

SCREENING OF RICE GERMPLASM FOR DROUGHT STRESS TOLERANCE USING MULTIVARIATE ANALYSIS

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Abstract

Drought stress reduces rice yields around the globe. For this, an investigation was carried out with rice landraces and cultivars to identify drought tolerant genotypes under drought stress conditions using multivariate statistical analysis. The experiment was conducted with 24 genotypes composed of 20 land races and four cultivars during October – March of 2016-17 season using strip-plot design with three replications under control and rainfed drought blocks. The results of the analysis of variance revealed significant differences among the genotypes, treatments and genotype \times treatment interactions for most of the characters studied. Comparing the mean values, control blocks produced superior performances than drought blocks for most of the traits except proline content. Based on the genetic distance, all the 24 genotypes were grouped into three different clusters. In both control and drought conditions, the cluster II contained some common genotypes viz. Kalamani and Burikatari with the highest number of relative means for number of tillers per hill, chlorophyll content, shoot fresh weight, shoot dry weight, root fresh weight and yield per plant. Principal component analysis revealed maximum variation by first three components. The biplot analysis indicated the maximum proximity of genotypes BRRI Dhan56, BRRI Dhan66 and BRRI Dhan71 with root traits and yield per plant. Drought susceptibility indices (DSI) indicated the superiority in the BRRI dhan66. Considering overall superior performances, the genotypes BRRI dhan71, BRRI dhan57, BRRI dhan56, Kalamani, Burikatari, Noyantara and Honumanjata might be useful for novel sources of drought tolerant genes and genotypes in future drought tolerance breeding.

Key words: Land races, Drought, Multivariate analysis, Proline content.

Introduction

Rice (*Oryza sativa* L.) is a major cereal crop that supplies food to about one third of the global population. Globally, the rice production was 483.1 million metric tons in 2015-16. In 2016-17, it is estimated as 481.04 million metric tons that could represent a decrease of 2.06 million metric tons or a -0.43% in rice production around the globe (USDA, 2017). The production is being decreased day by day due to several natural disasters especially for the drought affects in rice different growing periods.

Rice needs an ample amount of water during its life period as compared to other cereals. It is a major challenge in production of rice especially for rainfed and upland ecosystems (Pandey & Shukla, 2015). Moreover, rice plant suffers at different levels and stages of its life cycle due to water scarcity. It has been observed that under rainfed conditions, rice plant seriously affected at booting stage and eventually the plants are referred as susceptible plants that produced inferior yields (Agarwal *et al.*, 2016).

The term tolerance to drought stress means the expression of some phenotypic characters that withstand the plants and leads to higher productivity during the stress period. Here, no single trait might increase the plant performance and multiple traits like root and shoot parameters, heading, maturity, osmotic potentiality, proline content, chlorophyll content etc. are likely to increase the productivity in rice (Razak *et al.*, 2013). Thus, these mentioned traits might be considered in breeding programs to ensure sustainable production under water deficit conditions (Nguyen *et al.*, 1997).

Knowledge of clustering, principle component analysis and drought tolerant index are the essential tools in breeding that leads to improvement of any particular crop like rice through selection of a superior germplasm by analyzing its greater performance in a given conditions (Krishnaveni *et al.*, 2006). Among the breeding tools, multivariate cluster analysis and principal component analysis (PCA) are advanced biometric techniques. Cluster analysis is measured through Mahalanobis D² statistics (Mahalanobis, 1936) and used to quantify the amount of genetic divergence present in a given populations. In addition, PCA helps in computing the relative contribution of different quantitative traits having breeding value to the total divergence in their clustering pattern. Earlier workers had also reported the presence of substantial genetic diversity in rice (Sandhya *et al.*, 2015; Sowmiya & Venkatesan 2017). A drought tolerance index (DSI) is useful as an indicator to identify drought tolerant genotypes under drought environment. Some researchers reported that the genotypes which had the lowest DSI values were drought tolerant than the genotypes with the highest DSI values (Kumar *et al.*, 2014a).

Considering the global climate change, drought is becoming a serious threat in rice crop production. The available drought tolerant genotypes is still scare around the globe. So, the research hypothesis was the identification of potential drought tolerance genotypes. This might be achieved through the genetic study of morpho-physiological and biochemical traits using rice landraces and cultivars under drought stress conditions. Therefore, the present study was conducted to i) study the diverse germplasms and relationship of the characters through multivariate analysis in rice land races and cultivars and ii) identify drought tolerant genotypes through drought susceptibility index.

Materials and Methods

Plant materials: Twenty-four rice genotypes were used as plant materials in this research work. Among these, four were Bangladesh Rice Research Institute (BRRI) released variety and 20 were drought tolerant landraces. All these genotypes were collected from the Germplasm Centre of BRRI, Gazipur, Bangladesh.

Experimental setup: The experiment was conducted at the experimental farm of the Department of Genetics and Plant Breeding of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, from October 2016 to March 2017. Here, experiment was arranged in a strip-plot design with three replications. In each replication, the treatments (drought and control) were assigned as vertical strip-plot and the genotypes were allocated as horizontal strip-plot into each vertical strip. Overall, the experimental layout was 2 treatments (control and drought) × 24 plots × 3 replications. The individual vertical strip-plot size was 7.2 m × 3 m. In each vertical strip-plot, 24 genotypes were distributed in 24 lines where each line served as a horizontal strip-plot and each line contained 15 plants or hills. The distance between row to row was 30 cm and plant to plant was 20 cm. The control plots were irrigated 8 times from the tillering to reproductive stages of the plant at certain intervals as per recommended dose. For drought plots, the first irrigation dose was the same as control plot and afterwards, the irrigation was completely stopped and the plants were nourished only with rain water till the harvesting.

Data recording: Data were recorded on ten (10) random plants from each unit plot. In total fourteen morpho-physiological and biochemical characters viz. days to 50% flowering, plant height, number of tillers per hill, proline content, chlorophyll content, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, root length, root volume, number of grains per plant, 1000-grain weight and yield per plant were recorded. Here, proline extraction was done following the acid-ninhydrin method according to Bates *et al.*, (1973). This was

followed by UV-visible spectrophotometer analysis of the absorbance of the proline extract in toluene at a wavelength of 520 nm. Again, the chlorophyll content was measured as SPAD unit from the electronic device called chlorophyll meter.

Statistical analyses

The data obtained for different characters were recorded first on MS excel sheet. Afterwards, the data were analyzed using the software package R of version 3.4.2 (R Core Team, 2017) and Statistical Tool for Agricultural Research (STAR) of Version 2.0.1 (2014).

Results and Discussion

Analysis of variance and treatment performances comparison:

The positive outcome of any breeding program depends primarily on variability among the germplasms of a population. The results of the analysis of variance of 14 morpho-physiological and biochemical characteristics in rice are presented in (Table 1). Here, significant differences were observed among the genotypes and between the treatments for all the characters viz. days to 50% flowering, number of tillers per hill, plant height (cm), chlorophyll content, proline content ($\mu\text{g g}^{-1}$), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g), root length (cm), root volume (cm^3), number of grains per plant, thousand grain weight (g) and yield per plant (g). This clearly indicates the presence of variability among the genotypes studied for the mentioned traits. The similar findings were also observed by Ullah *et al.*, (2011), Seyoum *et al.*, (2012) and Abebe *et al.*, (2017). The genotype × treatment effect exhibited significant differences for most of the traits except root dry weight (g), root length (cm), root volume (cm^3) and thousand grain weight (g). Poli *et al.*, (2018) revealed highly significant interaction effect of 300 mutants and three environments (low P, water limited, normal condition) for plant height, tiller number and grain yield. Coefficient of variation (CVc) in all the characters was less than 20.00 except root dry weight and number of grains per plant.

Table 1. Mean squares (MS) derived from strip-plot design on morpho-physiological and biochemical characters in rice.

Character	Source of variation with mean square									
	Replication (2df)	Genotype (23df)	Error a (46df)	Treatment (1df)	Error b (2df)	Genotype × Treatment (23df)	Error c (46df)	CVa (%)	CVb (%)	CVc (%)
Days to 50% flowering	7.34	58.78***	4.70	5304.69***	2.17	43.85***	4.69	2.48	1.69	2.29
Number of tillers per hill	1.33	36.01***	2.62	483.64***	10.70	11.35***	1.77	17.89	36.09	14.71
Chlorophyll content	4.26	95.02***	12.93	41822.92***	0.13	35.67**	10.44	12.83	1.30	11.53
Proline content	0.01	0.45***	0.01	0.76***	0.04	0.19***	0.03	11.63	18.84	16.83
Plant height	139.04***	285.19***	10.60	41704.44**	0.57	168.14***	10.91	3.46	0.80	3.51
Shoot fresh weight	1.45	14.05***	1.41	448.32***	0.79	6.56***	1.20	20.90	15.61	19.27
Shoot dry weight	0.003	1.18***	0.11	52.95***	0.02	0.76***	0.07	21.09	17.20	1.64
Root fresh weight	16.06	78.25***	7.11	2077.08***	0.14	28.02***	3.92	18.14	2.62	13.46
Root dry weight	2.27	28.01***	1.37	157.68***	1.53	5.10	1.46	24.86	26.25	25.67
Root length	0.79	17.80***	3.44	610.99**	14.29	7.14	4.58	12.72	25.86	14.67
Root volume	3.54	207.66***	12.22	5521.23***	67.19	61.90	12.26	13.54	31.75	13.56
Number of grains per plant	5367.92	11232.72***	6687.50	70984.05***	5560.79	8090.23***	5623.71	27.40	20.98	25.66
1000-grain weight	11.32	55.52***	5.70	1151.52	23.40	7.54	6.34	10.50	21.26	11.07
Yield per Plant	0.04	1.14***	0.17	51.80	0.21	0.59***	0.17	19.02	21.63	19.49

Note. ** and *** indicates significant at 1% and 0.1% levels of probability, respectively. df = degrees of freedom, CV = Coefficient of variation

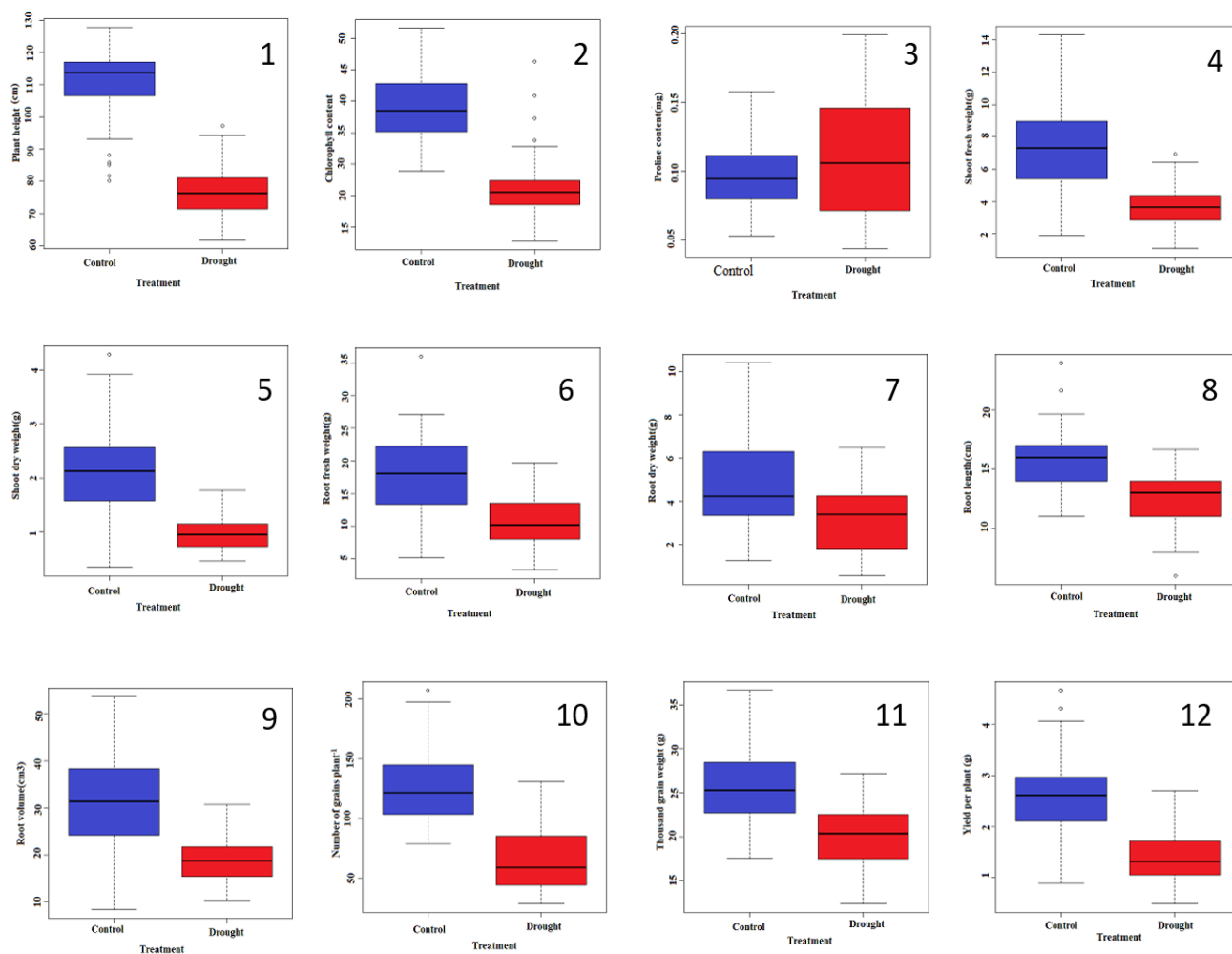


Fig. 1. Box plots showing comparison of selected trait performances between control and drought blocks. Here, 1. Plant height (cm), 2. Chlorophyll content, 3. Proline content, 4. Shoot fresh weight (g), 5. Shoot dry weight (g), 6. Root fresh weight (g), 7. Root dry weight (g), 8. Root length (cm), 9. Root volume (cm³), 10. Number of grains per plant, 11. Thousand grain weight (g) and 12. Yield per plant (g).

High levels of drought can significantly inhibit growth and development of rice plant. This leads to the lower productivity in plants by hindering the metabolism and various major functions of the plants. In our study, the box plots had given an idea that how different traits of rice were affected by drought stress. This affect also revealed in our study under drought stress conditions of the traits plant height, chlorophyll content, shoot fresh weight, shoot dry weight, number of grains per plant, 1000-grain weight and yield per plant significantly reduced (Fig. 1). The tendency of decreasing the range of most of the traits with the increasing of drought stress was confirmed by Agarwal *et al.*, (2016), Swapna *et al.*, (2017). In the study, marginal difference revealed for the root traits viz. root fresh weight, root dry weight and root length. Here, the median of root fresh weight (g), root dry weight (g) and root length (cm) was 18.06 cm, 4.24 cm and 16.00 cm in control conditions and 10.15 cm, 3.86 cm and 13.00 cm in drought conditions, respectively. This indicates that the underground parts of the rice plants were less affected that the above ground parts. In respect of drought tolerance breeding, these traits are very important and indicates some promising genotypes excels superior root traits.

Proline is an amino acid, synthesized in plants widely under abiotic stresses. In our study, proline content was increased in drought block compared to the control block genotypes. Here, the median in control conditions was 0.09 and in drought conditions, it was 0.11. Swapna *et al.*, (2017) reported that the proline content was increased in all 42 varieties of rice in drought blocks in their study. It has been suggested that the increasing rate of proline content helps the plant to combat with adverse effects of drought stress.

Cluster analysis: Cluster analysis was performed both control and drought conditions with relative mean values of each trait. Euclidian distance coefficients were calculated for all rice genotypes based on all traits studied. Dendrogram from UPGMA clustering indicated grouping of 24 genotypes of rice into three clusters for each control and drought treatments (Figs. 2 & 3). Under control conditions, cluster number II holding most tolerant genotypes (Kalamani, Burikatari and BRR1 Dhan71) with the highest number of relative mean for days to 50% flowering, plant height, number of tillers per hill, chlorophyll content, shoot fresh weight, shoot dry weight, root fresh weight,

root dry weight, root length, root volume, number of grains per plant, 1000-grain weight and yield per plant. The cluster II in drought conditions contained the genotypes Kalamanik, Burikatari, Honumanjata and BRR1 Dhan57. This cluster has given highest number of relative means for plant height, number of tillers per hill, proline content, chlorophyll content, shoot fresh weight, shoot dry weight, root fresh weight, root volume and yield per plant in drought conditions (Table 2). The genotypes from these clusters may be directly utilized for adaptation or may be considered as parents for future hybridization program of drought tolerance. Kumar *et al.*, (2015) found five clusters in 18 local and exotic rice genotypes and depicted that the Cluster II and III harbored maximum five while cluster V with two IRRI genotypes assuring comparative diversity of exotic material.

Principal Component Analysis (PCA): In the present study, the rotated component matrix of fourteen phenotypic traits of 24 rice genotypes were evaluated under control and drought stress conditions and are presented in (Table 3). Here, we have been followed a criterion given by Clifford & Stephenson (1975) and later that is supported by Guei *et al.*, (2005). According to them, the first three principal components are very important in most cases to observe the pattern of variations exist in the genotypes and the traits linked with these are more beneficial in differentiating the genotypes. The maximum proportion of variation were revealed by first three components (59.98 % in control and 62.11% in drought conditions). Again, the criterion given by Raji (2002) was considered to measure the cutoff limit for the coefficients. According to this, component matrix coefficients greater than 0.3 indicated an ample component effect and have importance of that component for a particular trait in the study. In our study, PCA revealed that the traits shoot dry weight (g), root volume (cm³), number of grains per plant and yield per plant (g) had greater influence (>0.3) on PC1, root dry weight (g) for PC2 and 1000-grain weight (g) for PC3 in control conditions. Under drought conditions, the traits yield per plant (g), root fresh weight (g), root volume, number of grains per plant had higher loadings (>0.3) on PC1, proline content and shoot dry weight (g) for PC2 and plant height (cm) and shoot dry weight (g) for PC3. Therefore, these characters should be given importance during selection. Anyaoha *et al.*, (2018) found about 64.55% of the total variation by the first three principal components and suggested that the grain yield, days to flowering, leaf area and plant height at maturity were the prime discriminatory traits in their study.

Biplot analysis: A genotype × traits biplot was created from a two-way matrix of eleven traits and 24 genotypes of rice for both control and drought stress conditions. The association among different characters and genotypes with respective principal components were later described by the principal component biplots in (Fig. 4) for both control and drought stress

conditions. According to the graph, the narrow angles between two adjacent vectors in same direction showed strong correlation of a character in terms of discerning genotypes. In control conditions, the first two axes of a PCA biplot summarizing the relationships between the variables and the genotypes explained 32.7% of the total variance by PC1 and 14.7% of the variance by PC2. In this condition, a higher number of genotypes were scattered in the negative side of the first and second principal component. The genotypes Pashpae (5), BRR1 Dhan57 = 22, Burikatari = 10 and Kalamanik = 9 have PCA1 score > 0 and positive for PCA 2. The genotypes 5 and 22 were clogged near the vicinity of the trait's days to 50% flowering and 1000-grain weight, chlorophyll content and number of tillers per plant. Again, the genotypes Burikatari (10) and Kalamanik (9) had higher loads for the traits plant height, proline content and number of grains per plant. In drought conditions, the first two axes of a PCA biplot summarizing the relationships between the variables and the genotypes explained 36.3% of the total variance by PC1 and 15.5% of the variance by PC2. Under drought conditions, the genotypes were scattered across all axes. The genotypes BRR1 Dhan56 (21), BRR1 Dhan66 (23) and BRR1 Dhan71 (24) contributed PCA1 > 0 and positive for PCA 2. These genotypes have proximity and have higher match with the traits 1000-grain weight, root length, number of grains per plant, root dry weight, root fresh weight, root volume and yield per plant. For proline content, the genotype Honumanjata (13) had higher positive loads in PC1 and negative for PC2. From the biplot analysis, Poli *et al.*, (2018) demonstrated that the rice mutant G17 of rice cultivar Nagina22 was the ideal genotype for yield and number of tillers at lower P, drought and well-watered conditions.

Drought susceptibility index (DSI): Significant variability was found amongst the genotypes for tolerance and rice genotype BRR1 Dhan66 exhibited the lowest DSI (-0.04) values exhibiting as highly tolerant. Genotypes BRR1 Dhan71, Noyantara, BRR1 Dhan57, Kalamanik, Hasikalmi, BRR1 Dhan56, Burikatari, Honumanjata, Pukhe, Surjamukhi, Dhalebaita and Kadamone were identified as tolerant genotypes giving with DSI value range from 0.24 to 0.99. The other genotypes Surjamukhe, Dharyl, Dular, Pabira, Begunbiche, Laksmelata, Pashpae, Begunbahar, Madabjata, Sreerampuri and Manikmodhu exhibited higher DSI values indicating susceptible genotypes under rainfed drought stress conditions (Table 4). Similar results were recorded by other workers for selections based on these indices (Khan & Dhurve, 2016; Kumar *et al.*, 2014b).

In our study, BRR1 Dhan66 revealed most tolerant genotype category followed by BRR1 Dhan71 and Noyantara exhibiting lower DSI value. These genotypes could be considered as a drought tolerant genotype and a donor for gene transfer in drought tolerance breeding.

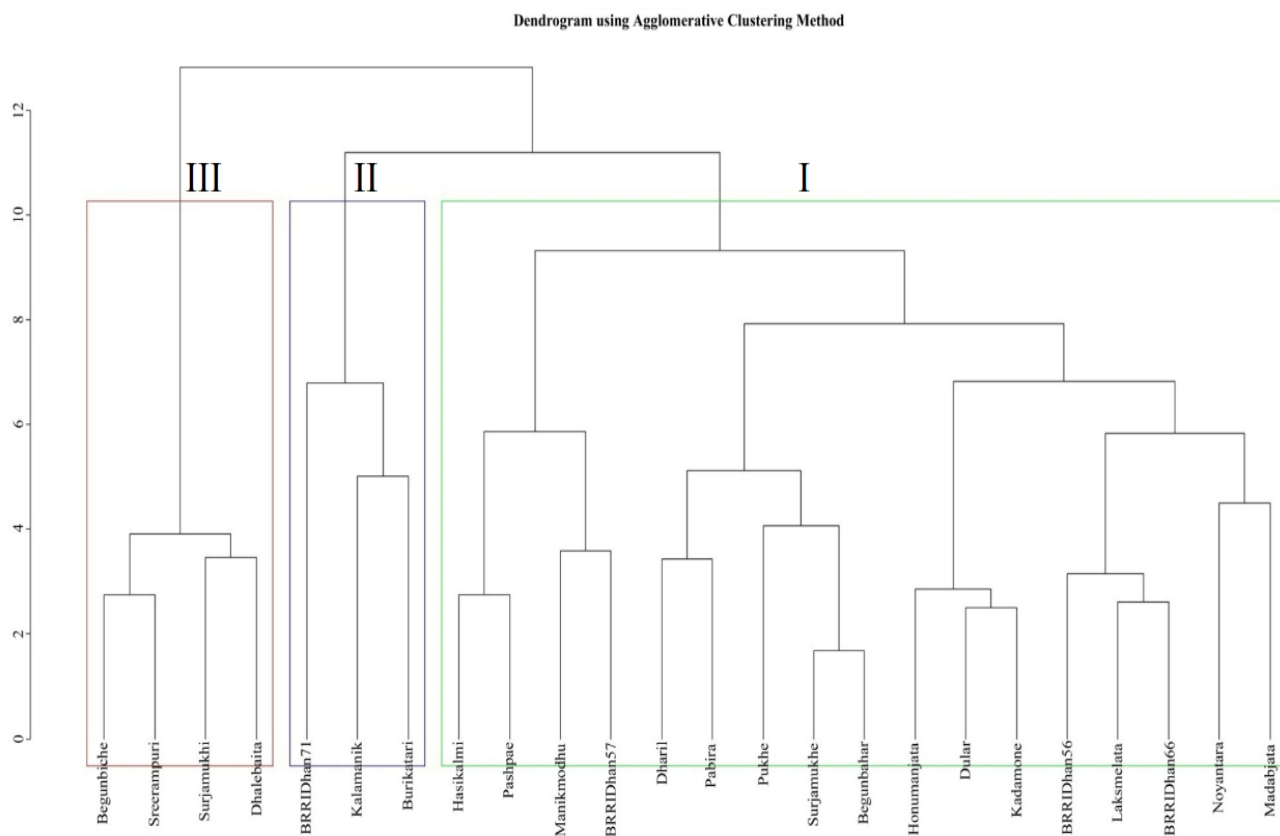


Fig. 2. Dendrogram from UPGMA clustering for 24 rice genotypes using Euclidean genetic distance based on all traits measured in control conditions.

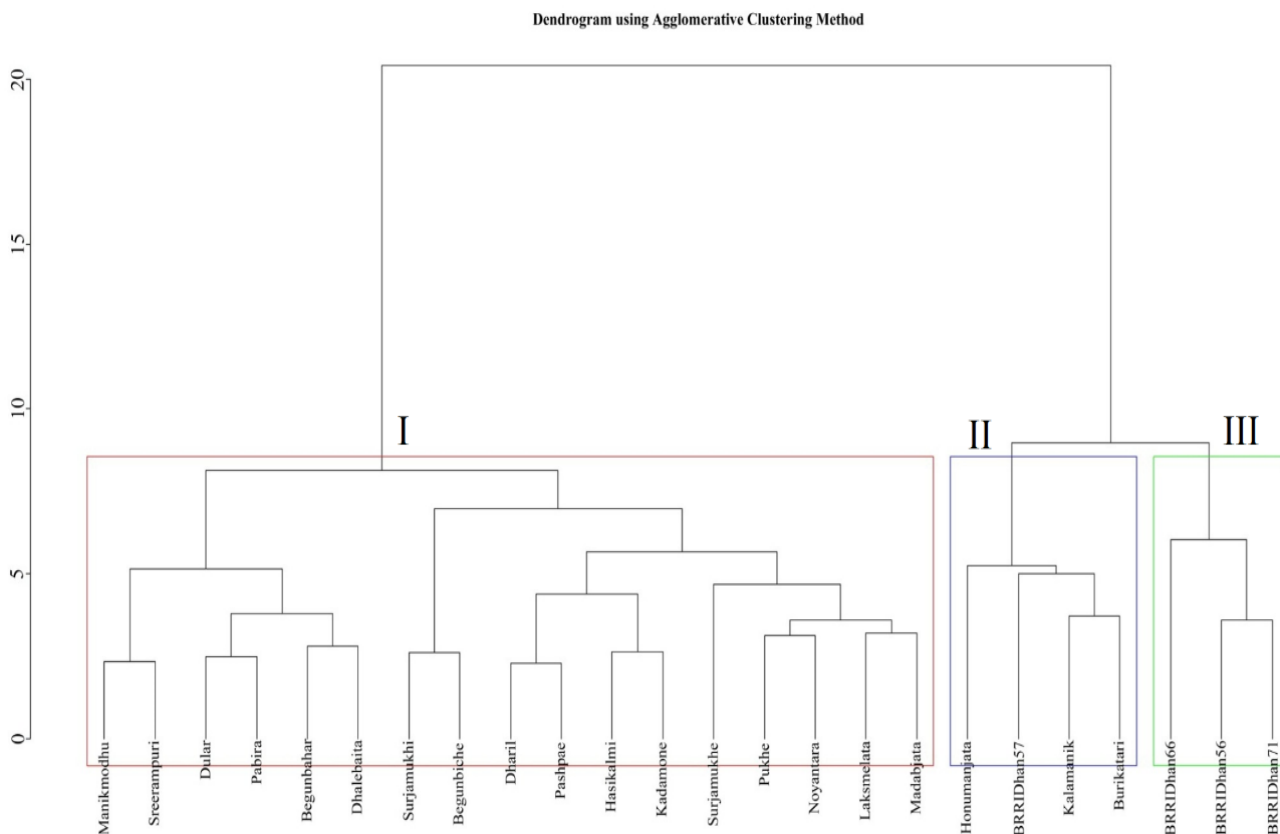


Fig. 3. Dendrogram from UPGMA clustering for 24 rice genotypes using Euclidean genetic distance based on all traits measured in drought conditions.

Table 2. Mean values for three clusters based on 14 quantitative traits of rice genotypes under control and drought stress conditions.

Characters	Cluster Mean					
	Control			Drought		
	I	II	III	I	II	III
Days to 50% flowering	81.20	82.89	80.92	94.35	94.08	87.89
Number of tillers per hill	10.90	13.08	9.25	6.56	10.43	6.76
Chlorophyll content (SPAD)	34.25	38.89	30.68	20.35	29.69	19.67
Proline content ($\mu\text{g g}^{-1}$)	0.10	0.09	0.09	0.11	0.15	0.08
Plant height (cm)	113.96	120.58	92.15	75.42	81.53	80.87
Shoot fresh weight (g)	7.68	10.27	4.42	3.71	5.13	3.58
Shoot dry weight (g)	2.28	3.30	1.28	0.96	1.33	1.06
Root fresh weight (g)	16.99	25.29	19.86	9.33	14.76	14.75
Root dry weight (g)	5.04	12.00	4.16	2.99	5.29	5.38
Root length (cm)	16.13	20.67	15.92	11.93	13.67	14.52
Root volume (cm^3)	32.63	40.89	22.70	17.32	26.39	23.67
Number of grains per plant	130.98	138.23	106.81	58.37	101.58	201.45
1000-grain weight (g)	25.95	26.27	23.53	19.00	21.37	23.29
Yield per Plant (kg)	2.84	2.89	2.36	1.30	2.29	2.14

Table 3. Rotated component matrix of fourteen phenotypic traits of 24 rice genotypes evaluated under both control and rainfed drought stress conditions.

Characters	Control			Drought		
	PC1	PC2	PC3	PC1	PC2	PC3
Days to 50% flowering	0.082	-0.415	-0.381	-0.198	-0.240	-0.238
Number of tillers per hill	0.176	-0.514	0.006	0.245	-0.247	-0.375
Chlorophyll content (SPAD)	0.156	-0.411	-0.110	0.281	-0.208	-0.130
Proline content ($\mu\text{g g}^{-1}$)	0.120	0.022	-0.565	0.035	0.450	-0.539
Plant height (cm)	0.397	-0.159	0.064	0.183	-0.295	0.441
Shoot fresh weight (g)	0.375	-0.030	0.044	0.061	-0.194	0.045
Shoot dry weight (g)	0.407	0.024	0.126	0.223	0.392	0.367
Root fresh weight (g)	0.200	0.257	0.126	0.325	0.100	-0.230
Root dry weight (g)	0.184	0.375	-0.058	0.297	0.174	0.112
Root length (cm)	0.185	0.014	-0.235	0.304	0.218	-0.221
Root volume (cm^3)	0.333	0.266	-0.115	0.321	0.009	-0.118
Number of grains per plant	0.330	-0.000	0.251	0.319	0.251	0.029
1000-grain weight (g)	0.089	-0.284	0.536	0.277	0.242	0.011
Yield per Plant (g)	0.389	0.087	-0.035	0.351	-0.031	0.177
Eigen value	5.249	2.435	1.525	5.253	2.471	1.206
Proportion of Variance (%)	32.7	14.7	12.5	36.3	15.5	10.4
Cumulative Proportion (%)	32.7	47.4	59.9	36.3	51.7	62.1

Table 4. Drought Susceptibility Index (DSI) showing degree tolerances in 24 rice genotypes considering yield performance in both control and drought conditions.

Genotype (DSI Value)	Level of tolerance
Surjamukhe (1.67), Dharil (1.62) Dular (1.51) Pabira (1.47) Begunbiche (1.41) Laksmelata (1.34) Pashpae (1.31) Begunbahar (1.30) MadabJata (1.18) Sreerampuri (1.02) Manikmodhu (1.01)	Susceptible
Kadamone (0.99), Dhalebaita (0.98), Surjamukhi (0.89), Pukhe (0.88), Honumanjata (0.84), Burikatari (0.74), BRRI Dhan56 (0.72), Hasikalmi (0.71), Kalamanik (0.58), BRRI Dhan57 (0.57), Noyantara (0.39), BRRI Dhan71 (0.24)	Tolerant
BRRI Dhan66	Highly Tolerant

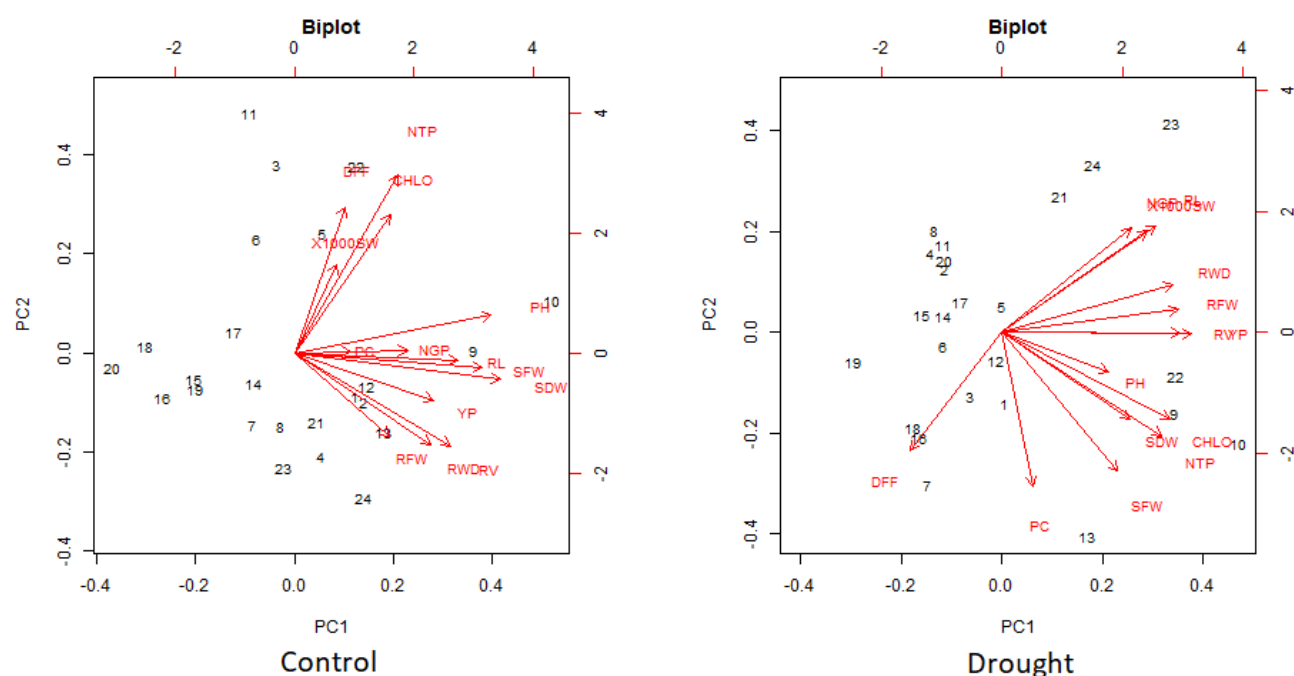


Fig. 4. Principal component biplot showing genotypic-traits grouping under control and drought stress conditions. Here, 1 = Dharil, 2 = Dular, 3 = Hasikalmi, 4 = Pabira, 5 = Pashpae, 6 = Pukhe, 7 = Surjamukhe, 8 = Laksmelata, 9 = Kalamani, 10 = Burikatari, 11 = Manikmodhu, 12 = Kadamone, 13 = Honumanjata, 14 = Begunbahar, 15 = Noyantara, 16 = Surjamukhi, 17 = MadabJata, 18 = Begunbiche, 19 = Dhalebaita, 20 = Sreerampuri, 21 = BRRi Dhan56, 22 = BRRi Dhan57, 23 = BRRi Dhan66, 24 = BRRi Dhan71.

Acknowledgments

We offer special thanks to Dr. Hasina Akter and Germplasm Centre of Bangladesh Rice Research Institute (BRRi), Gazipur for providing the germplasm. We are thankful to Mr. Alkasim Muhammad Sulaiman and Mst. Anamika Amzad for reading the manuscript. We are also grateful to Ministry of Science and Technology of Bangladesh for providing financial support for the research work.

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(Received for publication 19 March 2019)