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Estimates of Genetic Variability and Boxplot Analyses in F_2 Populations of Rice (*Oryza sativa* L.) for Yield-Related Traits

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Abstract

The present research was carried out to study genetic variability in 5×5 half diallel F_2 populations of boro rice. The F_2 populations of 10 bi-parental crosses, *viz.* P1 × P2, P1 × P3, P1 × P4, P1 × P5, P2 × P3, P2 × P4, P2 × P5, P3 × P4, P3 × P5, and P4 × P5, were evaluated, along with their parents, for grain yield and related characters. Significant variance revealed a wide range of genetic variability among the segregating progenies and parental genotypes for all the studied traits except grain breadth. The genotypic coefficient of variation (GCV) was smaller than the phenotypic coefficient of variation (PCV) for all the traits except the grain length-breadth ratio. Grain length exhibited high heritability (96.021%) and high genetic advance (22.137%), followed by the grain length-to-breadth ratio (95.308% and 40.7%), and 1000-grain weight (71.96% and 25.32%), respectively. Broader genetic variability for the genotypes was revealed based on boxplot analyses. The F_2 family $P_3 \times P_4$ showed the maximum transgressive segregation for the number filled grains per panicle and 1000-grain weight followed by $P_3 \times P_5$.

Keywords

GVC, PCV, genetic gain, transgressive segregation, boxplot analysis, grain yield

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Introduction

Rice (*Oryza sativa* L.) is the most important staple food for 65% of the global population. It also serves as the cheapest source of a staple food, energy, and protein (Padmaja *et al*., 2008). Rice is grown on more than 160 million hectares, with an annual production exceeding 750 million metric tons (FAOSTAT, 2022). Over the last three decades, Bangladesh has achieved significant advances in rice production and has become self-sufficient in rice (Mainuddin & Kirby, 2015, Tasmina *et al.*, 2018). The per capita rice consumption in Bangladesh is 134 kg year^{-1}, significantly higher than the global average of 57 kg year^{-1} (Mottaleb & Mishra, 2016). Rice is the leading cereal crop and contributes a significant portion to the incomes of farmers (Alam *et al*., 2016).

Genetic variability is considered a prerequisite for breeding programs and the selection of superior genotypes with desirable characteristics for any type of crop improvement. It is of utmost interest to plant breeders because it plays an important role in framing the breeding program. The improvement of any trait, whether it is morphological, yield, or yield-related, depends on the quantity of genetic variability present in the germplasm for the trait of interest (Nithya *et al.*, 2020). The existence of genetic variability in the segregating generation is essential for the improvement of any desirable traits through selection in recombinant inbred lines for the development of a cultivar (Reddy *et al.*, 2019).

Heritability estimation quantifies the proportion of trait variation that is genetically inherited from parents to offspring, and serves as a key analytical tool in crop breeding programs **(**Khan & Naqvi, 2011). The ultimate goal of a plant breeder is to develop or create a high-yielding variety having wider adaptability to the environment. As a result, a breeding program should be able to develop/identify high-yielding and well-adapted varieties by leveraging the good adaptability and stability of yield and its components in rice genotypes (Ogunbayo, 2011).

Utilizing genetic variability parameters such as the phenotypic and genotypic coefficients of variability (GCV and PCV, respectively), heritability, and genetic advance are crucial in future rice improvement research projections **(**Paikhomba *et al.*, 2014). Therefore, the present study aimed to estimate the genetic components of variance, GCV, PCV, heritability, and genetic advance for yield-associated traits in F_2 populations of rice.

Materials and Methods

Parental materials

Seeds of the parental generations were primarily collected from the Bangladesh Rice Research Institute (BRRI) and the Bangladesh Institute of Nuclear Agriculture (BINA). Four released boro (dry season) rice varieties (three by BRRI and one by BINA) and one IRRI developed line were used as the parental genotypes for the development of the F_2 generations (**Table 1**), namely BRRI dhan28 (P_1), BRRI dhan74 (P_2), BINA dhan10 (P_3), IR59418-7B-21-3 (P_4), and BRRI dhan67 (P_5). The parental genotypes included HYV mega varieties (BRRI dhan28) of rice cultivated in Bangladesh with defects in one or two characters. The remaining parent (BBRI dhan74) contained specific desirable traits (salinity tolerance and Zn content), which would be used in recombinations to develop better segregants.

Development of hybrids (F_1) and segregating populations (F_2)

A total of 10 bi-parental hybrids viz. $P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_3$, $P_2 \times P_4$, $P_2 \times P_5$, $P_3 \times P_4$, $P_3 \times P_5$, and $P_4 \times P_5$ were obtained through a 5 x 5 half diallel mating design using the five selected parents in the boro (dry) season of 2017. The F_1 generations were grown with their parents in the next boro season of 2018 to advance the generations as F_2.

Table 1. List of rice genotypes used for crossing with their salient features and sources

Parent	Rice Genotype	Features	Source
P_1	BRRI dhan28	Mega rice variety in Bangladesh, HYV, salt sensitive, also released in several states of India and vastly cultivated by farmers	BRRI
P_2	BRRI dhan74	HYV, Zn^+ rich variety, salinity tolerant	BRRI
P_3	BINA dhan10	HYV, salt tolerant variety, released and cultivated by farmers of several states in India	BINA
P_4	IR59418-7B-21-3	Resistant to salinity, developed by IRRI: IR 10198-66-2//IR 50404/AT 401	IRRI
P_5	BRRI dhan67	HYV, salt tolerant variety	BRRI

Evaluation of the experimental materials

The experimental materials comprised 10 F_2 families, along with the five parental genotypes, were evaluated during the boro season of 2019, following a randomized complete block design (RCBD) with three replications. The experiments were carried out at the field laboratory of the department of Genetics and Plant Breeding, Gazipur Agricultural University (formerly Bangabandhu Sheikh Mujibur Rahman Agricultural University), Gazipur, Bangladesh. All intercultural operations were done as per the recommendations of BRRI (Bangladesh Rice Research Institute, 2018). Data were recorded on 16 different yield and yield-related characters. The characters were days to panicle exertion (DPE), days to maturity (DMT), culm length (CLT), height of the plant (PLT), total tillers per plant (TPP), effective tillers per plant (ETP), non-effective tillers per plant (NTP), panicle length (PLT), number of filled grains (FGP), number of unfilled grains (UGP), grain length (GLT), grain breadth (GBD), grain length-to-breadth ratio (LBR), straw yield (SDT), 1000-grain weight (TGW), and grain yield per plant (YPP).

Data analysis

The recorded data were statistically analyzed according to the standard procedures described by Snedecor and Cochran (1967) and Clark (1973), and were subjected to analysis of variance (ANOVA) using the IRRI PB tools software. The means were compared using the least significant difference (LSD) at $P \leq 0.05$ and $P \leq 0.01$. R-software was employed to estimate the genetic components of variability such as mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genetic advance (GA), heritability (h^2b), and boxplot analyses for the representation of quartile distribution.

Results

Analysis of variance (ANOVA) and estimates of the genetic components

ANOVA results of the 16 yield-related traits of the 10 F_2 segregating populations along with their five parents are presented in **Table 2**, and they revealed the existence of significant differences among the genotypes, suggesting an adequate amount of variability was present in the studied traits. All the traits showed highly significant differences among the traits for the studied genotypes, revealing broader genetic variability except for grain breadth. As the variability study was carried out in the segregating populations together with their diverse parents, the accomplishment of wider variability for the characters is natural as well as mostly expected (Balat *et al.*, 2018).

The range, mean with standard error, and other estimates of genetic variability such as GCV (%), PCV (%), h^2b, genetic advance, and genetic advance as a percentage of the mean obtained from the population consisting of the 15 genotypes (10 F_2 progenies along with their five respective parental lines) in regard to the 16 agronomic traits under study are presented in **Table 3**.

Mean performance of the traits

Mean values for the 16 agronomic characters and their maximum and minimum values revealed widespread variability among the genotypes (**Table 3**). For instance, days to panicle exertion ranged from 62.65 to 82.55 with an average of 73.30 days. The maturity of the genotypes required a minimum of 136.39 to a maximum of 163.77 days, having an average of 152.48 days. The range for plant height varied from the shortest at 85.65cm to the tallest at 110.35cm, and the average height was 98.8cm.

Regarding culm length, the range was 61.22cm to 83.92cm, indicating the plants with the shortest as well as longest culm, respectively, and had an average culm height of 73.47cm. The panicle length exhibited a range between 15.78cm to 25.63cm with an average value of 21.53cm. The total number of tillers per plant varied between the lowest of 9.4 and the highest of 20.44 with a mean of 14.18. The number of effective tillers per plant, with the lowest value of 9.78 and the highest value of 18.22, representing the range for this trait, had a mean of 12.92. The number of non-effective tillers per plant was observed to be 0.033 as the minimum and 4.55 as the maximum with a mean value of 1.97 (**Table 3**).

Table 2. Analysis of variance (ANOVA) for the 16 yield-related traits in the F_2 populations and their parental genotypes of rice

No.	Characters	Mean sum of squares		
		Replication (df = 2)	Genotypes (df = 14)	Error
1	DPE	8.41	63.58**	12.56
2	DMT	38.33**	57.34**	46.54
3	PHT	6.01	78.83**	27.26
4	CLT	20.02**	19.67**	36.67
5	PLT	1.18	9.12**	7.96
6	TPP	1.16	13.73**	5.43
7	ETP	0.78	9.09**	2.27
8	NTP	0.39**	1.25**	2.26
9	FGP	50.63**	101.43**	77.39
10	UGP	11.74**	108.87**	73.41
11	GLT	0.03	2.28**	0.03
12	GBD	0.003	0.100	0.003
13	LBR	0.021*	1.20**	0.019
14	SDW	8.40	49.59**	19.19
15	TGW	7.26	53.79**	6.18
16	YPP	63.14**	46.80**	35.07

*Note: *, ** represent significance at the 5% and 1% levels, respectively; df = degrees of freedom. Days to panicle exertion (DPE), days to maturity (DMT), culm length (CLT), height of the plant (PLT), total number of tillers per plant (TPP), effective tillers per plant (ETP), non-effective tillers per plant (NTP), panicle length (PLT), number of filled grains (FGP), number of unfilled grains (UGP), grain length (GLT), grain breadth (GBD), grain length-to-breadth ratio (LBR), straw yield (SDT), 1000-grain weight (TGW), and grain yield per plant (YPP).*

Table 3. Estimates of the genetic components of the 16 related traits in the F_2 rice populations and their parental genotypes

Genetic parameters	Range	Mean ± SD	GCV (%)	PCV (%)	h^2b (%)	GA (%)	GAM in %
DPE	62.65 - 82.55	73.30 ± 5.35	5.63	7.42	57.51	6.44	8.79
DMT	136.39 - 163.77	152.48 ± 7.04	1.24	4.64	7.18	1.05	0.69
PHT	85.65 - 110.35	98.8 ± 6.53	4.19	6.74	38.67	5.31	5.37
CLT	61.22 - 83.92	73.47 ± 5.52	3.20	8.82	13.14	1.76	2.39
PLT	15.78 - 25.63	21.53 ± 2.83	2.89	13.42	4.63	0.28	1.28
TPP	9.4 - 20.44	14.18 ± 2.81	11.73	20.19	33.75	1.99	14.04
ETP	9.78 - 18.22	12.92 ± 2.09	11.67	16.51	49.93	2.20	16.99
NTP	0.033 - 4.55	1.97 ± 1.36	29.50	81.92	12.96	0.43	21.88
FGP	104.9 - 152.93	131.52 ± 9.16	2.15	7.03	9.38	1.789	1.36
UGP	6.66 - 40.9	20.66 ± 9.05	16.64	44.69	13.87	2.64	12.77
GLT	6.41 - 9.43	7.90 ± 0.865	10.97	11.19	96.02	1.75	22.14
GBD	2.22 - 2.91	2.45 ± 0.193	7.64	8.04	90.22	0.37	14.95
LBR	1.02 - 3.90	3.11 ± 0.631	20.24	20.73	95.31	1.27	40.70
SDW	11.11 - 39.06	27.50 ± 5.33	11.58	19.69	34.55	3.85	14.02
TGW	19.12 - 40.6	29.62 ± 4.62	14.49	17.08	71.96	6.96	25.32
YPP	21.33 - 41.58	27.50 ± 6.33	6.68	21.08	10.03	1.29	4.36

Note: Days to panicle exertion (DPE), days to maturity (DMT), culm length (CLT), height of the plant (PHT), total tillers per plant (TPP), effective tillers per plant (ETP), non-effective tillers per plant (NTP), panicle length (PLT), number of filled grains (FGP), number of unfilled grains (UGP), grain length (GLT), grain breadth (GBD), grain length-to-breadth ratio (LBR), straw yield (SDT), 1000-grain weight (TGW), and grain yield per plant (YPP).

Heritability and genetic advance

The estimate of heritability of a trait is very important to breeders, since its extent directs the phenotypes by their genotypes. The GCV with the heritability estimate of a trait could offer a clear picture of control of the trait. Burton and Devane (1953) suggested that the GCV with heritability estimates helps to make predictions through selection based on phenotype. The traits such as grain length (h^2b = 96.021%), grain breadth (h^2b = 90.215%), and the grain length-to-breadth ratio (h^2b = 95.308%) as well as the thousand grain weight (h^2b = 71.963%) revealed high heritability accompanied by high to moderate GCV and PCV (**Table 3**). The high value of heritability reflected the low or negligible influence of the environment on the genetic control of these traits. Hence, selection based on phenotypic expression of individual plants by implementing simple selection methods might be effective for the improvement of these traits. The higher heritability values of the above-mentioned traits were indicative of selection, supported by Matin *et al.* (2017) and Urichio *et al.* (2020).

High heritability along with high genetic advance helps in predicting traits and selecting superior varieties, but high heritability does not always indicate high genetic advance (Muchie & Fentie, 2016). High heritability estimates (> 60%) along with high genetic advance (> 20%) were observed for the traits of grain length (h^2b = 96.021%, GAM = 22.137%), grain length-to-breadth ratio (h^2b = 95.308%, GAM = 40.7%), and thousand grain weight (h^2b = 71.963, GAM = 25.316) (**Table 3**). The results indicated the scope for improvement of these traits through selection due to their additive genetic components. On the other hand, the traits with moderate heritability and genetic advance, such as days to panicle exertion, number of effective tillers per plant, and height of the plant, among others, revealed non-additive genetic components. The results suggested that improvement through simple selection may not be rewarding. In this case, the development of transgressive segregants through hybridization followed by selection would be a better option for improvement of the traits.

Boxplot analyses

Boxplots are used to visualize numerical data graphically, as well as skewness, by displaying the data quartiles or percentiles and averages (McLeod, 2019). Boxplots are non-parametric statistics which exhibit variation among the samples of a population. In the present study, the summary of the data set was represented by boxplots for the 16 agronomic characters in **Figure 1(A-D)**. Boxplots for panicle exertion time, maturity time, plant height, and culm length are shown in **Figure 1 (A-D)**. Days to panicle exertion ranged between 62.65 days and 82.55 days, where the first quartile (Q1) was about 68, indicating that only 25% of the genotypes required fewer than 68 days for panicle initiation. The rest of the 75% of the genotypes required more than 68 days for panicle initiation. Here, the middle quartile (Q2) value was 74, which partitioned the box into two equal parts, revealing a symmetrical and normal distribution of the data set for days to panicle exertion. The third (upper) quartile value was about 78 indicating that about 25% of the studied genotypes required more than 78 days for panicle initiation, and thus, were revealed as late flowering genotypes.

For days to maturity, the first quartile represented the 25% value for the studied genotypes, which required less than 136.39 days to reach maturity. While the second quartile represented 152.48 days, which revealed that 50% of the population needed more than 152.48 days to reach maturity. The box representing comparatively larger populations on the right of the middle quartile revealed a large number of populations and wider variability in this portion and the trait was positively skewed. There was an outlier (P3×P4) in the boxplot which required the minimum period to reach maturity among the populations and showed the highest trend of variability from the others for this trait.

The boxplot showed 85.65cm as the shortest and 110.35cm as the tallest genotypes in terms of plant height and the lower quartile represented 25% values of the trait, where genotypes possessing a plant height shorter than 94cm were short and dwarf statured plants. The remaining 75% of the values indicated relatively taller-

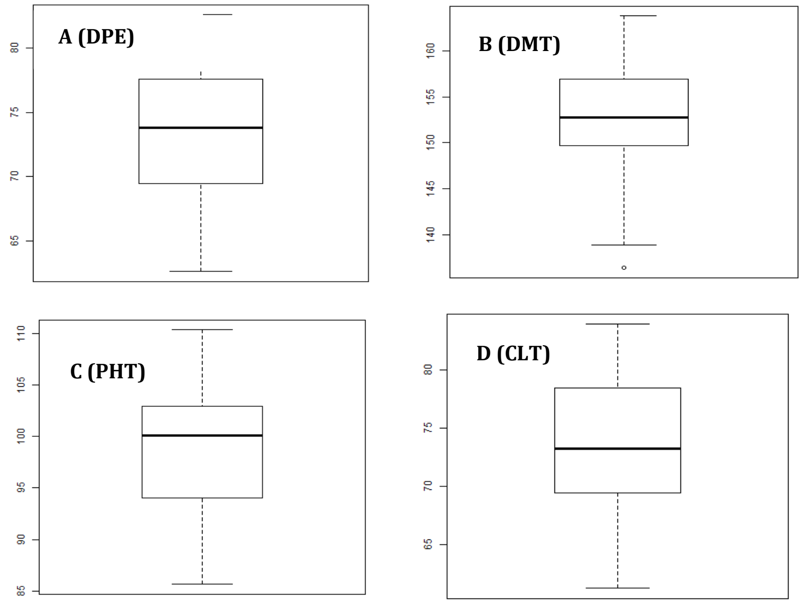

Figure 1(A-D). Boxplots showing the four summary distributions of the traits (A) days to panicle exertion, (B) days to maturity, (C) plant height, and (D) culm lenth.

statured plants. The second quartile value was 100 cm and revealed about 50% of the values, which represented plants taller than 100 cm in height. The box is wider on the left indicating a negative skewness in the distribution of data and also revealed more scattered distribution of genotypes in the left tail. The boxplot exhibited no outliers for plant height revealing less variation among the population.

In regard to culm length, the minimum value 61.22cm represented plants with shorter culms and the highest value 83.92cm indicated plants with the longest culms. The middle quartile value was 73.47cm, which indicated that 50% the boxplot represented the genotypes with a culm length less than 73.4cm and the remaining 50% represented the genotypes with a culm length more than 73.47cm. Here, the left portion of the box represented a slightly higher population than the right indicating that the data were slightly negatively skewed. The boxplot shows small variability among the genotypes and does not show any outliers.

The boxplot explanations of the traits of panicle length, total number of tillers per plant, and numbers of effective and non-effective tillers per plant are presented in **Figure 1 (E-H)**. The boxplot for panicle length revealed the values of 19.00, 21.53, and 25.90 for the lower, middle, and upper quartiles, respectively. The results conferred that 25% of the boxplot represented panicle lengths less than 19 cm, while 50% of the box exhibited panicle lengths more than 21.53 cm. The number of populations with comparatively longer panicles (≥ 21.53cm) was a bit more as the box was slightly larger to the right of the middle quartile and also suggested a slight positive distribution of the trait.

For the number of tillers per plant, the lower quartile value of 11.87 revealed that 25% of the boxplot represented the population having less than 11.87 tillers per plant and 75% of the boxplot showed the population with a comparatively higher number of tillers (≥ 11.87). Although, the boxplot revealed a slight positive distribution, it did not show much variability among the population as the size of the box was comparatively small and did not exhibit any outlier genotypes.

The boxplot for the number of effective tillers per plant revealed that the lowest value for the genotype was 9.78 and the highest number of effective tillers was 18.22 per plant. For the maximum number of effective tillers per plant, the upper quartile was 14.21, indicating that 25% of the values among the total portion of the

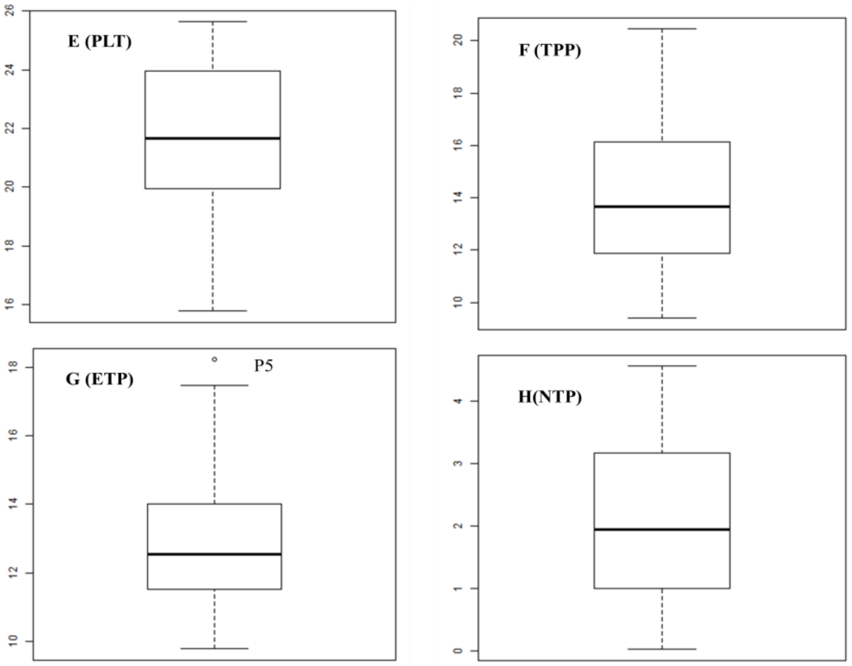

Figure 1(E-H). Boxplots showing the four summary distributions of the traits (E) panicle length, (F) tillers per plant, (G) effective tillers per plant, and (H) non-effective tillers per plant.

boxplot exhibited the population with more than 14.21 effective tillers per plant. The boxplot for the number of effective tillers per plant also showed a slight skewness in the positive direction of the data distribution of the highest number of effective tillers per plant with one outlier genotype (P5), which revealed a wider variability from the other genotypes. The boxplot also demonstrated a comparatively smaller variability among the genotypes of the studied population, since the size of the boxplot was comparatively smaller and conferred a less scattered distribution of genotypes for the trait.

The middle quartile value was 1.97 for the number of non-effective tillers per plant, which showed that 50% of the boxplot represented the population with fewer than 1.97 non-effective tillers per plant, while the remaining 50% had more than 1.97. Moreover, the boxplot was almost normally distributed for the trait and did not have any outliers, meaning the populations were almost symmetrically distributed within the boxplot.

Figure 1 (I-L) represents the boxplot descriptions for the traits of number of filled grains per panicle, number of unfilled grains per panicle, grain length, and grain breadth. The values between 104.9 to 152.93 demonstrated the genotypes with the minimum and the maximum number of filled grains per panicle, respectively. The upper, middle, and lower quartiles represented the trait values of 128.11, 134.2, and 138, respectively. Here, the number of populations with filled grains below 134.2 was comparatively larger, which created a slight negative skewness of the trait. The boxplot also revealed much smaller variations within this trait among the populations, although it showed two outliers on two opposite sides, which demonstrated a wider range of variability from others. On the other hand, the boxplot for unfilled grains depicted an undesired positive skewness, indicating a greater number of genotypes with a higher number (≥ 20.66) of empty grains per panicle. Here, 6.66 represented the genotypes with the minimum number of empty grains, while 40.9 represented the genotypes with the highest number of empty grains per panicle.

The boxplot for grain length showed the lower and middle quartile values of 7.1 and 8.3, respectively, and also depicted more populations with grain lengths more than 7.1mm and less than 8.3mm. The trait distribution was negatively

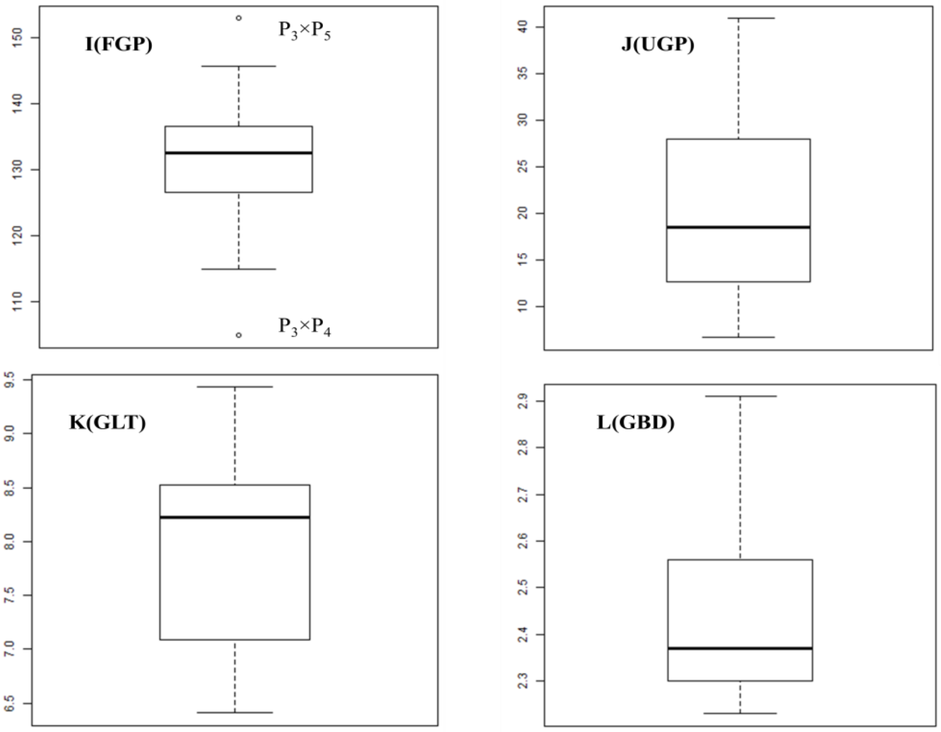

Figure 1(I-L). Boxplots showing the four summary distributions of the traits (I) number of filled grains per panicle, (J) number of unfilled grains per panicle, (K) grain length, and (L) grain breadth.

skewed in the plot, revealing a comparatively wider and scattered distribution on the opposite side of the median than both the filled grains and unfilled grains simultaneously. In comparison with grain length, the grain breadth exhibited more variability in the right portion, causing it to be positively skewed for the trait. The boxplot also revealed a comparatively less scattered distribution of the genotypes, which resulted in it being shorter than the boxplot for grain length, indicating less variability.

Variability explanations based on boxplots are presented in **Figure 1 (M-P)** for the traits of length-to-breadth ratio of the grain, dry weight of straw, 1000-grain weight, and grain yield plant. The boxplot for the grain length-to-breadth ratio showed a slight negative skewness with smaller variability among the studied rice genotypes. The outlier genotype, P4, showed a higher range of variability with the lowest (1.02) length-to-breath ratio compared to the other genotypes.

The boxplot for straw dry weight, however, depicted a normal symmetrical distribution. Here, 11.11g represented the genotypes with the lowest straw dry weight, while 39.06g represented the genotypes having the maximum straw dry weight. The second quartile (Q2) value (27.50) revealed that 50% of the values were genotypes with a straw dry weight less than 27.5g and similarly the remaining 50% had a SDW of more than 27.5g. Two genotypes, P2×P3 and P3×P4, exhibited larger dissimilarities for producing lower straw weights than the others, since they were located as lower outliers of the boxplot.

The boxplot of the 1000-grain weight ranged from 19.12g for the minimum weight to 40.6g for maximum weight. Here, 50% of the trait values fell below the middle quartile and the rest fell above the quartile. The second quartile divided the boxplot into two equal halves indicating a normal symmetrical distribution of the trait. Although the size of the plot revealed a less scattered distribution of genotypes with smaller differences, variability was depicted by two outliers (P2 and P3×P4) as they were located on the opposite outlier sides of the boxplot.

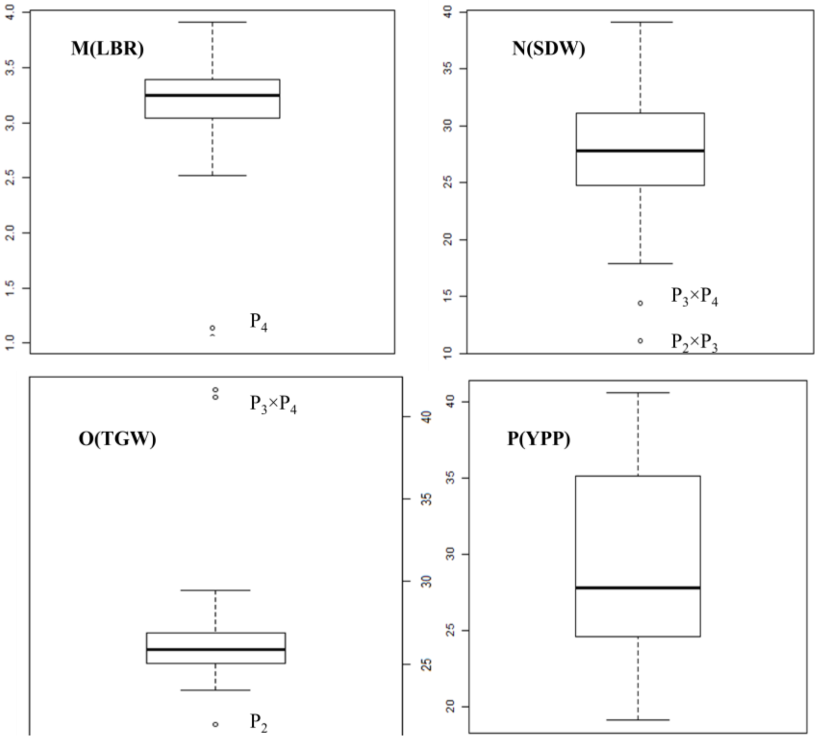

Figure 1(M-P). Boxplots showing the four summary distributions of the traits (M) length-to-breadth ratio of grain, (N) dry weight of straw, (O) 1000-grain weight, and (P) grain yield per plant.

Regarding the boxplot for grain yield per plant, it can be noticed that the populations were more scattered towards the upper whisker, indicating a greater number of genotypes with a higher than average yield (g) and also revealing a positively skewed distribution for this trait. The size of the boxplot also showed a diverse range of genotypes with wider variability and did not exhibit any outliers. The crosses involving the parental genotype P_3 (BINA dhan10) produced a wide range of segregants for effective tillers per plant, filled grains per panicle, panicle length, and grain length (**Table 3**). This result is also supported by the general combining ability of P_3 reported earlier by Ray *et al*. (2021).

Conclusions

The main goal of the present research was to create genetic variability utilizing elite and salinity tolerant genotypes for the development high yielding and salinity tolerant genotypes towards new varieties. The present research revealed significant genetic variability for all the studied characters except grain width among the genotypes. Lower GCV values were observed for the majority of the characters, where days to maturity exhibited the lowest GCV (1.244%) followed by filled grains (2.152%) and panicle length (2.888%). Small differences between GCV and higher PCV values indicated little influence of the environment and the predominant control of genetic factors towards the variability of these characters. The high heritability values of these traits indicated the low environmental influence on these characters. The traits such as grain length, grain width, length-to-breadth ratio of grain, and 1000-grain weight revealed high heritability coupled with moderate to high GCV and PCV, indicating the influence of both additive and non-additive genetic effects on these traits. Boxplot analyses also showed a diverse range of genotypic differences for all the studied characters of the rice genotypes. The traits of effective tillers per plant, filled grains per panicle, panicle length, and grain length exhibited prominent segregation among the F_2 population. The hybrid combination P_3 x P_4, among the ten evaluated crosses, showed the greatest potential for further use in breeding programs for improving yield, salinity tolerance, and Zn content in rice.

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