotypes for Fe and Zn content in rice grain at unpolished and polished conditions. (Table 8).

Table 8. Analysis of variance (ANOVA) of Fe and Zn content in rice grain at unpolished and polished conditions

Sources of variation	df	Unpolished Rice Zn	Polished Rice Zn	Unpolished Rice Fe	Polished Rice Fe
Replications	2	7.256	2.205	1.000	0.308
Genotypes	12	39.632***	207.641***	14.077***	18.590***
Error	24	7.979	53.128	2.750	0.224

*** indicates significant at 0.1% level of probability and df = Degrees of freedom

Least significant difference (LSD) on grain nutritional quality

LSD test separately analyzed to check the mean performance of the individual genotypes (Table 9). The genotypes showed significantly ($p < 0.05$) decrease in Fe and Zn content when rice grain were polished (Figure 4 and Figure 5). The Zn content in unpolished rice varied from 33.33 to 44.33 mg kg^{-1} with an average of 41.44 mg kg^{-1}. The lowest Zn content was found in IZSD-30 and the highest was found in IZSD-42 and EFSD-01. Average Zn content was found 23.97 mg kg^{-1} in polished rice grain where 27.67 mg kg^{-1} (EFSD-66) was the highest and 20.33 mg kg^{-1} (EFSD-21) was the lowest values. As like in unpolished rice, highest and lowest Fe content was found in EFSD-59 (13.33 mg kg^{-1}) and

Table 9. Combined effects of rice genotypes on unpolished and polished grain Fe and Zn content.

Genotypes	Unpolished rice grain Zn content (mg kg^{-1})	Polished rice grain Zn content (mg kg^{-1})	Unpolished rice grain Fe content (mg kg^{-1})	Polished rice grain Fe content (mg kg^{-1})
IZSD-10	38.67 bc	21.00 fg	8.33 c-e	4.33 c
IZSD-26	43.00 ab	24.00 c-e	13.00 a	7.33 a
IZSD-30	33.33 d	24.00 c-e	10.67 a-c	1.67 d
IZSD-42	44.33 a	23.00 d-f	9.67 b-d	1.00 de
IZSD-44	43.67 a	27.33 ab	10.67 a-c	5.33 b
IZSD-67	44.00 a	21.67 e-g	10.00 b-d	0.00 f
EFSD-01	44.33 a	21.33 fg	6.00 e	3.67 c
EFSD-21	36.00 cd	20.33 g	12.00 ab	0.00 f
EFSD-58	43.67 a	25.00 b-d	10.67 a-c	0.67 ef
EFSD-59	43.67 a	26.33 a-c	13.33 a	0.00 f
EFSD-66	38.33 bc	27.67 a	7.67 de	0.00 f
Binadhan-20	42.00 ab	25.33 a-d	8.67 c-e	4.00 c
BRRI dhan84	43.67 a	24.67 cd	12.33 ab	0.00 f
CV(%)	6.82	6.21	16.21	21.97
$LSD_{(0.05)}$	4.76	2.51	2.80	0.80
Mean	41.44	23.97	10.23	2.15
SD (±)	3.63	2.40	2.17	2.49
SE (±)	1.01	0.67	0.60	0.69
Maximum	44.33	27.67	13.33	7.33
Minimum	33.33	20.33	6.00	0.00

CV = Coefficient of variation, LSD = Least significant difference at 5% level of probability, SD = Standard deviation and SE = Standard error

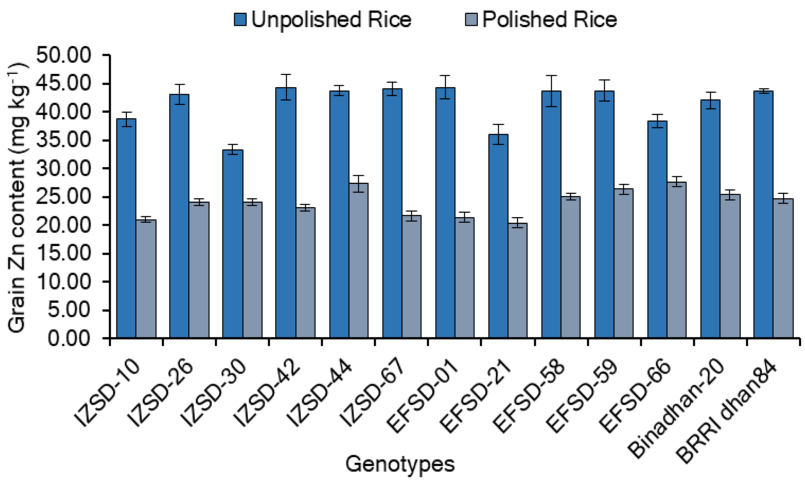

Figure 4. Grain Zn content in unpolished and polished rice of the eleven genotypes along with check varieties. Standard error indicated by error bars and lettering was done at 5% level of Tukey's honest significant difference test.

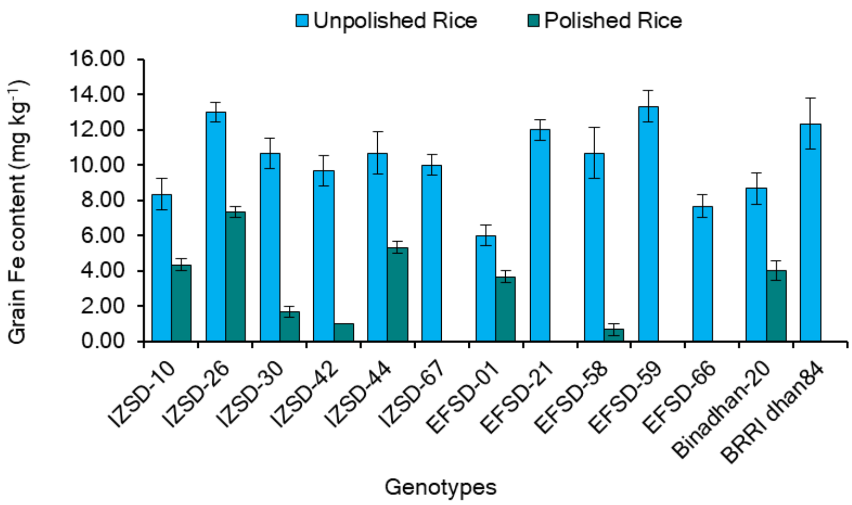

Figure 5. Grain Fe content in unpolished and polished rice of the eleven genotypes along with check varieties. Standard error indicated by error bars and lettering was done at 5% level of Tukey's honest significant difference test.

EFSD-01 (6.00 mg kg^{-1}), respectively with an average value 10.23 mg kg^{-1}. Besides, Fe content in polished rice was not found in the genotypes IZSD-67, EFSD-21, EFSD-59, EFSD-66 and BRRI dhan84 and the maximum value was 7.33 mg kg-1 found in IZSD-26. Higher heterogeneity in Fe and Zn levels between the genotypes were observed following polishing. In contrast, Fe loss (~60 to 80%) was almost double at 10% loading throughout the grain shapes compared to Zn (~20 to 40%). Gregorio (2002) also observed more loss of Fe than Zn during polishing. This might be owing to a loss of embryos and aleurons, partly or total, in the polishing process; the embryo has more Fe followed by aleuronic layer with endosperm (Gregorio, 2002), variation in aleuron or embryo layer thickness or both, etc. Almost all the genotypes had higher Fe and Zn content than the check varieties. Anuradha *et al.* (2012) reported that they analyzed brown rice of 126 accessions of rice genotypes for Fe and Zn content using Atomic Absorption Spectrophotometer (AAS). The analyzed results of 100 germplasm of rice for Fe and Zn content using ED-XRF method performed by Chandu *et al.* (2020). They found that Fe concentration varied from 1.6 to 15.2 ppm whereas Zn concentration ranged from 6.2 to 33.2 ppm of the tested germplasms.

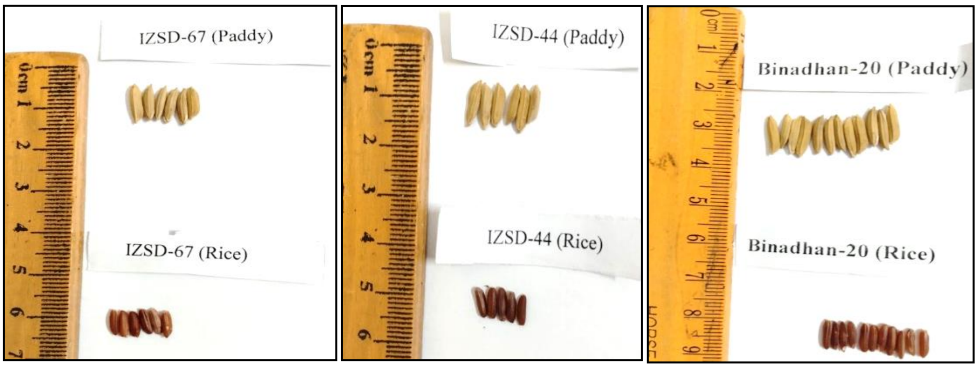

Figure 6. Pictorial view of paddy, brown rice of Fe and Zn enriched genotypes (IZSD-67, IZSD-44) along with check variety Binadhan-20.

Conclusions

Recently farmers are interested in new varieties that are comparably outstanding in yield as well as in improved grain quality, in order to preserve the integrity of new rice varieties. Significant variation was present among the studied rice genotypes. Heritability and genetic variability studies indicated that the grain physical properties of the genotypes were less influenced by the environment. PCA revealed that kernel length, kernel breadth as well as kernel length-breadth ratio contributed together to the formation of variation among the genotypes. Closely related clustered genotypes have good physical characteristics viz. long slender grain, milling yield percentage and head rice recovery percentage more than 60% etc. So, EFSD-59, IZSD-44, IZSD-67, IZSD-10, IZSD-30 and EFSD-66 could be selected for releasing a new variety in future. Widely distant clustered formed by IZSD-26, IZSD-42, EFSD-58, EFSD-21 and Binadhan-20 could be used as parents in hybridization programs aiming to improve the grain characters of rice, and direct progeny selection might be helpful to plant breeders.

Based on micronutrient (Fe and Zn) concentration analysis EFSD-66, IZSD-44, EFSD-59, EFSD-58 contains higher Zn and IZSD-26, IZSD-10, IZSD-44 contains higher Fe than the check varieties Binadhan-20 and BRRI dhan84 in polished rice. Considering physical grain quality and Fe, Zn content, the advanced lines EFSD-66, IZSD-44, EFSD-59, EFSD-58, IZSD-26, IZSD-67 and IZSD-10 could be used in breeding to develop premium quality nutrient enriched rice varieties in order to ensure nutritional security in Bangladesh. Furthermore, rest of the genotypes having good grain quality and Fe and Zn content can be used as plant breeding materials in future rice breeding programs.

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