Full Length Article

Resolution of Genetic Variability and Selection of Novel Genotypes in EMS Induced Rice Mutants Based on Quantitative Traits through MGIDI

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Abstract

Increased yield potential in rice has been a key genetic improvement problem that requires vast genetic variability and ethyl methanesulfonate (EMS) has the capability to advance plant breeding issues by creating new variants. The production of mutants followed by their characterization provides a powerful tool for selecting genotypes with desirable features. The goal of this work was to induce mutations in the background of a mega rice variety, BRRI dhan28 to create novel variants and to select high performing mutants with multiple traits. Descriptive statistics and analysis of variance reported a vast spectrum of heterogeneity among the mutants for all the 16 quantitative traits. Projected heritability and genetic variance figured that almost all the traits were highly heritable and had a complex inheritance pattern. The tillers hill^{-1}, primary branches hill^{-1}, grains panicle^{-1}, straw yield hill^{-1}, and harvest index were shown to be highly linked with grain yield, implying that direct selection based on these traits may be sufficient for improving other attributes. Besides, a heat map was employed to assess the mutants' similarity matrix. The first two principal components explicated around 41.62% of the overall variation, and the biplot of genotype by trait identified superior genotypes with favorable combinations of grain yield hill^{-1} with total tillers hill^{-1}, effective tillers hill^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, and straw yield hill^{-1}. The results of the multi-trait genotype-ideotype distance index (MGIDI) revealed that only ten mutants out of a hundred performed significantly better. These findings validate the efficiency of EMS in inducing mutations in rice, and the selected mutants can be exploited in future rice improvement efforts. © 2022 Friends Science Publishers © 2022 Friends Science Publishers

Introduction

Rice is the world's second most-produced cereal, and is serving as a staple food source for more than half of the world's population (Pandey 2020). The geometric growth rate of the global population compels increased rice production, predominantly in countries where rice is the principal food source (Hossain *et al.* 2021). Asian rice consumption is forecasted to account for 67% of the overall increase from 388 million tons in 2010 to 465 million tons in 2035 (Mohanty *et al.* 2013) and to around 750 million tons by 2050 (Pathak *et al.* 2018). So, rice production must be increased to meet the increased future demands. The production has increased by the expansion of genetic stocks through selection among improved cultivars or by improving mega varieties by modifying different characteristics. But, this frequently results in a narrow genetic base, limiting the possibilities for recombination and genetic segregation. Inducible mutation is a prime artificial source of variations

for crop improvement (Naeem *et al.* 2015) through generating variability in economically important traits among the mega varieties. Mutation efficiencies are higher in chemical or physical mutagens but chemical mutagens are frequently used for plant mutagenesis as this mutagen group could be useful as a complementary approach in creating useful heritable mutations (Ul-Allah *et al.* 2019). The most commonly used chemical mutagen is ethyl methane sulfonate (EMS; $CH_3SO_2OC_2H_5$) because of the high frequency of single nucleotide changes produced by alkylation of specific nucleotides (Viana *et al.* 2019). It differently affects the physiology, anatomy, biochemistry, and morphology of plants by generating a relatively high density of irreversible mutations (Talebi *et al.* 2012).

The BRRI dhan28 is the most popular and widely distributed rice variety in Bangladesh due to its short duration, resistance to pests, high nutrient use efficiency, medium slender grain (brri.org), fair market

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value, high yield with moderate tolerance to saline (Islam *et al.* 2016）and outstanding cooking and eating quality. Due to its high acceptance and popularity, it is a mega variety （Salam *et al.* 2019）. So, this variety deserves special attention in generating variation through mutagenesis for future breeding programs. Improving genetic variability in this variety would be beneficial in addressing some existing defects of this variety. Besides, its excellent combining ability (Hossain and Kamruzzaman 2013) will aid in the transfer of such traits into other varietal backgrounds. Since morphological mutants play an important role in modifying varietal characteristics and the development of new plant varieties, quantitative traits are thought to be an efficient way to detect changes and identify novel mutants.

Proficient plant breeders frequently try to integrate a set of exclusive traits into a single genotype that would result in superior performance and this genotype is treated as ideotype. Yield is a complex trait of any crop species and is directly associated with the performance of several traits. So, it must consider the efficiency of all yield-related traits to select a high yielded genotype. A potent selection scheme can save time and resources in plant breeding projects. Plant breeders investigated genetic variability, heritability, genetic advance, and other genetic parameters of rice (Sofian *et al.* 2019; Roy and Shil 2020; Okasa *et al.* 2021) but faced difficulty in selecting genotypes that combine multiple yield related attributes. Multivariate selection indices can be used to make this selection. Consequently, Smith (1936) and Hazel (1943) developed the SH index based on the inversion of the phenotypic covariance matrix but the index has multicollinearity issues, which lead to poor selection of desirable genetic traits. Aside from that, breeders are frequently faced with challenging decisions regarding how to articulate the economic value of features and convert them into practical economic weightings (Bizari *et al.* 2017). To overcome these constraints of the Smith-Hazel (SH) index, MGIDI has emerged as a novel tool for identifying superior genotypes based on multiple traits' information (Olivoto and Nardino 2020). It enables more efficient and precise genotype selection depending on anticipated or undesired crop attributes. Mutations can generate variability in rice and studying the level of accessible diversity in a crop improvement program is an important stage in genetic improvement of crop that can actually be achieved through the evaluation of the mutants. Thus, the present study was conducted to develop variants and to assess the variation of EMS-induced rice mutants towards the selection of promising mutants that exhibit genetic diversity for crop improvement programs using the multi-trait genotype-ideotype distance index.

Materials and Methods

Experimental site and climatic condition

The experiment was executed at Experimental Farm of Agrotechnology Discipline under Khulna University (latitude 22°79′88″ E, longitude 89°53′44″ N and elevation: 18 m above sea level), Bangladesh in 2021. The soil of the field was clay loam with pH 8.4. The nutrient status was OM = 4.68%, total nitrogen = 0.214%, P = 22.41 μg g^{-1} soil, K = 0.28 mg 100g^{-1} soil, S = 16.53 μg g^{-1} soil, Zn = 1.84 μg g^{-1} soil and B = 1.29 μg g^{-1} soil (Soil Resource Development Institute, Khulna). The prevailed climatic conditions are presented in Fig. 1 (Data source: Regional Weather Station, Khulna).

Achieving the mutant lines

The rice mutants were developed by treating the seeds of BRRI dhan28, collected from Bangladesh Rice Research Institute (BRRI), with EMS at 0.108 *M* concentration (LD$_{50}$). The lethal dose was determined in previous experiments with eight different concentrations based on the germination and survival parameters through the dose-response model curve (Mamun 2022). The mutagenesis treatment procedures have presented in Table 1.

Subsequently, the germinated seedlings were allowed to grow in rice field soil in pots (30 L) under a net house. The matured M$_2$ seeds from the panicles of main tillers of M$_1$ mutants were harvested that were used to grow the M$_2$ population for evaluation.

Experimental design and field evaluation

A total of 100 mutants (M$_2$) were arranged according to the augmented randomized complete block design with parent (BRRI dhan28) in three blocks. In each block, the parent repeated twice as parent-1 and parent-2 and all mutants replicated thrice giving total experimental plot of 306. One month old seedlings were transplanted with single seedling hill^{-1} maintaining 20 cm × 20 cm spacing. An isolation distance of 3m was maintained to check cross-pollination. Recommended fertilizer dose and agronomic operations were applied accordingly throughout the growing period.

Assessed traits and measuring procedure

The data recorded for sixteen quantitative traits from all the M$_2$ individuals have displayed in Table 2.

Statistical analyses

The data were analyzed by using the R (R Core Team 2018) package (2.14.0 program). The collected data for each trait were subjected to analysis of variance (ANOVA) employing the R package 'augmented RCBD'. Phenotypic variance (PV) and genotypic variance (GV) were assessed following Johnson *et al.* (1955) whereas genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) were calculated using the formula proposed by Singh and Choudhary (1985). Broad sense

Table 1: Mutagenesis steps involved in developing mutant lines

Soaking 750 seeds in the 500 mL distilled water	Room temperature	Over night
0.108 *M* concentrations of EMS (pH = 7.0)	Incubated (22 ± 1°C) in dark	12 h at 60 rpm
Washing under running tap water		3 h
Washing with autoclaved distilled water	Under laminar air flow cabinet	3 times, 3 min each
Sowing on soaked double layered filter paper (9 cm)	Incubated under 3000 lux light (16/8) at 27 ± 1°C	Data collection for 14 days
Measured germination (%), survival (%), shoot length, root number and root length.		

Table 2: List of considered 16 quantitative traits for evaluation

S. No.	Trait	Method of assessment
1.	Plant height	Height from base to tip of the tallest leaf
2.	Total tillers hill^{-1}	Number of total tillers per hill. The group of tillers produced by a single plant constitutes a rice hill.
3.	Effective tillers hill^{-1}	Number of panicle bearing tillers per hill
4.	Days to maturity	Number of days from seeding to maturing day
5.	Flag leaf length	Length from base to tip of the flag leaf
6.	Panicle length	Length from first node to tip of last grain of the panicle
7.	Primary branches panicle^{-1}	Branches devising from main axis of the panicle
8.	Secondary branches panicle^{-1}	Branches patenting from primary branches of the panicle
9.	Total grains panicle^{-1}	Dividing the number of total grains with total panicles
10.	Filled grains panicle^{-1}	Dividing the number of filled grains with total panicles
11.	1000-Grain weight	Counted 1000 seeds and weighed
12.	Grain length	Measure the length of filled grain using slide calipers
13.	Grain breadth	Measure the midpoint breadth of filled grain using slide calipers
14.	Straw yield hill^{-1}	Weight of straw in a hill after oven dry
15.	Grain yield hill^{-1}	Weight of total grains of a hill at 14% moisture content
16.	Harvest index	Dividing the grain yield with biological yield per hill and multiplied by 100
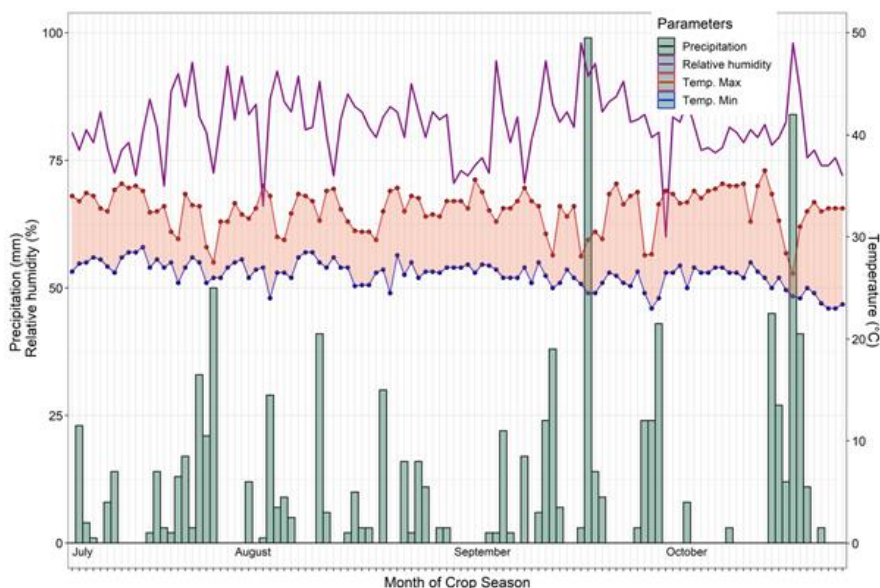
Fig. 1: Weather situations in the experimental field during the crop growing season

heritability (h^2) was calculated according to Falconer (1989). Genetic advance (GA) and genetic advance as a percentage of mean were estimated by the formula projected by Assefa *et al.* (1999). For heat map analysis, 'ggplot2' and 'heatmap.2' packages of R were used. Pearson's rank correlation algorithm was used to construct a correlation plot and principal component analysis (PCA) was accomplished using 'GGally', 'factoextra' and 'ggfortify' packages. A two-way matrix of the mutants and the traits was used to plot the first two PCs. Genotypes were plotted based on PC scores and each PC's eigenvectors were used to plot the traits.

To identify best performing genotypes based on quantitative traits, the multi-trait genotype-ideotype distance index (MGIDI) was computed according to Olivoto and Nardino (2020) as follows:

$$MGIDI_i = \left[\sum_{j=1}^{f} (\gamma_{ij} - \gamma_j)^2 \right]^{0.5}$$

Table 3: Analysis of variance of the studied quantitative traits for the mutants and their parents

Traits	Source of variation					
	Mutants with parents	Parents	Mutants vs. parents	Mutants	Adjusted block	Residuals
d.f	101	1	1	99	2	202
Plant height	86.29**	1.47ns	0.11ns	88.02**	1.19ns	11.08
Total tillers hill⁻¹	96.75**	10.67ns	38.85ns	98.20**	37.17ns	13.97
Effective tillers hill⁻¹	71.25**	6.00ns	61.88*	72.01**	3.50ns	10.42
Days to maturity	54.90**	6.00ns	113.88**	54.80**	10.67*	3.43
Flag leaf length	31.10**	0.67ns	20.90ns	31.51**	0.41ns	7.79
Panicle length	10.86**	5.61ns	1.54ns	11.01**	0.1ns	3.06
Primary branches panicle⁻¹	13.51**	6.00ns	9.44ns	13.63**	0.17ns	2.53
Secondary branches panicle⁻¹	63.69**	2.67ns	15.89ns	64.89**	0.50ns	10.59
Total grains panicle⁻¹	9851.23**	600.00ns	5668.76**	9986.92**	528.67ns	825.94
Filled grains panicle⁻¹	7031.11**	0.67ns	474.71ns	7168.35**	260.17ns	699.03
1000-grain weight	6.25**	0.39ns	5.10**	6.32**	1.04*	0.29
Grain length	0.22**	0.10ns	0.16ns	0.22**	0.03ns	0.09
Grain breadth	0.02**	0.02ns	0.25**	0.02**	0.0011ns	0.01
Straw yield hill⁻¹	96.59**	7.53ns	44.02ns	98.02**	25.67ns	35.34
Grain yield hill⁻¹	23.48**	0.88ns	19.19*	23.75**	0.69ns	3.53
Harvest index	80.03**	0.96ns	1.17ns	81.63**	18.16ns	27.92

d.f= degrees of freedom; ns= not significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$
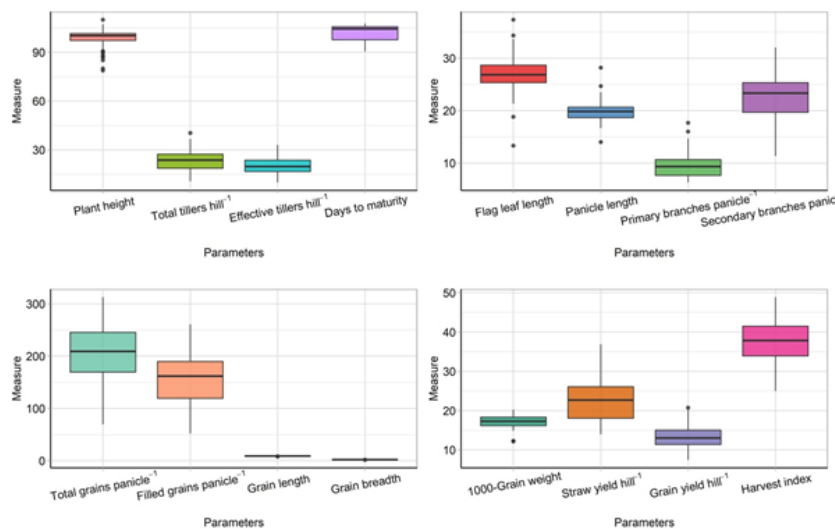

Fig. 2: Box plots depicting the pattern of the measured traits of the mutants

Where, $MGIDI_i$ is the multi-trait genotype-ideotype distance index for i^{th} genotype, γ_{ij} is the jth score of the ith genotype, and γ_j is the jth score of ideotype (i = 1, 2, ….., t; j = 1, 2, ….., f), being t and f the number of genotypes and traits.

The strength and weakness of the genotypes based on the proportion of the MGIDI of the ith genotype explained by j^{th} trait (ω_{ij}) was calculated as:

$$\omega_{ij} = \frac{\sqrt{D_{ij}^2}}{\sum_{j=1}^{f}\sqrt{D_{ij}^2}}$$

Where, D_{ij} is the distance between the ith genotype and ideal genotype for the jth trait. A trait with low contribution indicates that the genotypes within such trait are close to ideal genotype. The MGIDI and ω computation were carried out in R software with the function of 'gamam' and 'mgidi' of the package 'metan' (Olivoto and Lúcio 2020).

Results

Analysis of variance

The outcome of variance analysis demonstrated significant variation ($P \leq 0.01$) among the mutants and mutants with parents for all the quantitative traits considered. However, most of the traits revealed non-significant variations in mutants by parents except effective tillers panicle⁻¹, days to maturity, total grains panicle⁻¹, 1000-grain weight, grain breadth, and grain yield hill⁻¹. All the traits were non-significant for parents and adjusted blocks but days to maturity and 1000-grain weight for adjusted blocks (Table 3). Except for primary branches panicle⁻¹ that were skewed to the left and for plant height and days to maturity that were skewed to the right, all of the traits fit the normal distribution (Fig. 3). The variation in traits for all the genotypes is shown

Table 4: Descriptive statistics of the measured traits

Trait	Mean	Std. Error	Min	Max	Skewness	Kurtosis
Plant height	98.70	0.53	79.33	109.83	-1.29^{**}	5.29^{**}
Total tillers hill^{-1}	23.11	0.61	8.17	41.33	0.28^{ns}	2.87^{ns}
Effective tillers hill^{-1}	19.81	0.48	9.83	33.83	0.24^{ns}	2.90^{ns}
Days to maturity	101.66	0.50	87.67	108.33	-0.30^{ns}	1.95^{**}
Flag leaf length	27.02	0.32	13.4	37.75	-0.20^{ns}	6.07^{**}
Panicle length	19.83	0.19	14.23	27.95	0.78^{**}	5.79^{**}
Primary branches panicle^{-1}	9.42	0.21	6.00	17.83	0.98^{**}	4.72^{**}
Secondary branches panicle^{-1}	22.39	0.46	11.33	31.50	-0.48^{*}	2.73^{ns}
Total grains panicle^{-1}	202.93	5.89	51.67	317.67	-0.37^{ns}	2.47^{ns}
Filled grains panicle^{-1}	157.4	4.81	40.17	267.50	-0.05^{ns}	2.60^{ns}
1000-grain weight	17.34	0.16	11.33	20.45	-0.78^{**}	4.89^{**}
Grain length	8.82	0.03	8.04	9.35	-1.13^{**}	3.72^{ns}
Grain breadth	1.88	0.01	1.61	2.07	-0.41^{ns}	4.01^{ns}
Straw yield hill^{-1}	22.99	0.63	11.75	37.54	0.29^{ns}	2.30^{ns}
Grain yield hill^{-1}	13.46	0.27	7.92	20.95	0.72^{**}	3.32^{ns}
Harvest index	37.73	0.58	24.90	50.76	0.03^{ns}	2.36^{ns}

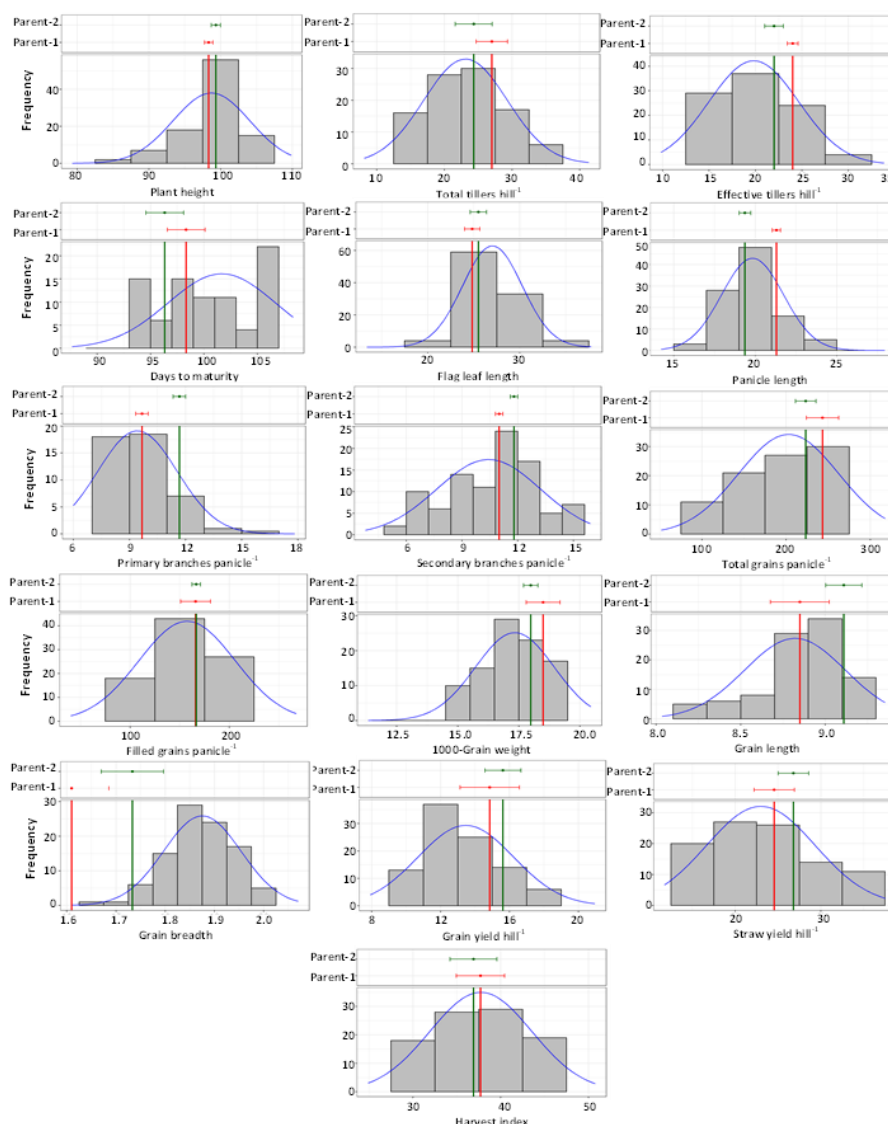Ns = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$

Fig. 3: Frequency distribution of the mutants based on 16 quantitative traits

Table 5: Estimation of genetic parameters of yield and yield contributing traits of the mutants

Trait	PV	GV	GCV		PCV		hBS		GA	GAM	
			Value	Category	Value	Category	Value	Category		Value	Category
Plant height	88.02	76.94	8.89	Low	9.51	Low	87.41	High	16.92	17.14	Medium
Total tillers hill^{-1}	98.20	84.23	39.71	High	42.88	High	85.77	High	17.54	75.88	High
Effective tillers hill^{-1}	72.01	61.58	39.61	High	42.83	High	85.52	High	14.97	75.57	High
Days to maturity	54.80	51.37	7.05	Low	7.28	Low	93.74	High	14.32	14.08	Medium
Flag leaf length	31.51	23.72	18.03	Medium	20.78	High	75.27	High	8.72	32.26	High
Panicle length	11.01	7.95	14.22	Medium	16.73	Medium	72.20	High	4.94	24.92	High
Primary branches panicle^{-1}	13.63	11.10	35.36	High	39.18	High	81.42	High	6.20	65.82	High
Secondary branches panicle^{-1}	64.79	54.20	32.88	High	35.95	High	83.66	High	13.89	62.05	High
Total grains panicle^{-1}	9986.92	9160.98	47.17	High	49.25	High	91.73	High	189.11	93.19	High
Filled grains panicle^{-1}	7168.35	6469.32	51.10	High	53.79	High	90.25	High	157.63	100.15	High
1000-grain weight	6.32	6.04	14.17	Medium	14.51	Medium	95.45	High	4.95	28.57	High
Grain length	0.22	0.13	4.12	Low	5.30	Low	60.36	High	0.58	6.60	Low
Grain breadth	0.02	0.01	5.09	Low	6.71	Low	57.46	Medium	0.15	7.96	Low
Straw yield hill^{-1}	98.02	62.68	34.44	High	43.06	High	63.94	High	13.06	56.81	High
Grain yield hill^{-1}	23.75	20.22	33.41	High	36.21	High	85.14	High	8.56	63.59	High
Harvest index	81.63	53.70	19.42	Medium	23.94	High	65.79	High	12.26	32.50	High

PV = phenotypic variance; GV = genotypic variance; GCV = genotypic coefficient of variation; PCV = phenotypic coefficient of variation; hBS = heritability in broad sense; GA = genetic advance, GAM = genetic advanced as a percentage of mean

as boxplots that represent information on the range, mean, and variation of the trait they represent (Fig. 2).

Descriptive statistics

The descriptive statistics of the evaluated quantitative traits are summarized in Table 4. A high degree of variability was exhibited for all the quantitative traits. Skewness and kurtosis were non-significant for the majority of the traits *viz.*, total tillers hill^{-1}, effective tillers hill^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, grain breadth, straw yield hill^{-1} and harvest index indicating maximum traits fashioned by a normal distribution. The panicle length, primary branches panicle^{-1}, and grain yield hill^{-1} were significant, and the distribution was positively skewed, implying that more genotypes were below the mean than would be anticipated in a normal distribution. Whereas, the plant height, secondary braches panicle^{-1}, 1000-grain weight, and grain length were not significant, and the distribution was negatively skewed, signifying that more genotypes above the mean than would be predicted in a normal distribution. The traits plant height, days to maturity, flag leaf length, panicle length, primary branches panicle^{-1}, and 1000-grain weight were positive and statistically significant for kurtosis indicating that the distribution was greatly leptokurtic (Fig. 3).

Genetic parameters

The estimates of genetic parameters of rice genotypes for quantitative traits revealed a greater value for GCV and PCV was observed for total and effective tillers hill^{-1}, primary and secondary branches panicle^{-1}, total and filled grains panicle^{-1}, straw yield hill^{-1}, and grain yield hill^{-1}, and among them filled grains panicle^{-1} exhibited the highest GCV (51.10%) and PCV (53.79%) (Table 5). Heritability was high for all the traits except grain

breadth indicating the possibility of upgrading these traits through selection. The GA for the traits had its peak with total grains panicle^{-1} (189.11) whereas the maximum GAM was for filled grains panicle^{-1} (100.15).

Correlation matrix

Correlation coefficient analyses were performed on all traits in all possible combinations to determine the nature of the relationship between them (Fig. 4). Positive correlation was observed among most of the traits. Grain yield hill^{-1} displayed a highly significant ($P \leq 0.01$) and positive correlation with total tillers hill^{-1}, effective tillers hill^{-1}, primary branches panicle^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, straw yield hill^{-1} and harvest index indicating selection based on these traits may be rewarding for high yielding mutants. The highest positive correlation (0.95) was found between total tillers hill^{-1} and effective tillers hill^{-1}. Conversely, grain yield hill^{-1} was negatively correlated with plant height, flag leaf length, panicle length, grain length, and grain breadth. The straw yield hill^{-1} and harvest index had the strongest negative correlation (-0.62).

Heatmap analysis

To demonstrate a chromatic assessment and relationship matrix of the genotypes, a heatmap analysis of the quantitative traits was obtained (Fig. 5). The red diagonal reflects each genotype's perfect relationship with itself whereas the relationship measures for a pair of genotypes are represented by the off-diagonal. Besides, presenting the performance of the genotypes for respected traits, the heatmap grouped the genotypes into two major groups through hierarchical clustering. The first group comprised 35 genotypes which further divided into two clusters-cluster I consisted of 10 genotypes and cluster II consisted 25.
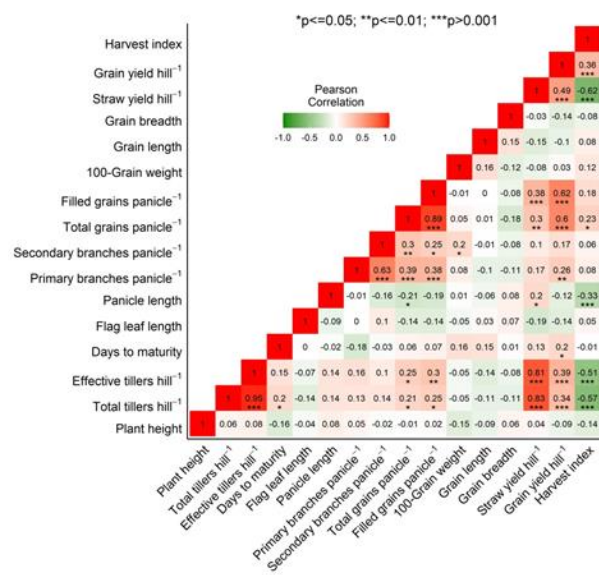
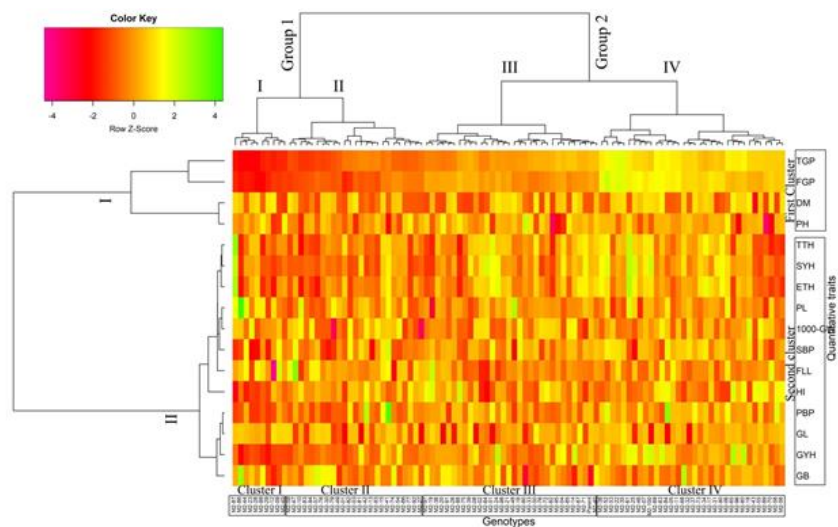**Fig. 4:** Pearson's correlation coefficient matrix of the 16 quantitative traits of the rice mutants

Fig. 5: Heatmap displaying the relationship matrix and the clustering pattern of the mutants and their parent with 16 quantitative traits
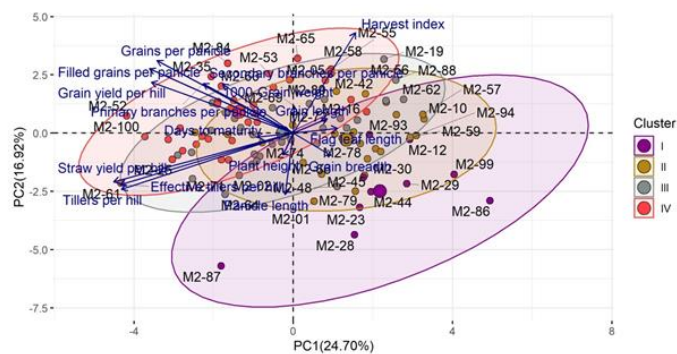

Fig. 6: PCA biplot representing the distribution of the mutants based on their genotypic variability and contribution of each trait to variation
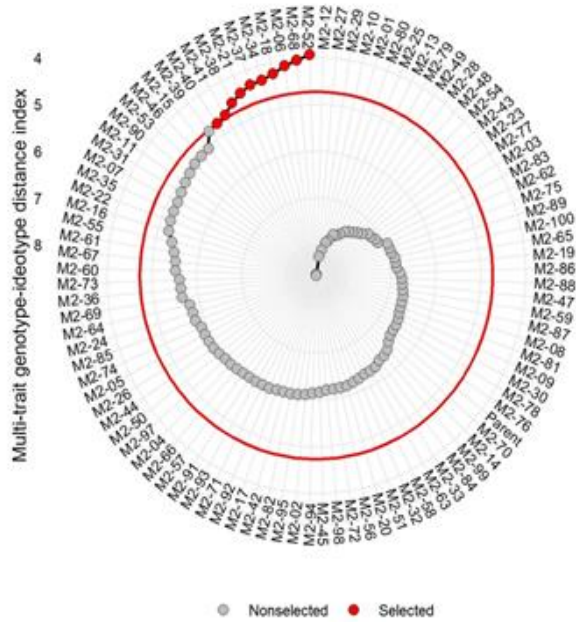
Fig. 7: Ranking of the mutants based on MGIDI index. The selected mutants based on MGIDI index considering 10% selection intensity are shown in red
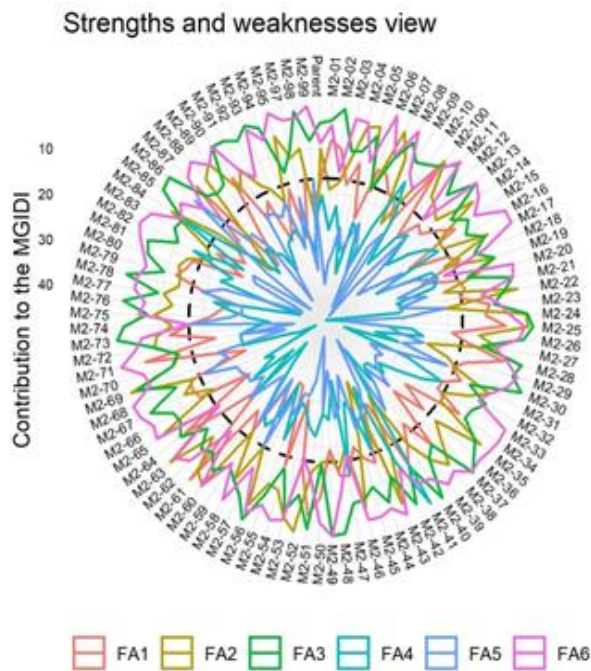

Fig. 8: The strength and weakness of all the mutants and their parent is shown as the proportion of each trait on the computed genotype-ideotype distance index (MGIDI). The dashed line shows the theoretical value if all the traits had contributed equally

The second group was also divided into two clusters- cluster II (32) and cluster IV (34). The heatmap also grouped the quantitative traits into two major clusters. The first cluster

comprised only 4 traits (total and filled grains panicle^{-1}, days to maturity, and plant height), whereas all other traits grouped into second cluster.

Principal component analysis (PCA)

The variability of the mutants was further validated by PCA to find out the pattern and relationships between several variables at the same time (Fig. 6). The two-dimensional plot of the first two principal components that contributed 41.62% of total variance portrayed the presence of wide variability among the genotypes by their widespread distribution throughout the plot. The biplot also measured the relationships among the traits and distinguished a strong relationship of grain yield hill^{-1} with total tillers hill^{-1}, effective tillers hill^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, and straw yield hill^{-1} as these traits had a small angle between the vector of grain yield hill^{-1} and the vector of their own.

Selection based on multi-trait index (MGIDI)

According to Gatten's lower bound principle, eigenvalues less than one were overlooked (Kumar *et al.* 2011). Six factors that presented eigenvalue greater than 1 were considered and they accounted for 73.35% of the total variation among the traits (Table 6). Hence, there were ways to reduce data dimensionality by ~27% maintaining only the data with high explanatory power. The 16 traits were grouped into six factors. Orthogonal rotation results to factor loadings which range from -1 to +1 and are the correlation coefficients among the traits and the factor. The MGIDI analysis assigns a rank to all the genotypes based on the desired value of the trait. The top 10 mutants were identified and selected based on 10% selection pressure. The selected mutants were M$_2$-06, M$_2$-18, M$_2$-21, M$_2$-34, M$_2$-37, M$_2$-38, M$_2$-40, M$_2$-41, M$_2$-52 and M$_2$-68 (Fig. 7). The mutant M$_2$-40 and M$_2$-41 were close to being cut (red line that separates the selected genotypes based on selection pressure), which suggests that these mutants may have some unique features. The selected mutants were further used to compute selection differentials. The MGIDI index forwarded the anticipated selection differential (SD) for 15 out of 16 studied traits with a success frequency of ~94% in selecting desired traits. The remaining trait was harvest index with undesired SD (-2.99) and SG (-5.19). The selected top 10 mutants showed desired values for most of the quantitative traits. The selection differential (SD) percentage for the traits ranged from -7.92% (harvest index) to 20.00% (total grains panicle^{-1}) with a mean of 8.16%. The traits with high heritability were 1000-grain weight (95.45%) followed by days to maturity (93.74%). But high heritability percentage in combination with high selection differential percentage was calculated for total and effective tillers hill^{-1}, total and filled grains panicle^{-1} and primary branches panicle^{-1}.

Table 6: Factorial loadings after rotation during factor analysis and selection differential and selection gain linked to the traits

Traits	FA1	FA2	FA3	FA4	FA5	FA6	SD (%)	SG (%)
Plant height	-0.08	0.00	-0.61	-0.04	0.13	-0.17	0.99	0.86
Total tillers hill[-1]	-0.93	0.17	0.07	-0.05	-0.09	-0.04	17.7	15.2
Effective tillers hill[-1]	-0.91	0.22	0.02	-0.05	-0.08	0.01	19.9	17.1
Days to maturity	-0.22	0.15	0.64	0.24	0.20	0.07	1.17	1.09
Flag leaf length	0.04	-0.23	0.05	-0.16	0.09	0.80	0.25	0.19
Panicle length	-0.29	-0.35	-0.14	0.00	0.07	-0.49	2.88	2.11
Primary branches panicle[-1]	-0.07	0.28	-0.19	-0.81	-0.09	-0.01	19.0	15.6
Secondary branches panicle[-1]	-0.06	0.15	0.06	-0.85	-0.01	0.17	15.2	12.8
Total grains panicle[-1]	-0.07	0.87	-0.01	-0.25	-0.01	-0.05	18.3	16.7
Filled grains panicle[-1]	-0.14	0.89	-0.07	-0.19	0.06	-0.04	20.0	18.0
1000-grain weight	0.12	-0.09	0.62	-0.41	0.04	-0.36	1.36	1.30
Grain length	0.15	0.01	0.34	-0.03	0.71	-0.09	0.92	0.57
Grain breadth	-0.01	-0.10	-0.27	0.12	0.75	0.13	1.88	1.10
Straw yield hill[-1]	-0.89	0.29	-0.03	-0.03	-0.04	-0.12	13.4	8.54
Grain yield hill[-1]	-0.21	0.81	0.15	-0.01	-0.15	-0.02	0.31	0.27
Harvest index	0.77	0.44	0.14	-0.01	-0.08	0.10	-7.92	-5.19
Eigenvalue	3.95	2.71	1.54	1.34	1.14	1.06	-	-
Variance (%)	24.70	16.92	9.62	8.39	7.11	6.61	-	-
Cumulative (%)	24.70	41.62	51.24	59.63	66.74	73.35	-	-

FA = factor analysis; SD = selection differential; SG = selection gain
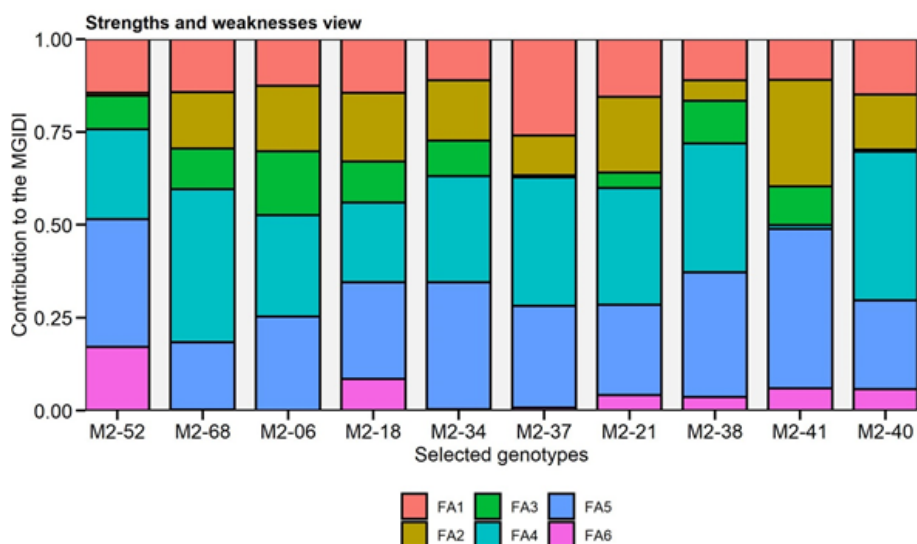
Fig. 9: The strength and weakness view of the selected mutants. The Y-axis shows the proportion of each trait on the computed MGIDI of the selected mutants

Strength and weakness of the mutants

The strength and weakness of the mutants are presented in Fig. 8 which accounted by the magnitude of each variable to the MGIDI of the mutants. Factor's contribution towards MGIDI was categorized into two as less and more contributing factors. The factors which contribute more were plotted close to center whereas lesser contributing factors were plotted towards the edge. Since the positive gains of all the traits in FA1 are desired, the selected mutants should have high values for total tillers hill[-1], effective tillers hill[-1], straw yield hill[-1], and harvest index. Grain yield belongs to the factor FA2 and had the smallest contribution for M_2-52 followed by M_2-38 mutant (Fig. 9) indicating that they were the most productive mutants

among the chosen ones. As total tillers hill[-1], and effective tillers hill[-1] were also grouped under FA2 factor, it indicated that yield was directly related to the tiller production efficiency of the mutants. The smallest contribution of FA3 for M_2-21 and M_2-37 suggested that these mutants had greater 1000-grain weight. Same interpretation can be extended to other traits as well.

Discussion

For a successful utilization of plant genetic resources, it is obvious to characterize the germplasm to identify superior genotypes through critical evaluation and selection. The traditional methods of genotype selection employed based on univariate and analysis of mean,

regression and deviation from regression (Benakanahalli *et al.* 2021). Multi-trait selection index is an advanced selection technique in plant breeding (Smith 1936; Hazel 1943), but the use of this technique is very adjuvant in selection of early or advanced breeding lines (Jahufer and Casler 2015). The efficiency of multivariate techniques is high when they were dealing with multi-traits (Zuffo *et al.* 2020). In this context, multi-trait genotype-ideotype selection index (MGIDI) technique was employed to select superior genotypes for future use in rice breeding program.

The analysis of variance being an additive technique fairly describes the main effects in stability analysis (Snedecor and Cochran 1980). For an efficient selection program, prevalence of genetic variation among the genotypes is one of the vital principles. The significant differences ($P \leq 0.05$) among the evaluated mutants reflected the presence of wide variability among the mutants. Frequency distribution and boxplots also depicted the presence of variation among the genotypes. This might be due to their genetic variation, environmental impacts or both. These findings substantiated with report of Wattoo *et al.* (2012), Ali *et al.* (2019) and Sharma *et al.* (2020) who found significant differences among the EMS induced rice mutants.

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was grouped as low (0–10%), moderate (10–20%), and high (> 20%) (Sivasubramanian and Madhavamenon 1973). In this study, the magnitude of PCV estimates was higher for all the traits compared to GCV indicating the impact of the environment on the expression of the traits (Ogunbayo *et al.* 2014). But the actual strength of variability can be achieved by comparing their coefficient of variation at both genotypic and phenotypic levels and in this study, the differences between GCV and PCV values for most of the traits were low signifying less influence of the environment that suggested the validity of quantitative trait-based selection. The proportion of phenotypic traits or total variance inherited from parents is heritability, and the heritability estimate was grouped into three major classes as low (< 30%), medium (30–60%), and high (> 60%) (Johnson *et al.* 1955). Heritability was high for all the traits but grain breadth, indicating the potential for these traits to be improved through selection. According to Brogin *et al.* (2003), traits with heritability estimates higher than 30% allow for genetic gains through selection in initial generations. High heritability values may be associated with less complexity in genetic control of the trait (Godoi and Pinheiro 2009) and dominance effects are likely to account for a greater proportion of total phenotypic variation with few genes involved in its expression. These findings may be attributed to high heritability values, which could be associated with high genetic variability among the genotypes tested and efficient environmental control achieved in the experimental field. The effects of dominance become extended by the advancement of inbred generations which subsequently reduces the rate of segregation as well.

But heritability alone does not always indicate the genetic gain unless it is studied concurrently with genetic advance as heritability comprises the effect of both additive and non-additive genes. Genetic advance is used to calculate the kind of gene action in polygenic traits and indicates the prospected progress as the result of selection (Pratap *et al.* 2014). Heritability and genetic advance are inexorable to select superior genotypes based on the performance of quantitative traits (Burton and Devane 1953). Thus, traits with high heritability accompanied by high genetic advance are the key to selection. Genetic advance as a percent of mean provides a more specific result in comparison to merely genetic advance (Adhikari *et al.* 2018). Moreover, high heritability with high genetic advance as a percentage of mean as well as high genotypic coefficient of variation give better hints for selecting suitable traits than the individual or twofold parameter(s). In this study, the traits having higher genotypic coefficient of variation have a higher value for genetic advance as a percentage of mean and heritability as well. Hence, selection relying on the phenotypic performance of these traits would result in a significant improvement in the population mean of rice for the next generation (Sumanth *et al.* 2017).

Correlation is the measure of relationship between two variables and the degree of association between them. It may be complicated to improve complex plant characters like yield based on direct selection of traits as the character is quantitatively inherited and influenced not only by genotypes but by the interaction between the genotype and environment also. Therefore, identification and selection of highly correlated traits is essential (Ahmadikhah *et al.* 2008). Significant, positive and high correlation coefficients of total tillers hill^{-1}, effective tillers hill^{-1}, primary branches panicle^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, straw yield hill^{-1}, and harvest with grain yield hill^{-1} was a clue that selection based on these traits would be suitable to obtain desired genotypes with better heterosis (Gyawali *et al.* 2018).

The heatmap depicted the genotype values for all traits in vibrant shades. The degree of high or low of the traits is indicated by the color intensity. A heatmap analysis of quantitative traits was carried out to decipher a chromic evaluation of rice mutants. The depth of correlation among the 16 quantitative traits of the mutants was visualized using a heatmap. The heatmap constructed double hierarchical dendrograms. The first dendrogram on the horizontal direction represents the arrangement of the genotypes, and the second dendrogram on the vertical direction representing the traits that influenced the diffusion. First dendrogram showed 4 clusters that indicates a degree of diversity among the mutants.

Principal component analysis redirects the significance of greater contributor to the total diversity at each axis of variation (Sharma 1998). The extensive phenotypic differences of the mutants were advanced by principal component analysis which specified that the overall

distinction was honestly distributed through all the quantitative traits studied rather than the contribution of some specific traits. The relationship between quantitative traits among the mutants was revealed by biplot analysis (Khan *et al.* 2021). The trait profiles of the mutants were displayed in a biplot, especially those mutants residing far from the source and the findings revealed a link between traits and mutants. The first principal component (PC1) elucidate utmost portion of total variation (Lezzoni and Pritts 1991). In our findings, PC1 accounted 24.70% of total variation and the PC2 16.92%. A positive correlation is indicated by a sharp angle between the vectors of two traits, while a negative correlation is implied by a blunt angle (Dehghani *et al.* 2008). The biplot of the current study summarized the matrix's values in principal components, where the correlation coefficient between traits is proportional to the cosine of the angle between the vectors linking those traits to the origin.

Plant breeders often practice traditional methods based on first-degree statistics to select desired genotypes from a mixed population. It is necessary to integrate several desired attributes into a genotype to screen out high-performing ones. Selection based on mean, regression, and deviation from regression parameters may not be adequate to identify an elite genotype from the ideotypes. Hence, various multivariate techniques *viz.,* PCA, heatmap analysis, cluster analysis, and factor analysis are widely used to select genotypes (Bhandari *et al.* 2017). We tried to connect traits and mutants through genetic analysis, PCA, heatmap analysis, and hierarchical clustering (Table 5, Fig. 5 and 6) but could not select any specific mutants. Therefore, we used an advanced quantitative genetic tool, the MGIDI technique of the 'metan' R package to select mutants with multiple desired traits. The MGIDI is a relatively new method for choosing a genotype consisting of multiple traits data suitable for all crop species (Olivoto and Nardino 2020). The data analyzed in the present study selected ten mutants as promising genotypes with desired values for the quantitative traits (Fig. 7). Besides, the M_2-39 mutant was quite close to the cut point, hinting that this mutant can bear desired features and deserves more attention from the researchers (Olivoto and Nardino 2020).

The proportion of the MGIDI index that each factor explains is an essential tool to identify the strength and weaknesses of all the mutants (Fig. 8) and also the selected mutants (Fig. 9). It allows identifying the traits that need to be improved for selected or even non-selected genotypes. The selected mutants had a high productive capacity not only for grain yield but for other desired traits also. This is, to the best of our knowledge, the first report regarding the selection of rice mutants based on multiple quantitative traits through the advanced selection strategy, MGIDI. A similar index was used to select the best performing genotypes for wheat (Pour-Aboughadareha and Poczai 2021), barley (Pour-Aboughadareha *et al.* 2021), strawberry (Olivoto *et al.* 2021), soybean (Maranna *et al.*

2021), eggplant (Uddin *et al.* 2021), guar (Benakanahalli *et al.* 2021), maize (Yue *et al.* 2022), *etc*.

Conclusion

The multi-trait framework provided by the multi-trait genotype-ideotype distance index (MGIDI) provides an easy way to select high performance rice mutants. The MGIDI provides a new framework of multivariate techniques to select desired genotypes that will optimize the use of resources and time. The MGIDI index used in the current study was found efficient in identification of promising mutants for higher yield. The selected mutants presented desired values for 15 out of 16 quantitative traits. The strength and weakness of the mutants in MGIDI indicated the importance of an ideal mutant with improved quantitative traits. The identified mutants could be regarded as the best genotype for a prospective breeding program to improve rice.

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Author Contributions

M.M. Islam and S.K. Adhikary developed the concept of the research and A.A. Mamun carried out the laboratory as well as field research. A.A. Mamun recorded and analyzed the data with the assistance of M.S. Sultana and prepared the manuscript. The manuscript was corrected and edited by M.M. Islam, S.K. Adhikary and M.S. Sultana. Finally, all authors read and approved the manuscript for publication.

Conflicts of Interest

For the present study, the authors proclaim no conflicts of interest.

Ethics Approval

The current study does not involve any animal and/or human experiments.

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