AGRO-MORPHOLOGICAL CHARACTERIZATION OF COLOURED RICE GENOTYPES FOR GRAIN YIELD

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Abstract

Coloured rice (*Oryza sativa* L.) has become increasingly popular recently as a functional food because of its nutritional and health benefits. Effective selection criteria can be developed using characteristics contributing to genetic diversity in a particular group. This study characterised and evaluated the yield performance of 33 Bangladeshi coloured rice genotypes, 31 traditional and two checks, in the *Kharif*-1 (March - July) season of 2021 following standard cultivation procedure. Eight of the 33 genotypes did not flower during the *Kharif*-1 season; all other genotypes showed substantial differences in yield and yield attributing descriptors. Based on the yield attributing traits, the genotypes were divided into two broad clusters. In the Principal component analysis, the traits - flag leaf length and width, panicle length, number of primary and secondary branches, and filled grains per panicle, had maximum contribution towards the total variability. Among the genotypes, Bandarban-3 produced the maximum grain yield (32.62 g/hill) , and some others had higher or comparable grain yield with the check Binadhan-20. These coloured rice genotypes might be added for multilocation trials to recommend for cultivation in the *Aus* season in Bangladesh.

Introduction

Coloured rice (*Oryza sativa* L.), often known as red, brown, pigmented, or black rice, has gained popularity recently as a functional food due to its nutritional and health benefits (Kushwaha 2016, Ghasemzadeh *et al*. 2018, Ito and Lacerda 2019, Husnah *et al*. 2024). Paddy consists of husk, bran/outer coating, embryo and endosperm. At the same time, the endosperm contains predominantly starch, bran and embryo that are rich in nutrients and phytochemicals of medicinal value. Endosperm is usually white or rarely appears black; while bran may be brown, red or black due to its high content of anthocyanin and other compounds which are reported to decrease in chronic and degenerative diseases due to their antioxidant, anti-inflammatory, anti-cancer and hypoglycemic activities (Hamid *et al*. 2010, Gani *et al*. 2012, Pratiwi and Purwestri 2017). Coloured rice is rich in proteins, fibre, vitamins, minerals, and antioxidants (Qiu *et al*. 1993, Gani *et al*. 2012, Ferdousi 2022). It also contains some essential amino acids, vitamins, and minerals (Qiu *et al*. 1993, Rachel *et al*. 2013, Chanu *et al*. 2016). Black rice bran contains a higher number of phenolic acids and flavonoids (Ghasemzadeh *et al*. 2018).

Although experiments on grain quality, chemical composition, and nutritional and phytochemical properties of coloured rice are available in the literature (Qiu *et al*. 1993, Rachel *et al*. 2013, Ziegler *et al*. 2017, Ito and Lacerda 2019, Priya *et al*. 2019), there is no notable published research works on nutritional composition and phyto-constituents related to health benefit of the above traits of coloured rice in Bangladesh. Although the evaluation of phenotypic diversity and the development of strategies to maximise rice yield potential have often relied on

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morphological and yield-associated features as criteria. Hitherto, little (of only one genotype) is known about the morphological and yield descriptors of Bangladeshi coloured rice cultivars (Kuddus *et al*. 2020, Nahar *et al*. 2021, Khatun *et al*. 2022). This research was, therefore, conducted to characterize and evaluate the yield performance of some of the coloured rice genotypes of Bangladesh, as a part of a project entitled "Collection and Evaluation of Coloured Rice Germplasm for Yield Quality, and Phytonutrients of Health Benefit".

Materials and Methods

A semi-structured questionnaire was used to collect basic information on coloured rice. Communication with the Department of Agriculture Extension personnel, scientists from Bangladesh Rice Research Institute (BRRI) and relevant farmers was established in 2020. Seeds of coloured rice genotypes were collected from southern & eastern parts, and hilly areas of Bangladesh, BRRI, and Bangladesh Institute of Nuclear Agriculture. The collected 33 paddy genotypes were tested for seed quality, dried and preserved for future investigations (Table 1).

A pot experiment was conducted at the Pot House, Department of Crop Botany, Bangladesh Agricultural University from 13 April to 26 July 2021 (*Kharif*-1 season) following a completely randomized design (CRD) with three replications. Standard cultural protocols were followed (BRRI 2020).

The "Descriptors for Wild and Cultivated Rice (*Oryza* spp.)" was followed for the characterization methodology (Anonymous 2007). Seed (spikelet) and grain morphological data *viz*. colour, presence or absence of awn, hairiness, length, width, and thickness of spikelet/grain, was observed and/or measured. Plant height, phenological data, yield and yield attributes were recorded.

Data were analyzed using Statistix 10 and means were presented by Duncan's Multiple Range test (DMRT). Agglomerative Hierarchical Clustering (AHC) dendrogram and Principle component analysis (PCA) were performed using XLSTAT software <<https://www.xlstat.> com/en/>.

Table 1. Spikelet and grain colour of 33 rice genotypes.

Results and Discussion

In rice, spikelet and grain colours are commonly golden yellow and white/translucent, respectively. In this study along with the common golden yellow colour, three more coloured, *viz*. reddish (5 genotypes), black (2 genotypes), and yellow with black stripped (1 genotype), spikelets

were observed among the collected rice genotypes (Table 1). Moreover, 4 genotypes had black, 20 red/reddish, and the rest 9 had white grains (Fig.1). The disparity in grain colour, and more especially in the colour of the pericarp of the kernel and aleurone pigments (Husnah *et al*. 2024), may be caused by variations in the presence (amount) of antioxidant, anthocyanin, and other pigments in grains (Ghasemzadeh *et al*. 2018).

Fig. 1. Grain colour of different rice genotypes.

Genotypic variation in the awn and hairiness of the spikelet were also observed (Table 2). An increased level of hairiness on the spikelet was present in six awned (having an awn) genotypes compared to the others. The presence or absence and size of hairs/trichomes could be used for varietal identification and classification (Chang and Bardenas 1965). Awns tend to enhance photosynthesis and supply assimilates for grain filling, thus increasing grain yield, particularly in situations where water and temperature stress are present (Ntakirutimana and Xie 2020). The presence of an awn on grain can also protect it from animals like birds; cultivars with a smaller awn are typically more extensively grown as they are easy to harvest (Kurniasih *et al*. 2019). The spikelet length (L), width (W) and thickness (T) varied from 7.2-11.2, 2.18-3.56 and 1.55-2.21 mm, respectively. The L/W, L/T and W/T ratios ranged from 2.57-4.73, 3.49-5.57 and 1.22-1.64, respectively (Table 2). The length and width of spikelets could be used for distinguishing modern and traditional cultivars; moreover, width and thickness were significantly correlated with 1000 grain weight (Sarwar *et al*. 1999). These criteria might be used as selection criteria for higher grain yield of rice.

In size classification, grains of all the rice genotypes could be classified as extra-long except Bandarban-7, Khagrachari-8 and 9. These have long (length) grains. In shape classification, grains of all the rice genotypes could be classified as slender except Bandarban-3, 4 and 7, Vojon, Faridpur-2, 3, 4 and 6, and Khagrachari-8 and 9. These are classified as medium grains (IRRI 1996). Both the (extra-)long and slender grains have good market acceptability and higher prices. Breeders can exploit these descriptors with higher yield potential for the development of new cultivars.

Plant height, days required to 50% panicle initiation and flag leaf size showed significant variation (Table 3). Among 33 rice genotypes, 8 didn't flower during the study period, which might be due to the photoperiod sensitivity of these genotypes. Plant stature is a genetically controlled feature however, it is affected by environmental and management variables. Plant height ranged from 80.18-129.54 cm, days required to 50% panicle initiation 73.00-96.33, flag leaf length 30.78-55.61 cm and flag leaf width 0.58-1.41 cm. Plant height is a significant characteristic as it is directly related to plant lodging. Generally, the majority of farmers like to grow shorter rice than taller genotypes, as taller rice collapses more readily than shorter rice. The Green Revolution was mainly associated with the development of cultivars with short stature (90-

110 cm) that were less susceptible to lodging when heavily fertilized especially, with nitrogen (Yoshida 1981). However, when contending with weeds, taller plants, as opposed to smaller ones, have an advantage. Most of the studied rice genotypes are intermediate stature (100-130 cm) which is considered desirable over short stature (90-110 cm) (Yoshida 1981). The days required for 50% panicle initiation are significantly correlated with the maturity duration of the respective genotype. Genotypes that required 75-83 days for 50% panicle initiation had a total lifespan of 98- 104 days (data not shown here). Moreover, genotypes required longer periods for 50% panicle initiation like Binadhan-20 possessed a longer lifespan of 125-130 days [\(https://bina.gov.bd/](https://bina.gov.bd/) accessed on September 20, 2024). Low sink capacity during grain filling becomes a primary cause

of the antagonistic relation between long duration and harvest index (Kakar *et al*. 2021). The flag leaf serves as the primary organ for photosynthesis and the primary source of absorption needed for plant growth and panicle formation (Tian *et al*. 2015). The longest and widest flag leaves were

found in Khagrachari-6 and 7, respectively (Table 3), though these were not the best yielders. The flag leaf's size, photosynthetic capability, and its association with yield-related features are determined by genetic variables (Tang *et al*. 2018). In rice, the weight of thousand grains, the weight of grains within a panicle, and other yield-related parameters are also correlated with the size of the flag leaf (Wang *et al*. 2020).

Fig. 2. Agglomerative Hierarchical Clustering dendrogram based on the average Euclidean Distance estimated from 13 quantitative agro-morphological traits of 25 coloured rice genotypes. Ban Bandarban, Far Faridpur, Kha Khagrachari, Paha Pahari, B Beguni, S Sada.

Significant variations in yield traits, *viz*. panicle length, number of primary (1º) and secondary (2º) branches per panicle, number of filled grains per panicle, 1000-seed weight, and seed yield per plant, were recorded. Panicle length varied from 21.73-32.83 cm, number of primary (1^0) branches per panicle 7.83-15.58, number of filled grains per panicle 73.42-227.47, 1000-seed weight from 15.52-35.55 g and grain yield 13.53-32.62 g/hill (Table 3). Panicles per unit area, number of spikelets per panicle, spikelet weight, and spikelet sterility or grain fullness are the primary components of yield. Among the yield components, the variation in grain yield is influenced by the order of panicle number > spikelet sterility > spikelet weight (Fageria 2007). The percentage of unfilled grains per panicle varied from 7.37-38.29%. Nine genotypes produced more than 20% of unfilled grains per panicle including the genotype Bandarban-3 (22.24%) which was much higher than the average maximum limit of 15% (Yoshida 1981); however, the presence of a much higher (54.45%) number of unfilled grains per panicle was reported by Husnah *et al*. (2024). Therefore, there is a huge scope for an increase in grain yields of these genotypes through source-sink manipulation. Among the genotypes, Bandarban-3 produced the highest grain yield

(32.62 g/hill) followed by Bandarban-4 (28.60 g/hill) and the lowest in Khagrachari-7 (13.53 g/hill). The presence of awn might be one of the causes of the higher yield of Bandarban-3, as discussed in the preceding paragraphs. Moreover, more than 50% of coloured rice genotypes produced comparable or higher yields with the check cultivar Binadhan-20 (Table 3). The variability in the current study material could be effectively applied through various breeding initiatives to enhance the genetic makeup of already available high-yielding varieties and/or in future. Therefore, there is a huge scope to exploit these potentials at the (commercial) field level for premium quality rice production.

Fig. 3. PCA graph of 25 coloured rice genotypes of 13 quantitative agro-morphological traits. Ban Bandarban, Far Faridpur, Kha Khagrachari, Paha Pahari, B Beguni, S Sada.

Genotype classification for plant improvement programs can be effectively achieved by using cluster analysis to examine group diversity (Thakur and Sharma 2023). Genetic distance between parents increases variability in segregating generations, making the genetic variety of parents crucial for producing variability. Two clusters were created in the Euclidean distance-based cluster analysis for the yield and yield attributing features (Fig. 2). The smaller Cluster I, which is made up of Khagrachari-10, Khagrachari-5, Bandarban-6, Khagrachari-2, Khagrachari-7, Khagrachari-6, and Bandarban-5, is one of the clusters that is more diversified. A mutation can alter an organism's phenotype, which is typically linked to genetic alterations in that organism (Husnah *et al*. 2024). Furthermore, the interaction of genes and environment can also result in disparities in outcomes.

Principal component analysis (PCA) is a statistical analysis technique that simplifies complex issues and makes their analysis understandable by reducing the dimensionality of numerous variables (Wang *et al*. 2022). PCA also measures the contribution and importance of each trait to the total variance, was used to investigate the connection between rice genotypes and yield and yield attributing attributes (Fig. 3). It also highlights the features with the greatest variability to carry out rigorous selection programs for the quick development of yield and quality-related attributes. Out of thirteen, five principal components (PCs) exhibited an Eigenvalue of more than 0.8 and accounted for 85.09% (PC1: 41.3%, PC2: 16.6%, PC3: 12.1, PC4: 9.2, PC5: 5.9) of the total variation in phenotype (Table 4). The number of genotypes and attributes considered for PCA may have contributed to the changing number of PCs needed to explain the total trait variation

Table 4. Contribution (Cont.) of the variables (%) and correlations (Cor.) between variables and factors to the principal components.

	F1		F2		F ₃		F4		F ₅	
Eigen value	5.781		2.321		1.689		1.289		0.831	
Variability (%)	41.296		16.581		12.064		9.207		5.939	
Cumulative %	41.296		57.876		69.940		79.148		85.087	
	Cont.	Cor.	Cont.	Cor.	Cont.	Cor.	Cont.	Cor.	Cont.	Cor.
Plant height	8.19	0.69	1.83	-0.21	0.01	0.01	0.91	-0.11	29.97	0.49
Total tiller	7.62	-0.66	17.93	0.65	3.11	0.23	0.56	-0.09	2.47	0.14
Effective tiller	5.16	-0.55	14.51	0.58	7.58	0.36	13.98	-0.42	1.40	0.11
Non-effective tiller	2.20	-0.36	2.360	0.23	4.07	-0.26	45.20	0.76	1.20	0.10
Panicle initiation	6.41	0.61	10.29	0.49	0.55	-0.10	10.56	-0.37	2.11	-0.13
Flag leaf length	12.29	0.84	0.03	0.03	0.60	-0.10	0.16	0.04	3.99	0.18
Flag leaf width	6.69	0.62	13.31	-0.56	0.66	-0.11	0.61	-0.09	7.41	0.25
Panicle length	8.57	0.70	0.71	0.13	18.73	0.56	0.85	-0.10	6.10	0.23
Primary branch	11.94	0.83	0.001	0.004	1.88	-0.18	4.36	-0.24	15.42	-0.36
Secondary branch	11.28	0.81	6.67	0.39	2.67	0.21	7.89	0.32	0.89	-0.09
Filled grain	9.31	0.73	4.92	0.34	6.90	0.34	9.92	0.36	0.99	0.09
Unfilled grain	9.37	0.74	8.22	0.44	0.59	-0.10	1.40	0.13	12.37	-0.32
1000 -seed wt.	0.11	0.08	15.52	-0.60	13.81	0.48	0.00	-0.00	14.25	-0.34
Seed yield	0.85	-0.22	3.71	-0.29	38.84	0.81	3.598	0.215	1.435	-0.109

(Rahangdale *et al*. 2021, Wang *et al*. 2022, Thakur and Sharma 2023). For example, Wang *et al*. (2022) reported that the top eight PCs represented 80.80% of the variation in the 18 quality-related traits. In terms of yield and yield attributes associated with genotype separation in rice, PC1 made a greater contribution. The PC1 contributed more towards yield and related traits to the separation of rice genotypes and captured the variability for flag leaf length (12.29%), primary branch count (11.94%), and secondary branch count (11.28%) per panicle. The PC2 was able to explain 16.6% of the difference across genotypes mostly contributing to the total number of tillers (17.93%), 1000-seed weight (15.52%), effective tiller (14.51%), flag leaf width (13.31%), and days to panicle initiation (10.28%). According to Thakur and Sharma (2023), the characteristics that contributed the most to the overall variability on the principal component analysis were days to 50% flowering, days to maturity, filled grains per panicle, biological yield, harvest index, and days to maturity. Plant height (0.69) , days to panicle initiation (0.61) , flag leaf length (0.84) and width (0.62), panicle length (0.7), number of primary branches (0.83), secondary branches (0.81), filled (0.73) and unfilled (0.74) grains per panicle were all positively correlated with PC1 (Table 4). On the other hand, the number of total (-0.66), effective (-0.55), and non-effective (-0.34) tillers per

hill were negatively correlated (with PC1). Positive correlations with PC2 were found for the total (0.65) , effective (0.58) , days to panicle commencement (0.49) , and unfilled (0.44) grains per panicle (Table 4). Spikelet density, spikelet fertility, number of tillers per plant, and panicle weight per plant could be considered for choosing high-yielding rice varieties (Rahangdale *et al*. 2021). The genotypes did not form separate groups, as seen by the scatter graph of yield and yield attributing features derived using principal components (Fig. 3), which revealed a wide dispersion among the genotypes (Kakar *et al*. 2021).

In general, the rice genotypes under investigation showed significant variation in the morphological and yield descriptors; however, none of the germplasms was shown to be better than the rest for all the studied descriptors. According to PCA analysis, yield-related traits are more significant than morphological variables when it comes to evaluating the total phenotypic variability and genotype characterization. Trait-based breeding may benefit from the utilized genotypes and diversity.

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