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RESEARCH ARTICLE

Inquest of climate-smart rice (*Oryza sativa* L.) genotypes considering multi-trait selection index and genotype-environment interaction at a variable duration of submergence

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Abstract

Submergence is a significant natural disaster that severely reduces rice yield. The research was conducted at Gazipur Agricultural University, involving 48 rice genotypes that were tested under 14 and 21 of Days of Submergence (DS) to identify rice submergence tolerance genotypes based on morpho-physiological characteristics in the F_2 population. The experiment employed a split-plot design with three replications. Significant variation was observed in all traits under submergence, with 21 DS showing a greater detrimental effect than 14 DS. The phenotypic coefficient of variance exceeded the genotypic coefficient of variance for all traits, with most traits exhibiting moderate to high heritability (30–60 %). High Genetic Advance (GA) was noted in days-to-maturity (54.02), Plant Height (PH) (61.57) and Plants per Hill (PPH) (87.36). Grain yield showed significant positive correlations with straw yield ($r = 0.571^{**}$, $r = 0.501^{**}$, $r = 0.516^{**}$), Filled Grain per Panicle (FGP) ($r = 0.564^{**}$, $r = 0.425^{**}$, $r = 0.770^{**}$) and PPH ($r = 0.729^{**}$, $r = 0.511^{**}$, $r = 0.509^{**}$) in control, 14 DS and 21 DS conditions. Principal components analysis revealed that five components accounted for 78.45 % of the total variability, with biplot illustrations depicting genotype-trait relationships. A hierarchical-clustering heatmap classified the genotypes into distinct clusters based on the stress tolerance index. The genotypes SR004, SR005, SR006, SR008, SR010, SR012, SR015, SR016, SR018, SR022, SR026, SR030, SR032, SR033, SR034, SR036, SR038, SR039, SR041, SR044, SR045 and SR047 exhibited tolerance under both 14 and 21 DS.

Keywords: F_2 generations; grain yield; submergence; tolerance; variation

Introduction

Rice (*Oryza sativa* L.) is the third most produced agricultural crop and over half of the world's population relies on rice as a staple food. It contains vital minerals, vitamins and bioactive phytochemicals and more than 20 % of calories consumed worldwide (1). Rice is grown in more than 100 countries worldwide, while 90 % of the world's rice is produced in Asian countries. Bangladesh is the third-largest producer of rice in the world, behind China and India, with 360 million tonnes produced on 11.55 million hectares of gross cultivated area. Here, rice was grown on approximately 80 % of the net cropped area to feed 169.04 million people (2,3). There were three distinct rice-growing seasons in Bangladesh: *Aus* (March to July), *Aman* (July to November) and *Boro* (December to May) (4). The contribution of *Boro* to the overall rice output is increasing at a rate of 0.97 % per year, while the production of *Aus* and *Aman* rice is annually declining at notable rates of 0.48 % and 0.49 %, respectively. Among them, the contribution of *Boro* rice to the total rice production is rising at a pace of 0.97 % per year, whilst the production of *Aus* and *Aman* rice is annually declining at notable rates of 0.48 % and 0.49 %, respectively (5). Despite all these achievements, climate change-related factors such as excessive temperature, drought, salinity, alkalinity, flooding, waterlogging, submergence and mineral shortage pose negative impacts on rice yield and quality (6).

Flooding has a significant impact on plant life and complete submergence is extremely harmful to rice. Under submergence, plants experience a variety of external difficulties concurrently or sequentially, which results in a range of internal pressures that affect the development, survival and production of the plants (7). Rice plants experience partial to complete submergence every year in several regions in our country and the submergence could last from a day to a few weeks (8). April through July and September through October are the peak months for water stagnation due to heavy or severe rainfall occurring quickly over a relatively limited region. The most severely impacted regions of Bangladesh are found in its Northeast and Southeast (9). According to flood duration, depth and floodwater conditions, yield loss might range from 10-100 % (5). Rice can deal with several forms of floods by either quiescence or escaping early. In the 1950s, low-yielding, submergence-tolerant landraces were discovered from Eastern India (10).

The ethylene-response factor known as the Sub1 gene enhances rice's ability to withstand submergence. BRRI dhan33, BRRI dhan56 and BRRI dhan57 performed better in submerged conditions and plant characteristics like weight of panicle, grain yield may be the desirable traits for rice submergence tolerance (11,12). BU dhan1 also survived and produced a significant amount of grain even after being totally submerged for 10 days (13). AZ7006 tolerates three weeks of submergence (14) and dry matter accumulation was one of the key determinants of submergence tolerance in rice (5). In the case of this hybrid, there is no variability among the progenies. In contrast, for F_2generation in inbreeding, there is more variability with more options to choose better progeny among the variation. Therefore, this experiment was conducted to screen out rice genotypes based on morpho-physiological characteristics and Stress Tolerance Index (STI) of some desirable traits for submergence tolerance of rice.

Materials and Methods

Experimental site and rice genotypes

The experiment was conducted in the submergence tank at the agronomy research field of Gazipur Agricultural University (GAU), Gazipur 1706, from November 2021 to April 2022. This location is part of the Madhupur tract in Agro-ecological Zone (AEZ) 28, situated at 24°09′ N latitude and 90°26′ E longitude, with an elevation of 8.4 m above sea level. The soil of the experimental site is silty clay on the surface and silty clay loam below and it experiences heavy rainfall during the monsoon season from May to September. The land in the submergence tank was prepared with a spade and levelled, with an 8–10 cm ridge built to prevent fertilizer loss due to runoff. The experiment involved growing 47 F_2 segregating rice genotypes (SR001 to SR047), with the AZ7006 hybrid from Bayer Crop Science, Dhaka, Bangladesh, serving as a check variety known for its waterlogging tolerance up to 21 days (14).

Experimental design and treatment imposition

The experiment employed a split-plot design with three replications, where genotypes were assigned to the main plots and submergence treatments to the subplots. Sprouted rice seeds were sown in the field on November 25 in 2021 and single 42-day-old seedlings were transplanted into the main field with a spacing of 25 × 15 cm. Fertilizers were applied according to the fertilizer recommendation guide (15), including urea (272 kg ha^{-1}), triple super phosphate (TSP) (102.5 kg ha^{-1}), muriate of potash (MoP) (166 kg ha^{-1}), gypsum (97 kg ha^{-1}) and zinc sulphate ($ZnSO_4$) (5.6 kg ha^{-1}). TSP, MoP, gypsum and zinc sulphate were applied as a basal dose during the final land preparation, while urea was applied in three splits: one-third at 5 Days After Transplanting (DAT) and the remaining at 7 and 21 Days After De-Submergence (DADS). Irrigation and other cultural practices were performed as needed. The F_2 genotypes were subjected to two levels of submergence: 14 days and 21 days, along with a control group with no submergence. Submergence treatments began 10 DAT. The water depth was kept at 10 cm above the plants during submergence. Tanks were de-submerged based on the treatment schedules. The water quality data, such as dissolved O_2,electrical conductivity, pH, turbidity and temperature, were monitored in the tank during submergence.

Data collection and statistical analysis

The crop was harvested when 80 % of the panicle grains turned golden yellow. After harvesting, Grain Yield (GY) per hill and Straw Yield (SY) per hill were measured. Data were collected from five randomly selected plants to assess various morpho-physiological traits.

Yield data under both stress and non-stress conditions were used to calculate the STI for each trait and yield-related feature at 14 and 21 DS, using the following equation.

$$\frac{\mathrm{Xc} \times \mathrm{Xs}}{(\bar{\mathrm{X}}\mathrm{c})^2}$$
(Eqn. 1)

where $\bar{X}_c$,Xs and Xc represent the average value of a specific trait across all genotypes under control conditions, the values of that trait in a particular genotype under submergence stress and control conditions, respectively (16).

Genotypic variance ($\sigma^2 g$) was calculated using the following equation

$$\frac{(\mathrm{MSG}-\mathrm{MSE})}{\mathrm{r}}$$
(Eqn. 2)

where MSG, MSE and r represent the mean square of genotypes, mean square of error and number of replications, respectively (17).

Phenotypic variance ($\sigma^2 p$) was calculated using the following equation

$$\sigma^2\mathrm{p} = (\sigma^2\mathrm{g}) + (\sigma^2\mathrm{e})$$
(Eqn. 3)

Where $\sigma^2 g$, genotypic variance and $\sigma^2 e$, environmental variance (17).

Genotypic Coefficient of Variation (GCV %) was calculated using the following equation (18).

$$\frac{(\sqrt{\sigma^2\mathrm{g}})}{\mathrm{Mean}}$$
(Eqn. 4)

Phenotypic Coefficient of Variation (PCV %) was calculated using the following equation (18).

$$\frac{(\sqrt{\sigma^2\mathrm{p}})}{\mathrm{Mean}}$$
(Eqn. 5)

Error Coefficient of Variation (ECV %) was calculated using the following equation (18).

$$\frac{(\sqrt{\sigma^2\mathrm{e}})}{\mathrm{Mean}}$$
(Eqn. 6)

Broad-sense heritability (H^2 %) was determined using the equation,

$$H^2 = \frac{(\sigma^2\mathrm{g})}{\sigma^2\mathrm{p}} \times 100$$
(Eqn. 7)

Where $\sigma^2 g$, $\sigma^2 p$ denote genotypic variance and phenotypic variance, respectively (19).

Expected GA was calculated using the following formula

$$\text{Genetic advance} = \mathrm{K} \times H^2 \times \sqrt{\sigma^2\mathrm{p}}$$
(Eqn. 8)

Where the selection differential (K) was 2.06 for a 5 % selection intensity (20).

The descriptive statistics for morpho-physiological traits and the correlations between various plant traits were analyzed using SPSS version 25. Analysis of Variance (ANOVA) among genotypes was performed with the same software at a 5 % significance level (21).

Multivariate analysis was conducted using the mean values of the studied morpho-physiological traits with the statistical software R version 4.2.2. A heatmap was generated using the "pheatmap" package and hierarchical cluster analysis was performed with the "factoextra" package. Principal Component Analysis (PCA) and biplots, based on the first two components (PC1 and PC2) that explained the most variance, were created using the "FactoMineR" package in R version 4.2.2.

Results

ANOVA of morphological traits

ANOVA indicated significant differences among the rice genotypes for all studied traits, except for PH, PPH, Panicle Length (PL), FGP, SY and GY. This implies that the variability in those traits is due to genetic sources (Table 1). The ANOVA also indicated considerable variance among the yield-related traits at 14 and 21 days submerged, except for PPH and Thousand Seed Weight (TSW). This shows substantial variation among rice genotypes for these traits under submerged conditions (Table 1). The study found a range of Coefficient of Variation (CV) from 0.6 % (DTM) to 45.1 % Unfilled Grains (UFG) per panicle, with UFG exhibiting the highest CV, followed by PPH, SY and GY. Other traits displayed modest CV values (≤20), indicating significant variability among rice genotypes for these characteristics (Table 1).

Analysis of range and mean of quantitative traits highlighted significant variation in 11 plant characteristics across 48 rice genotypes due to submergence. The traits TD7DS, DTM, PPH, PL and TSW showed narrower ranges of variation compared to the broader range observed in TDM after 21 days submerged. The traits PH, FGP, UFG, SY and GY exhibited notable genotypic variation strongly influenced by submergence.

Effect of submergence on morpho-physiological parameters

Submergence influences the growth and productivity of rice plants at various growth stages. In the current study, morpho-physiological parameters of 48 rice genotypes were observed under different submergence conditions (0, 7 and 14 days) and exhibited notable variations (Fig. 1). Submergence notably affected the PH of rice at both 7 and 21 DADS. Before submergence, PH ranged from 13 to 21 cm, 11.67 to 23.33 cm and 13.33 to 24 cm for control, 14 DS and 21 DS, respectively, with average height of 17 cm, 16.36 cm and 17.55 cm. At 7 DADS, PH ranged from 27 to 39.67 cm, 24 to 37.33 cm and 20.67 to 35.33 cm, with average height of 33.31 cm, 28.92 cm and 26.85 cm for control, 14 DS and 21 DS, respectively. Similarly, after 21 DADS, PH varied from 26.67 to 41 cm, 37.33 to 45.33 cm and 30.33 to 46.33 cm for control, 14 DS and 21 DS, respectively (Fig. 1a), indicating a decrease in height with prolonged submergence.

Submergence also significantly affected the SPAD value of rice plants. Before submergence, SPAD values ranged from 20 to 36, 19.1 to 35.17 and 18.07 to 37.67 for control, 14 DS and 21 DS, respectively, with average values of 28, 27.47 and 26.9. At 7 DADS, SPAD values ranged from 29.57 to 42.1, 22.9 to 30.47 and 22.03 to 30.67, with averages of 38.9, 28.82 and 26.17 for control, 14 DS and 21 DS, respectively. Following 21 DADS, SPAD values ranged from 43.37 to 46 and 36.03 to 41.6 for control and 14 DS, with averages of 45.05 and 39.28, respectively. Similarly, SPAD values were 45.4 to 51 and 41 to 44.73 for control and 21 DS, with averages of 47.44 and 42.91, respectively (Fig. 1b), indicating a significant decrease with increasing submergence duration.

Root Dry Matter (RDM) of rice was notably impacted by submergence. The RDM of all rice varieties notably decreased during the initial measurement at 7 DADS due to 14 and 21 DS, compared to the control. RDM ranged from 0.18 to 2.18 g hill^{-1}, 0.14 to 1.12 g hill^{-1} and 0.07 to 1.0 g hill^{-1}, with average dry matter of 0.76 g hill^{-1}, 0.36 g hill^{-1} and 0.33 g hill^{-1} for control, 14 DS and 21 DS, respectively (Fig. 1c), indicating lower RDM under submergence stress compared to control plants. The Shoot Dry Matter (SDM) varied significantly among different rice varieties due to flooding. SDM ranged from 0.38 to 2.44 g hill^{-1}, 0.26 to 1.28 g hill^{-1} and 0.095 to 0.61 g hill^{-1} for control, 14 DS and 21 DS, with average values of 1.24 g hill^{-1}, 0.64 g hill^{-1} and 0.32 g hill^{-1} (Fig. 1c). This indicates a considerable decrease in SDM for all kinds at 7 DADS due to 14 and 21 DS compared to control conditions. Additionally, RDM notably decreased in all rice varieties at 21 DADS due to 14 and 21 DS compared to the control. RDM ranged from 2.52 to 25.65 g hill^{-1}, 0.96 to 10.77 g hill^{-1} and 0.695 to 10.95 g hill^{-1} for control, 14 DS and 21 DS, with average values of 10.25 g hill^{-1}, 4.12 g hill^{-1} and 4.23 g hill^{-1} (Fig. 1d). This indicates a recovery trend in RDM at 21 DS, albeit with a decreasing trend compared to control plants subjected to submergence stress. Similarly, the output of SDM varied significantly among different rice varieties at 21 DADS, decreasing notably in 14 and 21 DS compared to the control. SDM ranged from 5.86 to 35.96 g hill^{-1} for control, with an average dry matter of 16.99 g hill^{-1}. During 14 DS, SDM varied from 4.72 to 32.94 g hill^{-1}, with an average of 11.34 g hill^{-1}. At 21 DS, SDM ranged from 3.39 to 19.12 g hill^{-1}, with an average of 9.32 g hill^{-1} (Fig. 1d), indicating a tendency toward a decrease in SDM at 21 DS compared to 14 DS and control plants exposed to submergence stress.

Table 1. Analysis of variance of 48 rice genotypes evaluated for growth and yield parameters

Source of variation	df	TD7DS	TD21DS	DTM	PH	PPH	PL	FGP	UFG	TSW	SY	GY
Replication (R)	1	0.22^{**}	8.79^{**}	1	818.44^{**}	13.35	58.41^{**}	31437.8^{**}	5573.92^{**}	10.76	13.94	3510.52^{**}
Genotypes (G)	47	0.98^{**}	181.25^{**}	16.86^{**}	84	12.63	4.8	1589.01	1173.52^{*}	8.36^{*}	100.67	125.58
Error-I	47	0.011	0.465	1.13	78.61	9.79	4.12	1239	807.47	5.03	76.74	116.03
Submergence (S)	2	46.67^{**}	5283.57^{**}	792.89^{**}	4030.39^{**}	13.56	108.74^{**}	21303.5^{**}	3852.78^{**}	2.68	2515.1^{*}	1384.07^{**}
G × S	94	1.12^{**}	103.51^{**}	8.83^{**}	79.03	13.46	3.75	1400.7	994.74	6.42	84.64	133.54
Error-II	96	0.015	0.43	0.61	76.24	12.76	3.16	1346.43	746.69	5.19	73.09	127.96
LSD		0.246	1.299	1.55	17.33	7.09	3.53	72.83	54.24	4.53	16.97	22.45
CV (%)		10.2	3.5	0.6	8	26.9	7	21.2	45.1	10.3	28.4	35.1

TD7DS: Total Dry Matter at 7 Days After De-Submergence; TD21DS: Total Dry Matter at 21 Days After De-Submergence; DTM: Days To Maturity; PH: Plant Height at Maturity; PPH: Plants per Hill; PL: Panicle Length; FGP: Filled Grains per panicle; UFG: Unfilled Grains per panicle; TSW: Thousand-Seed Weight; SY: Straw Yield per hill; GY: Grain Yield per hill; df: degrees of freedom; LSD: Least Significant Difference; CV: Coefficient of Variance; ** indicate significant at 1 % level; * indicate significant at 5 % level

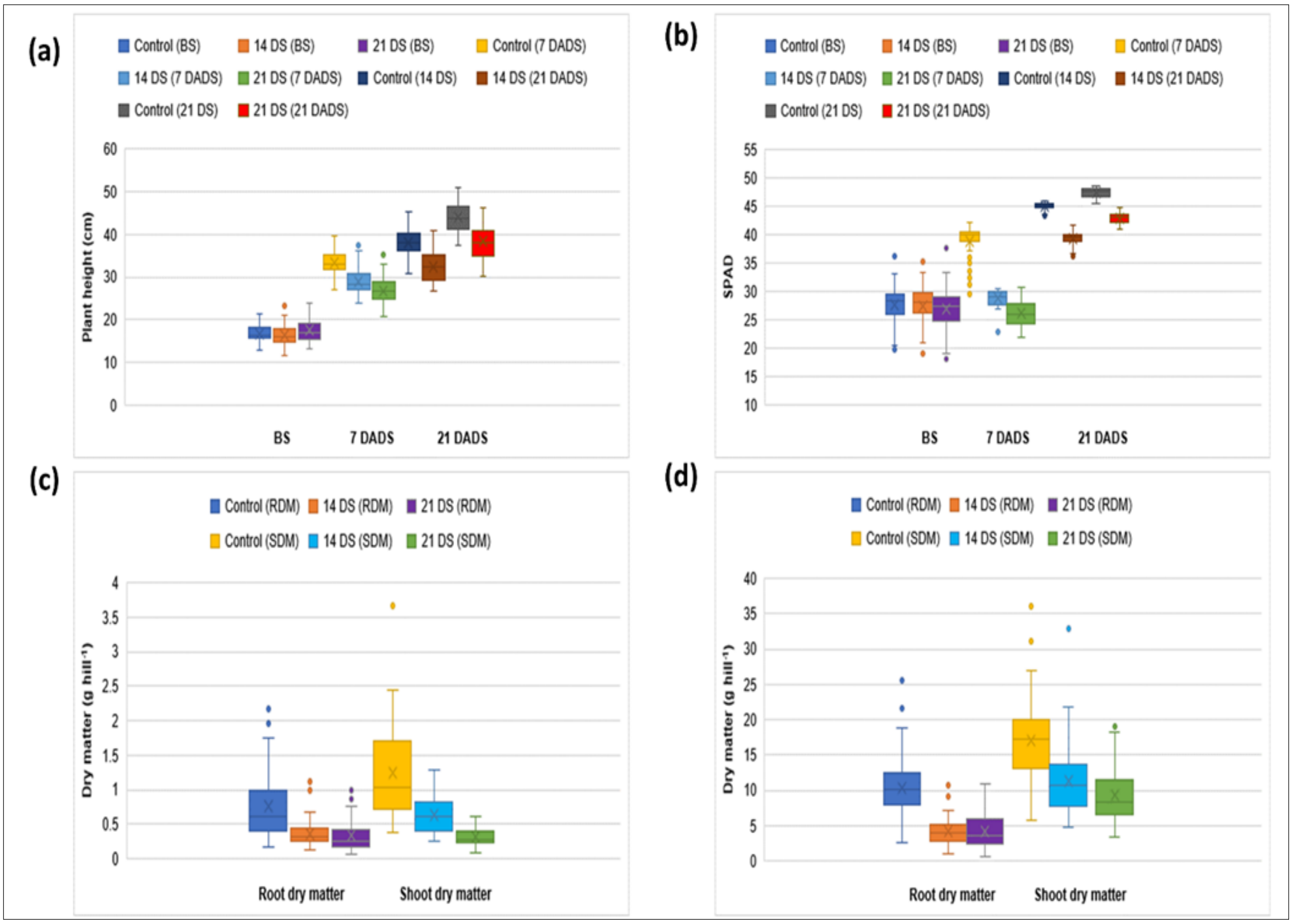

Fig. 1. Effect of variable duration of submergence on morpho-physiological traits of rice genotypes.

BS: Before Submergence; DADS: Days After De-Submergence; DS: Days of Submergence

Unraveling genetic variation

The mean values, ranges and variance components, including genotypic variance (σ^2g), environmental variance (σ^2e) and phenotypic variance (σ^2p), along with GCV, ECV, PCV, heritability (H^2) and GA for various traits, are presented in Table 2. The mean values and wide ranges of the studied traits indicate significant variations among the evaluated genotypes under submergence conditions. Notably high phenotypic and genotypic variances were recorded for UFG (990.5 and 183), FGP (1414 and 175) and TD21DS (90.86 and 90.39), while lower values were observed for TD7DS (0.50 and 0.49), PL (4.46 and 0.34), TSW (6.70 and 1.67) and PPH (11.21 and 1.42), respectively. Other traits displayed intermediate σ^2g and σ^2p. Generally, phenotypic variances exceeded genotypic variances for all traits, indicating the influence of environmental factors on trait expression. GCV values were smaller than PCV values for all traits, suggesting stronger environmental influence on trait expression. The highest GCV was observed for TD21DS (50.68 %), followed by TD7DS (45.45 %) and UFG (22.35 %), while the lowest GCV was observed for PH (1.51 %), DTM (2.06 %) and PL (2.31 %). GCV and PCV values varied little for traits TD7DS, TD21DS, DTM, PH, PL and TGW, indicating minimal environmental effects and better selection prospects for these traits. Significant variation among rice genotypes was evident from high PCV (34.14 %) and low GCV (6.79 %) reported for GY, suggesting potential yield enhancement through selection.

Table 2. Estimation of genetic variability components for growth and yield components rice genotypes

Traits	Mean	Range		σ^2e	σ^2g	σ^2p	ECV (%)	GCV (%)	PCV (%)	H^2 (%)	GA
		Min	Max								
TD7DS	1.54	0.17	5.84	0.011	0.49	0.50	6.81	45.45	45.91	98.00	14.28
TD21DS	18.76	4.39	57.52	0.465	90.39	90.86	3.64	50.68	50.81	99.49	19.53
DTM	135.97	128.5	142.5	1.13	7.87	9.00	0.78	2.06	2.21	87.44	54.02
PH	109.02	90.00	126	78.61	2.70	81.31	8.13	1.51	8.27	3.31	61.57
PPH	13.29	7.50	20.5	9.79	1.42	11.21	23.54	8.97	25.19	12.67	87.36
PL	25.25	13.35	28.5	4.12	0.34	4.46	8.04	2.31	8.36	7.62	33.17
FGP	172.89	46.50	238	1239	175	1414	20.36	7.65	21.75	12.38	9.58
UFG	60.53	20.50	156.5	807.5	183	990.5	46.95	22.35	51.99	18.48	11.97
TSW	22.18	17.43	26.89	5.03	1.67	6.70	10.11	5.82	11.67	24.87	13.30
SY	30.13	13.80	51.26	76.74	11.97	88.71	29.07	11.48	31.08	13.49	26.17
GY	32.19	7.00	51.67	116	4.78	120.8	33.46	6.79	34.14	3.95	8.95

Min: minimum; Max: maximum; σ^2e: environmental variance; σ^2g: genotypic variance; σ^2p: phenotypic variance; ECV: Error Coefficient of Variation; GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; H^2: broad sense heritability; GA: Genetic Advance of mean at 5 %; TD7DS: Total Dry Matter at 7 Days After De-Submergence; TD21DS: Total Dry Matter at 21 Days After De-Submergence; DTM: Days To Maturity; PH: Plant Height at maturity; PPH: Plants per Hill; PL: Panicle Length; FGP: Filled Grains per Panicle; UFG: Unfilled Grains per panicle, TSW: Thousand-Seed Weight; SY: Straw Yield per hill; GY: Grain Yield per hill

H^2 ranged from 3.31 % (PH) to 99.49 % (TD21DS). TD21DS, TD7DS and DTM were highly heritable traits (H^2 >60 %), indicating strong responses to selection for these traits. Conversely, PH, GY, PL, FGP, PPH, TSW and SY showed low heritability (H^2 >30 %), suggesting considerable environmental influence on trait variation. GA was highest for PPH (87.36), followed by PH (61.57), DTM (54.02), PL (33.17) and SY (26.17), with the lowest GA observed for GY (8.95) and FGP (9.58). Moderate GA was observed for other traits, indicating the potential for improvement through heterosis breeding, family selection and progeny testing methods.

Exploring trait correlations under submergence

The correlation coefficient plays a vital role in plant breeding programs for the indirect selection of plants. In this study, phenotypic correlation between grain yield and yield attributing traits of 48 rice genotypes in response to 14 and 21 DS was presented in Table 3. The results revealed significant positive correlations between PH and DTM in both control and 14 DS conditions (r = 0.290*, r = 0.289*), while PL exhibited significant positive correlations with PH in control and both 14 DS and 21 DS conditions (r = 0.302*, r = 0.494**, r = 0.428**). Furthermore, FGP showed positive correlations with PL in both control and submergence conditions (r = 0.345*, r = 0.304*, r = 0.407**), whereas UFG displayed significant negative correlations with FGP in control, 14 DS and 21 DS (r = -0.486**, r = -0.481**, r = -0.532**). The traits TSW had a significant negative association with PPH (r = -0.332*) and UFG (r = -0.370**) at 14 DS. The traits SY showed significant positive correlation with PPH (r = 0.432**, r = 0.678**, r = 0.722**) in both the control and submergence conditions and also positive correlation with PH (r = 0.471**), PL (r = 0.331*) and FGP (r = 0.500*) at only the control condition. The traits GY exhibited a significantly positive correlation with SY (r = 0.571**, r = 0.501**, r = 0.516**), FGP (r = 0.564**, r = 0.425**, r = 0.770**) and PPH (r = 0.729**, r = 0.511**, r = 0.509**) in control as well as both 14 and 21 DS, while UFG demonstrated a significantly negative correlation (r = -0.423**, r = -0.423**, r = -0.396**) with GY in both the control and submergence conditions. The remaining traits showed mostly insignificant positive and negative correlations with each other.

Genotype-trait relationship through PCA biplot

PCA was utilized to gather more robust insights into identifying groups of accessions with desirable yield traits for plant breeding purposes. In the PCA results encompassing 11 Principal Components (PCs), only five PCs exhibited eigenvalues >1 under 14 DS conditions, collectively contributing to 78.45 % of the total cumulative variability across various accessions. PC1 made the highest contribution to variability (22.81 %), followed by PC2 (19.14 %), surpassing other PCs. Similarly, in the case of 21 DS, the first four PCs displayed eigenvalues >1, contributing to a cumulative total variation of 71.53 %. Notably, PC1 accounted for 24.44 % of the total variation, while PC2 accounted for 19.21 %. In this study, the PCA biplot technique was employed to examine the interrelationships among variables and identify vectors exerting the greatest influence on total variation, considering eleven plant attributes and 48 rice genotypes to select genotypes based on improved agronomic performance. In 14 DS, the biplot graph revealed wider variability for traits such as GY, PPH, FGP, SY and PH compared to others. GY exhibited a more acute angle with SY, indicating a stronger positive correlation, followed by FGP, PPH, PH and PL. Genotypes like SR026, SR018, SR028, SR036, SR038, SR040 and SR006 were closely associated with GY, signifying superior yield performance, while genotypes like SR037 and SR020 showed a negative relation with GY (Fig. 2a). The biplot graph for 21 DS clearly indicated that GY, DTM, FGP and SY contributed significantly to total variations. GY showed

Table 3. Correlation analysis among the growth and yield-related traits of 48 rice genotypes subjected to submergence

Traits	Treatment	TD7DS	TD21DS	DTM	PH	PPH	PL	FGP	UFG	TSW	SY
TD21DS	Control	0.248									
	14 DS	0.008									
	21 DS	0.262									
DTM	Control	-0.080	-0.128								
	14 DS	-0.127	-0.391**								
	21 DS	0.076	-0.320*								
PH	Control	0.006	0.146	0.290*							
	14 DS	-0.158	-0.245	0.289*							
	21 DS	0.030	-0.126	0.220							
PPH	Control	-0.119	0.147	-0.033	-0.169						
	14 DS	0.018	0.167	0.046	-0.273						
	21 DS	-0.019	0.166	-0.009	-0.245						
PL	Control	0.122	0.070	0.096	0.302*	-0.410**					
	14 DS	-0.039	-0.110	-0.063	0.494**	-0.241					
	21 DS	-0.025	-0.224	0.166	0.428**	0.108					
FGP	Control	0.352*	0.310*	0.139	0.254	0.265	0.345*				
	14 DS	0.216	-0.331*	0.215	0.253	-0.083	0.304*				
	21 DS	0.159	-0.100	0.318*	0.274	0.108	0.407**				
UFG	Control	-0.192	-0.255	0.047	-0.228	-0.233	0.126	-0.486**			
	14 DS	-0.381**	0.060	0.122	0.095	0.138	0.017	-0.481**			
	21 DS	-0.232	-0.256	-0.048	-0.147	0.162	0.152	-0.532**			
TSW	Control	-0.040	-0.095	-0.028	0.115	-0.087	-0.016	-0.230	0.020		
	14 DS	0.151	-0.067	-0.157	0.147	-0.332*	0.209	-0.038	-0.370**		
	21 DS	0.033	-0.154	0.272	0.208	-0.128	0.080	0.177	-0.051		
SY	Control	-0.048	0.067	0.152	0.471**	0.432**	0.331*	0.500**	-0.119	-0.033	
	14 DS	-0.008	-0.028	0.146	0.276	0.678**	0.236	0.147	0.235	-0.218	
	21 DS	-0.100	-0.103	0.254	0.155	0.722**	0.350*	0.231	0.269	0.097	
GY	Control	0.184	0.213	-0.028	0.098	0.729**	-0.007	0.564**	-0.423**	0.165	0.571**
	14 DS	0.253	0.079	0.007	0.089	0.511**	0.168	0.425**	-0.264	0.090	0.501**
	21 DS	0.070	0.039	0.337*	0.108	0.509**	0.314*	0.770**	-0.396**	0.166	0.516**

**Significant at 1 % level of significance; *Significant at 5 % level of significance; TD7DS: Total Dry Matter at 7 Days After De-Submergence; TD21DS: Total Dry Matter at 21 Days After De-Submergence; DTM: Days To Maturity; PH: Plant Height at Maturity; PPH: Plants per Hill; PL: Panicle Length; FGP: Filled Grains per Panicle; UFG: Unfilled Grains per panicle; TSW: Thousand-Seed Weight; SY: Straw Yield per hill; GY: Grain Yield per hill more acute angle with FGP, suggesting a stronger positive correlation, followed by PPH, SY, DTM and PL, with a negative correlation observed with UFG. Genotypes such as SR004, SR033 and SR012, along with SR026 and SR036, exhibited higher performances concerning yield and related traits (Fig. 2b).

Heatmap hierarchical clustering for sorting desired genotypes

The hierarchical clustering heatmap was generated using Submergence Tolerance Index (STI) values for morphological and yield-related traits of evaluated rice genotypes under control and submergence stress conditions (Fig. 3). Each genotype's relation with STI was depicted by the color scale of the rectangles against STI values, while groups of genotypes with similar stability performance were easily identified by the dendrogram on the left side of Fig. 3. In this study, all 48 rice genotypes were arranged on the Y-axis, with 11 agronomical traits on the X-axis. The neighbor-joining dendrogram based on arithmetic means clustered, the 48 rice genotypes and eleven agronomical traits were grouped into two major clusters, further partitioned into five distinct sub-clusters denoted as 1, 2, 3, 4 and 5 in both treatments, 14 DS and 21 DS (Fig. 3). The row sub-clusters of 14 DS contained 3, 8, 18, 7 and 12 genotypes, respectively, while traits TSW were in column sub-cluster 1; PL, FGP, DTM and PH in sub-cluster 2; SY, PPH and GY in sub-cluster 3; UFG in sub-cluster 4; and TD7DS and TD21DS in sub-cluster 5, as depicted in Fig. 2a. Overall, rice genotypes in sub-cluster 5, including SR036, SR028, SR034, SR008, SR038, SR026, SR012, SR031, SR024, SR039, SR040 and SR010, exhibited higher STI values for GY among the 48 rice genotypes in 14 DS (Fig. 3a). For 21 DS, row sub-clusters contained 16, 8, 1, 7 and 16 genotypes, respectively, with traits PH and PL in column sub-cluster 1; UFG, DTM and TSW in sub-cluster 2; TD7DS and TD21DS in sub-cluster 3, FGP and GY in sub-cluster 4; and PPH and SY in sub-cluster 5 (Fig. 2b). Rice genotypes in sub-cluster 1, including SR032, SR018, SR030, SR015, SR024, SR026, SR033, SR031, SR024, check, SR036, SR006, SR008, SR004, SR012, SR009 and SR040, exhibited higher STI values for GY than other rice accessions, suggesting their potential for submergence-tolerant rice breeding programs (Fig. 3b).

Submergence tolerance ranking of rice genotypes

The ranking of rice cultivars based on their relative tolerance to submergence, assessed through growth and yield parameters, followed the IRRI standard evaluation system. Rice genotypes were classified into five groups according to the morphological changes induced by submergence (Table 4). In this study, the rice genotypes were sorted into five groups: highly tolerant (score ≤ 1), tolerant (1< score ≤3), moderately tolerant (3< score ≤5), susceptible (5< score ≤ 7) and very susceptible (score >7). This classification was based on the STI score. After 14 DS, thirty-five genotypes were identified as tolerant, while thirteen genotypes were moderately tolerant. Similarly, twenty-three genotypes were classified as tolerant and twenty-five genotypes as moderately tolerant after 21 DS. None of the genotypes were categorized as highly tolerant, susceptible or very susceptible under either 14 DS or 21 DS conditions (Table 4). Based on the STI score, genotypes such as AZ7006 (check), SR002, SR003, SR004, SR005, SR006, SR007, SR008, SR010, SR011, SR012, SR013, SR014, SR015, SR016, SR018, SR019, SR021, SR022, SR026, SR028, SR030, SR031, SR032, SR033, SR034, SR036, SR037, SR038, SR039, SR040, SR041, SR044, SR045 and SR047 were identified as tolerant rice genotypes under 14 DS conditions, maintaining their growth despite submergence (Table 4). Under 21 DS, the check,

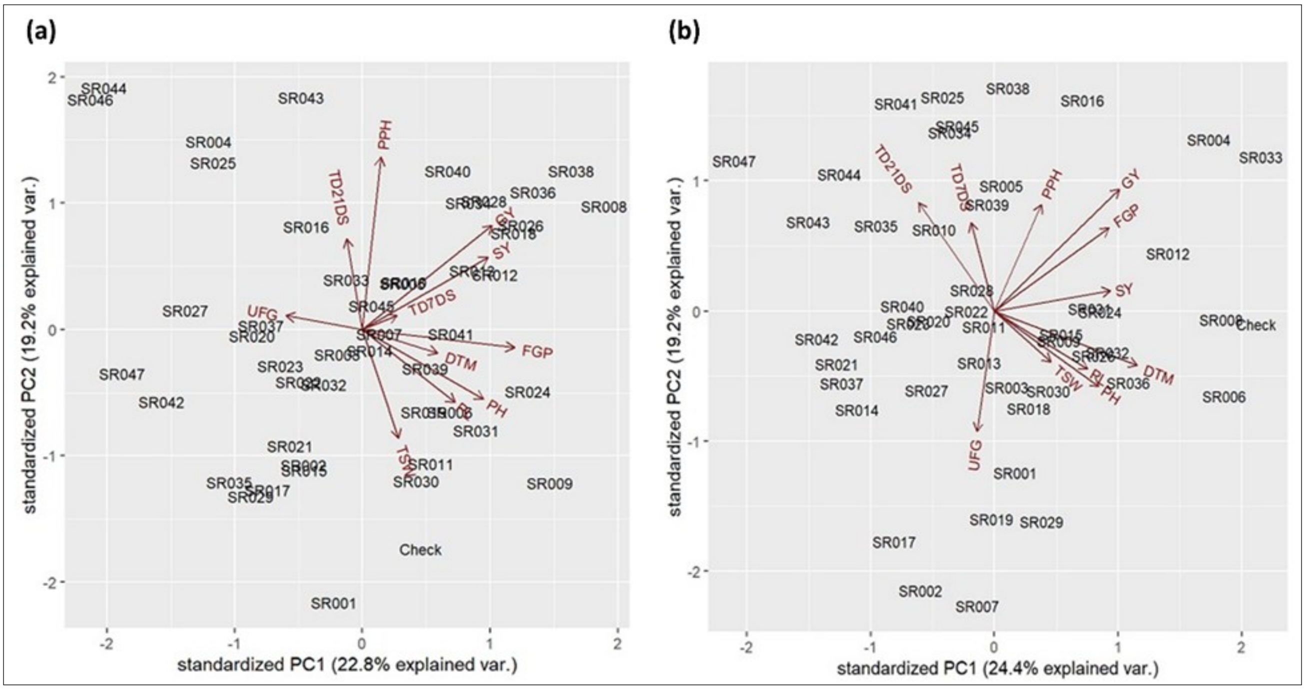

Fig. 2. Principal Component Analysis (PCA) biplot of rice genotypes showing trait contributions and relationships along the two main principal components under submerged stress.

(a) The two principal components explained maximum variability solely and cumulatively, 22.8 % and 19.2 % in 7 DS; (b) 24.4 % and 19.2 % of the variance in 21 DS, respectively to the total variation. The length and direction of the vector indicate the contribution of the individual traits to the total variation in the first two PCs. The biplot also showed the interrelationships among traits where the angle between the vectors stands < 90°, the correlation is equal to a positive one (+1). If the angle between the vectors of the attributes is equal to 90°, the correlation between the vectors of the attributes is zero (0). If the angle between the vectors is equal to 180°, the correlation is negative one (-1).

TD7DS: Total Dry Matter at 7 DADS; TD21DS: Total Dry Matter at 21 DADS; DTM: Days To Maturity; PH: Plant Height at Maturity; PPH: Plants per Hill; PL: Panicle Length; FGP: Filled Grains per Panicle; UFG: Unfilled Grains per panicle; TSW: Thousand-Seed Weight; SY: Straw Yield per hill; GY: Grain Yield per hill

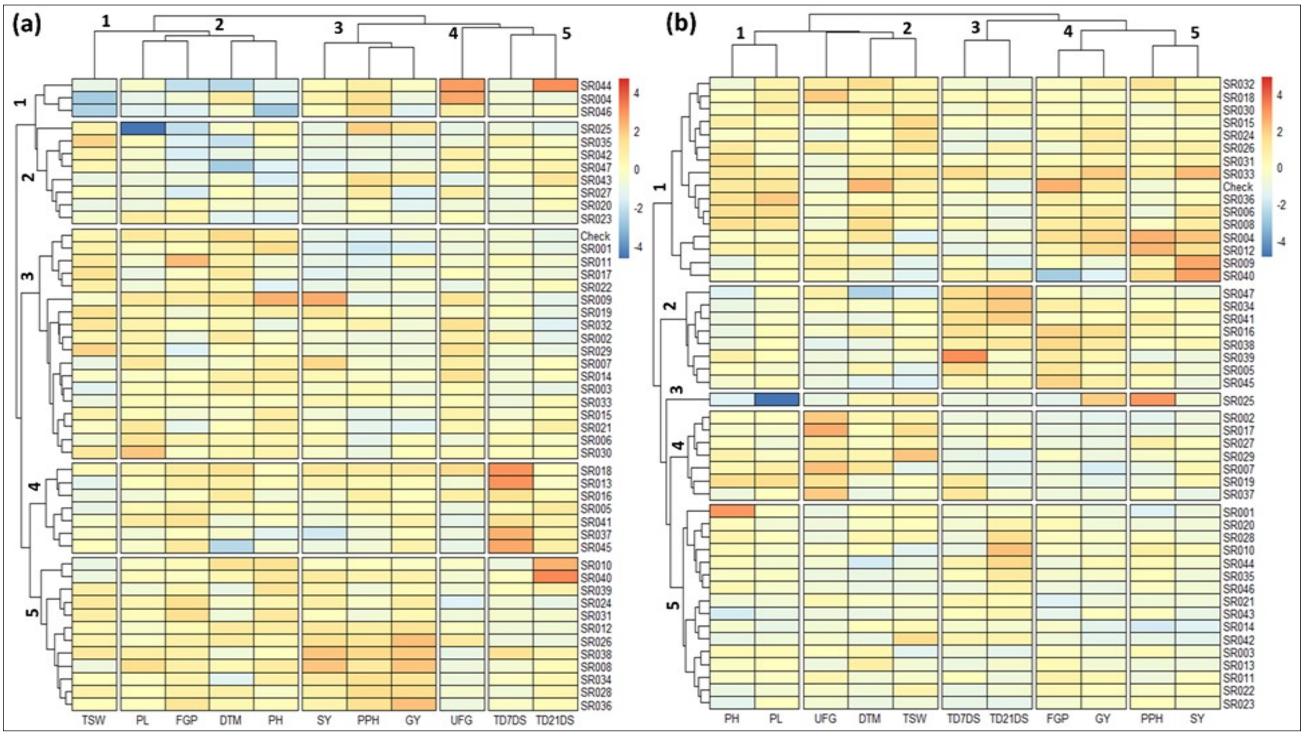

Fig. 3. Heatmap analysis of 48 rice genotypes based on submergence tolerance index.

(a) 14 DS; (b) 21 DS

Hierarchical clustering expresses the visual representation of accessions in different groups based on similarity as well as the correlation with different traits. Rows represent 48 rice genotypes and columns represent various traits, expressed as the difference between the individual genotypes with a different color scale (-4 to +4), indicated as blue to red.

TD7DS: Total Dry Matter at 7 DADS; TD21DS: Total Dry Matter at 21 DADS; DTM: Days To Maturity; PH: Plant Height at maturity; PPH: Plants per Hill; PL: Panicle Length; FGP: Filled Grains per Panicle; UFG: Unfilled Grains per panicle; TSW: Thousand-Seed Weight; SY: Straw Yield per hill; GY: Grain Yield per hill

SR004, SR005, SR006, SR008, SR010, SR012, SR015, SR016, SR018, SR022, SR026, SR030, SR032, SR033, SR034, SR036, SR038, SR039, SR041, SR044, SR045 and SR047 genotypes were classified as tolerant compared to others (Table 4).

Discussion

The development of rice varieties that can withstand submergence, maintaining high yield is challenging due to the potential changes in plant morphology. Submergence has widely acknowledged effects on both the physical structure and physiological processes of rice plants (22). Thus, identification of particular genotypes through precise morpho-physiological assessment is essential through utilizing the scope of genetic diversity. In this study, 48 rice genotypes were subjected to submergence for either 14 or 21 days, beginning 10 DAT. The impacts of submergence were compared to a control treatment. Significant variations in various agronomic traits were observed among the rice genotypes. Submergence led to reductions in growth and yield-related characteristics, including total dry matter, DTM, PH, number of PPH, PL, filled and unfilled grains per panicle, TSW, SY and GY, compared to the control (Table 1). Significant variations were noted among different rice genotypes when subjected to submergence, particularly in terms of PH, SPAD value and root and shoot dry weight at both 7 and 21 DADS, showing substantial reductions compared to the control condition without submergence (Fig. 1). This highlights the high sensitivity of rice plants

Table 4. Submergence tolerance ranking of 48 rice genotypes according to the mean ranking score subjected to 14 and 21 DS

Mean Score	Description	Genotypes
		14 DS
Score≤ 1	Highly tolerant (HT)	-
1< score ≤3	Tolerant (T)	Check, SR002, SR003, SR004, SR005, SR006, SR007, SR008, SR010, SR011, SR012, SR013, SR014, SR015, SR016, SR018, SR019, SR021, SR022, SR026, SR028, SR030, SR031, SR032, SR033, SR034, SR036, SR037, SR038, SR039, SR040, SR041, SR044, SR045 and SR047
3< score ≤5	Moderately tolerant (MT)	SR001, SR009, SR017, SR020, SR023, SR024, SR025, SR027, SR029, SR035, SR042, SR043 and SR046
5< score ≤7	Susceptible (S)	-
Score >7	Very susceptible (VS)	-
		21 DS
Score ≤1	Highly tolerant (HT)	-
1< score ≤3	Tolerant (T)	Check, SR004, SR005, SR006, SR008, SR010, SR012, SR015, SR016, SR018, SR022, SR026, SR030, SR032, SR033, SR034, SR036, SR038, SR039, SR041, SR044, SR045 and SR047
3< score ≤5	Moderately tolerant (MT)	SR001, SR002, SR003, SR007, SR009, SR011, SR013, SR014, SR017, SR019, SR020, SR021, SR023, SR024, SR025, SR027, SR028, SR029, SR031, SR035, SR037, SR040, SR042, SR043 and SR046
5< score ≤7	Susceptible (S)	-
Score >7	Very susceptible (VS)	-

to submergence, a finding supported a separate investigation which reported significant variations in agronomic traits among different *Aman* rice genotypes under submergence, leading to reductions in traits such as panicle number per hill, thousand-grain weight and overall rice yield (12). Similarly, a study found decreases in growth parameters, especially dry matter, in rice genotypes due to submergence (23). Analysis of genetic variability revealed a wide range of differences among genotypes for the studied parameters, indicating ample opportunity for effective selection in crop improvement, as noted in another study (24). In this study, phenotypic variance (δ^2p) and Phenotypic Coefficient Variance (PCV) exceeded their corresponding genotypic counterparts (δ^2g and GCV, respectively) for all traits, suggesting a stronger influence of the environment on trait expression. This finding aligns with previous studies in rice (25,26). Another study states that PCV and GCV values exceeding 20 % are considered high, while values below 10 % are deemed low and those falling between 10 and 20 % are categorized as moderate (17). Applying this classification to our study, most traits exhibited moderate to high PCV and GCV values, with exceptions such as DTM and PL, which had notably lower values (2.06 and 2.21 for DTM, 2.31 and 8.36 for PL) and traits like PPH, FGP and GY showed low GCV and high PCV values (Table 2). It is also reported that high PCV and GCV values for traits such as PH and the number of filled and unfilled grains per panicle, with low values for days to 50 % flowering, DTM, PL and fertility percent and medium values for the number of effective tillers per plant and TSW (27). Yield per panicle was associated with high PCV and moderate GCV values.

Similarly, the H^2 values were divided into three categories: low (<30 %), medium (30 to 60 %) and high (>60 %), while GA was classified as low (<10 %), medium (10 to 20 %) and high (>20 %), reflecting the extent of genetic influence on the observed phenotypic traits (28). Notably, traits such as TD21DS and DTM exhibited high H^2 values along with high GA values (99.49 and 19.53 for TD21DS, 87.44 and 54.02 for DTM), indicating that additive genetic effects primarily control these traits and are less affected by environmental factors (29). Therefore, these traits merit top priority in crop improvement programs. This finding aligns with the results of previous studies, which observed high GA along with high heritability in traits such as survival rate, shoot elongation, chlorophyll content and starch content in rice (30).

Correlation coefficient analysis measures the nature and strength of the relationship between two parameters, such as grain yield and shows potential for improving associated traits through selection (31). Understanding the degree of correlation between traits is crucial in plant breeding, as positive correlations allow for simultaneous improvement of related traits (32). Various plant traits are linked to submergence tolerance in rice and grain yield is particularly important for selecting submergence-tolerant varieties (33). In this study, ten agronomic traits were evaluated in 48 rice genotypes to examine the relationship between rice yield and its components under non-stress and 14 and 21 DS conditions (Table 3). The correlation analysis revealed significant (**; p-value ≤ 0.01 and *; p-value ≤ 0.05) positive and negative correlations among the studied traits, especially with grain yield. GY showed positive correlations with all traits except UFG. Moderate to high positive correlations of GY were found with SY ($r = 0.571^{**}$, $r = 0.501^{**}$, $r = 0.516^{**}$), FGP ($r = 0.564^{**}$, $r = 0.425^{**}$, $r = 0.770^{**}$) and PPH ($r = 0.729^{**}$, $r = 0.511^{**}$, $r = 0.509^{**}$) in control as well as both 14 and 21 DS. There was also a moderate negative correlation with UFG ($r = -0.423^{**}$, $r = -0.423^{**}$, $r = -0.396^{**}$) in both control and submergence conditions (Table 3). Similar findings were reported in another study which found that traits such as harvest index (0.70), PH (0.58), shoot elongation rate (0.44), shoot biomass (0.43), leaf sheath length for the third internodes, (0.43), flag leaf width (0.40) and leaf sheath length for the first internodes (0.40), leaf sheath length for, second internodes, (0.40), number of panicles (0.36), PL (0.31), flag leaf length (0.29) and 100 grain weight (0.29) were positively correlated with grain yield under stagnant flooding conditions, except for days to flowering (34). A previous study observed strong positive correlations of grain yield with panicle weight ($r = 0.51^{*}$) and significant positive relationships with panicles per hill ($r = 0.49^{*}$) under submergence conditions in rice (11).

Multivariate statistical analysis is a crucial technique for describing and summarizing genetic diversity and classifying germplasm collections based on this diversity (35). In this study, two multivariate techniques, PCA and heatmap hierarchical cluster analysis, were employed to describe trait contributions to diversity, explore trait relationships and form statistically homogeneous groups of rice genotypes subjected to submergence. PCA is widely used to identify and reduce duplicate genotypes, characterize different genotypes and classify numerous variables into distinct components while estimating their contribution to total variance (36). In this investigation, the first five PCs had eigenvalues greater than 1 in the 14-day submergence (14 DS) condition, cumulatively accounting for 78.45 % of the total variability. In the 21-day submergence (21 DS) condition, the first four PCs had eigenvalues greater than 1, contributing to 71.53 % of the total variation. A biplot was plotted between PC1 and PC2 using the variability of all quantitative traits to examine the interaction between these components. In the 14 DS condition, traits such as GY, PPH, FGP and SY exhibited greater variability and SY had a stronger positive correlation with GY followed by FGP, PPH, PH and PL. Genotypes SR026, SR018, SR028, SR036, SR038, SR040 and SR006 demonstrated higher yield performances due to their proximity to GY (Fig. 2a). Under the 21 DS condition, GY, DTM, FGP and SY had a significant influence on variations, with GY showing a strong positive correlation with FGP, followed by PPH, SY, DTM and PL. Genotypes SR004, SR033, SR012, SR026 and SR036 exhibited higher yield performances (Fig. 2b). Similar results were observed in another study where a biplot between PC1 and PC2 distributed the traits into four quadrants, showing a close relationship between grain yield and the number of total and effective tillers per hill under submergence conditions (37). Comparable findings were also reported in a separate investigation in relation to their evaluation of rice across three seasons (32).

The tested rice genotypes were grouped into several clusters based on their varying degrees of stress tolerance and the extent of tolerance for individual traits was displayed in a cluster heatmap (Fig. 3). The genotypes formed two major clusters, which were further divided into five distinct sub-clusters for both 14 and 21 DS treatments. These results indicate that genotypes in sub-cluster 5 had higher STI values for most quantitative traits, including GY, in the 14 DS. Similarly, genotypes in sub-cluster 5 exhibited higher STI values for GY and other yield-contributing traits in the 21 DS, highlighting their potential for use in submergence-tolerant rice breeding programs. This clustering outcome aligns with another experiment, which identified three cluster groups based on stress tolerance of rice genotypes under lowland conditions, categorizing them into susceptible, tolerant and intermediate clusters (38). Genotypes RD85 and RD31 were closest to the tolerant check, Azucena and the sensitive check, IR64, respectively. The evaluated genotypes were ranked based on STI for easier selection of tolerant rice genotypes and were divided into five categories under both submergence treatments (14 and 21 DS). No genotypes were found to be highly tolerant, susceptible, or very susceptible in either submergence treatment. However, genotypes SR004, SR005, SR006, SR008, SR010, SR012, SR015, SR016, SR018, SR022, SR026, SR030, SR032, SR033, SR034, SR036, SR038, SR039, SR041, SR044, SR045 and SR047, along with the check AZ7006, were identified as tolerant under both 14 and 21 DS (Table 4). These results are consistent with a previous investigation, which categorized genotypes into five cluster groups under 10 and 30 DS treatments, finding BRRI dhan33, Malshira, Sadapajam and Achin to be the most tolerant and Lalpayka to be the least tolerant among the 22 studied genotypes (12).

Conclusion

In conclusion, a wide genotypic variability in morphological traits was observed among the studied genotypes for submergence tolerance. Most yield-related traits showed moderate to high heritability, indicating additive gene action and suggesting potential for indirect selection. The multivariate approach highlighted genotype variations and their relationship to submergence-tolerant traits. Based on the STI, 35 genotypes were identified as tolerant and 13 as moderately tolerant under 14 DS, while 23 genotypes were tolerant and 25 moderately tolerant under 21 DS. Genotypes SR004, SR005, SR006, SR008, SR010, SR012, SR015, SR016, SR018, SR022, SR026, SR030, SR032, SR033, SR034, SR036, SR038, SR039, SR041, SR044, SR045 and SR047 were tolerant under both conditions. These genotypes may be beneficial for lowland rice areas affected by prolonged inundation.

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Authors' contributions

MAAM, MSR and MAK conceptualized the article and were involved in methodology. MAAM, AM and MTUM carried out the experimental work, formal analysis, validation, investigation and provided resources. MTUM and AM prepared the original draft and handled writing, review and editing. MAAM supervised the work, managed the project and secured funding. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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