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ASSESSMENT OF RICE (*ORYZA SATIVA* L.) GENOTYPES FOR DROUGHT STRESS TOLERANCE USING MORPHO-PHYSIOLOGICAL INDICES AS A SCREENING TECHNIQUE

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Abstract

Drought stress is one of the primary problem for agricultural crops which causes a great decline in crop production in Pakistan and worldwide. Rice is an economically main cereal crop affected by drought stress. In this study, twenty-one rice genotypes (including 19 mutants (M_5 generation) of super basmati and two varieties were subjected to various concentration of PEG-600 (10% and 15%) at seedling stage to explore the mechanism of drought stress tolerance. PEG_{-6000} induced drought stress caused a substantial decline in growth attributes and relative water contents (RWC), and increase the levels of electrolyte leakage (EL), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in all rice genotypes. A significant difference among the tested rice genotypes based on morpho-physiological indiceswas recorded. Based on morpho-physiological indices (RLSI, SLSI, PFWSI, PDWSI, EL, MDA and H_2O_2), genotypes HTT-119, HTT-74, HTT-92, HTT-97, HTT-104, HTT-119, HTT-125 and HTT-132 were categorized as drought tolerant, while HTT-19, HTT-39, Super basmati (Super Bas), HTT-81, and IR-64 performed poorly recognized as sensitive genotypes. In addition, remaining eight mutants were identified as moderate tolerant. According to present study findings, the screened rice genotypes for drought tolerance can also be suggested to farmers for the improved production and yield of crop on drought-affected area.

Key words: Cluster analysis; Polyethylene glycol; Physiological indices; Rice; Drought stress.

Introduction

Rice (*Oryza sativa* L.) is the 2nd major staple food in the world and commonly grown in the both tropical and temperate regions (Shivani *et al*., 2017). Aromatic rice is famous in Asia, Europe and the United States due to the sole characteristics like long grain, aroma, and amylose content (Ahmad *et al*., 2005). Rice being multiple product commodities is grown over ~163.3 million hectares in over a hundred countries (GRISP, 2013). Rice is a fundamental part of food for more than half of the world's population (Buffon *et al*., 2018), and its harvest yield is significantly influenced by worldwide environmental change, and constraint of water assets in the nature (Simova-Stoilova *et al*., 2008). In appraisal to other crops, rice yield is very water demanding and nearly 30.9% rice grown in areas of the globe is through rain-fed agriculture (Dixit *et al*., 2014). Rice germplasm is important to find the genetic potential for drought tolerance, which supports the breeding for high yield production and drought tolerant rice genotypes (Sahebi *et al*., 2018).

Abiotic stresses, including water shortage, salinity, heat stress, metals stress, etc. are the prime limiting factors in crop productivity (Du *et al*., 2013). Drought stress in a climate change scenario is one of the major threats for sustainable rice productivity (Bellard *et al*., 2012). For instance, IR-64 and Super basmati are considered susceptible to abiotic stresses particularly drought stress in the field and reduction in their yield (Kumar *et al*., 2014; Sabar *et al*., 2019). Yields in aerobic or upland rice are also quite low (Zhao *et al*., 2010). Climatic variability driven various biotic and abiotic factors have worsened the challenges related to global food security (Hussain *et al*., 2016). Water scarcity is considered the single most critical factor globally and shortage of water resources pose serious threat to food security (Shiferaw *et al*., 2011). Drought stress is becoming more serious problem in crop production, which becoming more severe with increasing of population and climatic change (Yang & Liu, 2008). These unfavorable changes cause the recurrence of extreme events like flooding and drought (Rosenzweig *et al*., 2001). Drought stress generally curtails the life cycle, decreasing the photosynthesis and hasten the senescence process in plant (Simova-Stoilova *et al*., 2009). Seed germination and early seedling stage is a main subject to develop a crop stand against environmental stress; therefore, these characters may be used to select the genotypes for drought stress tolerance (Rana *et al*., 2017). Inhibitory effects of drought stress on plants are partly due to oxidative injury because of excessive ROS accumulation (Noctor *et al*., 2014). Reactive oxygen species include H_2O_2, OH and singlet oxygen ($^1O_2$) (Choudhury *et al*., 2017; Foyer & Shigeoka, 2011; Gill & Tuteja, 2010). ROS are highly reactive in nature altering the normal cellular metabolism by inducing substantial injury to proteins, lipids, pigments and nucleic acid (Sharma *et al*., 2012). Plants have developed a conspicuous antioxidant system to mitigate ROS-induced oxidative damage to organelles and cell membranes (Foyer & Shigeoka, 2011).

Plant breeders produce and recognize the genotypes that are tolerant to drought tress (Todaka *et al*., 2015). They suggested that screened genotypes based on drought tolerance potential are valuable for the cultivation in lands facing to water deficit conditions (Kausar *et al*., 2012).

Among many osmotica, PEG is frequently used to stimulate the osmotic stress, which inhibit the seed germination (Zafar *et al.*, 2015). PEG has higher molecular weight, inert, non-ionic and impermeable, therefore used to adjust the water potential (Mendhulkar & Nisha, 2015). Thus, the aims of this study was assessment of drought-tolerant rice genotypes through effective screening techniques under drought or water stress which in future may be helpful for selection of rice genotypes with better performance to varying degree of drought stress and also to calculate the negative impacts of drought stress on the rice plants.

Materials and Methods

Plant material, growth conditions, and stress treatment: Seeds of twenty one including 19 mutants (M_5 generation) of super basmati (HTT-18, HTT-19, HTT-25, HTT-29, HTT-31, HTT-39, HTT-51, HTT-53, HTT-74, HTT-81, HTT-92, HTT-97, HTT-98, HTT-104, HTT-114, HTT-119, HTT-125, HTT-132, HTT-138 and two varieties (Super basmati and IR-64) were obtained from Plant Breeding and Genetics Division, NIAB Faisalabad, Pakistan. The study was conducted at Stress Physiology Lab of NIAB under laboratory conditions (30±2°C). Different levels (0 (control), 5, 10, 15 and 20%) of glycol-6000 (PEG_{-6000}) solution were prepared to select the most effective levels for screening purpose. Then osmotic potential (ψs-0.088, 0.280, 0.425, 0.605 and 0.763–MPa) of these PEG_{-6000} solutions (0, 5, 10, 15 and 20%) were determined by Osmometer (Wescor, Model-5520), respectively according to the method of Michel and Kaufmann (1973). Healthy seeds of 21 genotypes were initially checked for their viability, treated with 5% NaOCl solution (10%) for 5 min, rinsed with distilled water and then air dried for 1h before sowing. Ten sterilized seeds of each genotype was sown in each Petri-plates (100 mm×15 mm) containing three layered filter papers and subjected to 12 mL of 10% and 15% PEG-6000 solution for osmotic stress and distilled deionized water using as control under laboratory conditions. Each Petri-plate containing ten seeds incubated in the darkness (30°C) at 60% relative humidity. The five-rice seedling in each Petri-plate were maintained and then transferred in to controlled environment (Plant growth chamber, Sanyo-Gallenkamp, UK) at 30±2°C. The other growth conditions were light/dark period (12/12h), and photo synthetically active radiation (PAR-520-µmol cm^{-2} S^{-1}). The design of experiment was completely randomized with three replications per treatment (Three Petri-plates per treatment). Different morpho-physiological indices like RLSI, SLSI, PFWSI, PDWSI, RWC, EL, MDA and H_2O_2 contentswere used to determine the drought tolerance in rice genotypes. Data were also analyzed to measure the difference for drought tolerance by correlation and clustering methods. After ten days, for root length (cm) determination, seminal roots used and plant fresh and dry weight (g) were determined. The plants were dried in an oven for 72 hours at 70°C and their dry weights were measured. The physiological indices such as RLSI, SLSI, PFWSI and PDWSI were calculated by using following formula as described by Fernandez (1992).

$$\text{RLSI} = \frac{\text{Root length of stress seedling}}{\text{Root length of non-stress seedling}} \times 100$$

$$\text{SLSI} = \frac{\text{Shoot length of stress seedling}}{\text{Shoot length of non-stress seedling}} \times 100$$

$$\text{PFWSI} = \frac{\text{Plant fresh weight under stress}}{\text{Plant fresh weight under non-stress}} \times 100$$

$$\text{PDWSI} = \frac{\text{Plant dry weight under stress}}{\text{Plant dry weight under non-stress}} \times 100$$

Drought tolerance index (DTI) percentage: The DTI percentage was measured by using the individual scores of attributes and used for grouping the genotypes according to their relative tolerance. DTI (%) was calculated by following formula:

$$\text{DTI (\%)} = \frac{\text{Sum of individual scores for each parameter}}{\text{Sum of highest score for all parameters}} \times 100$$

Determination of water status: Leaf discs (1.0 cm in diameter) of third leaves were sampled. Leaf relative water content (LRWC) was calculated in these leaf discs as follows:

$$\text{LRWC} = [(\text{fresh wt}-\text{dry wt}) \div (\text{turgid wt}-\text{dry wt})]*100 \text{ (Cornic, 1994)}$$

Fresh weight (fresh wt) of ten leaf discs were recorded immediately. Turgid weight (turgid wt) was measured by immersing them in distilled water for overnight in darkness at 4°C for 24 h. Afterward, the leaf discs were oven-dried at 70°C for 48 h for determination of dry weight (dry wt).

Measurement of electrolyte leakage (EL): The membrane permeability was expressed in terms of EL from the leaves under stress. Electrolyte leakagewas assayed by using the procedure of Korkmaz *et al.*, (2010). Ten leaf discs (1.0 cm in diameter) were taken from third leaf from top of plant and washed with distilled water to remove the surface contamination. The leaf discs were placed in test tube containing 10 mL distilled water, vortex them for five second and placed them for 24 h by keeping them at 4°C. The samples were placed at room temperature, then electrical conductivity (EC_1) of the filtrate was obtained. Then same sample were kept in an autoclaved 120°C for 15 min. The electrical conductivity (EC_2) after cooling the solution at room temperature. The percentage of EL was calculated as follows:

$$\text{EL (\%)} = [EC_1/EC_2] \times 100$$

Measurement of malondialdehyde content (MDA) contents: Lipid peroxidation was quantified by the estimation of MDA content, which was assessed spectrophotometrically using thiobarbituric acid assays (Heath & Packer, 1968). Fresh leaf tissue (0.25 g) was homogenized in 5 mL of 5% (w/v) TCA and centrifuged at 12,000 *g* for 15 min to get supernatant. Then 0.5 mL supernatant was mixed with 20% TCA (1 mL) containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was heated at 95°C for 30 min, cooled down on ice bath and then centrifuged the reaction mixture at 7500 *g* for 5 min absorbance read at 532 and 600 nm. The absorbance was recorded at 532 nm and 600 nm, whilst 5% TCA used as blank. MDA contents were calculated using an extinction coefficient of 155,000 nmol mol^{-1} fresh weight.

$$MDA\ (nmol\ mL^{-1}\ FW) = [(A532-A600)/155000]10^{6}$$

Measurement of hydrogen peroxide (H_2O_2) contents: H_2O_2 contentswere estimated using the method of Velikova *et al*., (2000). 0.25 g fresh leaf tissues were grinded with 0.1% chilled TCA (5 mL). The homogenate was centrifuged at 12000 g for 15 min to get supernatant. The supernatant was mixed with 500 µL of chilled potassium phosphate buffer (50 mM; pH 7.5) and 1 mL of KI (1 M) and vortexed and the absorbance was measured at 390 nm, while 0.1% TCA used as blank The H_2O_2 content was determined from a standard curve and the values are expressed as µmolg^{-1} fresh weight.

Statistical analysis

Experiment were performed in completely randomized design (CRD) with factorial arrangement. There were three replicates of each treatment. The difference among means was determined with least significant difference at 5% probability level ($p\leq0.05$) by using STATISTIX software (version 8.1). The MStatC and Minitab-6 was used for cluster analysis and coefficient of variation analysis.

Results

Root lengths stress tolerance index (RLSI): Root lengths stress tolerance index of 21 rice genotypes declined ($p\leq0.001$) considerably due to PEG_{-6000} induced drought stress (Table 1). Off all rice genotypes, HTT-138 showed the highest rate of RLSI (91.30%) at all concentration of PEG_{-6000}. In contrast, HTT-39 and Super Bas genotypes had lowest RLSI (50% and 58.92%) at all levels of PEG_{-6000}, respectively. Under 10% concentration of PEG_{-6000}, all rice genotypes responded inversely; however, the highest RLSI (97.69%) was shown by HTT-138 closely followed by HTT-114 (90%) and HTT-104 (89.83%), minimum RLSI was found in HTT-39 (56.21%). At 15% concentration of PEG_{-6000}, HTT-138 (84.91%), HTT-51 (79.76%), and HTT-97 (78.13%) kept maximum RLSI closely followed by HTT-19 (75.78%), HTT-29 (75.44%) and HTT-19 (75.65%), while the minimum score obtained in HTT-39 (43.79%). In addition, HTT-25 (63%), HTT-74 (69.75%), HTT-81 (64.21%), and HTT-98 (69.26%) genotypes were intermediate in this index at all levels of PEG_{-6000}. On mean percent of control basis, HTT-138 (91.30%), HTT-51 (81.66%) and HTT-19 (78.68%) was assigned as tolerant (T) and ranked as first, second and third position, while HTT-39 (50%) belong to moderate sensitive (MS) and was placed at 21st position. Other genotypes like Super Bas (58.92%), HTT-53 (61.88%), and IR-64 (61.94%), were placed at 20th, 19th, and 18th positions belong to moderate sensitive (MS) and tolerant (MT) group, respectively (Table 2).

Shoot lengths stress tolerance index (SLSI): A significant decrease ($p\leq0.001$) in SLSI from untreated plants was evident in rice plants raised under drought stress. The genotypes of rice was differ in SLSI at various levels of PEG_{-6000} (Table 1). Maximum RLSI was recorded in HTT-18 (99.92%), HTT-119 (92.39%), and HTT-119 (90.71%) at 10% concentration of PEG_{-6000} and closely followed by HTT-81(89.70%), HTT-29 (88.01%), HTT-29 (75.44%) and HTT-19 (75.65%), while the minimum score was obtained in HTT-39 (74.05%). Under 15% concentration of PEG_{-6000}, the highest RLSI (83.03%) was shown by HTT-29 closely followed by HTT-53 (82.33%) and HTT-25 (81.49%), while minimum RLSI was found in IR-64 (57.69%) and Super Bas (59.64%). Moreover, HTT-81 (82.82%), HTT-51 (82.76%), HTT-74 (82.29%), HTT-92 (82.24%), HTT-19 (81.77%), HTT-119 (81.42%), HTT-25 (81.41%) and HTT-31 (80.27%) were intermediated in this index at all levels of PEG_{-6000} induced drought stress. On other hand, the genotypes HTT-29 (85.52%) was observed as highly tolerant and ranked as first on mean percent of control basis, while IR-64 (67.68%), HTT-39 (68.25%) and Super Bas (68.50%) genotypes were assigned as moderately tolerant (MT) placed at 21st, 20th and 19th position (Table 3).

Table 1. Mean square values of rice genotypes from analysis of variance for primary data of stress indices.

SOV	df	RL	SLSI	PFWSI	PDWSI	RWC	EL	MDA	H_2O_2
Treatments (T)	2	35.392***	24.981***	0.430***	0.008***	114112***	34528.4 ***	328.719 ***	9679.50 ***
Genotypes (G)	20	1.299***	0.823***	0.0099***	0.0001***	1126***	219.2***	14.613 ***	204.29***
G× T	40	0.242***	0.345***	0.0051***	0.00009***	216***	84.3***	3.478***	61.06***
Error	126	0.1206	0.0997	0.0011	0.00002	58	5.9	0.517	7.00
Total	188								

* Significant at $p<0.05$; ** highly significant at $p<0.01$; *** very high significant at $p<0.001$ Abbreviations: SOV= Source of variance; df= Degree of freedom; RLSI= Root length stress tolerance indices; SLSI= Shoot length stress tolerance indices; PFWSI= Plant fresh weight stress tolerance indices; PDWSI= Plant dry weight stress tolerance indices; RWC= Relative water contents; EL= Electrolyte leakage; MDA= Malondialdehyde; H_2O_2= Hydrogen peroxide

Table 2. Root lengths (cm) stress tolerance index (RLSI) in 21 genotypes of rice.

Genotypes	(PEG-6000) Treatments (%)					Ranking
	Control (0)	10%	15%	Means*	Group	
HTT-18	4.167	3.50(84.00)	2.80(67.20)	75.60 bcd	T	8
HTT-19	3.400	2.77(81.57)	2.58(75.78)	78.68 bc	T	3
HTT-25	4.653	3.32(71.28)	2.55(54.73)	63.00 efg	MT	16
HTT-29	3.813	3.11(81.56)	2.88 (75.44)	78.50 bc	T	4
HTT-31	4.040	2.77(68.56)	2.24(55.53)	62.05 efg	T	17
HTT-39	4.667	2.62(56.21)	2.04(43.79)	50.00 h	MS	21
HTT-51	3.590	3.00(83.57)	2.86(79.76)	81.66 ab	T	2
HTT-53	4.167	2.62(62.96)	2.53(60.80)	61.88 fg	MT	19
HTT-74	4.193	3.07(73.29)	2.78(66.22)	69.75 def	MT	13
HTT-81	4.713	2.69(57.14)	3.36(71.29)	64.21 efg	MT	15
HTT-92	4.233	3.50(82.76)	2.76(65.12)	73.94 cde	T	10
HTT-97	3.993	2.67(66.86)	3.12(78.13)	72.50 cde	T	11
HTT-98	3.047	2.23(73.09)	1.99(65.43)	69.26 def	MT	14
HTT-104	3.900	3.50(89.83)	2.62(67.09)	78.46 bc	T	5
HTT-114	4.100	3.69(90.00)	2.23(54.39)	72.20 def	T	12
HTT-119	3.573	2.92(81.62)	2.45(68.66)	75.14 bcd	T	9
HTT-125	3.693	2.95(79.96)	2.68(72.65)	76.31 bc	T	7
HTT-132	3.607	3.16(87.52)	2.44(67.65)	77.59 bc	T	6
HTT-138	4.043	3.95(97.69)	3.43(84.91)	91.30 a	HT	1.0
Super Bas	4.480	2.56(57.04)	2.53(60.79)	58.92 g	MT	20
IR-64	4.427	2.73(61.75)	2.72(60.12)	61.94 fg	MT	18
Mean	3.89	3.02 (75.63a)	2.75 (66.55b)			
CV (%)	6.67	6.89	9.25			

Score	10	8	6	5	3
Tolerance index (%)	Highly tolerant (100-90)	Tolerant (89-70)	Moderate tolerant (69-51)	Moderate sensitive (50-40)	Sensitive ≤30

Notes: Means sharing similar letter did not differ significantly ($p>0.05$) in rows and column; HT= High tolerant, T= Tolerant, MT= Moderately tolerant, MS= Moderately sensitive, S= Sensitive, CV = Coefficient of variation; ( ) = Percent of control,* = Mean percent of control of both drought treatments

Table 3. Shoot lengths (cm) stress tolerance index (SLSI) in 21 genotypes of rice.

Genotypes	(PEG-6000) Treatments (%)					Ranking
	Control (0)	10%	15%	Means*	Group	
HTT-18	5.45	5.44 (99.92)	3.77 (69.22)	84.55 ab	T	4
HTT-19	6.15	5.21 (84.62)	4.86 (78.93)	81.77 bc	T	11
HTT-25	6.00	4.88 (81.32)	4.89 (81.49)	81.41 bcd	T	13
HTT-29	5.95	5.24 (88.01)	4.94 (83.03)	85.52 a	T	1
HTT-31	6.13	5.30 (86.41)	4.55 (74.13)	80.27 bcd	T	14
HTT-39	6.99	5.17 (74.05)	4.36 (62.45)	68.25 e	MT	20
HTT-51	5.36	4.63 (86.50)	4.23 (79.03)	82.76 b	T	8
HTT-53	6.45	5.60 (86.82)	5.31(82.33)	84.57 ab	T	3
HTT-74	6.43	5.47 (85.11)	5.11 (79.46)	82.29 bc	T	9
HTT-81	6.54	5.87 (89.70)	4.97 (75.94)	82.82 b	T	7
HTT-92	5.42	5.00 (92.19)	3.92 (72.28)	82.24 bc	T	10
HTT-97	5.98	5.60 (93.70)	4.40 (73.58)	83.64 b	T	5
HTT-98	5.94	4.53 (76.32)	3.61 (60.83)	68.57 e	T	18
HTT-104	6.34	5.23 (82.49)	4.31 (67.93)	75.21 cde	T	16
HTT-114	5.28	4.37 (82.70)	4.03 (76.39)	79.55 bcd	T	15
HTT-119	5.87	5.42 (92.39)	4.13 (70.45)	81.42 bcd	T	12
HTT-125	5.45	4.95 (90.71)	4.31 (78.97)	84.84 ab	T	2
HTT-132	6.03	4.95 (82.04)	4.02 (66.63)	74.34 de	T	17
HTT-138	6.87	6.00 (87.38)	5.41 (78.74)	83.06 bc	T	6
Super Bas	6.89	5.33 (77.37)	4.11 (59.64)	68.50 e	MT	19
IR-64	6.66	5.17 (77.67)	3.84 (57.69)	67.68 f	MT	21
Mean	6.10	5.21 (85.59a)	4.43 (72.82b)			
CV (%)	3.79	5.43	7.25			

Score	10	8	6	5	3
Tolerance index (%)	Highly tolerant (100-90)	Tolerant (89-70)	Moderate tolerant (69-51)	Moderate sensitive (50-40)	Sensitive ≤30

Notes: Means sharing similar letter did not differ significantly ($p>0.05$) in rows and column; HT= Highly tolerant, T= Tolerant, MT= Moderately tolerant, CV = Coefficient of variation; ( ) = Percent of control,* = Mean percent of control of both drought treatments

Plant fresh weight stress tolerance index (PFWSI): PEG_{-6000} induced drought stress and caused an extreme decline ($p \leq 0.001$) in fresh weight of all rice genotypes seedlings (Table 1). Under 10% concentration of PEG_{-6000}, HTT-51 (95.80%), HTT-138 (95.69%) and HTT-92 (93.19%) maintained the maximum PFWSI, while minimum in Super Bas (58.22%), HTT-31 (59.25%) and HTT-98 (64.77%) and lowest values of PFWSI for HTT-39 (50.94%) and IR-64 (49%) were measured. At 15% concentration of PEG_{-6000}, maximum value of PFWSI was recorded for HTT-138 (81.66%) and HTT-19 (74.94%) and HTT-119 (74.87%). The HTT-39 genotype showed poor performance (50.94% and 42.05%) for PFWSI, respectively at 10% and 15% level of PEG_{-6000}. In addition, HTT-25 (75.69%), HTT-114 (73.89%), HTT-125 (70.55%), HTT-29 (68.52%), HTT-92 (75.96%) and HTT-81 (67.27%) rice genotypes were intermediated in this index at all levels of PEG_{-6000}. On mean percent of control basis, the genotype HTT-138 (88.67%) and HTT-51 (83.48%) were observed tolerant (T) and got maximum points for FWSTI and ranked as first and second position, respectively, while HTT-39 (46.50%) and IR-64 (48.31%) were found moderately sensitive (MS) got 21st and 20th position (Table 4).

Plant dry weight stress tolerance index (PDWSI): Drought stress considerably ($p \leq 0.001$) decreased PDWSI in 21 all rice genotypes (Table 1). At 10 % level of PEG_{-6000}, maximum PDWSI was recorded for HTT-138 (95.90%) followed by HTT-29 (88.94%), HTT-119 (87.43%) and HTT-125 (87.33%) while the lowest value of DWSTI was evident in HTT-39 (47.27%), HTT-39 (48.09%), HTT-104 (49.61%) and Super Bas (50.90%). Under 15% level of PEG_{-6000}, the highest PDWSI was observed for HTT-138 (76.59%) closely followed by HTT-119 (69.78%) and HTT-51 (60.97%), while minimum value of it were recorded in HTT-98 (33.07%), HTT-31 (40.91%), and HTT-81 (43.28%). In addition, HTT-114 (64.98%), HTT-132 (64.51%), HTT-25 (63.49%), HTT-92 (63.48%), HTT-18 (60.54%), HTT-53 (56.20%), HTT-74 (56.68%) and HTT-81 (52.87%) genotypes were intermediate in PDWSI. On mean percent of control basis, HTT-138 (86.25%), HTT-119 (78.61%), and HTT-125 (71.66%) genotypes were observed drought tolerant (T) and ranked as first, second, and third position, respectively. While HTT-39 (47.30%), HTT-31 (45.25%), HTT-104 (40.82%) and HTT-98 (40.58%) genotypes has minimum mean percent of control for PDWSI and categorized as moderate sensitive and ranked on 18th, 19th, 20th and 21st position, respectively (Table 5).

Table 4. Plant fresh weights (g) stress tolerance index (PFWSI) in 21 genotypes of rice.

Genotypes	(PEG_{-6000}) Treatments (%)					Ranking
	Control (0)	10%	15%	Means*	Group	
HTT-18	0.461	0.36 (78.15)	0.26 (55.38)	66.75 def	MT	15
HTT-19	0.360	0.32 (89.14)	0.27 (74.94)	82.04 abc	T	6
HTT-25	0.415	0.32 (77.76)	0.31 (73.63)	75.69 bcd	T	9
HTT-29	0.500	0.43 (86.83)	0.25 (50.22)	68.52 def	MT	12
HTT-31	0.450	0.27 (59.25)	0.24 (53.83)	56.04 fg	MT	19
HTT-39	0.482	0.25 (50.94)	0.20 (42.05)	46.50 h	MS	21
HTT-51	0.424	0.41 (95.80)	0.30 (71.15)	83.48 ab	T	2
HTT-53	0.414	0.38 (92.90)	0.30 (73.29)	83.09 ab	T	4
HTT-74	0.488	0.40 (82.81)	0.36 (73.76)	78.29 bcd	T	7
HTT-81	0.473	0.37 (77.47)	0.27 (57.07)	67.27 def	MT	14
HTT-92	0.426	0.40 (93.19)	0.18 (42.75)	67.97 def	MT	13
HTT-97	0.446	0.41 (92.03)	0.33 (74.84)	83.43 ab	T	3
HTT-98	0.505	0.33 (64.77)	0.27 (52.96)	58.86 efg	MT	16
HTT-104	0.507	0.30 (59.84)	0.27 (53.02)	56.43 fg	MT	18
HTT-114	0.475	0.44 (92.59)	0.26 (55.18)	73.89 cde	T	10
HTT-119	0.446	0.40 (89.25)	0.33 (74.87)	82.06 abc	T	5
HTT-125	0.417	0.36 (85.33)	0.23 (55.78)	70.55 de	T	11
HTT-132	0.400	0.33 (82.92)	0.28 (69.01)	75.96 bcd	T	8
HTT-138	0.433	0.41 (95.69)	0.35 (81.66)	88.67 a	T	1
Super Bas	0.458	0.27 (58.22)	0.25 (55.09)	56.66 fg	T	17
IR-64	0.537	0.26 (49.00)	0.26 (47.62)	48.31 g	MS	20
Mean	0.44	0.35 (78.76a)	0.28 (61.3b)			
CV(%)	5.58	9.00	8.03			

Score	10	8	6	5	3
Tolerance index (%)	Highly tolerant (100-90)	Tolerant (89-70)	Moderate tolerant (69-51)	Moderate sensitive (50-40)	Sensitive ≤30

Notes: Means sharing similar letter did not differ significantly (p>0.05) in rows and column; HT= Highly tolerant, T= Tolerant, MT= Moderately tolerant, MS= Moderately sensitive, S= Sensitive, CV = Coefficient of variation; ( ) = Percent of control,* = Mean percent of control of both drought treatments

Table 5. Plant dry weights (g) stress tolerance index (PDWSI) in 21 genotypes of rice.

Genotypes	(PEG_{6000}) Treatments (%)					Ranking
	Control (0)	10%	15%	Means*	Group	
HTT-18	0.052	0.04 (78.22)	0.02 (42.86)	60.54 defg	MT	12
HTT-19	0.049	0.04 (79.47)	0.03 (54.52)	67.00 cd	MT	7
HTT-25	0.051	0.04 (71.04)	0.03 (55.94)	63.49 cdef	MT	10
HTT-29	0.056	0.05 (88.94)	0.03 (48.33)	68.63 cd	MT	6
HTT-31	0.051	0.03 (49.58)	0.03 (40.91)	45.25 k	MS	19
HTT-39	0.046	0.02 (47.27)	0.02 (47.34)	47.30 hijk	MS	18
HTT-51	0.045	0.04 (81.42)	0.03 (60.97)	71.19 b	T	4
HTT-53	0.049	0.03 (67.30)	0.02 (45.10)	56.20 efgh	MT	13
HTT-74	0.056	0.03 (58.37)	0.03 (50.98)	54.68 fghi	MT	14
HTT-81	0.056	0.03 (62.45)	0.02 (43.28)	52.87 ghij	MT	15
HTT-92	0.043	0.04 (83.45)	0.02 (43.51)	63.48 cdef	MT	11
HTT-97	0.047	0.04 (79.47)	0.03 (58.17)	68.82 cd	T	5
HTT-98	0.059	0.03 (48.09)	0.03 (33.07)	40.58 l	MS	21
HTT-104	0.056	0.03 (49.61)	0.02 (40.04)	44.82 k	MS	20
HTT-114	0.051	0.04 (79.14)	0.03 (50.82)	64.98 cde	MT	8
HTT-119	0.045	0.04 (87.43)	0.03 (69.78)	78.61 b	T	2
HTT-125	0.039	0.03 (87.33)	0.02 (56.00)	71.66 b	T	3
HTT-132	0.038	0.03 (75.24)	0.02 (53.77)	64.51 cde	MT	9
HTT-138	0.051	0.05 (95.90)	0.04 (76.59)	86.25 a	T	1
Super Bas	0.053	0.03 (50.95)	0.025 (49.49)	50.22 ij	MS	17
IR-64	0.054	0.03 (55.81)	0.03 (48.54)	52.18 hij	MT	16
Mean	0.050	0.04 (70.31 a)	0.03 (50.95 b)			
CV (%)	8.240	7.87	6.98			

Score	10	8	6	5	3
Tolerance index (%)	Highly tolerant (100-90)	Tolerant (89-70)	Moderate tolerant (69-51)	Moderate sensitive (50-40)	Sensitive ≤30

Notes: Means sharing similar letter did not differ significantly (p>0.05) in rows and column; Super Bas= Superbasmati, HT= Highly tolerant, T= Tolerant, MT= Moderately tolerant, MS= Moderately sensitive, S= Sensitive, CV = Coefficient of variation; ( ) = Percent of control,* = Mean percent of control of both drought treatments

Relative water contents (RWC): Drought stress markedly ($p \leq 0.001$) reduced RWC in all rice genotypes. At 10 % level of PEG_{6000}, higher RWC was determined for HTT-31 (91.74%) and then followed by 88.43% and 86.70% increase in HTT-19 and HTT-98, respectively. In this context, HTT-39 exhibited less RWC (52.85%). Under 15% level of PEG_{6000}, a significant increased in RWC of HTT-25 (49.71%) followed by HTT-138 (48.88%) and HTT-104 (47.22%), while the lowest value of it recorded in HTT-132 (27.78%) and IR-64 (28.60%). Furthermore, on mean percent of control basis, response of HTT-29 (62.55%), HTT-81 (60.85%), HTT-97 (60.14%), HTT-125 (60.85%), HTT-81 (59.90%), HTT-18 (58.33%) and HTT-74 (56.70%) genotypes were intermediate with respect to this attributes. Of 21 rice genotypes, HTT-31 has shown higher tolerance behavior (RWC-69.27%), while HTT-39 (43.78%) got lowest score in this context under drought stress and categorized as moderate sensitive. Super Bas (51.68%), HTT-132 (51.77%), and HTT-51 (55.50%), which are near to borderline between tolerant and sensitive genotypes (Table 6).

Electrolyte leakage (EL): Drought stress-induced oxidative damage considerably ($p<0.001$) in all rice genotypes. The higher increase in EL was recorded (768% and 1027%) due to the application of PEG_{6000} (10% and 15%) in HTT-39, respectively. Rice genotypes HTT-39, HTT-104, Super bas, and IR-64 were more affected by increasing the drought stress resulted in maximum EL by exhibiting highest scores of percent control (898%, 641%, 558% and 547%), respectively because these mutants along with HTT-53 (536%) were categorized as moderate drought sensitive. Nevertheless, HTT-138 having minimum mean value of EL (302 %) of control and kept in drought-tolerant group. Furthermore, on mean percent of control basis, response of HTT-25 (465%), HTT-97 (464%), HTT-119 (463%), HTT-114 (442%), HTT-18 (417%) and HTT-132 (409%) genotypes were intermediate with respect to this attributes and were designated as moderately tolerant (MT) to drought stress. HTT-39 is most sensitive mutant, which showed maximum scores (898%) followed by Super bas (558%) and IR-64 (547%) as shown in table 6.

Table 6. Effect of drought stress on relative water contents (%) and electrolyte leakage (%) in 21 genotypes of rice.

	Relative water contents (RWC-%)						Electrolyte leakage (EL-%)					
Genotype	**Control (0)**	**PEG (10%)**	**PEG (15%)**	**Mean***	**Group**	**Ranking**	**Control (0)**	**PEG (10%)**	**PEG (15%)**	**Mean***	**Group**	**Ranking**
HTT-18	136.00	108.67 (79.88)	50.00 (36.76)	58.33 def	MT	12	12.28	43.34 (353.02)	59.08 (481.18)	417.10 efg	MT	11
HTT-19	144.00	127.33 (88.43)	54.00 (37.50)	62.96 abc	MT	4	15.44	55.48 (359.42)	62.50 (404.89)	382.15 hij	T	15
HTT-25	115.33	92.67 (80.35)	57.33 (49.71)	65.03 ab	MT	3	9.68	39.01 (402.95)	51.00 (526.79)	464.87 cde	MT	7
HTT-29	175.33	147.33 (84.03)	72.00 (41.06)	62.55 bcd	MT	8	13.08	35.03 (267.76)	53.22 (406.82)	337.29 kl	T	20
HTT-31	145.33	133.33 (91.74)	68.00 (46.79)	69.27 a	MT	1	12.87	42.40 (329.54)	53.39 (414.90)	372.22 ijk	T	16
HTT-39	128.67	68.00 (52.85)	44.67 (34.72)	43.78 h	MS	21	6.51	50.06 (768.48)	66.92(1027.22)	897.85 a	S	1
HTT-51	145.33	104.00 (71.56)	57.33 (39.45)	55.50 def	MT	16	12.27	38.22 (311.64)	58.75 (478.98)	395.31 ghi	T	14
HTT-53	130.00	96.00 (73.85)	49.33 (37.95)	55.90 defg	MT	15	9.78	43.26 (442.16)	61.55(629.01)	535.59 bcd	MS	5
HTT-74	139.33	108.00 (77.51)	50.00 (35.89)	56.70 defg	MT	13	14.49	37.23 (256.93)	61.69 (425.69)	341.31 jkl	T	19
HTT-81	126.00	104.00 (82.54)	49.33 (39.15)	60.85 cde	MT	9	10.29	49.77 (483.76)	53.15(516.58)	500.17 cd	MS	6
HTT-92	138.67	93.33 (67.31)	48.00 (34.62)	50.96 fg	MT	19	13.72	43.36 (315.92)	56.93 (414.81)	365.36 jkl	T	17
HTT-97	138.00	113.33 (82.13)	52.67 (38.16)	60.14 cde	MT	10	9.01	31.22 (346.45)	52.44 (581.88)	464.17 cde	MT	8
HTT-98	145.33	126.00 (86.70)	62.67 (43.12)	64.91 abc	MT	5	13.79	41.49 (300.97)	59.24 (429.73)	365.35 jkl	T	18
HTT-104	144.00	122.67 (85.19)	68.00 (47.22)	66.20 ab	MT	2	10.73	58.11(541.66)	79.33 (739.42)	640.54 b	MS	2
HTT-114	131.33	105.33 (80.20)	60.00 (45.69)	62.94 bcd	MT	6	10.85	43.31 (399.24)	52.72 (485.93)	442.58 def	MT	10
HTT-119	134.67	104.67 (77.72)	48.00 (35.64)	56.68 defg	MT	14	10.08	33.47 (331.91)	59.91 (594.16)	463.04 cde	MT	9
HTT-125	134.67	116.00 (86.14)	45.33 (33.66)	59.90 def	MT	11	12.33	47.11 (382.07)	50.71 (411.30)	396.68 ghi	T	13
HTT-132	132.00	100.00 (75.76)	36.67 (27.78)	51.77 efg	MT	18	11.73	40.37 (344.04)	55.79 (475.41)	409.73 fgh	MT	12
HTT-138	148.67	113.33 (76.23)	72.67 (48.88)	62.56 bcd	MT	7	14.19	38.45 (270.96)	47.17 (332.37)	301.66 l	T	21
Super Bas	158.67	104.67 (72.27)	49.33 (31.09)	51.68 efg	MT	17	10.04	47.91 (477.05)	64.11 (638.25)	557.65 bc	MS	3
IR-64	157.33	114.67 (66.53)	45.00 (28.60)	47.56 g	MS	20	6.18	21.54 (348.65)	45.98 (744.25)	546.65 bc	MS	4
Mean	140.41	109.68(78.04a)	54.30 (38.74b)				11.40	41.91 (382.60 b)	57.41 (531.41a)			
CV (%)	136.00	7.39	13.12				14.20	5.37	4.91			

Notes: Means sharing similar letter did not differ significantly ($p>0.05$) in rows and column; T= Tolerant, MT= Moderately tolerant, MS= Moderately sensitive, S= Sensitive, CV= Coefficient of variation; ( ) = Percent of control,* = Mean percent of control of both drought treatments

Table 7. Effect of drought stress on MDA contents (nmolmL^{-1} FW) and H_2O_2 contents (µmolg^{-1} Fw) in 21 genotypes of rice.

	Malondialdehyde contents (MDA-nmol mL^{-1} FW)						Hydrogen peroxide contents (H_2O_2-µmol g^{-1} Fw)					
Genotype	**Control (0)**	**PEG (10%)**	**PEG (15%)**	**Mean***	**Group**	**Ranking**	**Control (0)**	**PEG (10%)**	**PEG (15%)**	**Mean***	**Group**	**Ranking**
HTT-18	3.34	3.68 (111.12)	6.61 (200.55)	155.84 efg	MT	16	9.18	22.78 (250.03)	27.25(300.82)	275.43 c	MS	6
HTT-19	2.74	4.97 (182.08)	7.11 (260.10)	221.09 abc	MS	4	7.52	20.51 (273.12)	29.31 (390.14)	331.63 b	S	5
HTT-25	4.46	5.30 (119.24)	6.02 (135.71)	127.47 fg	T	20	12.25	21.86 (180.05)	24.84 (205.14)	192.59 ef	MT	20
HTT-29	3.54	5.71 (163.53)	6.38 (183.98)	173.76 de	MT	9	9.75	23.56 (242.58)	26.33 (271.86)	257.22 cd	MS	11
HTT-31	2.22	2.80 (127.72)	4.94 (225.28)	176.50 cde	MT	8	6.10	11.57 (191.83)	20.37 (337.18)	264.51cd	MS	9
HTT-39	3.35	6.85 (204.76)	10.86 (324.57)	264.67 a	MS	1	9.23	28.25 (313.73)	44.78 (496.98)	405.36 a	S	1
HTT-51	5.32	6.30 (123.94)	8.76 (172.85)	148.39 efg	MT	18	14.62	25.97 (178.47)	36.12 (249.51)	213.99 def	MT	19
HTT-53	4.52	6.18 (136.57)	9.45 (208.90)	172.73 de	MT	10	12.44	25.48 (204.86)	38.96 (313.35)	259.10 cd	MS	10
HTT-74	3.78	6.19 (172.28)	8.62 (233.93)	203.11 bcd	MS	5	8.41	25.55 (308.45)	35.55 (430.82)	369.63 ab	S	3
HTT-81	3.68	7.12 (216.21)	9.79 (299.59)	257.90 a	MS	2	10.12	29.38 (294.62)	40.38 (407.15)	350.88 ab	S	4
HTT-92	4.66	6.38 (142.09)	8.62 (193.24)	167.67 def	MT	12	12.82	26.31 (209.36)	35.55 (283.90)	246.63 cde	MS	15
HTT-97	5.33	6.18 (118.81)	11.44 (214.08)	166.45 def	MT	14	14.67	25.48 (171.31)	47.19 (325.35)	248.33 cd	MS	14
HTT-98	5.13	6.06 (118.44)	9.48 (185.83)	152.13 efg	MT	17	14.10	24.98 (178.34)	39.10 (280.38)	229.36 cdef	MS	17
HTT-104	5.23	8.45 (162.42)	8.34 (160.61)	161.52 defg	MT	15	14.38	34.85 (246.47)	34.42 (242.92)	244.70 cde	MS	16
HTT-114	5.64	6.64 (120.24)	9.20 (165.73)	142.99 efg	MT	19	15.52	27.39 (181.08)	37.97 (250.49)	215.79 def	MT	18
HTT-119	4.75	6.00 (128.84)	11.13 (240.60)	184.72 cde	MT	6	13.06	24.77 (192.10)	45.92 (356.07)	274.09 c	MS	7
HTT-125	5.64	7.14 (127.17)	11.58 (206.35)	166.76 def	MT	13	15.52	29.45 (189.95)	47.76 (308.63)	249.29 cd	MS	13
HTT-132	5.28	7.47 (142.93)	10.32 (198.31)	170.62 def	MT	11	14.52	30.80 (214.39)	42.58 (297.47)	255.93 cd	MS	12
HTT-138	4.30	4.92 (114.35)	5.20 (120.92)	117.64 g	T	21	11.83	20.30 (171.50)	21.43 (181.27)	176.38 f	MT	21
Super Bas	5.20	6.23 (119.89)	12.15 (233.63)	176.76 cde	MT	7	14.29	25.69 (182.04)	50.10 (353.66)	267.85 cd	MS	8
IR-64	3.58	6.93 (194.60)	10.70 (300.57)	247.59 ab	MS	3	9.84	28.60 (294.26)	44.14 (454.59)	374.43 ab	S	2
Mean	3.14	6.07 (145.11b)	8.89 (212.64a)				1191	25.41 (222.31b)	36.67 (320.84a)		MS	
CV (%)	12.72	16.05	13.12				14.20	11.22	4.91			

Notes: Means sharing similar letter did not differ significantly (p>0.05) in rows and column; T= Tolerant, MT= Moderately tolerant, MS= Moderately sensitive, S= Sensitive, CV = Coefficient of variation; ( ) = Percent of control,* = Mean percent of control of both drought treatments

Table 8. Scores obtained by various rice genotypes.

	Scoring on basis of morpho-physiological attributes								Scoring on basis ofphysio-chemical attributes					
Genotypes	**SLSI**	**RLSI**	**PFWSI**	**PDWSI**	**RWC**	**Average scores**	**Group**	**Ranking**	**EL**	**MDA**	**H_2O_2**	**Average scores**	**Group**	**Ranking**
HTT-18	10.11	8.26	7.47	6.88	8.05	8.15	T	11	4.6	5.76	7.08	5.83	MS	11
HTT-19	9.85	8.64	9.24	7.70	8.59	8.81	T	4	4.3	8.47	8.38	7.05	S	4
HTT-25	9.83	6.87	8.57	7.36	9.38	8.40	T	8	5.2	5.00	4.98	5.06	MT	20
HTT-29	10.31	8.62	7.61	7.79	8.71	8.61	T	6	3.7	6.73	6.68	5.71	MS	14
HTT-31	9.65	6.78	6.33	5.26	9.71	7.54	MT	17	4.2	6.53	6.49	5.73	MS	13
HTT-39	8.20	5.46	5.23	5.55	6.37	6.16	S	21	10.0	10.00	10.00	10.00	HS	1
HTT-51	9.97	8.97	9.36	8.23	7.87	8.88	T	2	4.4	5.45	5.41	5.07	MT	19
HTT-53	10.20	6.80	9.34	6.45	7.84	8.13	T	12	5.9	6.57	6.50	6.33	MS	8
HTT-74	9.92	7.65	8.84	6.37	7.83	8.12	T	13	3.7	7.53	9.30	6.86	S	5
HTT-81	9.96	7.12	7.54	6.08	8.44	7.83	T	15	5.7	8.85	8.84	7.78	S	3
HTT-92	9.87	8.07	7.48	7.19	7.15	7.95	T	14	4.1	6.21	6.21	5.50	MS	16
HTT-97	10.03	8.02	9.39	7.94	8.31	8.74	T	5	5.1	6.15	6.11	5.78	MS	12
HTT-98	8.23	7.59	6.62	4.67	9.06	7.23	MT	18	4.0	5.75	5.71	5.17	MS	18
HTT-104	9.03	8.55	6.37	5.20	9.39	7.71	MT	16	7.1	6.42	6.40	6.65	S	6
HTT-114	9.58	7.81	8.21	7.44	8.97	8.40	T	9	5.0	5.40	5.40	5.26	MT	17
HTT-119	9.76	8.22	9.24	9.11	7.82	8.83	T	3	5.1	6.72	6.68	6.15	MS	9
HTT-125	10.21	8.37	7.87	8.21	8.08	8.55	T	7	4.5	6.27	6.20	5.65	MT	15
HTT-132	8.93	8.46	8.55	7.43	6.92	8.06	T	12	4.6	6.48	6.46	5.83	MS	10
HTT-138	10.00	10.00	9.99	10.00	9.07	9.81	HT	1	3.4	4.67	4.63	4.23	T	21
Super Bas	8.21	6.50	6.41	5.89	7.07	6.82	MS	19	6.2	6.55	6.49	6.42	MS	7
IR-64	8.11	6.82	5.47	6.08	6.50	6.60	MS	20	5.9	9.37	9.32	8.19	S	2

Notes: HT= Highly tolerant, T= Tolerant, MT= Moderately tolerant, HS= Highly sensitive, MS= Moderately sensitive, S= Sensitive

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents: Drought stress-induced oxidative damage significantly (p<0.001) in all rice genotypes (Table 1). The degree of oxidative damage in the form of H_2O_2 and MDA was more in HTT-39as compared to HTT-138. PEG$_{-6000}$ (10% and 15%) produced maximum MDA (204% and 324%) and H_2O_2 (306% and 485%) contents in HTT-39 under stress conditions. Moreover, IR-64 (246% and 370%), HTT-81 (230% and 345%), HTT-19 (221% and 331%), HTT-74 (196% and 363%) and Super Bas (177% and 265%) exhibited transitional response with respect to MDA and H_2O_2 under drought stress, respectively. On other hand, for MDA and H_2O_2 contents the minimum mean percent of control (118% and 176%) was recorded in HTT-138 succeeded by HTT-25 (127% and 191%) allocated as drought tolerant genotypes. Among the moderate tolerant genotypes, HTT-114 (140% and 211%) was better performing which are followed by HTT-51 (142% and 212%), whereas, drought-stressed plants of HTT-39 (264% and 396%), HTT-81 (230% and 345%), HTT-19 (221% and 331%) and HTT-74 (196% and 363%) exhibited maximum values for MDA and H_2O_2 contents respectively, thus considered as sensitive (Table 7).

From the data concerning to physiological indices like RLSI, SLSI, FWSI and DWSI, it is manifested that these indices may be used to select the rice genotypes for drought stress tolerance. Out of total 21, HTT-138 got the average highest score (9.81) for morphological indices and less score for EL, MDA and H_2O_2 indices. While HTT-39 (6.16), IR-64 (6.60) and Super Bas (6.82) mutants obtained the lowest scores for RLSI, SLSI, PFWSI, PDWSI and RWC contents. In addition, HTT-98 (7.23), HTT-31 (7.54) and HTT-104 (7.71) genotypes were intermediate in scorer. Among all tested genotypes, HTT-138 was found to be the more drought tolerant mutant having drought tolerant value (9.81), while HTT-39 has the lowest drought tolerant indices (6.16). This mutant was sensitive for RLSI, SLSI, PFWSI, PDWSI, RWC, EL, MDA and H_2O_2 under drought stress (Table 8).

Pearson correlation coefficients of stress tolerance indices revealed a positive correlation among SLSI, RLSI, PFWSI, PDWSI and RWC during correlation coefficient analysis. A strong negative and highly significant correlation of EL, MDA and H_2O_2 with SLSI, RLSI, PFWSI, PDWSI and RWC was also observed. This indicates that oxidative stress was the foremost cause of growth decline in rice genotypes (Table 9).

The cluster analysis based on complete linkage and correlation coefficient distance accomplished in this screening experiment, which divided the 21 rice genotypes into three clusters (Fig. 1). Some genotypes (mutant/variety) like HTT-18, HTT-25, HTT-29, HTT-31, HTT-51, HTT-98, HTT-114 and HTT-138 in cluster 1, maintained higher values for morpho-physiological indices and can be considered as drought stress tolerant check. Cluster 2 includes mutants (HTT-119, HTT-74, HTT-92, HTT-97, HTT-104, HTT-119, HTT-125 and HTT-132) performed satisfactory under drought stress and can be used as a moderately tolerant check. In cluster 3, genotypes like HTT-19, HTT-39, Super Bas, HTT-81, and IR-64 obtained lower scores in morpho-physiological indices and considered as sensitive check for drought stress in screening experiments at early seedling stage (Fig. 1).

Table 9. Pearson correlation coefficients among various screening techniques.

Techniques	RLSI	SLSI	PFWSI	PDWSI	RWC	EL	MDA	H_2O_2
RLSI	1							
SLSI	0.4139***	1						
PFWSI	0.5023***	0.5476***	1					
PDWSI	0.5899***	0.5789***	0.8174***	1				
RWC	0.3376***	0.5622***	0.5218***	0.5493***	1			
EL	-0.4186***	-0.3821***	-0.3474***	-0.4082***	-0.4670***	1		
MDA	-0.2197**	-0.3955***	-0.3310***	-0.3349***	-0.5539***	0.3674***	1	
H_2O_2	-0.2251**	-0.3565***	-0.3154***	-0.3358***	-0.5712***	0.4318***	0.8881***	1

*, ** and *** significant at p≤0.05, 0.01, and 0.001 respectively; RLSI=root lengths stress tolerance index; SLSI= Shoot lengths stress tolerance index; PFWSI= Plant fresh weight stress tolerance index; PDWSI= Plant dry weight stress tolerance index; RWC = Relative water contents; EL= Electrolyte leakage; MDA= Malondialdehyde; H_2O_2= Hydrogen peroxide

Discussion

The establishment of a crop in unfavorable conditions generally based on early seedling stage of plant (Farooq *et al.*, 2009). This fact has been effectively found in soybean (Hamayum *et al.*, 2010), wheat (Khan *et al.*, 2013), sorghum (Kausar *et al.*, 2012) and maize (Khan *et al.*, 2003). The variations in morphology of seedling affected the *Zea mays* yield potential at maturity stage (Akram *et al.*, 2013). Therefore, it becomes essential to evolve well-organized screening methods and appropriate frequent screening criteria at germination and initial seedling stage obtain optimum yield (Cooper *et al.*, 2014). The above-mentioned results reflect the morpho-physiological indices, whichcan be used to appraise the drought stress tolerance in rice genotypes during screening (Tables 2-7). The present study showed that HTT-138 performed better and thus can be categorized as tolerant one i.e., it has genetic potential to utilize water more economically. The performance of HTT-51, HTT-19, HTT-29, HTT-125, HTT-18, HTT-53, HTT-97 and HTT-119 was very close to tolerant mutant (HTT-138) thus may be termed as tolerant ones (Tables 2-7). The HTT-39 mutant performed very poor and attained minimum scores; therefore, it can be considered as drought sensitive mutant (Tables 2-7). These findings correlates with those of Zafar *et al.*, (2015) and Ashraf *et al.*, (2008) who documented that same physiological indices were used to select the wheat germplasm for salt and drought tolerance, respectively. Therefore, it is concluded that HTT-138 has great potential for drought tolerance and can be sowing directly in soils with low moisture contents.

Rice genotypes showed better performance under stress conditions (Saxena & Toole, 2002). The genotypes with strong root systems can be recognized as tolerant one under stress environment that can provide a higher grain yield due to their better genetic make-up, which showed the expression under drought stress condition. The length and other features of the root are controlled by dominant allele that can easily be used for drought resistance in breeding (Vijendradas, 2000). In this study, root length reduced with increased of osmotic stress ranging from 10 to 15% PEG regimes (Table 2). The results of our study are in agreements with the results of earlier researcher where PEG-induced drought stress caused decrease in root length of wheat (Jajarmi *et al.*, 2009) and rice (Sabesan&Saravanan, 2016). Drought stress was reported as a great threat for seedling (Ashraf *et al.*, 2002), seed germination, growth and development (Almaghrabi & Abdelomoneim, 2012). Dhanda *et al.*, (2004) reported that seed vigor and seedling growth are highly susceptible to water deficient condition. In this study, shoot length of 21 rice genotypes reduced with increasing the level of PEG (10% to 15%) (Table 3). Similar to our results, Govindaraj *et al.*, 2010 and Ashraf *et al.*, 2008 have demonstrated that decreased shoot length in drought-stressed pearl milletand wheat due to increasing of drought stress, respectively. Osmotic stress caused by PEG influenced the plant growth resulting reduced fresh biomass of plant (Pirdashti *et al.*, 2003). In present study, plant fresh and dry weights of 21 rice genotypes were reduced under PEG_{-6000} (10% and 15%) regimes (Tables 4-5). This reduction is directly proportional to the intensity of PEG concentration (Saghafikhadem, 2012). Some other researchers have also described similar results where higher levels of PEG caused more reduction in fresh weight of rice genotypes (Farooq *et al.*, 2009; Luis *et al.*, 2012).

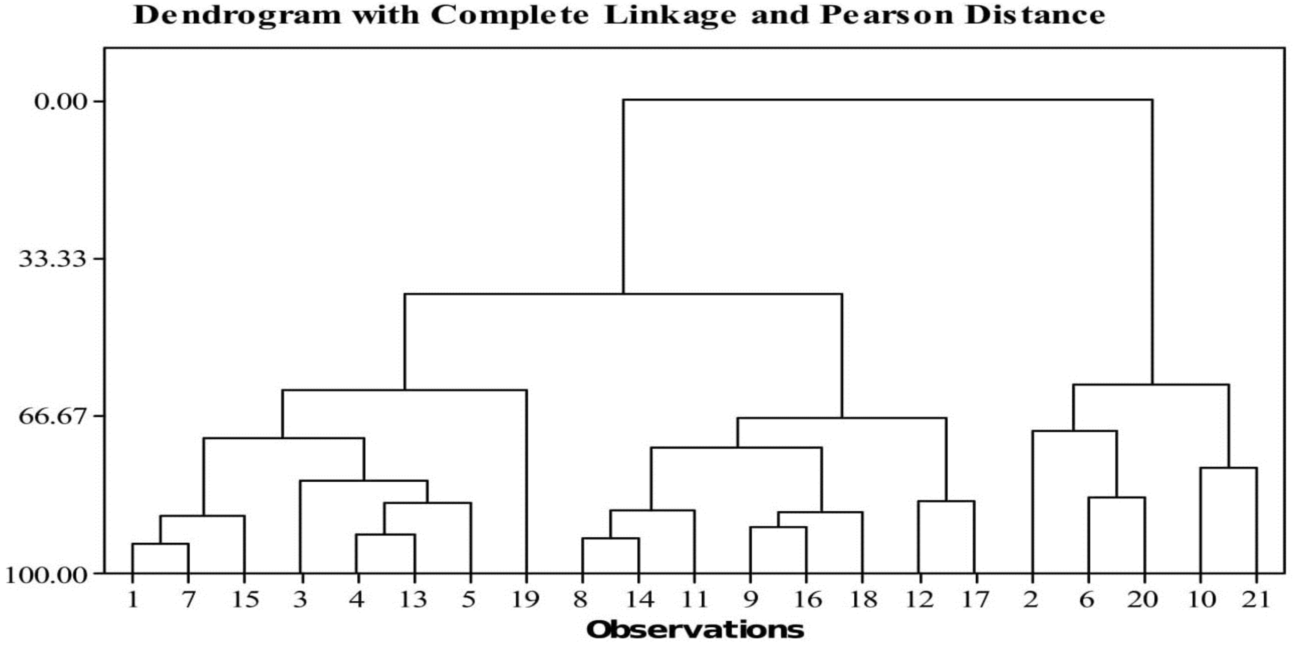

Fig. 1. Custer 1 includes 8=HTT-53, 9=HTT-74, 11=HTT-92, 12=HTT-97, 14=HTT-104, 16=HTT-119, 17=HTT-125, and 18=HTT-132, cluster 2 consists of 1=HTT-18, 3=HTT-25, 4= HTT-29, 5=HTT-31, 7=HTT-51, 13=HTT-98, 15=HTT-114, and 19=HTT-138; cluster 3 consists of 2=HTT-19, 6=HTT-39, 10=HTT-81, 20=Super Bas and 21=IR-64.

Maintenance of water status is primary challenge for plants facing water deficit stress. Imbibition increases the seed water contents to a certain level at initial stages, which may gradually decrease after radicle emergence (Ajouri *et al.*, 2004). Water scarcity at seedling stage impairsthe field performance and healthy growth of seedlings (Ahmad *et al.,* 2015). Thus, there is establishing a relationship between RWC and biomasses. Obviously, our study exhibited that RWC reduced in all rice genotypes under drought stress (Table 6). Reduction in RWC was also documented in other crops like barley and wheat under drought stress (Sallam *et al.*, 2019). RWC is also good indicator and has positive relationship with photosynthetic rate (Toscano *et al.*, 2016). Electrolyte leakage (EL) was influenced by stress, season, age, sampling part and plant species (Agarie *et al.*, 1995). In our study, EL raised from 22 to 79% under 10 to 15% level of PEG_{-6000}, respectively. Maximal increased in EL (897.85%) were recorded in HTT-39, while the minimum value in HTT-138 (301.66%) as shown in Table 6. The EL has considerable negative correlation (r = -0.4186***; -0.3821***; -0.3474***; -0.4082***; -0.4670***) with RLSI, SLSI, PFWSI, PDWSI and RWC which were considered as main indicators of drought tolerance (Table 9). Less membrane injury in present study was associated with rise of sugars accumulation in the leaves during drought stress (Bajji *et al.*, 2002).

Increased lipid peroxidation due to the drought-induced accumulation of hydrogen peroxide has been reported to inhibit photosynthetic potential (Sharma *et al.*, 2012). Drought stress enhanced the generation of toxic ROS (H_2O_2) and lipid peroxidation (MDA). Plants with more ROS generation under drought stress exhibited greater injury to membranes as evident in the form of more MDA and RMP levels (Anjum *et al.*, 2017). In present work, there was a substantial difference in MDA contents among the genotypes. The MDA contents was elevated under drought stress in many genotypes, however it remain constant in some genotypes. The maximum MDA recorded in HTT-39, which was drought sensitive genotype (Table 7). In the present investigation, we have observed a considerable negative correlation of MDA with RLSI(r = -0.2197***), SLSI (r = -0.3955***), PFWSI (r = -0.3310***), PDWSI (r = -0.3349) and RWC (r = -0.5539***) which were considered as main indicators of drought tolerance (Table 9). Wang *et al.*, (2009) determined a negative correlation between MDA and herbage yield in alfalfa.

From the data of RLSI, SLSI, PFWSI, PDWSI, RWC, EL, MDA and H_2O_2, it is apparent that morpho-physiological indices can be used to select the rice genotypes for drought tolerance. Several rice mutants like (HTT-119, HTT-74, HTT-92, HTT-97, HTT-104, HTT-119, HTT-125 and HTT-132) maintained intermediate scores and can be used as a moderately tolerant check, which pooled them in cluster 1 in dendogram. The genotype HTT-138 was the maximum scores for indices followed by HTT-18, HTT-25, HTT-29, HTT-31, HTT-51, HTT-98, HTT-114, which pooled them in cluster 1 in dendrogram, can be considered as tolerant one. While some rice mutants like HTT-19, HTT-39, Super Bas, HTT-81, and IR-64 maintained below average scores, considered them as sensitive for drought stress, and grouped them in cluster 3 in dendogram (Fig. 1). These results correlates with the findings of Ashraf *et al.*, (2008) and Kausar *et al.*, (2012). Therefore, the selected rice mutants have a genetic potential for drought tolerance and HTT-138 can be cultivated in lands directly with low moisture contents.

An analysis of correlations between different morpho-physiological indices revealed that there is significant and positive correlation of RWC with RLSI (r = 0.3376***), SLSI (r = 0.5622***), PFWSI (r = 0.5218***) and PDWSI (r = 0.5493***) that are recorded in Table 9, which showed that these indices might be used to select the rice genotypes for drought tolerance in this work. Ashraf *et al.*, (2008) and Kausar *et al.*, (2012) determine a positive correlation between physiological indices in wheat are good screening techniques for drought tolerance. Assembly of crops for the enhancement of drought tolerance is a good strategy to achieve cost-effective yields (Marium *et al.*, 2019). The positive and significant correlation between RLSI, SLSI, PFWSI, PDWSI and RWC suggested that these indices could be a consistent and effective method for evaluating drought tolerance in rice genotypes. The evidence about significant correlations among growth attributes is essential for beginning of any breeding program because it gives a chance for screening of desired genotypes with desired characters together (Ali *et al.*, 2009).

Many workers have made different groups of wheat genotypes using cluster analysis based on various characteristics (Nookra & Khaliq, 2007; Zafar *et al.*, 2015). Many reports and research work suggested that cluster analysis are used to select the genotypes for stress tolerance (Noorifarjam *et al.*, 2013). In present study, over all cluster 1 included eight mutants (HTT-119, HTT-74, HTT-92, HTT-97, HTT-104, HTT-119, HTT-125 and HTT-132) considerably performed well than others for all tested physiological indices and considered as drought tolerant ones. Cluster 2 contained eight mutants (HTT-18, HTT-25, HTT-29, HTT-31, HTT-51, HTT-98, HTT-114 and HTT-138) performed intermediate and considered as moderately tolerant. While cluster 3 comprised of five genotypes (HTT-19, HTT-39, Super Bas, HTT-81, and IR-64) performed below average therefore, considered as sensitive. Screened genotypes can be used in breeding programs for drought stress tolerance further.

In conclusion, results suggested that the genetic potential for drought stress tolerant rice genotypes could be assessed by using physiological indices at an early seedling stage. Positive and significant correlations between different indices and cluster analysis also demonstrated that screening of rice genotypes based on physiological indices are considered as drought tolerant. The genotypes HTT-138 and HTT-51 are drought tolerant can be further used in drought areas to increase the development and yield of rice genotypes in drought-hit areas of the world. Tolerant genotypes can be recommended to cultivate on lands directly with low moisture contents.

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