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EFFECTS OF PEG INDUCED WATER STRESS ON GROWTH AND PHYSIOLOGICAL RESPONSES OF RICE GENOTYPES AT SEEDLING STAGE

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Abstract

Studies were conducted to evaluate the effect of water deficit imposed by three different concentrations of polyethylene glycol-6000 (PEG-6000; w/v - 10, 15 & 20% equivalent to osmotic potential -0.19, -0.36 and -0.58 MPa along with non treated control) on plant growth and physiological traits of eight rice genotypes at seedling stage. The results showed that all growth and physiological responses of rice genotypes were affected with varying intensities under water stress conditions. These effects were comparatively low at 0.19and 0.36 MPa, PEG-6000. The concentration of 0.58 MPa, PEG-6000 drastically affected germination (up to 50%) and growth (more than 80%) in some genotypes. The results of physiological attributes revealed that relative water contents (RWC) and chlorophyll were significantly reduced in leaves with increased concentration of PEG-6000. On the contrary to this electrolyte leakage, proline, and potassium contents increased with varying intensities among rice genotypes. Genotypic comparison has shown that genotypes IR-50, IR-72, DR-92 and IR-6 exhibited tolerance potential against water stress. Tolerant genotypes exhibited differential osmo-regulatory responses in term of solute production. Correlation studies among growth and physiological traits have revealed significant positive correlation of shoot growth with relative water contents and chlorophyll. The parameters of proline, electrolyte leakage and sugars were negatively related with growth attributes. Thus, these attributes can be used as screening tool for drought tolerance in rice.

Key words: Rice, Water stress, Relative water contents, Chlorophyll, Electrolyte leakage, Proline.

Introduction

Abiotic stresses (drought, salinity, temperature and nutrient deficiency) are the main constraints, negatively affect crop productivity and create food insecurity worldwide. Among these stresses water shortage / drought is of foremost nature for reducing crop productivity up to 70% (Lum *et al.,* 2014). Rice (*Oryza sativa* L.) is second most important cereal and source of food for more than 50% of world (Wang *et al.,*2014) is a high water requiring crop (3000-5000 L water / kg rice). It is reported that more than 70 m ha of rice growing areas is affected worldwide due to water shortage (Ahmed *et al*., 2014). In future the impact of this stress will further increase on rice productivity due to increase population pressure and climatic changes in rainfall and temperature (Lesk *et al.,* 2016 & Kashmir *et al.,* 2016). These conditions will further intensify water shortage problem for cultivation of rice crop. According to an estimate 53% of world rice growing regions suffer from climate variability causing yield reduction approximately at the rate of 0.1 t/hm^2/ year (Ray *et al.,* 2015). To reduce the effects of such climatic risk there is a need for the identification/development of water stress tolerant rice genotypes suitable to thrive with sustainable yield under these stressed environments. For this comprehensive understanding of stress responsive traits of adaptive nature is prerequisite for tailoring of genotypes under stressed environments.

Generally it is considered that in rice, seedling and flowering stages are more sensitive to stresses (Sridevi & Chellamuthu, 2015). The responses of rice to drought are complex, varied widely with genotypes, duration of stress and stage of growth (Ji *et al*., 2012). Under the stressed conditions rice plants exhibit different alterations in biochemical and physiological processes. To date studies have indicated that drought / water stress reduces growth through affecting many metabolic processes including water relations, nutrient uptake, enzymatic activities, photo assimilate synthesis and its partitioning (Yousfi *et al.,* 2016; Fahad *et al.,* 2017; Khan *et al.,* 2017). All these factors cumulatively results in poor plant growth and reduction in yield. Under water stress conditions low water potential of the rhizosphere is the major limiting factor which hampers water absorbing capability of seeds/plants. Polyethylene glycol (PEG) is a non ionic, inert polymer widely used for simulating water stress conditions and is a well known established technique generally used for evaluating genotypes under laboratory conditions.

Quantification of water stress tolerance at initial stages of plant development is of primary importance, because the seeds with good germination and better seedling growth under water deficit conditions may indicate tolerance potential at later growth stages and is expected to achieve in higher yields (Petrovic *et al.,* 2016).

In this study, germination, growth and physiological responses of rice genotypes were studied at early seedling stage by applying different concentrations of PEG-6000.Understanding of these responses at physiological and biochemical level may be helpful for developing pragmatic inferences through better insight into phenomena to improve water stress tolerance in rice.

Materials and Methods

Laboratory experiments were conducted in factorial completely randomized design (CRD) with 3 replicates. Eight rice lines (IR 83142-B-60-B, GML-507, IR-6, IR-50, IR-72, DR-92, and IR-8) including international drought tolerant check (IR04L191) was studied to evaluated germination, growth and physiological responses at seedling stage. Experiments were conducted in germinators (Naqvi *et al.,* 1994). Seeds were sterilized with 3% NaOCl for 20 minutes and washed thoroughly with distilled water then were planted on nylon nets fitted in germinators (size:8 cm Ø and 7cm height) containing treatments solution of PEG-6000 supplemented with Yoshida nutrient culture solution adjusted at pH 5.5 (Yoshida *et al.,* 1976). Three levels of water stress were induced through PEG-6000 (i.e 10, 15 and 20% equivalent to osmotic potential -0.19, -0.36 and -0.58 MPa) along with non treated controls (-0.05 MPa). These germinators were covered with polyethylene sheets to minimize evaporation and were placed under darkness in programmed controlled incubator at temperature 30/28°C day and night cycle with 14 hrs photoperiod (irradiance 22 Wm^{-2}). Culture solutions were replaced twice a week to maintained required stress. Germination was recorded on 5th day after planting. Following growth and physiological parameters were measured after giving exposure of treatments for the period of 10 days.

Growth parameters: Ten randomly selected plants were harvested and separated into shoot and root and measurements were made for shoot & root lengths and fresh weights. The samples were dried in hot air drying oven at 80 ºC for 72 hrs for determining their dry weights.

Chlorophyll: From each sample 0.1g chopped shoot fresh weight were taken into 10 ml acetone (80%) and kept overnight. The leaves extracts were then centrifuge at 4000xg for 5 min. chlorophyll a and b weremeasured (Lichtenthaler, 1987) by taking absorbance at 663.2, 646.8, 470 nm at spectrophotometer (Hitachi double beam 150, Japan).

Relative water contents (RWC): were measured according to method of Bonnet *et al*., (2000). Ten fully expanded leaves were sampled from each replicate. The leaf segments were weighed (FW), kept for 10 hrs in distilled water at 4ºC, after that turgid weight (TW) were taken. The leaves were oven dried for 72 hrs at 80ºC to obtained dry weight (DW). The RWC (%) were calculated by formula:

$$RWC= [(FW-DW)/ (TW-DW)] X100$$

Proline: 0.5g freshly chopped shoot samples were homogenized in 10 ml sulphosalicylic acid (3%) and then filtered. Two ml filtrate was reacted with 2ml acid ninhydrin and 2 ml of glacial acetic acid at 100 ºC for one hr. The reaction was stopped in ice bath. Four ml toluene was added to this reacted filtrate and mix for 15-20 seconds on vortex mixer. Toluene layer was aspirated and read at 520 nm on double beam spectrophotometer (Hitachi 150, Japan). Proline concentrations were calculated on fresh weight basis using formula (Bates *et al.,* 1973).

$$(\mu \text{ moles proline /g FW} = [(\text{ug proline / ml x 4 ml toluene})/115.5 \text{ ug / } \mu \text{ moles}]/[0.5 \text{ g/5}].$$

Electrolyte leakage (EL): Electrolyte Leakagewas measured by following method of Wu *et al.,* (2017). 0.1 g fresh leaves cutting (5 mm segments) were placed in 10 ml de-ionize water and incubated in water bath at 32ºC. After 2 hours electrical conductivity (EC) of medium was recorded (EC 1). The samples were autoclaved at 121ºC for 20 minutes to release electrolytes. The samples were cooled at room temperature and electrical conductivity was recorded (EC 2). The rate of electrolyte leakage was calculated according to formula:

$$EL = EC (1) / EC (2) x100$$

Potassium: 0.1 g shoots samples were extracted in 10 ml of 100 mM acetic acid (CH_3COOH) in water bath at 90ºC for an hr according to method of Yeo & Flowers, (1993). The extracted solution was filtered, and read at flame photometer (Jenway, Model PFP7).

Total sugars: one g chopped fresh leaf sample was shaken in 10 ml of 80 % ethanol (v/v) for overnight. The 0.1 ml of ethanolic extract was then mix with 3ml anthrone (150 mg in 100 ml of 72% sulfuric acid) heated at 97ºC for 10 min then cooled in ice bath (Riazi *et al.,* 1987).The samples were read at 625 nm wavelength in double beam spectrophotometer (Model: Hitachi 150-20, Japan).

Statistical analysis: The data was statistically analyzed using two way ANOVA for genotypes, treatments and genotypes treatments interactions followed by Tukey HSD test to compare treatments means (at α 0.05). Correlation co-efficient (Pearson's) studies for different growth and physiological traits were done by statistix 8.1[analytical software Inc., Tallahassee, FL, USA] software.

Results: The results of ANOVA have shown that genotypes, treatments and their interactions were statistically significant at p α 0.01 for all growth and physiological attributes studied (Table 1). All growth parameters were significantly reduced with varying intensities under water stress conditions (Fig. 1).

Germination and growth responses: The water stress induced by PEG-6000 variably affected germination of rice genotypes. Germination % reduces significantly ($p \leq 0.05$) with increase in water stress (Fig. 1A). These effects were comparatively low at 0.19 and 0.36 MPa PEG-6000. The concentration of 0.58 MPa PEG-6000 drastically reduced germination up to 50% in some genotypes. However the genotypic comparison at all treatments of water stress has shown that highest germination with minimum relative reduction was observed in DR-92 (23%) followed by IR-6(28 %), IR-72 (30%) and IR-50 (35%). The data of shoot and root

lengths displayed significant differences among rice genotypes (Fig. 1B & 1C). Comparison on the basis of relative reduction under water stressed conditions in relation to their non stressed controls have exhibited adverse effects of water stress on the shoot and root length and were more than on germination at each levels of water stress. The least relative reduction was observed in drought tolerant check at all treatments whereas, genotypic comparison for shoot and root length have shown that genotypes IR-50, IR-72 and DR-92 were significantly different from rest of the genotypes in having maximum shoot lengths at all treatments. These genotypes have also displayed comparatively less relative reduction under stress conditions. The data of root length have shown that GML-507 and IR-50 exhibited maximum root length at each level of stress. Whereas, with respect to relative reduction under stress, least reduction was observed in drought tolerant check (15%) followed by IR-50. Shoot and root fresh weights (Fig. 1 D & I F) were drastically reduced at highest treatment of water stress (0.58 MPa) with comparatively more reduction in shoot than in root fresh weights. The data of shoot fresh and dry weights have revealed that IR-50 was found best followed by IR-72 and DR-92 at all treatments of water stress. These three genotypes also exhibited comparatively less relative reduction in their shoot fresh weight at highest treatment of water stress (0.58 MPa, PEG-6000) when compared to their respective controls (Fig. 1D).

The responses of genotypes IR-6, IR-50, IR-72 and DR-92 were observed more or less similar for dry weights (Fig. 1E) as were observed for fresh weights of shoot. The genotype IR-8 was observed as sensitive as this genotype exhibited more than 50% reduction in their shoot fresh and dry weights at treatment of 0.36 MPa. At 0.58 MPa, PEG-6000 the growth affected more severely as it was observed that four genotypes (Drought tolerant check, IR 83142-B-60-B, GML-507 and IR-8) exhibited more than 90% reduction in their shoot weights. Among these four genotypes IR-8 exhibited comparatively more reduction in their growth (fresh & dry weight of shoot) at all levels of treatment.

Physiological responses: Tukey HSD all pair wise comparison tests of electrolyte leakage (EL) for treatments have shown significant differences. The value of electrolyte leakage increased gradually with increase in water stress (Table 2a). The effects were more pronounced at 0.36 & 0.58 MPa, PEG-6000. Genotypic comparison has shown that the highest leakage was observed in IR 83142-B-60 –B followed by IR-8 at highest concentration of PEG-6000 (0.58 MPa). The genotypes IR-6 exhibited least leakage at this level followed by IR-72, DR-92 and IR 04L191 (Drought tolerant check). At the level of 0.36 MPa, PEG-6000, the response of IR 83142-B-60 –B, IR-8 and GML-507 were more or less similar.The values of electrolyte leakage under different treatments when compared to their respective controls, variable degree of increase was observed under different levels of water stress. IR 04L191 (Drought tolerant check) exhibited least relative increase at all treatment of water stress whereas, among the tested genotypes, IR-72 & DR-92 exhibited comparatively less increase under water stress conditions.

Relative water contents (RWC) decrease gradually with the increase in water stress. Genotypic differences were not so obvious at 0.19 MPa PEG-6000. The values of RWC declined drastically at 0.58 MPa, where pronounce differences were observed among genotypes (Table 2a). The genotypes IR-6 and IR-50 were observed significantly different from rest of the genotypes in having more RWC values with comparatively less relative reduction of 9.6 & 13% respectively at highest treatment PEG-6000 (0.58 MPa).

Proline concentrations (µ mole /g FW) increased significantly with variable intensities among genotypes under PEG induced water stress conditions (Table 2a). The drought tolerant check (IR 04L191) has accumulated highest proline concentration at 0.19 and 0.36 MPa treatments of PEG-6000. At the highest treatment of PEG (0.58 MPa), the genotypes IR-72 and IR-50 have exhibited highest value of proline followed by drought tolerant check (IR 04L191).

Table 1. Mean square values of growth and physiological parameters of rice genotypes under different levels of water stress at early seedling stage.

Growth parameters	Genotypes d.f= 7	Treatments d.f= 3	GxT d.f= 21	Error d.f= 42	CV
Germination	379.71**	6348**	63.29**	12.24	4.27
Root length	8.3413**	35.8248**	4.3608**	0.9586	14.38
Shoot length	85.214**	241.3**	3.898**	2.084	12.04
Root (FW)	120424**	142358**	2285**	825	14.74
Root (DW)	125.84**	1357**	7.19**	8.23	12.67
Shoot(FW)	90043**	500944**	5482**	1552	12.40
Shoot (DW)	1045**	7603**	261**	48.81	12.77
Electrolyte leakage	5143.1**	29373.4**	1877.4**	66.5	11.04
RWC	214.70**	3206.02**	127.71**	61.54	9.56
Proline	36.653**	844.249**	24.752**	1.314	16.91
Chlorophyll	0.28942**	0.60691**	0.02862**	0.0103	8.35
Potassium	0.14164**	0.50225**	0.04583**	0.0033	9.89
Sugars	2037.04**	3133.59**	86.15**	86.15	11.22

** = Significant @ 0.01 probability

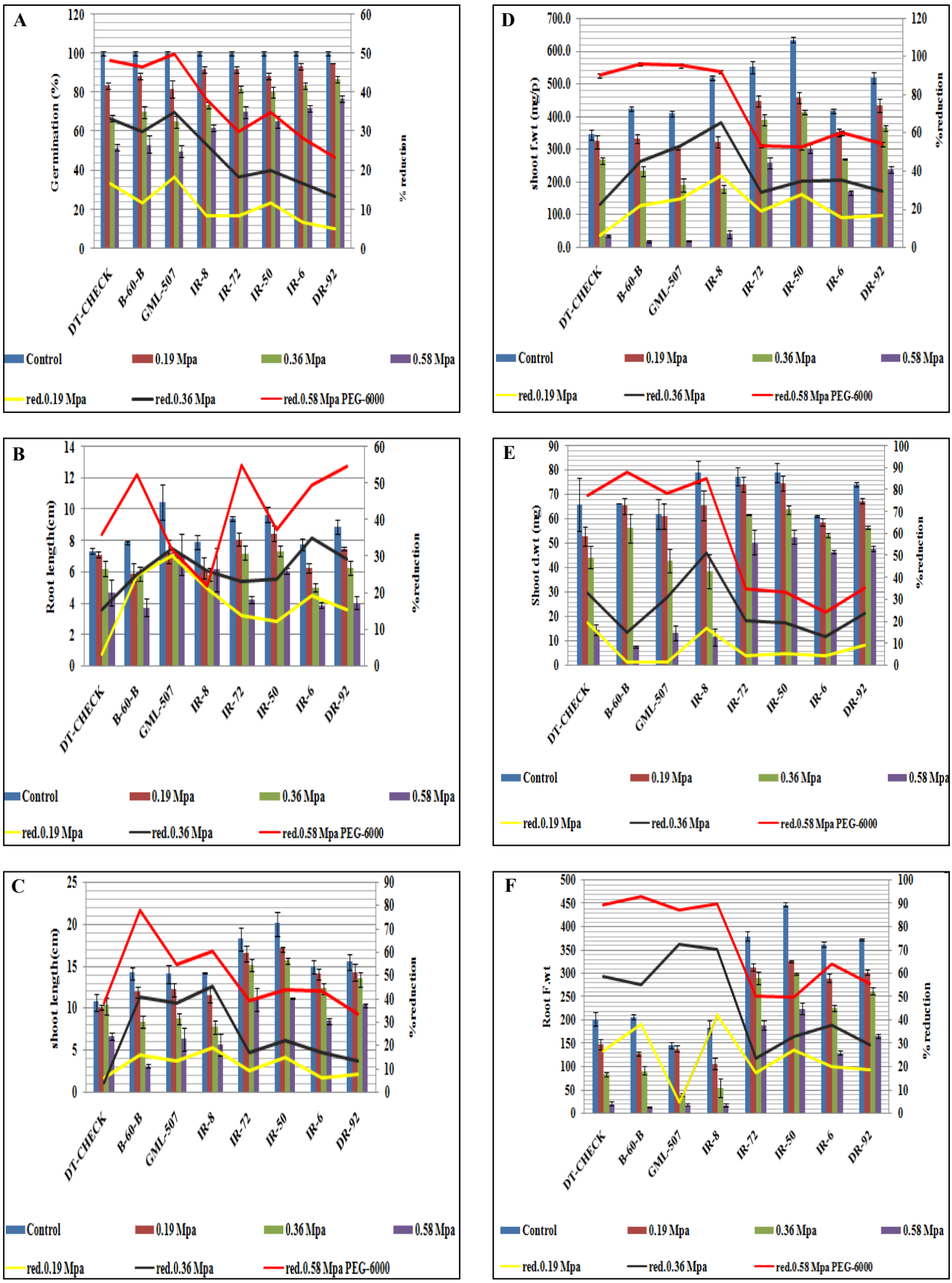

Fig. 1. Relative growth responses of eight rice genotypes at seedling stage under different treatments of PEG-6000. A) Germination, B) Root length, C) Shoot length, D) shoot fresh weight, E) Shoot dry weight, F) Root fresh weight. Control: non-stressed conditions; 0.19 MPa, 0.36 MPa and 0.58 MPa, PEG-6000 treatments. Bars indicate ± SE. Means ± SE were computed from individual values of 3 plants per treatment and per genotype.

Table 2a. Physiological traits of rice genotypes under different levels of water stress at early seedling stage.

Genotypes	Electrolyte leakage (%)					Relative water contents (%)				Proline (µ moles proline /g FW)			
	Control	PEG-6000 (MPa)			Control	PEG-6000 (MPa)			Control	PEG-6000 (MPa)			
		0.19	0.36	0.58		0.19	0.36	0.58		0.19	0.36	0.58	
IR 04L191	33.5 H (0.00)	55.0 FG (64.2)	66.6 BCDEF (98.8)	74.7 ABCDE (123)	85.6 ABC (0.00)	85.0 ABCD (-0.7)	82.5 ABCDE (-3.5)	60.2 DEF (-29.7)	1.4 L (0.00)	4.2 HIJKL (204.8)	8.3 DEFG (506.9)	14.8 B (986)	
IR 83142-B-60	25.5 H (0.00)	81.7 ABCD (220.4)	83.1 ABC (225.9)	89.3 A (250.2)	93.1 A (0.00)	88.4 AB (-5.1)	87.1 AB (-6.5)	61.1 CDEF (-34.4)	1.2 L (0.00)	2.4 KL (105.6)	4.4 HIJKL (285.1)	13.8 BC (1097)	
GML-507	35.7 GH (0.00)	59.9 EF (67.8)	84.9 AB (137.7)	88.0 A (146.5)	92.8 A (0.00)	89.9 AB (-3.1)	79.8 ABCDEF (-14)	55.7 F (-40)	1.5 L (0.00)	3.1 JKL (113.1)	7.2 DEFGH (391.3)	14.1 BC (867)	
IR-8	38.2 GH (0.00)	74.7 ABCDE (95.5)	82.7 ABCD (116.5)	88.3 A (131.2)	91.7 A (0.00)	86.8 AB (-5.4)	78.1 ABCDEF (-14.8)	56.1 F (-38.9)	1.8 L (0.00)	3.6 HIJKL (96.6)	8.2 DEFG (349.9)	10.5 CDE (474)	
IR-72	31.5 H (0.00)	61.8 EF (96.2)	70.5 ABCDEF (123.8)	71.7 ABCDEF (127.6)	96.6 AB (0.00)	91.7 AB (-5.1)	66.7 BCDEF (-31)	59.2 EF (-38.7)	3.0 KL (0.00)	3.9 HIJKL (31.4)	6.8 EFGHI (131.7)	25.7 A (772)	
IR-50	19.8 H (0.00)	64.6 CDEF (225.9)	68.3 BCDEF (244.7)	77.8 ABCDE (292.5)	94.4 A (0.00)	92.7 AB (-1.8)	83.8 ABCDE (-11.2)	81.8 ABCDE (-13.3)	2.8 KL (0.00)	3.6 HIJKL (29.9)	6.7 FGHIJK (140.0)	22.4 A (702)	
IR-6	20.2 H (0.00)	63.3 DEF (214.1)	65.0 CDEF (222.4)	65.0 CDEF (222.4)	94.1 A (0.00)	88.1 AB (-6.3)	86.2 ABC (-8.4)	85.0 ABCD (-9.6)	2.0 KL (0.00)	3.3 IJKL (63.9)	5.7 GHIJK (180.1)	10.8 CD (432)	
DR-92	28.1 H (0.00)	67.2 BCDEF (139.3)	68.3 BCDEF (143.2)	73.9 ABCDEF (163.1)	92.5 A (0.00)	88.4 AB (-4.4)	76.4 ABCDEF (-17.4)	73.6 ABCDEF (-20.5)	2.1 KL (0.00)	3.8 HIJKL (83)	4.2 HIJKL (101.3)	9.7 DEF (365)	
HSD values for genotypes at α 0.05		7.7772				10.032				1.4659			
HSD values for treatment at α 0.05		4.6318				5.9744				0.8730			
HSD values for G x T at α 0.05		19.644				25.338				3.7025			

-Values with the same letters are not significantly different at ($p \leq 0.05$)

* Figure in parenthesis indicates % increase (+) and decrease (-) over control

Table 2b. Physiological traits of rice genotypes under different levels of water stress at early seedling stage.

Genotypes	Total chlorophyll (mg/g FW.)				Potassium (%)				Total soluble sugars (mg/g FW)			
	Control	PEG-6000 (MPa)			Control	PEG-6000 (MPa)			Control	PEG-6000 (MPa)		
		0.19	0.36	0.58		0.19	0.36	0.58		0.19	0.36	0.58
IR 04L191 (DT-CHECK)	1.34 BCDEFG (0.00)	1.11 EFGHIJ (-17.2)	1.18 DEFGHI (-11.6)	1.08 FGHIJK (-19.6)	0.36 L (0.00)	0.49 GHIJKL (37.2)	0.53 FGHIJKL (48.7)	0.66 DEFG (83.4)	18.60 IJKLMN (0.00)	36.85 FG (98)	46.99 CDE (153)	57.25 AB (208)
B-60-B	1.26 DEFGH (0.00)	1.05 GHIJK (-17.2)	1.08 FGHIJK (-14.9)	0.77 K (-39.3)	0.40 JKL (0.00)	0.42 IJKL (4.6)	0.55 FGHIJK (34.9)	0.62 EFGH (53.9)	21.36 IJKL (0.00)	34.74 GH (62.6)	46.36 DEF (117)	54.83 ABCD (157)
GML-507	1.30 CDEFGH (0.00)	1.28 CDEFGH (-1.8)	1.18 DEFGHI (-9.6)	1.14 EFGHI (-12.3)	0.37 KL (0.00)	0.46 HIJKL (23.7)	0.55 FGHIJK (48.9)	0.75 CDE (104)	19.34 IJKLM (0.00)	37.18 EFG (92)	48.34 BCD (150)	59.31 A (207)
IR-8	1.30 CDEFGH (0.00)	0.99 HIJK (-24)	0.88 IJK (-32.7)	0.80 JK (-39)	0.39 JKL (0.00)	0.46 HIJKL (18.6)	0.56 FGHIJ (45.6)	0.68 DEFG (75.3)	20.29 IJKLM (0.00)	35.57 GH (75.3)	47.26 BCDE (133)	56.89 ABC (180)
IR-72	1.43 BCDE (0.00)	1.17 DEFGHI (-18.1)	1.20 DEFGHI (-16.3)	1.07 FGHIJK (-25.2)	0.54 FGHIJKL (0.00)	0.59 EFGHI (9.3)	1.09 A (102)	0.99 AB (83.6)	11.75 LMNO (0.00)	11.98 KLMNO (2.0)	18.75 IJKLMN (59.6)	25.70 HI (119)
IR-50	1.39 BCDEF (0.00)	1.29 CDEFGH (-7.3)	1.18 DEFGHI (-15.4)	1.15 EFGHI (-17.6)	0.50 GHIJKL (0.00)	0.62 EFGH (24)	0.88 BC (76)	0.47 HIJKL (-6)	6.97 O (0.00)	11.06 MNO (58.8)	22.04 IJK (216)	28.13 GHI (304)
IR-6	1.76 A (0.00)	1.55 ABC (-11.7)	1.28 CDEFGH (-27.1)	1.23 CDEFGH (-30.2)	0.43 IJKL (0.00)	0.43 IJKL (0.5)	0.84 BCD (95.3)	0.96 AB (123.3)	6.03 O (0.00)	11.81 LMNO (96)	13.76 JKLMNO (128)	23.53 IJ (290)
DR-92	1.64 AB (0.00)	1.48 ABCD (-9.8)	1.32 BCDEFG (-19.6)	1.06 GHIJK (-35.3)	0.41 IJKL (0.00)	0.42 IJKL (0.5)	0.70 CDEF (69)	0.46 HIJKL (11.6)	9.07 NO (0.00)	12.09 KLMNO (33)	20.91 IJKLM (130)	19.06 IJKLMN (110)
HSD values for genotypes at α 0.05		0.1300				0.0734				4.0081		
HSD values for treatment at α 0.05		0.0774				0.0437				2.3871		
HSD values for G x T at α 0.05		0.3283				0.1855				10.124		

-Values with the same letters are not significantly different at ($p \leq 0.05$)

* Figure in parenthesis indicates % increase (+) and decrease (-) over control

Table 3. Pearson's Correlation coefficient among growth and physiological traits of rice genotypes under water stress.

	Electrolyte leakage	Proline	RWC	Shoot FW	Shoot DW	Chlorophyll	Potassium
Proline	0.4865**						
RWC	-0.7338**	-0.7316**					
Shoot FW	-0.8132**	-0.5957**	0.7933**				
Shoot DW	-0.8421**	-0.6071**	0.8469**	0.9465**			
Chlorophyl	-0.5898**	-0.376*	0.4477**	0.5595**	0.424**		
Potassium	0.2348 ns	0.5017**	-0.5466**	-0.2934 ns	-0.3033 ns	-0.217 ns	
Sugars	0.8009**	0.4356*	-0.6652**	-0.8443**	-0.8223**	-0.6869**	0.1125ns

** = Significant @1% prob., * = Significant @ 5% probability, ns= Non-significant

Chlorophyll contents have shown highest values in IR-6 and DR-92 under non stress conditions. Chlorophyll contents were variably reduced under stress conditions. Genotypic comparison at 0.58 MPa PEG has shown that IR-6, IR-50 and GML -507 had comparatively highest chlorophyll values with less relative reduction as compared to their respective controls (Table 2b).

Potassium concentrations in all genotypes increased under water stress conditions (Table 2b). This increased was more pronounced at 0.36 and 0.58 MPa. Overall genotypic comparison irrespective of treatments has shown that more potassium concentrations were observed in IR-72, IR-50 and IR-6. These three genotypes were also distinctly different from rest of the genotypes at the highest level of water stress (0.58 MPa, PEG-6000) where these have shown comparatively higher potassium concentrations along with higher relative increase.

Pearson's correlations: Correlation co-efficient studies among physiological traits (Table 3) have revealed that electrolyte leakage (EL) was negatively correlated with relative water contents, chlorophyll, and shoots fresh and dry weights and positively correlated with sugars. Proline was significantly positively correlated with electrolyte leakage($r = 0.4865$) and negatively correlated with fresh ($r = -0.5957$) and dry weights ($r = -0.607$). The parameters RWC & chlorophyll were significantly positively correlated with fresh and dry weights ($r = 0.8469$).

Discussion

Drought hinders crop productivity though alteration in many physiological processes and thereby affecting growth and yield. The results of present studies have displayed significant differences among rice genotypes studied under different concentrations of PEG-6000 induced osmotic stresses at seedling stage. It was generally observed that all growth parameters reduced with varying intensity in concentration depended manner. Highest concentration of PEG-6000 (0.58 MPa) reduced germination up to 50% in some genotypes. Highest germination with minimum relative reduction was observed in DR-92 followed by IR-6, IR-72 and IR-50. Present study also revealed significant variable reduction in all growth parameters (Fig. 1) under different PEG concentrations. However under present experimental conditions the genotypes IR-50, IR-72 and DR-92 performed better in having maximum shoot lengths and shoot fresh weights in all treatments of PEG-6000 (Fig. 1B, C, D & E). The decline in germination and growth under stress condition is a general response and this has been reported by many workers (Gampala *et al.,* 2015; Nurhayati *et al.,* 2017; Kosar*et al.,* 2018). The main causative factor under water stressed conditions is loss of turgor (Fahad *et al.,* 2017; Zaefizadeh *et al.,* 2011). Moisture deficit under high osmotic stress can reduced initially water absorption capability of seed, nutrient transfer to embryo (Fahad *et al.,* 2017) and activity of enzymes involved in hydrolysis of stored material in seed which may results in reduced germination, cell division, cell elongation and subsequently results in poor growth.

In the present study variable reduction in growth parameters of different rice genotypes were also reflected in their physiological responses. Chlorophyll a, b and total chlorophyll contents were reduced under PEG induced water stress conditions. Decrease in the chlorophyll contents by water stress has been observed in all genotypes with variable intensities. The genotypes GML-507, IR-50 and IR 04L191 have shown comparatively less reduction. The reduction in total chlorophyll contents under stress have also been observed in studies reported earlier for rice (Purbajanti *et al.,* 2017, Shereen *et al.,* 2017), wheat (Saleem *et al.,* 2017), sunflower (Kosar *et al*., 2018), peanut (Mehar *et al.,* 2018). Chlorophyll is a membrane bounded, light absorbing key pigment involved in photosynthetic process. The reduction in chlorophyll under stress is mainly result of damage to chloroplast which could be related to formation of reactive oxygen species (O^2& H^2O^2) produces under stress can cause lipid peroxidation of chloroplast membrane, which affect membrane stability and causes chlorophyll degradation (Gupta & Huang, 2014).

Relative water contents (RWC) is considered as an effective parameter to measure water status in plants which helps the plant to grow under water deficit conditions through maintaining cell turgor, which may have positive effects on enzyme and membrane integrity (Chutia *et al*., 2012). The data revealed that the shoots RWC decreased variably under different treatments of water stress. Most of the genotypes maintained their RWC up to 0.36 MPa, PEG-6000. The decline in RWC among genotypes became pronounced at 0.58 MPa

where, IR-6, IR-50 and DR-92 exhibited significantly higher RWC with less relative reduction (Table 2a). This indicates that these genotypes have greater ability to retain water under stress. Decrease in RWC was also observed earlier by other workers (Kumar *et al*., 2014; Kunder *et al*., 2016; Meher *et al*., 2018). Puangbut *et al*., (2018) were of the opinion that high root length coupled with maintaining high RWC likely improves photosynthetic capacity and plant growth during water stress. Gupta & Huang (2014) reasoned that this ability to retain a larger amount of water at any given leaf water potential, may be due to rigidness of cell wall or due to osmolytes accumulation in cells. As in the present study differential osmo-regulatory responses of tolerant rice lines were observed in solute accumulation i.e. the genotypes IR-50 have exhibited higher RWC with greater potential of cell membrane stability and accumulated greater quantities of proline (Table 1), IR-72 showed higher potassium along with higher proline (Table 2). Whereas, in contrast to these genotypes IR-6 displayed comparatively higher RWC and cell membrane stability with least accumulation of proline and high potassium under water stress (0.58 MPa). This suggests that these genotypes adjusting osmotically with different solutes. The accumulation of different osmolytes under stressed conditions is well known osmo-regulatory phenomena observed in different plants. Many earlier studies confirms that these factors may contribute to drought tolerance (Cha-um *et al.*, 2010; Akram *et al*., 2013; Kumar *et al*., 2014; Wang *et al*., 2014; Alter *et al*., 2015; Kunder *et al*., 2016; Purbajanti *et al.*,2017). Blum (2016) described osmotic adjustment through accumulation of potassium in dehydrating cells due to turgor derived signal induced upregulation of K transporter genes. This creates an increasing K influx. On the other side some strain responsive gene like P5CR and betA express which regulate production/accumulation of compatible organic solutes protective in function for cellular proteins and organelles under dehydration. He regarded it cellular dehydration strain tolerance mechanism instead of protection mechanism and osmotic adjustment through K and total soluble sugars accumulation as a cellular dehydration strain avoidance mechanism.

Conclusions

Comparative growth and physiological responses of these genotypes under water stress have revealed that IR-50, IR-72 & IR-6 were better in growth and most of the physiological attributes studied. The tolerant genotypes have exhibited least electrolyte leakage with more RWC, chlorophyll and potassium contents. The parameters RWC & chlorophyll were significantly positively correlated with fresh and dry weights($r = 0.8469$). These traits may be used as a screening tool for evaluating water stress tolerance. Tolerant genotypes also exhibited differential osmo-regulatory responses in term of solute production. Furthermore, comprehensive understanding of stress responsive traits particularly adaptive physiological traits is necessary for trait based characterization of rice genotypes.

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