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Research Article

Phenotypic Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Drought and Low Phosphorus Stress Tolerance

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Abstract

Drought and phosphorus (P) deficiency stress are two significant natural abiotic stresses restricting rice growth and yield worldwide. Developing rice varieties tolerant to drought and low P stress is crucial for sustainable agricultural production. To address these issues, two separate experiments were conducted using selected advanced rice breeding lines to study the impact of drought and low P stress on yield-attributing traits. The first experiment evaluated the drought stress tolerance of five advanced lines (Lines 14, 16, 20, and 22) under pot culture conditions by applying drought stress and not providing water throughout the reproductive stages (late booting to ripening). All genotypes under study exhibited a significant reduction in the yield of grain, ranging from 75.68% to 39.52%, as well as spikelet fertility and SPAD value when drought stress was applied; however, Line 20, BRRI dhan71, and Nerica 10 showed a less significant decrease. Conversely, days to first flowering, days to maturity, and the number of unfilled grains increased significantly in all of the genotypes studied; however, Line 20, BRRI dhan71, and Nerica10 showed a slight increase. Based on stress tolerance indices, Nerica 10, BRRI dhan71, and Line 20 are the best performers. In the second experiment, four advanced lines (Lines 3, 4, 6, and 20) were evaluated for low phosphorus (P) stress tolerance under conditions where no P fertilizer was applied. The application of low P stress significantly harmed all of the yield characteristics (excluding days to first flowering, days to maturity, and number of unfilled grains) of most of the genotypes, while Binadhan-17, Line 4, and Line 20 displayed a milder reduction, with overall grain yield reductions across genotypes ranging from 40.74% to 8.78%. Binadhan-17 and Line 20 showed higher stress tolerance indices and were classified as low P-tolerant genotypes. Considering both experimental results, the advanced breeding Line 20 was categorized as a promising advanced line. Therefore, Line 20 could be a potential donor parent for breeding drought and P deficiency-tolerant rice varieties.

Keywords

Rice; drought; phosphorus deficiency; yield reduction; stress tolerance

1. Introduction

With its widespread consumption, Rice (*Oryza sativa* L.) acts as the main sustenance for more than 50% of the world's population, meeting approximately 80% of their caloric needs, particularly in Asian nations [1]. In 2022, approximately 28.89 million acres of land were used to produce 38.14 million metric tons of rice in Bangladesh alone, making it the most important food crop, accounting for 95% of all cereals consumed [2]. It contributes to half of all agricultural income and one-sixth of national revenue in Bangladesh [3]. It was predicted that by 2050, rice output must quadruple to feed the world's fast-growing population [4]. However, several biotic and abiotic stressors prevent this expected rise in rice output [5, 6]. Abiotic stresses, including salt, drought, and phosphorus (P) deficiency stresses, constantly threaten rice yield and sustainability in Bangladesh [7, 8]. The increasing global temperature and other climatic factors make these abiotic stresses harder to fight against.

Bangladesh is quite vulnerable to the effects of climate change, as it is expected to experience a rise in temperatures of 1°C by 2030 and 4°C by 2050 [9]. Consequently, approximately 2.7 million hectares are susceptible to annual droughts, with a 10% possibility that 41-50% of the country might face drought in any given year [9]. This can substantially undermine agricultural production in these drought-prone regions. Under such conditions, the nation's primary agricultural crop, rice, a water-loving plant, is extremely vulnerable to drought stress, which significantly affects its grain yield and causes an annual loss of production of almost 18 million tons worldwide [10]. In Bangladesh, during 1978-79, drought stress resulted in a significant loss (around 2 million tons) of rice production [11]. Drought affects rice at many developmental growth stages, with the flowering and grain-filling phases being the most drought-sensitive, leading to significant output losses [12, 13]. Drought also impacts leaf membrane shape, photosynthesis, and pigment concentration [14]. Even grain's physiology, morphology, anatomy, and biochemistry are all significantly impacted by drought, negatively impacting yield production [15]. Rice yield loss from mild drought ranges from 10–30%, while severe drought can cause yield losses of up to 70–90% [16]. While it is possible to overcome the crop development retardation brought on by water stress during the seedling stage, water stress during the reproductive stage can significantly lower rice yield [17]. International Rice Research Institute (IRRI) has shown that rice plants growing under a range of water applications transpired 500–1,000 liters of water to produce 1 kg of rough (unmilled) rice [18]. With the world's population on the rise and water resources dwindling, meeting such a colossal water demand is not only economically and environmentally unsustainable but, in some instances, simply unattainable. In light of the increasing demand for food, the depletion of resources, and the high variability of climate, drought-tolerant rice varieties are essential for ensuring food security.

Phosphorus (P) is an essential plant macronutrient and the second-most important nutrient for rice production. Being indispensable, P is recognized as a primary factor driving optimal crop productivity on arable lands worldwide. Insufficient P in plants limits growth and decreases the dry matter content of multiple plant organs indicating retardation in crop health and overall yield. Moreover, P deficit impairs nutrient absorption and dramatically lowers photosynthesis efficiency, producing reactive oxygen species [19]. Globally, 5.8 billion hectares of arable land are thought to be lacking P [20, 21]. It is estimated that 41% of the soils of Bangladesh contained P below the critical level and 35% of the soils contained P above the critical level but below the optimum level and the available P of Bangladeshi soils ranged from 2 to 14 ppm with a mean value of 12 ppm [8, 22]. Due to the soil's diminished P availability, the plant's ability to absorb soluble phosphate is confined. Studies proved that P is more susceptible to end up as being the limiting nutrient among the three major nutrients, namely nitrogen (N), P, and potassium (K) [23]. In Bangladesh, the primary chemical fertilizer for adding P to the soil, diammonium phosphate (DAP), has been used more than four times as much during the past ten years, from 4.03 lakh metric tons in the 2011–12 fiscal year to 16.85 lakh metric tons in the 2021–22 fiscal year [2]. In order to lower production costs, fertilizer import costs, and environmental contamination caused by P fertilizer runoffs, breeding for low P tolerant or resistant with higher uptake and assimilation efficient rice variety is the best alternative for sustainable production [24, 25].

To provide sustainable rice production in response to climate change and fulfill the demands of an expanding population, future rice varieties must be resilient to environmental stresses and exhibit moderate responses and good performance under pressure. Since rice was first domesticated thousands of years ago, breeding has been subject to high selection pressure, reducing the crop's genetic variability [26]. Therefore, research groups and breeders have been interested in improving rice genetic variability. Genetic variants can arise from hybridization, natural mutation, and/or artificial modification. As a rapid method to boost agricultural productivity, improve crop quality, and increase genetic variability, mutation breeding has gained popularity recently [27]. We have produced few advanced high-yielding mutants and cross-breeding lines in our lab. Therefore, it would be worthwhile to investigate their response against drought and low P stress based on morphological traits. Breeding for nutrient-efficient and drought-tolerant rice varieties will also be accelerated by identifying the morphological determinants that regulate stress tolerance at different plant growth stages, association studies, component analyses, and selection based on multiple stress tolerance indices. Given the issues above, the current research experiments were conducted to fulfill the following objectives: (i) to study the effects of drought and low P stress on yield and yield attributing traits in a few advanced rice mutants and cross-breeding lines (ii) to identify suitable advanced lines for future plant breeding program.

2. Materials and Methods

2.1 Experimental Site and Climatic Conditions

Two distinct experiments were carried out at the Department of Genetics & Plant Breeding's net house at Bangladesh Agricultural University, Mymensingh-2202, between July 2022 and May 2023. The experimental area is situated in the agro-ecological zone (AEZ 9) of the Old Brahmaputra Floodplain, at coordinates 24.72225 latitude and 90.42327 longitude. Medium-high land makes up around 35% of the total area; the soil is primarily silty with low fertility and organic matter levels. The monthly weather data on relative humidity, temperature and rainfall of the growing area are presented in Figure 1.

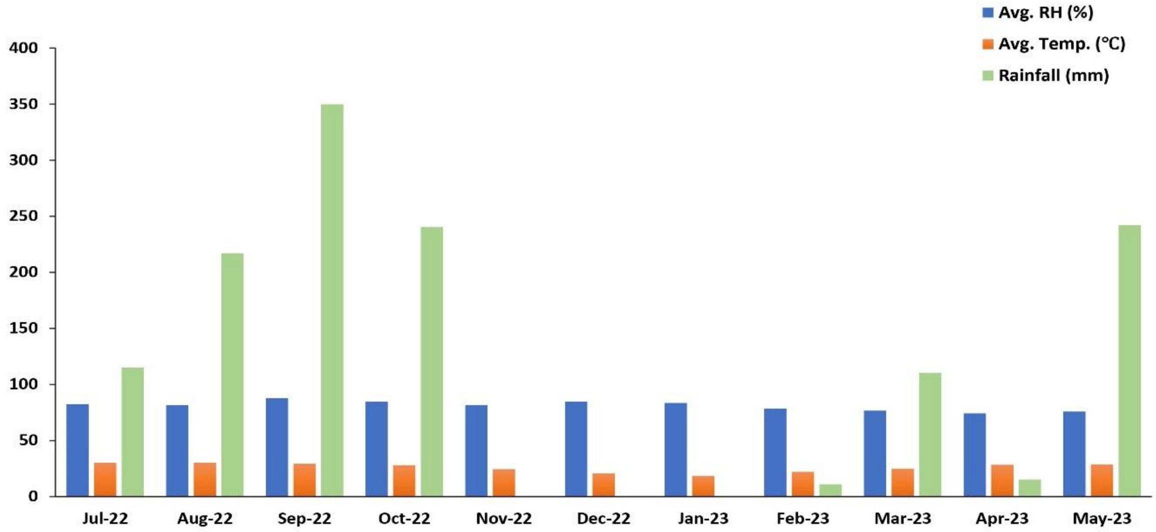

Figure 1 Monthly relative humidity, temperature and rainfall data of the growing region from the period July 2022 to May 2023. *Source:* Weather station, BAU Campus.

2.2 Effect of Drought Stress on Yield and Yield-Related Traits of Rice

2.2.1 Plant Materials and Experimental Design

One drought-tolerant rice variety, BRRI dhan71, one drought-susceptible variety, BRRI dhan49, and four advanced (M_4 generation) mutants (Line 14, Line 16, Line 20, and Line 22) developed from Nerica 10 were utilized as plant materials. The seeds of the genotypes were collected from the Bangladesh Rice Research Institute (BRRI) and the Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh. The Experiment was conducted following a randomized complete block design (RCBD) with three replications and two treatments (control and drought stress).

2.2.2 Preparation of Soil for Tray and Transplanting of Seedling

The experiment was conducted using medium-size (55 cm × 30 cm × 17 cm) plastic trays filled with field soil. The soil was prepared by puddling and mixing with cow dung (5000 kg/ha) and synthetic fertilizers (Urea, TSP, MP, and Gypsum @ 130, 50, 80, and 45 kg/ha, respectively) to provide proper nutrition for seedling establishment. Twenty-six-day-old seedlings were transplanted into the trays on July 28, 2022, maintaining a plant-to-plant distance of 20 cm.

2.2.3 Imposition of Drought Stress

The plants were initially grown under standard agronomic practices to ensure optimal growth and development. At the late booting stage, drought stress was imposed by withholding water application and continued up to the ripening stage, maintaining soil moisture levels around 10% and 12%. In contrast, following normal cultural practices, the control pots continued receiving sufficient water. The experiment was conducted under a rainout shelter to avoid rainwater.

2.2.4 Intercultural Operation

The first weeding was performed 15 days after transplanting, followed by the second at 25 days, and the last at 45 days. Weeds were carefully uprooted and removed from the trays without harming the rice plants. Basudin @ 3.33 kg/ha was applied to prevent nematode and rice yellow stem borer. Other intercultural practices were carried out as needed following standard practices.

2.3 Effect of Low P Stress on Yield and Yield-Related Traits of Rice

2.3.1 Plant Materials and Experimental Design

As plant materials, the Department of Genetics and Plant Breeding at Bangladesh Agricultural University (BAU) provided three advanced (F_3 generation) cross-breeding lines (Lines 3, 4, and 6), one advanced mutant (Line 20), and two cultivated varieties (BRRI dhan49 and Binadhan-17) from the Bangladesh Rice Research Institute (BRRI) and Bangladesh Institute of Nuclear Agriculture (BINA).Using three replications and two treatments (low P stress and control), the experiment was carried out using a randomized complete block design (RCBD).

2.3.2 Preparation of Soil for Tray, Transplanting of Seedling and Imposition of Low P Stress

The experiment was conducted using a large plastic tub filled with field soil, each tub measuring 135 cm × 90 cm × 24 cm. Control trays were amended with cow dung (5000 kg/ha) and fertilizers (Urea, TSP, MP, and Gypsum @ 130, 50, 34, and 45 kg/ha) to ensure optimal soil nutrient conditions. The tray marked with low P stress received the same rate of fertilizers as applied in the control trays except TSP fertilizer for creating a low P stress condition. The native soil P level was 9.21 ppm, and available P (according to Olsen method) below 15 ppm for rice-growing soil is categorized as P-deficient soil [28]. Seeds of six genotypes were sown in a seedbed on December 28, 2022, and transplanted into the plastic-tubes on February 18, 2023, maintaining a plant-to-plant distance of 20 cm and row-to-row distance of 25 cm.

2.3.3 Intercultural Operation

The first weeding was done 15 days after transplanting, the second was done 25 days after transplant, and the last was given 45 days after transplanting. Basudin @ 3.33 kg/ha was applied to prevent nematode and rice yellow stem borer. Other intercultural operations were done as per requirement.

2.4 Data Collection on Yield and Yield-Attributing Traits

In both experiments, information was recorded regarding the following: days to first flowering (DFF), days to maturity (DM), plant height (PH), panicle length (PL), number of filled grains/panicle (NFG), number of unfilled grains/panicle (NUG), spikelet fertility (%) (SF%), 1000 seed weight (100-SW), and grain yield/plant (Y/P). Furthermore, in the experiment I measured the SPAD value (SV). Data were gathered from five randomly chosen plants for every genotype and replication.

2.5 Estimation of Stress Tolerance Indices

The stress-tolerant indices were calculated using the following formulas:
Stress susceptibility index, $\mathrm{SSI} = \frac{1-Y_s/Y_p}{1-\bar{Y_s}/\bar{Y_p}}$ [29]
Stress tolerance, $\mathrm{TOL} = Y_p - Y_s$ [30]
Mean productivity, $\mathrm{MP} = \left(Y_s + Y_p\right)/2$ [30]
Stress tolerance index, $\mathrm{STI} = \frac{(Y_s)(Y_p)}{({Y_p}^2)}$ [31]
Geometric mean productivity, $\mathrm{GMP} = \sqrt{Y_p \times Y_s}$ [31]
Yield index, $\mathrm{YI} = \frac{Y_s}{\bar{Y_s}}$ [32]
Here, Y_p = Yield under normal conditions, and Y_s = Yield under stressed conditions.

2.6 Statistical Analysis

For both studies, a comprehensive tabulation and compilation of the data acquired for various parameters was done in preparation for statistical analysis using the Minitab 19 statistical software tool (Minitab Inc. State College, Pennsylvania). To evaluate the varietal performance of the genotypes under study, a number of statistical tests were run, including the two-factor ANOVA test, mean performance, combined effects of genotype treatment, reduction percentage, and PCA analysis.

3. Results

3.1 Effect of Drought Stress on Yield and Yield-Related Traits of Rice

3.1.1 Analysis of Variance for Drought Stress on Yield and Yield-Related Traits

The analysis of variance (ANOVA) for DFF, DM, PL, NFG, NUG, SF, SV, 100-SW, and Y/P showed a highly significant variation at 0.1% probability level. In comparison, PH showed variation at 1% level among the studied genotypes (Table S1). In treatment, the characters DFF, DM, PL, NFG, NUG, SF, SV, 100-SW, and Y/P also showed significant variation at a 0.1% probability level, whereas PH showed at 5% probability. In G × T interaction, DFF, DM, NFG, NUG, and SF showed significant variation at 0.1% level of probability, PH and Y/P showed considerable variation at 1% level of probability, and PL, SV, and 100-SWnshowed significant variation at 5% level of probability (Table S1).

3.1.2 The Mean Performance of the Rice Genotypes for Yield-Attributing Morphological Traits Studied under Drought Stress

Days to First Flowering (DFF). The present study found a remarkable variation in DFF. On average, DFF was maximum in BRRI dhan49 (91 days) and minimum in Line 16 (71 days) under control conditions (Table 1). Drought stress caused a significant delay in DFF for most genotypes, except two mutants, Line 14 and Line 22. The maximum delay in first flowering was found in Line 20 (10.09%), followed by Line 16, Nerica 10, BRRI dhan49, and BRRI dhan71 (7.08%, 5.07%, 4.67%, and 3.14%, respectively) (Table 1). Conversely, Line 14 and Line 22 exhibited earlier flowering by 4.20% and 2.46%, respectively, under drought conditions.

Table 1 Mean performances of seven rice genotypes based on different yield and yield-related traits grown under control and drought conditions.

Genotypes	Treatment	DFF	DM	PH	PL	NFG	NUG	SF	SV	100-SW	Y/P
BRRI dhan71	Control	85.00 CD	111.33 DE	95.38 A-C	24.53 B-E	128.02 B	12.33 F	91.22 A	37.68 A	2.28 AB	35.65 AB
	Drought	87.67 BC	114.33 C	81.28 BC	23.51 C-F	78.40 CD	63.67 C	55.19 C	24.12 D	2.12 B-D	17.52 DE
BRRI dhan49	Control	91.00 AB	116.67 B	78.21 C	21.74 EF	134.33 AB	15.00 EF	89.96 A	34.81 A-C	1.81 EF	33.22 A-C
	Drought	95.00 A	121.33 A	88.96 A-C	20.77 F	55.33 E	84.33 A	39.62 G	23.58 DE	1.68 F	9.46 EF
Line 14	Control	71.33 GH	117.33 B	98.87 AB	23.25 C-F	124.33 B	24.67 D	83.46 B	33.39 BC	2.26 AB	26.40 C
	Drought	68.00 H	120.33 A	94.82 A-C	22.81 C-F	64.41 DE	73.33 B	46.74 EF	21.46 D-F	2.23 AB	9.33 EF
Line 22	Control	81.33 DE	107.33 G	81.36 BC	24.81 B-D	129.67 B	17.33 E	88.22 AB	33.93 BC	2.31 A	27.96 BC
	Drought	79.33 EF	113.67 C	83.87 BC	22.09 D-F	49.67 E	68.00 C	42.13 FG	20.69 EF	2.18 A-C	6.80 F
Nerica 10	Control	72.33 GH	108.67 FG	104.85 A	27.17 AB	116.67 B	15.00 EF	88.59 AB	31.97 C	2.33 A	26.02 CD
	Drought	76.00 FG	112.67 CD	82.14 BC	25.13 A-C	85.33 C	67.33 C	55.88 C	20.06 F	2.13 B-D	15.71 E
Line 16	Control	70.67 H	109.33 F	91.00 A-C	24.18 C-E	118.67 B	12.00 F	90.78 A	33.07 C	2.29 AB	27.99 BC
	Drought	75.67 FG	112.67 CD	89.54 A-C	22.14 D-F	63.72 DE	66.00 C	48.88 DE	23.76 DE	2.01 CD	11.93 EF
Line 20	Control	76.00 FG	109.67 EF	92.18 A-C	27.83 A	149.48 A	17.33 E	89.62 A	36.28 AB	2.19 AB	38.99 A
	Drought	83.67 C-E	113.67 C	86.83 A-C	23.69 C-E	77.33 CD	66.00 C	53.84 CD	23.95 D	1.99 DE	16.37 E

Notes: Different letters are significant at 5% level of significance following Tukey’s method. Here, DFF = days to first flowering, DM = days to maturity, PH = plant height (cm), PL = panicle length (cm), NFG = no. of filled grains, NUG = no. of unfilled grains, SF = spikelet fertility (%), SV = SPAD, 100-SW = 100-seed weight (g), Y/P = yield/plant (g).

Days to Maturity (DM). According to (Table 1), the average range of DM among the genotypes was 107 days to 117 days under control conditions. Line 14 required a maximum number of DM (117 days), and Line 22 required a minimum no. of DM (107 days). Drought stress led to a significant delay in DM among all the genotypes studied. The highest increase was observed in Line 22 (5.91%) and the lowest was in Line 14 (2.56%) (Table 1).

Plant Height (PH). Drought stress showed a significant impact on PH as compared to their control. The maximum value was observed in Nerica 10 (104.85 cm) and the minimum in BRRI dhan49 (78.21 cm) under control treatment. Drought stress imposition significantly reduced PH compared to control in most of the studied genotypes. Nerica 10 exhibited the highest reduction at 21.66%, while BRRI dhan71, Line 20, Line 14, and Line 16 showed 14.78%, 5.80%, 4.10%, and 1.60%, respectively (Table 1).

Panicle Length (PL). When exposed to drought stress, there was a significant variance in the genotypes for panicle length (Table 1 and Figure 2). While BRRI dhan49 had the lowest PL (21.74 cm), Line 20 had the highest (27.83 cm) in the control condition. A significant decrease in PL was observed in rice plants exposed to drought stress; the most critical loss was observed in Line 20 (14.88%), which was followed by Line 22, Line 16, Nerica 10, BRRI dhan49, BRRI dhan71, and Line 14 (Table 1).

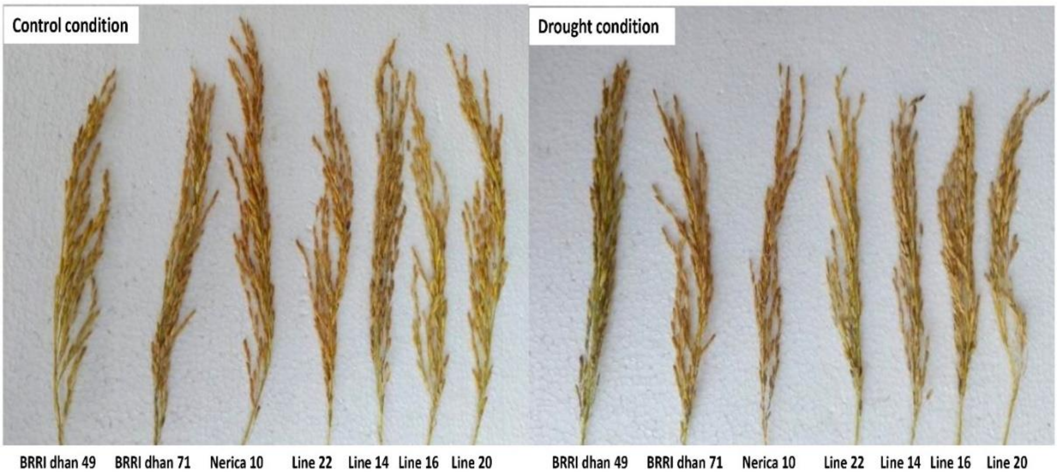

Figure 2 Phenological appearances of rice panicles grown under control (left picture) and drought stress conditions (right picture).

No. of Filled Grains/Panicles (NFG). The imposition of drought stress resulted in a significant decrease in filled grains among all genotypes compared to the control group (Table 1). Under control conditions, the highest NFG was found in Line 20 (150) and the lowest in Nerica10 (117), whereas BRRI dhan71 (78.40) had the maximum number of NFG and Line 22 (49.67) had the minimum number of NFG in a stressed condition. Drought stress resulted in a considerable decrease in NFG. A 61.70% reduction in NFG was observed in Line 22, followed by 58.81% in BRRI dhan49, 48.27% in Line 20, 48.19% in Line 14, 46.3% in Line 16, 38.76% in BRRI dhan71, and 26.86% in Nerica10 (Table 1).

No. of Unfilled Grains/Panicle (NUG). In this study, the effects of drought stress treatments showed a significant variation in unfilled grains among the genotypes (Table 1). The highest NUG was noted in Line 14 (25) and the lowest was recorded in Line 16 (12). Drought stress imposition resulted in significant induction of NUG as compared to control. The highest induction was found in BRRI dhan49 (462.20%), followed by Line 16, BRRI dhan71, Nerica 10, Line 22, Line 20 and Line 14 (450%, 416.38%, 348.87%, 292.38%, 280.84%, and 197.24%, respectively) (Table 1).

Spikelet Fertility (SF). There was significant variation in the SF (%) case, with an average range of 39.62 to 91.22. BRRI dhan71 showed the highest value (91.22) for SF (%), whereas Line 14 showed the lowest value (83.46) for SF (%) in the control condition (Table 1). Drought stress led to a significant decrease in SF (%) among all the genotypes studied. The highest reduction was 55.96% in BRRI dhan49, followed by 52.24% in Line 22, 46.16% in Line 16, 44% in Line 14, 39.92% in Line 20, 39.5% in BRRI dhan71, and 36.92% in Nerica 10 (Table 1).

SPAD Value (SV). Drought stress showed a significant impact on SPAD value as compared to their control. The highest value was observed in BRRI dhan71 (37.68) and the lowest in Nerica 10 (31.97) under the control treatment (Table 1). Drought stress imposition resulted in a significant reduction of SPAD value compared to control. The highest reduction was found in Line 22 (39.02%), followed by Nerica 10, BRRI dhan71, Line 14, Line 20, BRRI dhan49, and Line 16 (36.92%, 35.99%, 35.73%, 33.99%, 32.26% and 28.15%, respectively) (Table 1).

100-Seed Weight (100-SW). The present study found a significant variation in 100-SW. According to Table 1, the highest 100-SW was recorded in Nerica 10 (2.33 g) and the lowest in BRRI dhan49 (1.81 g) under control conditions. Drought stress causes a considerable reduction in 100-SW. The maximum reduction was found in Line 16 (12.23%), followed by Line 20 (9.13%), Nerica 10 (8.58%), BRRI dhan49 (7.18%), BRRI dhan71 (7.02%), Line 22 (5.63%), and Line 14 (1.33%) (Table 1).

Grain Yield/Plant (Y/P). The effect of drought stress causes significant variation among the genotypes for grain Y/P. The highest grain Y/P was found in Line 20 (38.99 g), whereas the lowest was found in Nerica 10 (26.02 g) under control conditions. The highest grain Y/P was found in BRRI dhan71 (17.52 g), followed by Line 20 (16.37 g) and Nerica 10 (15.71 g), whereas the lowest was found in Line 22 (6.80 g) under drought conditions. Drought stress resulted in a significant reduction in grain Y/P among all of the genotypes; the highest decrease was observed in Line 22 (75.68%), with subsequent reductions in BRRI dhan49 (71.52%), Line 14 (64.66%), Line 20 (58.01%), Line 16 (57.38%), BRRI dhan71 (50.86%), and Nerica 10 (39.52%) (Table 1).

3.1.3 Principal Components (PCs) for Ten Morphological Traits in Seven Rice Genotypes from Principal Component Analysis (PCA)

The principal component analysis of the ten studied morphological parameters was conducted on seven rice genotypes under both unstressed (control) and drought-stressed conditions. This analysis extracted three principal components (PC) with eigenvalues higher than unity (Table 2). These three principal components (PCs) accounted for 87.7% of the total variation. The first component, PC1, accounted for 58.7% of the total variance, with SF (0.398), NFG (0.386), Y/P (0.373), SV (0.370), PL (0.290), 100-SW (0.248), and PH (0.123) resulting positive contributions, while DFF (-

0.125) and DM (-0.289) generating negative contributions (Table 2; Figure 3). The PC2, which explains 19.5% of the variation, with the majority of the contribution attributed to the more significant positive loadings of DFF (0.596), Y/P (0.246), SV (0.239), NFG (0.190), DM (0.162), and SF (0.145), as well as the more significant negative loadings of 100-SW (-0.466), PH (-0.370), PL (-0.272), and NUG (-0.126) (Table 2; Figure 3).

Table 2 Principal components (PCs) for ten morphological traits in seven rice genotypes from principal component analysis (PCA) under control and drought treatment.

Variable	PC1	PC2	PC3
DFF	-0.125	0.596	-0.104
DM	-0.289	0.162	0.544
PH (cm)	0.123	-0.370	0.737
PL (cm)	0.290	-0.272	-0.236
NFG	0.386	0.190	0.053
NUG	-0.395	-0.126	-0.065
SF (%)	0.398	0.145	0.065
SV	0.370	0.239	0.206
100-SW (g)	0.248	-0.466	-0.196
Y/P (g)	0.373	0.246	0.059
Eigenvalue	5.8655	1.9510	0.9520
Proportion	0.587	0.195	0.095
Cumulative	0.587	0.782	0.877

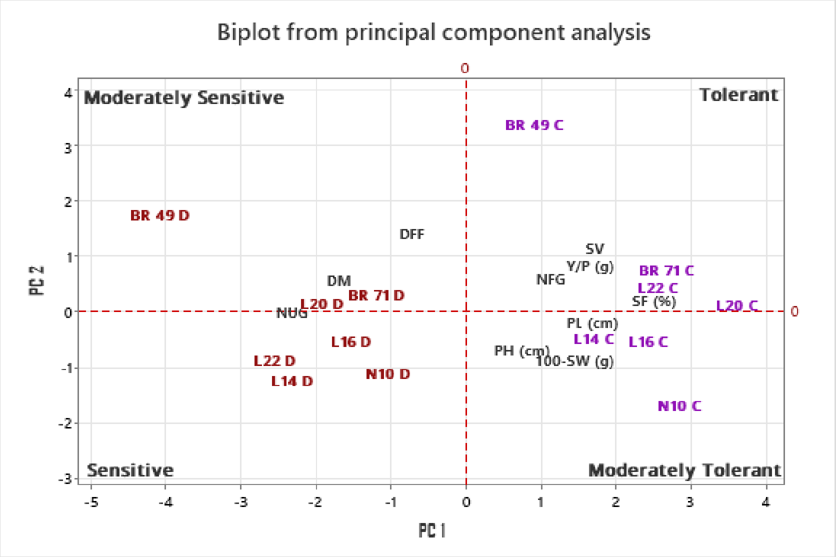

Figure 3 Biplot from principal component analysis of morphological traits of seven rice varieties under control and drought treatment. Here, DFF = days to first flowering, DM = days to maturity, PH = plant height (cm), PL = panicle length (cm), NFG = no. of filled grains, NUG = no. of unfilled grains, SF = spikelet fertility (%), SV = SPAD value, 100-SW = 100-seed weight (g), Y/P = yield/plant (g), BR 49 = BRRI dhan49, BR 71 = BRRI dhan71, L 14 = Line 14, L 16 = Line 16, L 20 = Line 20, L 22 = Line 22, N 10 = Nerica 10, 'C' and 'D' indicate treatment under control and drought condition, respectively.

To visually demonstrate the placements of the variables, the coefficients of PC1 and PC2 were projected in two dimensions, as shown in Figure 2. The biplot revealed that the PC1 scores of BRRI dhan49 under the low P stress treatment were entirely distinct from those of Line 20 under control treatments. The variation between BRRI dhan49 under low P conditions and Line 20 under control treatments was due to a higher negative coefficient of the traits NUG, DFF, and DM. This was in contrast to the positive coefficients of the characteristics of PL, PH, SF (%), and Y/F, shown in Figure 2. Similarly, the PC2 scores of Binadhan-17 in the control group exhibited a complete separation from those of Line 6 in the low P group. This difference is attributed to the higher positive coefficients associated with the traits NFG, DFF, DM, and SF (%) as opposed to the higher negative coefficients associated with 100-SW and NUG.

3.1.4 Estimation of Stress Tolerance Attributes in Rice Genotypes, Estimated from Grain Yield/Plant Obtained from Control and Drought Stress Conditions

Table 3 displays several stress tolerance indices for different rice genotypes calculated from yields under normal and drought stress conditions. For mean productivity (MP) values, Line 20 was recorded as the highest (27.68) followed by BRRI dhan71 (26.59), BRRI dhan49 (21.34), Nerica 10 (20.87), Line 16 (19.96), Line 14 (17.87) and Line 22 (17.38) (Table 3). The highest value of geometric mean productivity (GMP) was obtained for the genotype Line 20 (25.26), followed by BRRI dhan71 (24.99), Nerica 10 (20.22), Line 16 (18.27), BRRI dhan49 (17.73), Line 14 (15.69) and Line 22 (13.79). Regarding the stress susceptibility index (SSI), Line 22 had the highest value (1.27), followed by BRRI dhan49 (1.20), Line 14 (1.08), Line 20 (0.97), Line 16 (0.96), BRRI dhan71 (0.85), and Nerica 10 (0.66). In terms of tolerance (TOL), BRRI dhan49 led with a value of 23.73, with Line 20 (22.62), Line 22 (21.16), BRRI dhan71 (18.13), Line 14 (17.07), Line 16 (16.06), and Nerica 10 (10.31) following. The maximum stress tolerance index (STI) was recorded for Line 20 at 0.67. This was followed by BRRI dhan71 (0.65), Nerica 10 (0.43), Line 16 (0.35), BRRI dhan49 (0.33), Line 14 (0.26), and Line 22 (0.20). Nerica 10 exhibited the highest yield stability index (YSI) at 0.60, with BRRI dhan71 following at 0.49, Line 16 at 0.43, Line 20 at 0.42, Line 14 at 0.35, BRRI dhan49 at 0.28, and Line 22 at 0.24 (Table 3).

Table 3 Stress Tolerance Indices in rice genotypes, estimated from grain yield/plant obtained in control & drought stress conditions.

Genotypes	MP	GMP	SSI	TOL	STI	YSI
BRRI dhan71	26.59	24.99	0.85	18.13	0.65	0.49
BRRI dhan49	21.34	17.73	1.20	23.76	0.33	0.28
Line 14	17.87	15.69	1.08	17.07	0.26	0.35
Line 22	17.38	13.79	1.27	21.16	0.20	0.24
Nerica 10	20.87	20.22	0.66	10.31	0.43	0.60
Line 16	19.96	18.27	0.96	16.06	0.35	0.43
Line 20	27.68	25.26	0.97	22.62	0.67	0.42

Here, MP: Mean productivity; GMP: Geometric mean productivity; SSI: Stress Susceptibility Index; TOL: Tolerance Index; STI: Stress Tolerance Index; YSI: Yield stability index.

3.2 Effect of Low P Stress on Yield-Related Traits of Rice Genotypes

3.2.1 Analysis of Variance for Low P Stress on Yield and Yield-Related Traits

The result of the analysis of variance for all the characters (DFF, DM, PH, PL, NFG, NUG, SF (%), 100-SW, and Y/P) showed highly significant ($P \leq 0.01$) variation among the genotypes and treatments. In G × T interaction, DFF, PH, NFG, NUG, SF, 100-SW, and Y/P showed significant variation at a 0.1% level of probability, where DM showed considerable variation at a 1% level of probability, and PL showed significant variation at a 5% level of probability (Table S2).

3.2.2 The Mean Performance of the 09 Qualitative Traits Studied in Six Rice Genotypes under Low P Stress is Described Below

Days to First Flowering (DFF). The present study found a remarkable variation in DFF in response to low P stress. On average, DFF was maximum in Binadhan-17 (60.33 days) and minimum in Line 6 (47.67 days) under control conditions. Under low P stress conditions, the highest number of days (61.33 days) required for first flowering was recorded in the variety Binadhan-17, whereas the lowest number required (51.33 days) was found in Line 6. Low P stress resulted in a considerable delay in DFF, with maximum delay found in BRRI dhan49 (14.47%). The minimum delay in DFF was recorded in Binadhan-17 (1.66%) (Table 4).

Table 4 Mean performances of six rice genotypes based on different yield and yield-related traits grown under control and low P conditions.

Genotypes	Treatment	DFF	DM	PH	PL	NFG	NUG	SF	100-SW	Y/P
BRRI dhan49	Control	53.00 C	86.67 DE	58.53 H	24.15 E	136.67 F	9.77 H	93.33 BC	1.82 G	31.82 AB
	Low P	60.67 A	92.67 B	51.06 I	21.19 F	125.67 H	27.68 D	81.96 H	1.68 H	23.01 DE
Binadhan-17	Control	60.33 A	96.33 A	65.88 F	25.43 DE	176.51 A	6.96 I	96.21 A	2.02 E	28.11 BC
	Low P	61.33 A	98.33 A	61.71 G	24.38 DE	171.93 B	19.77 E	89.68 F	1.96 F	26.73 CD
Line 20	Control	49.33 DE	85.67 EF	89.96 A	30.58 A	174.32 AB	16.50 F	91.35 DE	1.98 EF	35.45 A
	Low P	51.33 CD	88.33 CD	65.11 F	26.16 CD	115.00 I	35.33 B	76.50 J	2.27 C	17.50 F
Line 6	Control	46.67 E	84.00 F	69.36 DE	29.31 A	129.40 G	8.93 HI	93.55 B	2.47 A	29.53 BC
	Low P	51.33 CD	88.33 CD	65.11 F	26.16 CD	115.00 I	35.33 B	76.50 J	2.27 C	17.50 F
Line 3	Control	51.67 CD	85.33 EF	67.05 EF	25.39 DE	153.33 D	12.60 G	92.41 CD	2.31 C	28.45 BC
	Low P	56.33 B	90.33 C	58.34 H	23.91 E	144.33 E	41.67 A	77.60 I	2.12 D	17.22 F

Line 4	Control	49.33 DE	86.00 EF	74.67 C	27.31 BC	118.00 I	11.83 G	90.89 E	2.38 B	26.60 CD
	Low P	53.00 C	89.00 C	69.38 D	25.20 DE	106.33 J	31.00 C	77.43 IJ	2.28 C	22.24 E

Notes: Different letters are significant at 5% level of significance following Tukey's method. Here, DFF = days to first flowering, DM = days to maturity, PH = plant height (cm), PL = panicle length (cm), NFG = no. of filled grains, NUG = no. of unfilled grains, SF = spikelet fertility (%), 100-SW = 100-seed weight (g), Y/P = yield/plant (g).

Days to Maturity (DM). The variety Binadhan-17 required a maximum of days (96.33 days) to mature, whereas Line 6 required a minimum of days (84 days) to mature under control conditions. Under low P stress, Binadhan-17 required the maximum (98.33 days), and Line 6 required a minimum no. of days to mature (88.33 days). Low P stress led to a significant delay in maturity among all the genotypes studied, with BRRI dhan49 experiencing the maximum delay (6.92%) and Binadhan-17 showing the minimum delay (2.08%) (Table 4).

Plant Height (PH). The maximum PH was observed in Line 20 (89.96 cm) and the minimum in BRRI dhan49 (58.53 cm) under control treatment (Table 4). Low P stress imposition resulted in a significant reduction of PH as compared to control. The highest reduction was found in Line 3 (12.99%) followed by BRRI dhan49 (12.76), Line 4 (7.08), Binadhan-17 (6.33), and Line 6 (6.13) whereas the lowest reduction was found in Line 20 (3.48%) (Table 4) (Figure 4).

Figure 4 Phenotype of rice plant grown under control (left picture) and low P stress conditions (right picture).

Panicle Length (PL). The highest PL under the control condition was found in Line 20 (30.58 cm), whereas the lowest was found in BRRI dhan49 (24.15 cm) (Table 4). In stress conditions, the highest PL (28.97 cm) was recorded in the advanced Line 20, whereas the lowest length (21.19 cm) of the panicle was found in the variety BRRI dhan49 (Table 4). Rice plants grown under low P stress showed a considerable reduction in PL and the highest reduction was observed in BRRI dhan49 (12.26%) and the lowest reduction was observed in Binadhan-17 (4.13%) (Table 4).

No. of Filled Grains/Panicle (NFG). Under control condition, the highest NFG was found in Binadhan-17 (176.51) and the lowest in Line 4 (118), but in a stressed condition, the highest NFG was found in Binadhan-17 (171.93) and the lowest in Line 4 (106.33) (Table 4). A significant reduction in NFG was observed in plants grown under low P stress conditions. According to values, Line 6 experienced the maximum decline in NFG, with a decrease of 11.13%. This was followed by Line 4 (9.89%), BRRI dhan49 (8.05%), Line 20 (7.26%), Line 3 (5.87%), and Binadhan-17 (2.59%).

No. of Unfilled Grains/Panicle (NUG). The highest NUG was noted in Line 20 (16.50), and the lowest was recorded in Binadhan-17 (6.96) at the control condition (Table 4). Under low P stress conditions, the highest NUG was noted in Line 3 (41.67), and the lowest was recorded in Binadhan-17 (19.77). Low P stress resulted in a significant increase in NUG. The highest induction in NUG was observed at 295.63% in Line 6, followed by Line 3 at 230.71%, Binadhan-17 at 184.05%, BRRI dhan49 at 183.32%, Line 4 at 162.05%, and Line 20 at 30.48%.)

Spikelet Fertility (SF). Binadhan-17 showed the highest value (96.21) for SF, whereas Line 4 showed the lowest value (90.89) for SF in the control condition (Table 4). In stress, Binadhan-17 still showed the highest value (89.68) for SF, whereas Line 6 showed the lowest value (76.50) for SF. Plants grown under low P stress showed a significant decrease in NUG. Line 6 exhibited the highest reduction at 18.23%, with Line 3 following at 16.03%, Line 4 at 14.81%, BRRI dhan49 at 12.18%, Binadhan-17 at 6.79%, and Line 20 at 3.39% (Table 4).

100-Seed Weight (100-SW). The highest 100-SW was recorded in Line 6 (2.47 g), while the lowest was in BRRI dhan49 (1.82 g) under control conditions. Under low P stress conditions, the highest 100-SW was recorded in Line 4 (2.28 g) and the lowest in BRRI dhan49 (1.68 g). Maximum reduction was found in Line 3 (8.23%) followed by Line 6, BRRI dhan49, Line 20, Line 4, and Binadhan-17 (8.10%, 7.69%, 6.06%, 4.20%, and 2.97%, respectively) (Table 4).

Grain Yield/Plant (Y/P). The highest Y/P was found in Line 20 (35.45 g), whereas the lowest was found in Line 4 (26.60 g) under control conditions. Under low P stress conditions, the highest grain (26.73 g) yield was recorded in Binadhan-17, whereas the lowest was recorded in Line 3 (17.22 g). Rice plants grown under low P stress resulted in a considerable reduction in grain yield. The highest reduction was found in Line 6 (40.74%), followed by Line 3, Line 20, BRRI dhan49, Line 4, and Binadhan-17 (39.47%, 35.74%, 27.69%, 16.39%, and 8.78%, respectively) (Table 4).

3.2.3 Principal Component Analysis

Principal component analysis (PCA) was conducted to determine which morphological parameters contributed most significantly to low phosphorus (P) tolerance among six rice genotypes. The first three principal components explained 88.0% of the overall variation observed among the six rice genotypes (Table 5). PC1 accounted for 44.50% of the total variance, where PL (cm) had the highest positive coefficient (0.425) followed by Y/P (0.392), PH (0.377), SF (0.358), 100-SW (0.164), NFG (0.146) and DM (-0.297), DFF (-0.357) and NUG (-0.363) had negative coefficient (Table 5; Figure 5). For the second component PC2, which explained 31.00% variation, which was attributed to the positive loading of NFG (0.467), DFF (0.398), DM (0.391), SF (0.357) and Y/P (0.284) and the positive loading of 100-SW (-0.398), NUG (-0.283), PL (-0.139) and PH (-0.074) (Table 5; Figure 5).

Table 5 Principal components (PCs) for nine morphological traits in six rice genotypes from principal component analysis (PCA) under control and low P treatments.

Variable	PC1	PC2	PC3
DFF	-0.357	0.398	0.037
DM	-0.297	0.391	0.189
PH (cm)	0.377	-0.074	0.560
PL (cm)	0.425	-0.139	0.381
NFG	0.146	0.467	0.387
NUG	-0.363	-0.283	0.431
SF (%)	0.358	0.357	-0.300
100-SW (g)	0.164	-0.398	-0.239
Y/P (g)	0.392	0.284	-0.147
Eigenvalue	4.0032	2.7931	1.1258
Proportion	0.445	0.310	0.125
Cumulative	0.445	0.755	0.880

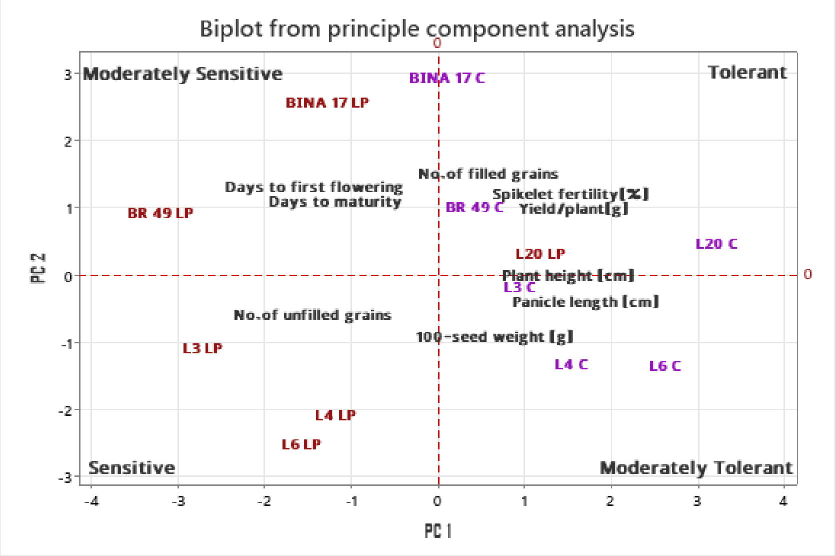

Figure 5 Biplot from principal component analysis of morphological traits of six rice varieties under control and low P treatments Here, DFF = days to first flowering, DM = days to maturity, PH = plant height (cm), PL = panicle length (cm), NFG = no. of filled grains, NUG = no. of unfilled grains, SF = spikelet fertility (%), 100-SW = 100-seed weight (g), Y/P = yield/plant (g), BR 49 = BRRI dhan49, BINA 17 = Binadhan-17, L4 = Line 4, L6 = Line 6, L3 = Line 3, L20 = Line 20, 'C' and 'LP' indicate treatment under control and low P condition, respectively.

3.2.4 Stress Tolerance Indices Based on Seed Yields of Six Rice Genotypes Obtained from Control and Low P Stress

Line 20 showed the highest MP value (30.62), followed by Binadhan-17 (27.42), BRRI dhan49 (27.42), Line 4 (24.42), Line 6 (23.52), and Line 3 (22.84). Similarly, the highest GMP value (30.23) was recorded for Line 20, followed by Binadhan-17 (27.41), BRRI dhan49 (27.06), Line 4(24.32), Line

6(22.73), and Line3(22.13). The genotype Line 6 had the highest SSI (1.54), whereas Binadhan-17 had the lowest (0.19). Line 6 exhibited the highest TOL at 12.03, followed by Line 3 at 11.23, Line 20 at 9.67, BRRI dhan49 at 8.81, Line 4 at 4.36, and Binadhan-17 at 1.38. Line 20 achieved the highest STI at 1.02, while Line 3 showed the lowest STI at 0.54. YSI was highest for Binadhan-17 (0.95), succeeded by Line 4 (0.84), Line 20 (0.73), BRRI dhan49 (0.72), Line 3 (0.61), and Line 6 (0.59) (Table 6).

Table 6 Stress Tolerance Indices in rice genotypes, estimated from grain yield/plant obtained in control & low P stress condition.

Genotypes	MP	GMP	SSI	TOL	STI	YSI
BRRI dhan49	27.42	27.06	1.05	8.81	0.81	0.72
Binadhan-17	27.42	27.41	0.19	1.38	0.84	0.95
Line 20	30.62	30.23	1.03	9.67	1.02	0.73
Line 6	23.52	22.73	1.54	12.03	0.57	0.59
Line 3	22.84	22.13	1.50	11.23	0.54	0.61
Line 4	24.42	24.32	0.62	4.36	0.66	0.84

Here, MP: Mean productivity; GMP: Geometric mean productivity; SSI: Stress Susceptibility Index; TOL: Tolerance Index; STI: Stress Tolerance Index; YSI: Yield stability index.

4. Discussion

4.1 Effect of Drought Stress on Yield and Yield-Related Traits of Rice

In Bangladesh, during the pre-Kharif season, approximately 12.49 million hectares of "Aman" rice land and 9.32 million hectares of winter (Rabi) cropland were affected by drought [16]. This water scarcity significantly disrupts rice cultivation, impacting growth, physiology, and yield. Under normal drought conditions, Aus rice yields can decline by 3% to 22%, with losses increasing to 10% to 30% in moderate drought and reaching reductions of 70% to 90% in severe drought [16, 33]. In the global climate change, drought-tolerant rice varieties urgently need to be developed capable of withstanding water deficit stress, recovering swiftly, and thriving upon the return of soil moisture to maximize yield. The current study evaluated several advanced rice breeding lines under drought stress based on yield-attributing morphological traits. ANOVA showed significant variation for genotype (G), treatment (T), and G × T interactions for the studied traits (Table S1), reflecting the scope of trait improvement through selection in the subsequent generation.

4.1.1 Mean Performance of Yield and Yield-Related Traits of Rice Genotypes under Drought Stress

Rice is especially susceptible to drought stress during its flowering and grain-filling stages. Severe drought stress can prolong the flowering and maturity cycle and significantly impact yield reduction [34]. In the present study, we found a significant delay in flowering and maturing in most of the studied genotypes. Similar to our findings, rice has also reported a delay in flowering and maturity [35-37]. Plants that bloom later than usual respond positively to drought stress by adapting to their surroundings. Additionally, longer maturation times for specific cultivars may be linked to higher water usage [37]. Drought stress during the flowering stage of rice can significantly delay blossom and maturation, impacting yield performance. This delay is primarily attributed to prolonged expression of the flowering repressor gene Ghd7 and delayed expression of florigen genes Hd3a and RFT1 [38, 39].

Plant height in rice is influenced by both genetic and environmental factors, such as root structure, culm elongation, internode number, and leaf growth. In this study, drought stress significantly reduced PH, with Nerica 10 showing the most significant reduction and least reduction in Line 16. Previous studies have also noted a decrease in PH under drought conditions [40, 41]. Drought limits metabolic activity and turgor pressure, inhibiting cell division and elongation, which ultimately reduces PH [42]. A slight increase in PH was found in BRRI dhan49 and Line 22 in response to drought stress. However, the increase was non-significant with control.

Panicle length is one of rice's most important yield-related traits influencing grain yield. Longer panicles will bear an increased number of spikelet resulting in enhanced grain yield. In response to drought stress, PL decreased considerably and showed variation. A maximum decrease in PL was found in genotype Line 20 and a minimum reduction in Line 14. Previous researchers found similar results of decreasing PL in response to drought stress [43, 44].

Drought impacts grain development throughout the reproductive stage, and spikelet infertility leads to unfilled grains [45, 46]. Water stress during the grain-filling stage accelerates leaf senescence, reducing the plant's capacity to fill the grain [47] and lowering output [48]. In this study, drought treatment resulted in a significant decrease in NFG, whereas Line 22 showed the highest reduction, and Nerica 10 showed the lowest reduction. In the case of NUG, BRRI dhan49 showed the highest increase, whereas the lowest increase was recorded in Line 14 (Table 1; Figure 2).

The reduction in rice production can be attributed to the negative impacts of drought on different elements of reproductive growth, including spikelet fertility, pollen cell function, seed set, and maturity of newly formed seeds [49, 50]. The drought-induced inhibition of panicle exertion is due to a reduction in peduncle elongation, which usually accounts for 70 to 75% spikelet sterility under water deficit [51]. The current study also revealed that drought stress led to a significant decline in SF compared to control. The genotype BRRI dhan49 showed the highest reduction, and the genotype Nerica 10 showed the lowest reduction (Table 1). Other researchers also reported a similar decline in spikelet fertility [46, 52]. The better performance of Nerica 10 is probably due to better reactive oxygen species management [53].

Moisture deficiencies impact photosynthesis, essential for crop growth and production [54]. Effective photosynthesis is highly correlated with the presence of chlorophyll content. Plants absorb light and transfer energy through leaf chlorophyll [55]. Researchers have observed that chlorophyll is degraded, and its content is reduced due to water scarcity [56]. In this study, SPAD value was also observed to be reduced in drought stress. The highest reduction was noted in Line 22 whereas the lowest was recorded in Line 16. Other researchers also reported a decrease in SPAD value in response to drought stress [57].

Imposition of drought stress resulted in a significant decrease in 100-SW for all the genotypes compared to control. The maximum reduction was found for genotype Line 16, and the minimum was found for genotype Line 14. Others also reported a decrease in 100-seed weight in response to drought stress [58, 59]. A research group reported that high drought stress hampered spikelet formation, which subsequently reduced 100-GW as well as the grain yield [59].

Yield per plant is one of the most important attributes for developing drought-stress-tolerant rice varieties. Imposing drought stress severely decreased all the genotypes' yield per plant over control. The highest grain yield per plant was found in BRRI dhan71 (17.52 g), followed by Line 20 (16.37 g) and Nerica 10 (15.71 g). However, the highest yield reduction was found for genotype Line 22 and the lowest for genotype Nerica 10. Others also reported a decrease in yield per plant in response to drought stress in rice [58, 60]. A decrease in grain yield in rice could be attributed to the inhibition of photosynthesis rates and the reduced translocation of assimilated products. This reduction is primarily driven by lower soil moisture levels, which negatively impact the plant's overall productivity [56].

4.1.2 Principal Component Analysis

Principal component analysis has been extensively applied to various crops to evaluate morphological variation and identify genetic relationships among genotypes. PCA determines the significance of each component and its contribution to total variation. In the present study, PCA revealed three principal components (PCs) with eigenvalues greater than one, accounting for 87.7% of the total variance in the data (Table 2). PC1 and PC2 indicated that SF, NFG, Y/P, SV, PL, 100-SW, PH, DFF, and DM were the most crucial traits responsible for the observed variation (Table 2; Figure 3). In terms of the quantitative features under study, this conclusion implies that the research accessions were highly diverse. Previous studies have documented the significance of characteristics including YPP, SF%, SV, NFG, DM, and 100-SW in explaining variance in drought tolerance [61]. PC1 distinguished the stressed samples from the unstressed samples in the biplot, which is noteworthy (Figure 3). The tolerant check (BRRI dhan71) and the susceptible check (BRRI dhan49) were the unstressed samples that were placed in the same quadrant of the biplot with the greatest distance between them (Figure 3). A comparable location was noted in the opposite quadrant for the stressed samples of these two dissimilarly tolerant examinations. Line 20, one of the lines evaluated for salinity tolerance, showed some drought tolerance and was aligned with the resistant check BRRI dhan 71 for both stressed and unstressed samples in their respective quadrants of the biplot (Figure 3). The noticeable separation of the susceptible check BRRI dhan49 from the tolerant lines can be attributed to its substantial decrease in parameters such as NFG, SF, and YPP under the stressed condition (Table 1).

4.1.3 Stress Tolerance Indices (STI)

STI, an advanced index for pinpointing high-yielding genotypes under both stress and normal conditions [31, 62], is part of a range of indices developed to identify tolerant genotypes under various abiotic stresses. Based on their trait contributions, these indices are classified into tolerant (higher MP, GMP, STI, YSI values) and susceptible (lower TOL and SSI values) groups. Based on the current results of MP under drought stress, Line 20 had the highest value, followed by tolerant check variety BRRI dhan71. Because MP and Ys (stressful environment) have been shown to correlate positively, selection based on MP will increase average yields in stressful and non-stressful environments [30]. Thus, high MP can be applied to the process of genotype selection.

The study of GMP showed that the genotype Line 20 had the highest value, followed by tolerant check variety BRRI dhan71 and Nerica 10. Based on this index, genotypes with higher values were considered tolerant and had high yields under both normal and stress conditions [63]. The SSI study revealed that genotype Line 22 exhibited the highest sensitivity, while the lowest was recorded for Nerica 10. Higher SSI values, as indicated by other researchers [64], signify increased sensitivity and greater yield reduction under stress, whereas lower readings suggest decreased susceptibility. SSI has often been utilized in moisture-restricted environments to identify genotypes with stable yields [65, 66]. Therefore, based on SSI, Nerica 10 showed maximum tolerance. Additionally, the TOL index results indicated that BRRI dhan49 had the highest TOL value, with Nerica 10 recording the lowest. Lower TOL indices are associated with greater stress tolerance [67]. With the use of this criterion, genotypes with low yield potential in non-stressful conditions and high potential in stressful ones can be chosen [31]. Greater stress resilience and yield potential are associated with a genotype's higher STI value in a stressful environment [31]. The genotype Line 20, which has a higher STI value than BRRI dhan71 and Nerica 10, indicates their resistance to drought stress. YSI is particularly relevant to differentiate tolerant and sensitive varieties of drought stress. In this study, YSI discriminated Nerica 10, BRRI dhan71, and Line 20 as the most tolerant genotypes. According to the stress tolerance indices findings and the tolerant check genotypes Nerica 10 and BRRI dhan71, advanced mutant Line 20 emerged as a potential drought-tolerant genotype, performing well under drought-stress conditions.

4.2 Effect of Low P Stress on Yield-Related Traits of Rice Genotypes

Phosphorus is an essential food production element and cannot be substituted [68]. At the plant level, it promotes seed germination, root development, stalk and stem strength, flower and seed formation, crop yield, and quality [69]. Low P levels in soil can significantly reduce rice production by limiting root growth, delaying maturity, increasing sterility, reducing photosynthesis efficiency, and lowering potential yield [70]. Nutrient depletion of N, P, and K in soil is imminent, necessitating the development of rice varieties that can thrive under such conditions without significant yield losses through improving P uptake and use efficiency. This study examined the effects of low P stress on seed yield and related traits in six rice genotypes. ANOVA showed significant variation in the characteristics for Treatments (T), genotypes (G), and G × T interactions (Table S2).

4.2.1 Mean Performance of Yield and Yield-Related Traits of Rice Genotypes under Low P Stress

Stages like early flowering assure the breeder of getting sufficient yield as the varieties will receive an adequate period of grain development. Delayed flowering is a common consequence of low P stress in rice, and we have observed a significant delay in DFF in most of the studied genotypes. However, under low P stress, Line 6 took less time to flowers while BRRI dhan49 took the highest period. This result was supported by the findings of others [8, 71]. Researchers reported that P significantly affects days to 50% flowering [72]. There is a positive correlation between P levels and gibberellin levels [73], and low gibberellin levels have been found to delay flowering, suggesting that gibberellin signaling might be responsible for delayed flowering in response to low P [74]. Similarly, late maturity is a common consequence of low P stress, and maturity can be delayed by one week to 20 days [75]. Insufficient P impairs chlorophyll production, reducing photosynthesis efficiency and extending the time needed for the plant to reach full maturity [76]. Under the condition of low P stress, Line 6 emerged as an early maturing genotype less affected by low P stress whereas Binadhan-17 was found late maturing genotype, which is consistent with other researchers [8, 72]. This delay is mediated by reduced transcription of key flowering genes, including Ehd1, Hd3a, and RFT1, which integrate photoperiodic signals [38]. The delayed flowering response may be adaptive, allowing plants more time for P acquisition and utilization, as demonstrated in *Arabidopsi*s

[77]. Molecular mechanisms underlying P deficiency responses in rice involve complex regulatory pathways, including the interaction between phosphate over-accumulator (PHO2) and GIGANTEA, which links P homeostasis and flowering time regulation [78].

Plant height was seen to decrease with low P stress. Line 20 had the lowest PH reduction in the current study, whereas Line 3 had the largest. This could be due to another adaptation mechanism that aids the plant in acquiring more P for growth and maintenance, thereby slowing down cell proliferation [79]. Others have also observed a drop in PH in response to low P stress, consistent with our findings [80, 81]. Low P stress increases rice's auxin concentration and OsPIN5a expression, leading to increased root growth and decreased shoot growth. The transcription factor MYB110 negatively regulates plant height in rice under low P stress [82].

In response to low P stress, PL decreased significantly and exhibited considerable variation in our study. A maximum decrease was found in the genotype BRRI dhan49 (12.26%) and a minimum decrease in genotype Binadhan-17 (Table 4). Similar results of decreasing PL were found by previous researchers [81, 83]. It was discovered that a decrease in the size of the RuBP pool and insufficient ATP synthesis led to a drop in photosynthetic C assimilation, which hindered plant growth [84].

This study's low P stress significantly affected NFG, NUG, SF%, and 100-SW. Regarding NFG, the highest reduction was found in Line 6 whereas the highest increase in NUG was also observed in the same genotype. In contrast, a limited increase in NUG and a limited decrease in SF were recorded in Line 20. These results are by the results of others [8, 81]. Low P stress induces several physiological changes, including membrane disruption, compromised formation of biomolecules and high-energy compounds, nutrient imbalance, altered enzyme activity, reduced cell division, and impaired metabolic functions, necessitating increased energy investment, which subsequently led to decreasing fertility as well as the weight of grains [85]. Phosphorus deficiency in rice can significantly impact grain yield by reducing the number of filled grains per panicle [86]. During grain filling, P uptake from soil contributes 40-70% of total plant P, with the panicle being the main P sink [87]. Down-regulation of *OsSPX1*, a gene involved in P sensing, can lead to semi-male sterility and reduced seed-setting rates by affecting carbohydrate metabolism and sugar transport [88].

Imposition of low P stress significantly decreased the Y/P of all genotypes compared to the control, with the highest reduction observed in genotype Line 6 and the lowest in genotype Binadhan-17. This decline in rice yield under low P stress has been documented by other researchers [72, 81, 83]. This reduction in rice yield under low P stress may be attributed to decreased photosynthetic efficiency in filling developing seeds because P plays a crucial role in grain formation by regulating various metabolic functions [85]. However, a few cultivars showed less decline in yield and yield-related characteristics under low P stress conditions, most likely because of their greater P uptake or P utilization efficiency [80]. Low P availability limits tillering, shoot biomass, leaf area, and photosynthesis [89]. Low P stress decreases rice yield by reducing photosynthesis and inducing oxidative stress [90].

4.2.2 PCA

In the present study, PCA identified three principal components with Eigen values exceeding unity, accounting for 88.0% of the total variance in the data (Table 5). PC1 and PC2 differentiated genotypes with higher NFG, SF, PH, PL, DM, and Y/P, indicating that these traits were primarily responsible for the observed variation among the studied genotypes. Similarly, another research group highlighted the importance of these traits in their study on low P stress tolerance [8]. Therefore, selecting these traits that contribute to maximum variability would be advantageous. Interestingly, except for Line 20, the biplot demonstrated that PC1 effectively separated the stressed genotypes from the unstressed ones (Figure 5). Under both stressed and non-stressed conditions, the advanced mutant Line-20 remained in the same quadrant, indicating less affected by low P stress than the control condition and showed its potential for low P tolerance. The lowest reduction in SF and relatively lower reductions in PL and NFG in Line 20 may explain its consistent positioning in the same quadrant (Table 4).

4.2.3 STI

The study on STI aimed to identify genotypes resilient to low P, employing criteria based on responses under normal and low P stress conditions. MP analysis revealed that Line 20 exhibited the highest value, followed by Binadhan-17, BRRI dhan49, Line 4, Line 6, and Line 3. These higher values indicate increased average yield potential in stress and non-stress environments. MP analysis highlighted Line 20 as having the highest value, signifying tolerance and high yields under normal and stress conditions [67]. Regarding SSI and TOL values, Line 6 exhibited the highest, while Binadhan-17 showed the lowest. Lower SSI and TOL values suggest genotypes with greater tolerance [64]. Based on these findings, Binadhan-17 emerged as more tolerant than others, which is supported by the findings of other researchers [8]. They also reported Binadhan-17 as tolerant to low P. The genotype Line 20, followed by Binadhan-17 and BRRI dhan49 genotypes, have higher values of STI and indicate their tolerance to low P stress. YSI, which differentiates between tolerant and sensitive genotypes, discriminated Binadhan-17, Line 4 and Line 20 as the most tolerant genotypes. According to these stress tolerance indices and Binadhan-17, our developed mutant Line 20 showed potential as a low P stress-tolerant genotype due to its better yield performance under low P conditions.

5. Conclusions

Drought and phosphate availability are the two main abiotic variables limiting rice productivity in rainfed upland environments. This study evaluated a few advanced rice breeding lines for drought and low P stress tolerance based on yield-attributing morphological traits. Significant reductions in yield and yield-attributing were observed under drought stress in most of the studied mutants, including referred positive and negative controls. However, Line 20 maintained a higher yield under drought, close to drought tolerant variety. PCA analysis reflected that the first three principal components were responsible for explaining 88.0% of the overall variation observed among the six rice genotypes. Stress tolerance index studies also reflected the better performances of line 20 compared to other lines. A significant reduction in yield-attributing traits was also observed in the studied advanced lines and mutants grown under low P stress; however, a smaller decrease was observed in Line 20. PCA identified three principal components with Eigen values exceeding unity, accounting for 88.0% of the total variance in the data. Stress tolerance index studies also showed the desirable range of the values of line 20. However, further studies should be required to evaluate the genotypes under direct field conditions. In addition, breeding initiatives should be undertaken to develop drought and low-P-tolerant varieties using this advanced line.

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Author Contributions

Conceptualization, Sopnil Ahmed Jahin, Mohammad Anwar Hossain; **design and methodology,** Sopnil Ahmed Jahin, Mohammad Abu Kawsar Sarower Siddique, Mohammad Anwar Hossain; **investigation,** Sopnil Ahmed Jahin; **data analysis,** Sopnil Ahmed Jahin, Mohammad Abu Kawsar Sarower Siddique; **writing—original draft preparation,** Sopnil Ahmed Jahin, Biswajit Das, Adrita Abdullah, Sadia Akter; **writing—review and editing,** Biswajit Das, Mohammad Abu Kawsar Sarower Siddique and Mohammad Anwar Hossain; **project administration,** Mohammad Anwar Hossain.

Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded to the page of this paper.

1. Table S1: Analysis of variance on yield and yield-related traits of seven rice genotypes grown under control and drought conditions.
2. Table S2: Analysis of variance on yield and yield-related traits of six rice genotypes grown under control and low P condition.

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