Original Research Article

Identification of *in-vitro* PEG mediated drought tolerance genotype in rice (*Oryza sativa* L.)

ABSTRACT

Drought is a serious bottleneck in the production of rice globally. For this, an experiment was conducted *in-vitro* on six rice genotypes viz. BRRI dhan-28, Begunbahar, Burikatari, Pashpai, Dular and Begunbichi to investigate the effect polyethylene glycol (PEG) mediated artificial drought on morpho-physiological parameters such as germination percentage, shoot length, root length, fresh weight, dry weight, turgid weight, relative water content, and proline accumulation. Here, different doses of PEG-6000 viz. 0 gL^{-1}, 15gL^{-1}, 30gL^{-1}, 45gL^{-1} and 60gL^{-1} were used with Murashige and Skoog (MS) medium. The results demonstrated that BRRI Dhan-28, Burikatari and Dular revealed greater performance at control conditions but at the highest degree of water stress conditions only Burikatari showed higher mean value for all parameters studied. Again, Begunbichi followed by BRRI Dhan-28 exhibited the lowest mean value for almost all traits except for proline accumulation. Here, water stress decreased performance of morpho-physiological characters except proline accumulation in rice. The cluster analysis was performed and distributed into three groups where there was a significant variation among the clusters at different water stress conditions. Here, the genotype Burikatari is more diverse giving maximum Euclidian distances in drought treatments. It could be considered as a parent in the hybridization program against Begunbahar, Dular and Paspai. Therefore, considering the mean performances and cluster analysis, Burikatari exhibited greater performances against the highest degree of

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drought conditions. This genotype may bear drought-tolerant gene for which could be utilized for further development of drought tolerant variety and gene transfer.

Key words: *In vitro*, water stress, PEG (Polyethylene Glycol), rice.

1. INTRODUCTION

Rice has been referred to as "Global Grain" and considered as a model cereal crop in the world [1]. It belongs to genus *Oryza* that contains 25 recognized species, of which 23 are wild species and two; *O. sativa* and *O. glaberrima* are cultivated [2]. *O. sativa* is the most widely grown cultivated species. In the year 2017-18, the rice production around the world was estimated at 484.7 million tons and was 0.5 % below the year-earlier record [3] that was cultivated at least 114 countries. Asia is the leader in rice production accounting for about 90% of the world's production where about 75% of rice is consumed by the Asian people [4,5].

Rising temperature as a form of climate change and altered soil moisture is projected to decrease the yield of food crops over the next 50 years [6]. In recent years, drought and salt stress reduces rice production worldwide [7,8]. However, depends on crop growth period and stress intensity, drought reduces the yields by 15 to 50 percent [9]. It has been reported that main constrain to crop yield is ~~for the~~ precarious rainfall or scarcity of the water in soil deep layer due to presence~~t~~ of hardpan that resists accessing water [10, 11]. Rice is one of the most sensitive cultivated species to water stress. So, the farmer is more likely to access ~~the plant breeding brought~~ tolerant~~ce~~ genotypes rather than expensive agronomic practices [12].

Plant responses to drought involve ~~and engaging lots of~~ physiological, biochemical and molecular changes [13]. So, it seems impossible to increase crop yield ~~remain~~ in water-deficient condition during crop cultivation [14]. Morphological characters viz., shoot and root length [15],

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leaf fresh, turgid, dry weight and relative water content [16] and seed germination and seedling growth [17] and biochemical: proline accumulation [16] are adversely affected by water stress.

In vitro, PEG (polyethylene glycol) are known to cause osmotic stress which alters the osmotic potential of the cell and hence these will be as useful selection agents for drought tolerance. It resists both water and mineral upliftment by root by forming hydrogen bond with water and also decrease the water potential in the culture medium~~ by root~~. Thus, the osmotic agent acts in lowering the water potential in a way similar to soil drying [18,19].

Cluster analysis based on Mahalanobis D^2 statistic [20] is the possible quantifier for amounting the degree of genetic variability among the genotypes. The numerous cluster group demonstrated the highest degree of variability present in the materials evaluated. Earlier workers had also reported the presence of substantial genetic diversity in rice [21,22].

Considering the above aspects, one of the best policies would be the development of water stress tolerance genotypes to increase the rice yield in drought-prone area. So, the research hypothesis might be the identification of potential drought tolerance genotypes. This might be achieved through the genetic study of morphophysiological and biochemical traits *in- vitro* conditions using PEG treatment (under drought stress conditions). Therefore, the major objectives of the present research work was (i) to evaluate the result of drought-induced seed germination and seedling growth parameter of rice genotypes, (ii) to mold a quick and effective strategy for rice against drought conditions and (iii) to determine the most drought tolerant the genotype of rice.

2. MATERIALS AND METHODS

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Plant materials: Seeds of six rice genotypes comprised of drought-tolerant landraces (Burikatari, Begunbahar, Dular, Pashpai, Begunbichi) and an elite cultivar BRRI Dhan- 28 were used in the present investigation. These materials were collected from the Genetic Resources and Seed Division of Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

Experimental set-up: During the period of 2017 to December 2017 the experiment was carried out at tissue culture Laboratory of the Genetics and Plant Breeding and C~~c~~hemistry Laboratory of the Hajee Mohammad Danesh Science Technology University (HSTU) Dinajpur, Bangladesh. Here we used [23] Murashige and Skoog, media for culturing the seeds. Firstly, seeds were sterilized in mercuric chloride for five minutes then sterilized in 70 % ethyl alcohol for three minutes and washed with double distilled water. Afterward, sterilized matured seeds were inoculated into the test tube containing 10ml MS solution with PEG-6000 at the different concentrations such as T_0 = 0gL^{-1} (Control condition), T_1 = 15gL^{-1}, T_2= 30 gL^{-1}, T_3 = 45 gL^{-1}, T_4 =60 gL^{-1} with four replications and lab temperature was controlled at 25º C with ~~under the correspondence of~~ sixteen hours light period and eight hours dark period.

Data collection: The following data was~~ere~~ recorded during the experimental period. The procedures of measurement of these data are described here below.

Percentage of germination:

$$\text{Percentage of germination} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds inoculum}} \times 100$$

Shoot length, root length, fresh weight, dry weight, turgid weight of plant~~, and shoot root ratio~~:

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Seedling was taken out with the help of forceps at the 17th days of inoculation and shoot length, root length and total length were measured in cm by a graduated scale. And also, the fresh, turgid and dry weight was calculated in gram by electrical balance.

Relative water content of leaf: The relative water content [24] of leaf was determined as follows:

$$\text{Relative water content} = \frac{\text{Fresh weight - dried weight}}{\text{Fully turgid weight - dried weight}} \times 100$$

Leaf fresh weight was taken, then l~~L~~eaf~~ves~~ was~~ere~~ submersed into distilled water in the darkness at 4º C to minimize the respiration losses until ~~they~~ reached the constant weight that is 12 hours, a~~.~~nd weighed as turgid weight. After leaf was dried ~~the leaves~~ in the oven for 48 hours at 70º C, dry weight was taken in gram.

Determination ~~Four times weight were obtained for each treatment.~~

of p~~P~~roline content of leaf~~determination~~: Sulfosalicylic acid was used ~~for the extracted of~~to extract proline from the leaves, and the filtrated solution was mixed with an equal amount of ninhydrin reagent and glacial acetic acid that is 1.25g ninhydrin, 20ml $6NH_3PO_4$ and 30ml glacial acetic acid and incubated at 100º C for 1hour. All test tubes placed in coll~~o~~d water to cool the sample and toluene mixed with it and vigorously shaken ~~it~~ for complete mixing~~ completely~~. Color was read at 520nm ~~light was passed thorough the sample~~ using Pharmacia LKB-Novaspace spectrophotometer~~ to determine the light absorption of toluene~~. Standard curved was used to measure the concentration of the proline that was expressed as mg/100g of plant parts [25].

Cluster analysis: The statistical software – Agricultural Research (STAR) Version 2.0.1 (2014) was used for estimating of Euclidian distance of coefficients. Euclidean distance matrix

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generated from seedling data was used as input data for cluster analysis based on the un-weighted pair-group method of arithmetic average (UPGMA). To estimate the level of relatedness among the genotypes based on the Euclidean genetic distances a UPGMA was drawn.

3. RESULTS AND DISCUSSION

The experiment was conducted to analyze morpho-physiological traits of six rice genotypes under control and different water stress conditions. Mean performance and other analyses were done on eight morpho-physiological traits like shoot length, root length, fresh weight, turgid weight, dry weight, relative water content, and germination percentage. The results have been presented and discussed under the following headings:

The analysis of variance for different yield and morpho-physiological characters: The analysis of variance was accomplished to assess the variability pertained for a character among the six rice genotypes (Table 1). For all traits, the analysis of variance showed a significant difference among the genotypes indicated that the genotypes have inherent genetic variation among themselves with respect to the characters studied. The treatment effect was also highly significant for all the studied traits which was also reported earlier. [26,27]. Interaction between genotype and treatment showed highly significant differences for all most all of the traits and the mean squares against the replications found significant for all the characters.

Genotype x treatment interactions on morpho-physiological characters on rice genotypes:

The interaction effect of –genotypes and treatments on eight morpho-physiological traits are presented in Table 2. Significant variations were observed in the different treatments on shoot length, root length, fresh weight, turgid weight, relative water content and percentage of seed germination. Shoot length varied from 7.40 to 21.55cm. Maximum shoot length was recorded in

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BRRI dhan-28 (21.55cm), when the seed was inoculated in MS medium supplement with 0 gL^{-1} PEG (Figure 1(a)). The lowest shoot length was found in Begunbichi (7.40 cm) followed by ~~BRRI dhan-28~~Pashpai (8.2~~7~~5 cm) when those were treated with 60 gL^{-1} PEG (Table 2, Figure 1(b)). Therefore, the interaction effect of varieties and treatments were highly significant for ~~root~~ shoot length. The maximum value of root length was found in BRRI dhan-28 (6.80 cm) followed by Dular ~~Burikatari~~ and the lowest was recorded in Begunbichi (2.28 cm). For drought effect, shoot length, root length, for most of the plants were decreased compared to the control conditions which is a common adverse effect of drought that was similar to previous study [14]. However, reduction of shoot length, root may ~~be occurred due to implementation of~~also occur due to decreased cell division under the stress condition. Moreover, the relative water content was calculated from fresh weight, turgid weight and dry weight, which was varied from 3.82% to 16.75%. Maximum value of relative water content 16.75% was found in Dular followed by Burikatari 15.64% when seeds were inoculated on MS medium with 0gL^{-1} PEG and lowest water content was found in Begunbahar 3.82% followed by Begunbichi, BRRI dhan-28 with 5.05% and 5.36% respectively with 60 gL^{-1} PEG. The interaction effect of genotypes and treatments differ significantly on relative water content. However, the relative proline varied from

Table 1. Mean squares (MS) derived from CRD (two Factor) model on morphophysiological and biochemical characters in rice.

Characters	Source of variation with mean square				
	Genotype (5df)	**Treatment (4df)**	**Replication (3df)**	**Genotype × Treatment (20df)**	**Error (87df)**
Shoot Length	81.260***	320.27***	0.360***	5.820***	0.830***

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Root Length	6.340***	18.428***	0.253***	0.568***	0.127***
Fresh Weight	0.002***	0.005***	0.001	0.001***	0.001
Turgid Weight	0.005***	0.015***	0.001***	0.001*	0.001***
Dry Weight	0.002***	0.005***	0.001	0.001***	0.001
Relative Water Content	60.545***	286.488***	0.569	3.156***	0.695
Germination	2465.000***	4047.300***	0.800***	932.800*	0.600***
Proline Content	6.710***	327.710***	0.150***	0.57***	0.050***

Here, * and *** indicates significant at 5% and 0.1% levels of probability, respectively and df indicates degrees of freedom.

3.89 mg to 15.28mg. Maximum proline content was observed in BRRI-28 (15.28 mg) with 60g L^{-1} of PEG and lowest proline content was observed in Dular (3.89 mg) followed by Begunbichi (3.98 mg) with 0 gL^{-1} PEG supplement. It was observed that a significant increase of proline content with the increasing of water stress conditions [28,29]. The highest proline content was found [16] at the 9% PEG supplement on MS medium. The germination percentage was varied 25% to 99.75% with a different concentration level of PEG. ~~The germination percentage showed by Burikatari with 0gL^{-1} PEG and the L~~lowest germination percentage was found in Begunbichi followed by BRRI Dhan-28 with 60gL^{-1} PEG. According to previous studies [30] water stress decreased the germination percentage.

Cluster analysis: Cluster analysis showed the significances difference among the rice for the rice genotypes that reveled the variability among the genotypes. Cluster analysis was performed for 0gL^{-1} PEG (control), 15gL^{-1} PEG, 30gL^{-1} PEG, 45gL^{-1} PEG and 60gL^{-1} PEG treatment and Euclidian distance of coefficients were studied~~s~~ for all rice genotypes based on all traits. Dendrogram from UPGMA clustering indicated the grouping of six genotypes of rice into three

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clusters. In control conditions (0gL^{-1} PEG), Cluster I, II and III, comprised of 2, 1 and 3 genotypes, respectively (Figure 2). Among the three clusters, cluster number II revealed the highest distance by the genotype BRII Dhan 28 and the lowest distance was exhibited by the cluster III with genotypes Begunbahar, Paspai and Begunbichi. But with the increasing of water stress (increasing PEG amount in MS medium), the cluster arrangement becomes changed. Here, BRRI Dhan 28 revealed the highest distance in ~~cluster 2 of~~ 30gL^{-1} PEG treatment ~~. Moreover, this genotype revealed~~but moderate distance in 45gL^{-1} PEG conditions ~~and~~ but lower distance in 15gL^{-1} PEG and 60gL^{-1} PEG conditions. Again, the genotypes Begunbahar, Dular and Paspai revealed lowest euclidian distance under all treatment conditions. In contrast, Burikatari exhibited the highest distance in treatments 15gL^{-1}, 45gL^{-1} and 60gL^{-1} PEG conditions and moderate in 3~~6~~0gL^{-1} PEG conditions that was similar to previous study. [31]. Therefore, this genotype is more diverse and could be considered as a parent against the genotypes Begunbahar, Dular and Paspai in hybridization program.

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Table 2. Interaction effect of genotypes x treatments on eight morphological and physiological traits in six rice genotypes.

Genotype	Treatment combination	Shoot length (cm)	Root length (cm)	Fresh weight of the plant(g~~m~~)	Turgid weight of the plant (g)	Dry weight of the plant (g)	Relative Water Content % of leaf	Proline Content of leaf (g~~m~~/100g)	Germination %
V1	V1T0	21.55A	6.80A	60.75G	112.25C	53.2H-J	12.68D-F	4.65q	99.50a
	V1T1	18.95B	5.00D-F	50.25K	78.25J	46.80M	10.96G-I	6.90m	74.50b
	V1T2	17.55B-D	4.55F-H	38.50N	58.77Q	36.75O	7.95JK	8.92j	74.25b
	V1T3	12.835D-F	4.23H-J	32.75O	45.82S	31.85P	6.44M-P	11.71f	74.75b
	V1T4	8.75HI	3.65KL	28.50Q	37.25U	28.00R	5.36OP	15.28a	49.50c
V2	V2T0	21.23A	4.80D-Q	76.00B	128.00A	66.35B	15.64AB	4.22q	99.75a
	V2T1	18.63BC	4.50F-H	73.50C	106.75D	67.35B	15.58AB	6.20n	99.75a
	V2T2	15.98E-Q	4.35GH	65.25F	92.50H	61.32EF	12.60EF	8.46k	99.25a
	V2T3	13.46IJ	3.75I-L	67.25E	92.70H	63.92C	11.54F-H	11.36g	99.25a
	V2T4	12.75J	3.78J-L	56.00H	72.00L	54.50H	8.53J	13.84b	99.25a
V3	V3T0	18.83BC	4.90D-F	68.75D	113.12C	62.82CD	11.79E-Q	4.17q	99.25a
	V3T1	13.40IJ	4.43F-H	64.50F	98.45F	60.80F	9.82I	5.63o	99.00a
	V3T2	13.25IJ	3.50K-M	56.00H	81.00I	53.95HI	7.58J-L	8.11l	99.50a
	V3T3	10.55K	3.40K-M	46.75L	62.00O	45.70M	6.43L-P	10.97h	74.75b
	V3T4	9.25LM	3.30K-M	42.75M	55.35R	42.25N	3.82Q	13.47c	74.25b
V4	V4T0	19.03B	6.25AB	118.50A	125.55B	117.10A	16.75A	3.89q	99.25a
	V4T1	15.03GH	5.88BC	67.50DE	102.00E	62.35DE	13.00DE	5.88op	99.00a
	V4T2	13.08IJ	4.73E-H	51.50JK	71.62L	49.07L	10.761-I	7.99l	99.50a
	V4T3	12.33K-M	4.58F-H	46.75L	60.32P	45.62M	7.67J-L	10.20i	74.75b
	V4T4	10.75L-N	3.70J-L	37.00N	45.25S	36.47O	6.01NOP	12.47e	74.25b
V5	V5T0	17.33C-E	5.13EF	67.50DE	102.25E	61.82D-F	14.03CD	4.01q	99.25a
	V5T1	17.78B-D	3.65KL	64.75F	95.15G	60.65F	11.82E-Q	5.60o	74.25b
	V5T2	17.025DE	3.50K-M	60.00G	81.82I	58.25G	7.30JK-M	7.91l	74.25b
	V5T3	12.375IJ	3.33K-M	52.25J	69.37M	50.95K	7.04K-N	10.42i	49.25c
	V5T4	8.250MN	2.95M	30.50P	37.05U	30.07Q	6.09M-P	13.06d	49.00c
V6	V6T0	16.900DE	5.40CD	60.75G	97.10F	54.27H	15.15BC	3.98q	99.75a
	V6T1	15.525FQ	4.36G-I	56.00H	79.00J	52.60IJ	12.90D-F	6.01n	99.50a
	V6T2	13.525H-J	3.75J-L	54.25I	75.00K	51.87JK	10.286HI	7.07m	74.25b
	V6T3	10.25KL	3.15LM	52.00J	66.12N	50.97JK	6.77K-O	10.21i	49.50c
	V6T4	7.40N	2.28N	30.00PQ	39.82T	29.47QR	5.05PQ	12.35e	25.00d
LSD $_{(0.05)}$		0.57	0.225	0.0005	0.0005	8.4052	0.5238	0.1351748	0.497625
CV (%)		6.25	8.414	1.623	1.726	0.523	8.405	1.987608	0.947033

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Here, V1= BRRI dhan-28, V2= Burikatari, V3= Begunbahar, V4= Dular, V5= Pashpai, V6= Begunbichi

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4. CONCLUSION

From this study, it ~~was disclosed~~can be concluded that moisture stress ~~imposed in all genotypes~~ causes differential responses ~~of~~ in rice genotypes ~~to impose water stress conditions~~thus may indicate ~~the~~ differential drought tolerance ability of rice genotypes. Based on the findings of the present investigation, it was found that the genotype Burikarari showed the best performance in control conditions followed by BRRI Dhan 28 and Dular based on parameters. But with the increasing the degree of drought stress, the percentage of the morpho-physiological characters were less affected ~~by~~ in Burikatari. On the contrary, BRRI Dhan-28 were affected significantly higher followed by Begunbichi based on parameters. So, these findings suggested that the genotype Burikatari could be considered as more tolerant than the other genotypes against drought conditions.

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List of figures:

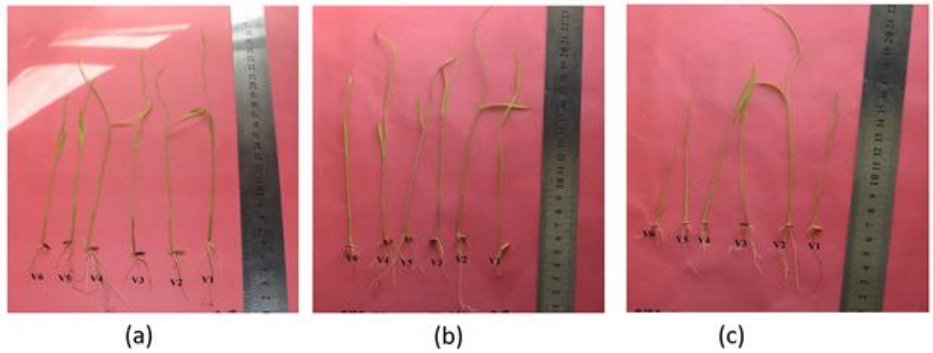

Figure 1. Effect of different water stress (a. 0gL^{-1} PEG treatment, b. 30gL^{-1} PEG treatment and c. 60gL^{-1} PEG treatment) at *in vitro* conditions on six rice genotypes (V1= BRRI dhan-28, V2= Burikatari, V3= Begunbahar, V4= Dular, V5= Pashpai, V6= Begunbichi) after seventeen days of sowing.

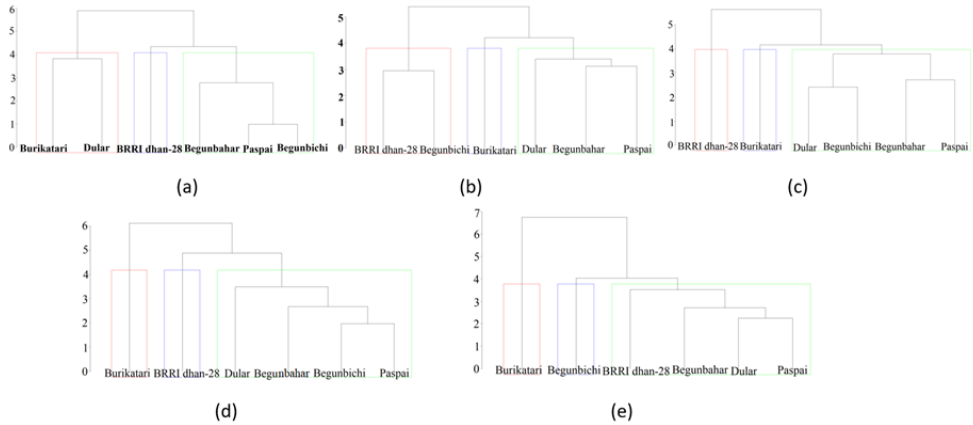

Figure 2. Dendrogram from UPGMA clustering for six rice genotypes using Euclidean genetic distance based on all traits measured in different stress water conditions (a. $0gL^{-1}$ PEG treatment, b. $15gL^{-1}$ PEG treatment, c. $30gL^{-1}$ PEG treatment, d. $45gL^{-1}$ PEG treatment and e. $60gL^{-1}$ PEG treatment).

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