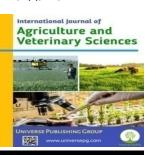


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Phenotyping for the Seedling Stage of the Drought Stress Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

The ever-increasing demand for rice raises the need to increase productivity by developing drought-tolerant rice varieties. Drought tolerance is a complex polygenic trait that largely depends upon plant developmental stages and showed genotype specific variability. The experiment was conducted using drought tolerant (Binadhan-19, BRRI dhan83) and drought susceptible (BRRI dhan26, BRRI dhan48) rice genotypes at the glasshouse of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh to characterize the seedling stage drought tolerance as well as disclose the variability of the genotypes for morphological and biochemical traits. A completely randomized design (CRD) with three replications and treatments (control and drought stress imposed by 20% PEG) was used for the experiment. Data on shoot length, root length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, chlorophyll content (SPAD value), MDA and H₂O₂ were recorded and analyzed through appropriate breeding tools. Significant variation (p<0.01) was observed for genotype, treatment and genotype (G) × treatment (T) interactions viz., shoot length, root length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, chlorophyll, H₂O₂ and MDA. Drought stress leads to a significant decrease in root and shoot growth whereas the level of H₂O₂ and MDA increased significantly. A greater decrease in root and shoots growth was observed in susceptible genotypes (BRRI dhan28, BRRI dhan48) compared to tolerant genotypes (BRRI dhan71, Binadhan-19). In contrast, a limited increase in H₂O₂ and MDA was recorded in tolerant genotypes compared to susceptible genotypes. H₂O₂ showed a significant positive correlation with root and shoot characteristics under control conditions, whereas H₂O₂ showed a significant negative correlation with chlorophyll content under drought conditions. MDA showed a significant negative correlation with most of the studied traits under well-watered conditions. Considering all of the traits at the seedling stage, the genotype Binadhan-19 is considered a drought-tolerant genotype both under well-watered and drought conditions and this genotype was selected for further study under direct field conditions as well as for genetic improvement against drought stress.

Keywords: Drought stress, Rice, Morphological characterization, Biochemical marker, and PEG.

INTRODUCTION:

Rice (*Oryza sativa* L.) is the most widely consumed staple food for a large part of the world's human population, especially in Asia (Samal *et al.*, 2018). Asia is on the top in terms of production and con-

sumption of rice. According to FAO report (2016-2017), average production of rice is estimated as 499.1 million tones and due to rise in population, the requirement is expected to increase up to 2000 million metric tons by the year 2030. Climate change is

a major threat to agriculture particularly in developing nations. Climate change globally, influences the regularity and level of hydrological fluctuations, and causes various abiotic stresses for plants (Turral *et al.*, 2011). Amongst the abiotic factors, drought is the most imperative and a major limitation for rice production in rainfed ecosystems (Pandey & Shukla, 2015; Alam *et al.*, 2022).

Rice is the principal food crop of Bangladesh as well as second important food grain in the world. About 34.7 million tons of rice is produced in Bangladesh per annum and 75% of the cultivable land is used for rice production (BBS, 2016). Rice sector contributes one-half of the agricultural gross domestic products and one-sixth of the national incomes. In Bangladesh, rice productivity and sustainability are continually threatened by abiotic stresses like drought, heat and cold stresses. The situation becomes worse due to climate change that may multiply the frequency and severity of such abiotic stresses. Drought associated with high temperature plays a key role for impacting negatively on crop yield. Yield loss of rice due to slight drought is from 10-30% and might be up to 70-90% under severe drought (Rahman, 2011). Importantly, climate change model predicts 33% rice yield decrease in 2100 in Bangladesh (Karim et al., 2012) whereas we need to increase rice yield to feed the ever-increasing population. Any decline in rice production through climate change would thus critically impair food security in the country. Hence, there is an urgent need to develop improved rice varieties or management techniques that are more resilient to drought stress to ensure food security and also for sustainable agricultural production. Considering the above facts, the present research work was therefore undertaken to fulfil the following objectives: to characterize rice genotypes for drought stress tolerance and to establish physiological and biochemical markers linked to drought stress tolerance.

MATERIALS AND METHODS:

Experimental materials

Four rice genotypes including two drought tolerant genotypes (Binadhan-19 and BRRI fhan71) and two drought sensitive genotypes (BRRI dhan28 and BRRI dhan48) were used as plant materials. The seeds were collected from Bangladesh Rice research institute (BRRI) and Bangladesh Institute of Nuclear agriculture (BINA).

Design and treatments of the experiment

The experiment was conducted in completely randomized design (CRD) with three replications and treatments used for these experiments were as follows: control (C) and drought stress imposed by 20% PEG.

Time and experimental site

The experiment was conducted in the glasshouse of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, and executed during the period of January to March 2022. Seedlings establishment and growth in hydroponic system the rice seeds were surfaced sterilized soaked in distilled water for 24 h and germinated on wet filter paper embedded in petri dishes. Sprouted seeds were sown in a line on Styrofoam sheet floating in trays containing normal water under glasshouse condition. The seedlings of each line were arranged in two sets of floats (one for stress treatment and the other for control). The seedlings in this setup were kept in water at 28 °C for 3 days. On the 4th day, the seedlings were transferred to grow in Peter's nutrient medium solution and grown under open glasshouse conditions by adjusting pH 5.1 to 5.2 NaOH and/or HCl whenever necessary.

Hydroponic drought treatment

Under hydroponic condition, 14 days old seedlings were subjected to drought stress grown primarily in Peter's nutrient solution, containing 20% PEG-6000 with osmotic potential (OP) of -4.05 to -4.95 MPa for 5 days at (28±2) °C. However, the non-stressed plants were grown in a normal strength of the hydroponic Peter's nutrient solution.

Data on morphological and biochemical traits at the seedling stage

The following data on shoot length (cm), root length (cm), fresh root weight (mg), and fresh shoot weight (mg) were calculated from five seedlings per genotype for each replication. Data on dry root weight (mg) and dry shoot weight (mg) were estimated after oven drying of the samples at 60 °C for 3 days. Data on biochemical parameters viz., chlorophyll content (SPAD value), MDA and H₂O₂ were measured from flag leaf tissues after 7 days of drought treatment.

Determination of Hydrogen Peroxide (H₂O₂)

Hydrogen peroxide (H_2O_2) from flag leaf tissue was measured the following method (Velikova *et al.*, 2000) and the H_2O_2 content was calculated by uti-

lizing extinction coefficient (0.28 $\mu M^{\text{-1}} \text{cm}^{\text{-1}}$) and expressed as nmolg⁻¹FW.

Determination of Malondialdehyde (MDA)

MDA was measured from leaf tissue following the standard method as described by Heath and packer (1968) and the MDA content was determined using 155 mM⁻¹ cm⁻¹ extinction coefficient and expressed as nmolg⁻¹FW.

Determination of Chlorophyll Content (SPAD value)

Chlorophyll content of flag leaf, 1st, 2nd and 3rd leaves was measured at the end of the drought stress using SPAD meter (Model: SPAD 502).

Statistical analysis

(%) Reduction = $\frac{\text{(value of stressed plants)} - \text{(value of control plants)}}{\text{value of control plants}} \times 100$

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RESULTS:

Effect of drought stress on rice genotypes at the seedling stage

Morphological traits

The result of analysis of variance (ANOVA) for all the characters (viz., shoot length, root length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, chlorophyll, H₂O₂, MDA) showed highly significant (P≤.001) variation among the genotypes studied. The present study found a considerable variation in shoot length. On average, shoot length was the highest in BRRI dhan48 (31.84 cm) and the lowest in BRRI dhan71 (19.65 cm) under control condition (Table 1). Drought stress resulted in a remarkable decrease in shoot length and the highest decrease was observed in BRRI dhan48 (36.99 %) followed by BRRI dhan28, Binadhan-19 and BRRI dhan71 (25.08, 21.06, and 15.12%, respectively) compared to control (Fig. 1). Drought stress lead to a significant decrease in root length among all the genotypes studied. The highest reduction was observed in drought susceptible BRRI dhan28 (25.58 %) whereas the lowest reduction was found in BRRI dhan71 (9.53 %) (Fig. 1). The result found that the highest fresh shoot weight was noted in BRRI dhan48 (1066.78 mg) and the lowest was recorded in BRRI dhan71 (343.00 mg) (Table 1). Drought stress resulted in significant reduction in fresh shoot weight among all of the genotypes; the highest reduction was found in BRRI dhan48 (77.18 %) whereas the lowest reduction was found in BRRI dhan71 (44.73 %) as compared to control (Fig. 1).

Data recorded for different parameters were compiled and tabulated in proper form for statistical analysis which was carried out in Minitab 17 statistical software package (Minitab Inc. State College, Pennsylvania) and software R, version 3.3.2. Various statistical tests such as two-factor ANOVA test, mean performance, combined effects of genotype treatment, percent reduction and correlation coefficient were performed to assess the varietal performance of the studied genotypes.

Calculation of percent yield reduction

For comparing stressed plants with control plants, the percent reduction was calculated by the following formula:

For shoot dry weight, the highest reduction was

observed in drought susceptible BRRI dhan48 (66.55%) whereas the lowest reduction was found in BRRI dhan71 (17.22%) compare to their control (**Fig. 1**). Drought stress resulted in a sharp decrease in root fresh weight among all genotypes; the highest reduction was found in BRRI dhan48 (72.21%) and the lowest reduction was found in BRRI dhan71 (53.30%) related to their control (**Fig. 1**). Under drought stress condition, maximum decrease was recorded in BRRI dhan48 (64.26%) and the lowest in BRRI dhan71 (22.69%) as compared to control (**Fig. 1**).

Biochemical traits

In the study, the treatment effects showed a significant variation in MDA content among the genotypes. MDA was noted maximum in BRRI dhan48 (25.98 nmolg⁻¹) and minimum in Binadhan-19 (22.17 nmolg⁻¹) under control condition (**Table 1**). Drought stress lead to a significant increase in MDA content in all of the genotypes; the highest induction was found in BRRI dhan28 (185.76 %) and the lowest increase in Binadhan-19 (104.23 %) as compared to control (Fig. 1). Drought stress resulted in a sharp increase in H₂O₂ level, the highest increase was recorded in susceptible variety BRRI dhan28 (63.77 %) & the lowest induction in tolerant variety Binadhan-19 (34.43 %) as compared to control (Fig. 1). Imposition of drought stress resulted in a significant decrease in chlorophyll content (SPAD) among all of the genotypes compared to control. Due to drought stress, the highest decrease in SPAD was found for BRRI dhan28 (56.49 %) and the lowest in

Binadhan-19 (39.51%) (**Fig. 1**).

Table 1: Mean performances of 4 rice genotypes based on different morphological and biochemical traits grown under control and drought stress conditions at the seedling stage.

Genotypes	Trt	Shoot	Root	Shoot	Shoot	Root	Root	MDA	H_2O_2	Chlorophyll
		length	length	fresh wt.	dry wt.	fresh wt.	dry wt.	(nmolg ⁻	(nmolg ⁻	(SPAD)
		(cm)	(cm)	(mg)	(mg)	(mg)	(mg)	¹ FW)	¹ FW)	
Bina dhan-	С	26.01 c	15.61 b	612.44 c	110.96 с	327.78 c	44.40 b	22.17 g	26.11 f	75.69 a
19	D	20.53 d	13.17 d	279.50 e	75.50 d	188.17 e	28.03 c	45.27 d	35.33 d	48.73 d
BRRI	С	19.65 d	14.37 с	343.00 d	55.65 f	250.83 d	29.82 с	22.52 g	25.21 g	76.36 a
dhan71	D	16.57 e	12.38 e	181.83 g	45.90 h	125.50 g	20.48 d	47.31 c	37.49 c	50.90 с
BRRI	С	27.49 b	14.69 с	723.33 b	120.63 b	466.89 b	43.61 b	24.19 f	25.86 f	73.13 b
dhan28	D	20.08 d	10.83 f	274.67 e	62.23 e	173.00 f	21.67 d	69.12 b	43.78 b	39.10 e
BRRI	С	31.84 a	18.53 a	1066.78 a	151.12 a	617.89 a	60.51 a	25.98 e	27.10 e	75.50 a
dhan48	D	20.12 d	14.13 с	243.33 f	49.30 g	175.00 f	21.58 d	20.48 a	46.60 a	38.77 e
Max.		31.84	18.53	1066.78	151.12	617.89	60.51	69.12	46.60	76.36
Min.		16.57	10.83	181.83	45.90	125.50	20.48	22.17	25.21	38.77
CV		2.23	3.06	2.09	2.16	1.95	3.83	0.77	0.79	1.06

Here, Trt= Treatments, C= Control condition, D= Drought condition.

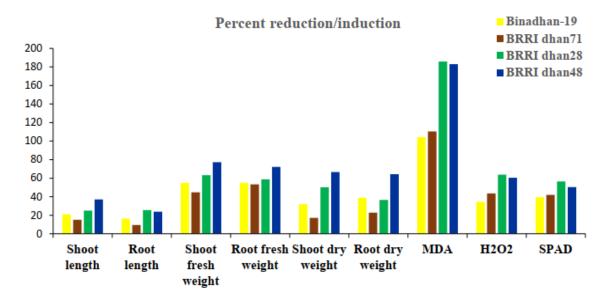


Fig. 1: Percent reduction/induction due to drought stress in morphological and biochemical traits among 4 rice genotypes at the seedling stage.

Estimation of phenotypic correlation co-efficient among nine characters of rice genotypes under control and drought stress conditions at the seedling stage

Correlation co-efficient is a measure of intensity or degree of linear relationship between two variables. In the present study, relationship among morphological and biochemical traits were studied through analysis of correlation between them. Phenotypic correlation co-efficient among 9 traits of 4 rice geno-

types are presented in **Table 2**. In the present study out of 72 associations, 27 associations were significant at phenotypic level in control condition and 12 associations were significant at phenotypic level in drought stress condition (**Table 2**). The positive and non-significant associations referred information of inherent relation among the pairs of combination. The negative and non-significant association referred a complex linear linkage among the pair of combinations.

Table 2: Phenotypic correlation coefficient among nine characters of rice genotypes under control and drought stress condition at the seedling stage.

Traits		Shoot length	Root length	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.	MDA	H ₂ O ₂
Root length	С	0.790							
	D	0.123							
Shoot fresh	С	0.978***	0.859***						
wt.	D	0.929***	-0.090						
Shoot dry	С	0.994***	0.760**	0.966***					
wt.	D	0.652**	-0.145	0.822***					
Root fresh	С	0.936***	0.792**	0.974***	0.915***				
wt.	D	0.955***	0.204	0.942***	0.736**				
Root dry	С	0.968***	0.907***	0.976***	0.954***	0.915***			
wt.	D	0.565	0.249	0.631**	0.881***	0.657***			
MDA	С	0.799**	0.753**	0.883***	0.763**	0.954***	0.797**		
	D	0.419	-0.008	0.273	-0.309	0.307	-0.484		
H ₂ O ₂	С	0.917***	0.922***	0.932***	0.902***	0.858***	0.961***	0.738**	
	D	0.317	0.049	0.139	-0.438	0.200	-0.567	0.988***	
SPAD	С	-0.385	0.182	-0.284	-0.383	-0.369	-0.188	-0.273	-0.071
	D	-0.578	0.068	-0.472	0.091	-0.484	0.313	-0.973***	-0.931***

Here, C= Control condition and D= Drought condition.

DISCUSSION:

The ultimate goal of plant breeding is to develop superior crop varieties with potential to give maximum yield under optimum growth condition as well as able to produce a satisfactory yield under any stressful conditions. The negative effects of drought stress are well studied but the variability of drought stress response at various plant developmental stages by using tolerant and sensitive genotypes has not been well documented. In the present study, an attempt was made to investigate the impact of drought stress on morphological and biochemical traits by using drought sensitive and drought tolerant rice genotypes. A detail discussion of the results found is presented below.

Root length was severely affected by the induction of drought. As drought decreases the growth of plants, the decrease in root length might be due to the negative consequence of drought stress. The reduction in root length due to drought was also found by other researchers (Lanna *et al.*, 2021; Sourour *et al.*, 2017; Omisun *et al.*, 2018; Susanto *et al.*, 2018). Shoot length was also significantly reduced by the imposition of drought stress. The highest reduction of shoot length was found in drought susceptible genotypes compared to their tolerant

genotypes. Similar to our results, the reduction of shoot length due to drought was also reported by others (Lanna *et al.*, 2021; Ahmed *et al.*, 2019; Omisun *et al.*, 2018; Susanto *et al.*, 2018).

Root fresh weight was also significantly affected by the imposition of drought stress. The highest reduction of root fresh weight was observed in drought susceptible genotypes compared to tolerant genotypes. The reduction of root fresh weight due to drought was found by Lanna *et al.* (2021), Sourour *et al.* (2017), and Ahmed *et al.* (2019). Shoot fresh weight was severely affected by drought. The reduction of shoot fresh weight was highest in drought susceptible genotypes compared to tolerant genotypes. The reduction of shoot fresh weight due to drought was found by Lanna *et al.* (2021), Sourour *et al.* (2017), and Ahmed *et al.* (2019).

Root dry weight was also significantly affected by the imposition of drought stress. The reduction of root dry weight was highest in drought susceptible genotypes compared to tolerant genotypes. The reduction of root dry weight due to drought was found by Lanna *et al.* (2021), Sourour *et al.* (2017), and Ahmed *et al.* (2019). Shoot dry weight was severely affected by drought. The reduction of shoot dry

weight was highest in drought susceptible genotypes compared to tolerant genotypes. The reduction of shoot dry weight due to drought was found by Lanna *et al.* (2021), Sourour *et al.* (2017) and Ahmed *et al.* (2019).

Chlorophyll concentration was also significantly affected by the imposition of drought stress. The reduction of chlorophyll concentration was highest in drought susceptible genotypes compared to tolerant genotypes. The reduction of chlorophyll content due to drought was found by other researchers (Beena *et al.*, 2012; Sourour *et al.*, 2017; Susanto *et al.*, 2018; Ray *et al.*, 2022).

H₂O₂ level was significantly increased due to drought. The reduction of H₂O₂ was highest in drought susceptible genotypes compared to tolerant genotypes. Similar results were also indicated by other researchers (Nahar *et al.*, 2018; Beena *et al.*, 2012; Susanto *et al.*, 2018, Sourour *et al.*, 2017; Omisun *et al.*, 2018). MDA level was significantly increased due to drought. The reduction of MDA was highest in drought susceptible genotypes compared to tolerant genotypes. Similar results were also indicated by others (Nahar *et al.*, 2018; Beena *et al.*, 2012, Sourour *et al.*, 2017; Omisun *et al.*, 2018; Susanto *et al.*, 2018).

In a nutshell, the decrease in growth and development of drought stressed seedlings might be due to the negative effects of high osmotic potential of the nutrient solution that lowered absorption of water and nutrient (Shrivastava *et al.*, 2015). The morphological parameters of all genotypes usually decreased under drought stress though some drought tolerant genotypes, namely BRRI dhan71 and Binadhan-19 maintained higher growth by the adjustment of drought stress (Sourour *et al.*, 2017). Reduction/induction level could be used as an important physiological assortment criterion for drought tolerance in many plant species, such as tomato (Juan *et al.*, 2005; Nahar *et al.*, 2018), wheat (Oyiga *et al.*, 2016) and barley (Chen *et al.*, 2005).

The results of the study also reflected that drought treatment led to the enhancement of H_2O_2 and MDA contents in all genotypes but the accumulations of H_2O_2 and MDA were lower in drought tolerant genotypes compared to drought susceptible genotypes (**Table 2**). The lower accumulations of MDA and H_2O_2 in drought tolerant genotypes imply

protection against oxidative damage by better regulating mechanism to control the formations of more MDA and H₂O₂, and therefore, these genotypes displayed more drought tolerance (Akram *et al.*, 2017; Sourour *et al.*, 2017). In contrast, the higher accumulations of H₂O₂ and MDA contents in drought susceptible genotypes probably due to higher rate of ROS production as well as higher inactivation of antioxidant enzymes (Sourour *et al.*, 2017) leading to oxidative stress and membrane permeability by assaulting membrane lipids (Sourour *et al.*, 2017; Saini *et al.*, 2018; Karim *et al.*, 2021).

Association among nine characters of rice genotypes under control and drought stress condition at the seedling stage

Phenotypic correlation provides the information about the relationship between the two or more than two independent variables. In plant breeding correlation analysis can be used to estimate the value of different traits (Ahmad *et al.*, 2012).

Phenotypic correlations of morphological and biochemical traits at the seedling stage

In the present experiment, the relationship among morphological and biochemical traits were studied through phenotypic correlation (Table 2). Results reflect a significant positive correlation between shoot length with most of the morphological traits under both control and saline condition. This finding may help to conclude that shoot length and morphological traits might be the better descriptors of drought tolerance of genotypes. However, in saline condition, root length, H₂O₂ content and chlorophyll have not found such significant association. It may be suggested that insignificant relation of root length, H₂O₂ content and chlorophyll under drought stress may not be a good descriptor of drought tolerance. MDA with morphological traits showed significantly negative association indicate that higher the MDA negatively affects the root and shoot growth characteristics. Similar result was also found in H₂O₂ level with chlorophyll content. Thus for screening the tolerant genotypes at seedling stage, its shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, MDA may be considered as selection criteria.

CONCLUSION:

Drought in the field of agriculture is a major obstacle which detrimentally affects rice in terms of growth and productivity leading to low yield potential and food insecurity. Drought tolerance is a complex polygenic trait that is genotype specific and largely depends upon plant developmental stage. The study was attempted to identify the potential determinates of drought tolerance at seedling stage in rice through the assessment of morphological and biochemical traits. The experiment was conducted following a CRD design with three replications and two treatments (control and drought) to fulfill the proposed objectives. The results of this experiment have been summarized as follows:

One group of seedlings was subjected to drought stress (20% PGE) for 72 hrs. After the completion of drought stress treatment, data on nine morphological and biochemical parameters were recorded and analyzed through appropriate breeding tools. The results of analysis of variance showed significant variation due to genotypes, treatments and genotype × treatment interaction for most of the studied traits. In response to drought stress, a significant decrease in root and shoot growth characteristics were recorded whereas the level of H2O2 and MDA increased significantly. A greater decrease in root and shoot characteristics was observed in the sensitive genotypes (BRRI dhan28, BRRI dhan48) as compared to tolerant genotypes (BRRI dhan71, Binadhan-19). In contrast, a limited increase in H₂O₂ and MDA content were recorded in the tolerant genotype as compared to tolerant genotype. H₂O₂ showed significant positive correlation with root and shoot characteristics whereas it showed non-significant negative correlation with chlorophyll content under control conditions. Under drought stress conditions, H₂O₂ showed significant negative correlation with chlorophyll content. MDA showed nonsignificant correlation for most of the studied traits under controlled conditions whereas it showed significant negative correlation with most of the studied traits. Considering all of the traits at the seedling stage, the genotype Binadhan-19 considered as drought tolerant genotype both under control and drought condition.

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CONFLICTS OF INTEREST:

There is no conflict of interest

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