Research Article

Screening of Elite Coarse Rice Lines for Drought Stress Simulated by Polyethylene Glycol (PEG) at Seedling Stage

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Abstract | Among various abiotic stresses causing serious impact on crop growth, drought poses a significant reducing stress towards crop yield and its stability over the time and locality. In terms of sensitivity to this environmental stress, rice, which is a drought-sensitive crop, exhibits notable varietal variability. for the estimation of elite coarse rice lines to withstand against the drought stress especially during early plant developmental phases and germination and seedling stage, an experiment was carried out from June to July 2021 at the Rice Molecular Breeding Laboratory of the Rice Research Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad. Ten elite coarse rice lines were subjected to varying degrees of drought stress as 0, 10, 15 and 20% through the application of Polyethylene glycol (PEG-6000). To carry out experimentation, carried out it following completely randomized design. Germination was found to be negatively associated with stress level as germination was 100% in the control and 0% in the highest stress level (20% PEG). All attributes examined were shown to differ significantly across all levels of drought stress. For treatment, traits such as germination rate, emergence percentage, vigor index, seedling height, fresh weight and dry weight of seedling are significant at 10% alpha, likewise the interaction of genotypes x treatments (G x T) is significant for seedling height at 1% alpha in all rice lines, seedling height and dry weight decreased along with elevated stress levels. Four lines, NRPC-9, NRPC-7, NRPC-6, and NRPC-1 were shown to be tolerant at 15% PEG 6000 water stress level while all lines tolerated 10% PEG induced water stress. It was concluded from our findings that elite coarse green super rice (GSR) lines viz; NRPC-9, NRPC-7, NRPC-6, and NRPC-1 are good drought tolerant and can be exploited in future breeding initiatives for variety development with increase drought stress.

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Introduction

Abiotic stresses, which are increasing throughout the world, have a negative impact on agriculture's productivity by affecting the growth of plants, which lead to the failure of crop and pose a serious threat to crop production (Wu *et al*., 2011; Musclo *et al*., 2014). Due to drastic change in climate and increased in the population of world, it is becoming difficult to meet the human requirements (food, shelter, and other necessities) (HongBo *et al*., 2005; Akram, 2007). Rice plant is sensitive to drought conditions, as it exhibit negative impact when subjected to the moisture stress condition at critical stages of development (Cha-Um *et al*., 2010) and approximately 50% of the world's rice crop production is impacted by water shortage conditions (Mostajean and Rahimi-Eichi, 2009). The impact of water stress was greater during the germination and seedling stages, which shows the crop sensitivity to moisture stress at these stages, effect the overall success of a crop (Tsago *et al*., 2014; Khayatnezhad *et al*., 2010). Water is a limiting abiotic stress that lowers plant yield, drought stress cause reduction in the development process of rice plant (Purbajanti *et al*., 2017). The water scarcity cause drought condition which triggers the numerous plant responses include cell division, cell growth, stomata conductress, photosynthesis rate, transpiration rate and eventually yield of plant (Chaves and Oliveira, 2004; Cattivelli *et al*., 2008; Tuna *et al*., 2010). Plants respond differently under water deficit conditions, some shows tolerance and some shows avoidance mechanism (Nazar *et al*., 2015).

Rice crop is significantly affected by the drought stress which limits its production per unit area in Pakistan. Several factors can contribute to drought stress in rice crops, the major factor is climate change that results in increased temperatures and changing rainfall pattern, which can lead to drought stress in rice crop (Faroq et al., 2015). Second one is the water scarcity and limited access to irrigation water at the time of transplanting or even lateral crop stages specially at flowering time dry weather and heat stress conditions also cause evapotranspiration losses of water both in the file and affect crop physiology. Soil conditions including high salinity and poor crop management practices for irrigation such as less irrigation due to fear of increase cost of production due to power expenditures for ground water irrigation also led to drought stress in rice crop. On an average almost 1500 liters of irrigation water is utilized to gain 1 Kg of rice, major outflow goes in the form of evapotranspiration, percolation, and seepage. Under the present scenario of climate change and increased population pressure water availability to raised crops is challenging. Screening of rice genotypes resilient to climatic stresses specially drought stress is prerequisite for drought tolerant variety development.

Plants are screened for moisture stress using a substance called polyethylene glycol (PEG). The rice crop's stunted development is caused by a lack of water, and this condition also affected the rate of photosynthesis and the length of the charging period, which in turn reduced the grain yield of rice (Sayar *et al*., 2008). Breeders should give careful consideration to morphological analyses of the traits linked to tolerance and yield improvement when selecting genetic components for the development of new variety. In breeding projects, choosing, and using features related to yield and tolerance could result in the development of sustainable varieties in stress-prone areas (Masud *et al*., 2022).

Water scarcity shows strong associated connection to limit the rice crop productivity and quality, and its effects are expected to become more severe with increasing climate variability and change. Despite extensive research efforts, we could not reveal a concise and open information related to drought tolerance mechanism of rice.

There is a need to unveil the impact of drought on rice crop which may be achieved by artificially inducing drought stress by the application of polyethylene glycol (PEG) as it produces almost similar level of hindering effects on plant. PEG treatment induces osmotic stress in plants, simulating drought stress, and which ultimately helps to figure out the genotypes with tolerance potential to withstand drought levels.

PEG-induced drought stress has been widely adopted method as it is reliable and repetition of similar stress is possible for the confirmation of genetic potential of genotypes and to test the tolerance efficacy as well. reproducible method for screening rice germplasm for drought tolerance. PEG treatment has also been used to identify key characters which may pose a significant role towards the drought tolerance by improving water use efficiency and normalizing the osmotic adjustment.

The basic objective of this experiment was to reveal the potential of rice germplasm for their genetic capability to withstand the severity of drought stress being induced at various levels by polyethylene glycol (PEG) during early growth stages. The study aims to identify the most suitable parent plants with higher tolerance to PEG, an indicative of drought tolerance, which further may be employed in our breeding program in different crossing schemes. The use of PEG as a stress inducer in this experiment provides an opportunity to assess the ability of rice plants to survive under limited water availability conditions, which is an essential trait for rice cultivation in regions with limited water resources. By identifying the genotypes with higher PEG tolerance, the study can provide insights into the genetic mechanisms lying behind the ability to tolerate drought stress. Exploitation of the potential parent genotypes having higher degree of tolerance may lead towards the development of improved drought tolerant rice verities.

Materials and Methods

Experimental site

Experiment was carried out at Rice Molecular Breeding Laboratory of Rice Research Program, Crop Sciences Institute, National Agriculture Research Centre, Islamabad between June to July 2022.

Material

In this experiment 10 coarse rice GSR lines obtained from PGRI, NARC were used against various levels of drought stress (Table 2) at germination and seedling stage.

Table 1: *Germplasm used in the present study.*

S. No.	Genotypes	S. No.	Genotypes
1	NRPC-1	6	NRPC-6
2	NRPC-2	7	NRPC-7
3	NRPC-3	8	NRPC-8
4	NRPC-4	9	NRPC-9
5	NRPC-5	10	NRPC-10

Table 2: *Different level of drought stress (PEG induced).*

S. No.	Treatments
1	T0= Control
2	T1= 10% PEG
3	T2=15% PEG
4	T3=20% PEG

Method

The rice lines were tested against 4 concentrations of Polyethylene glycol 6000 (PEG-6000) at 0, 10, 15 and 20% induced drought stress. For the execution of experiment, completely randomized design was adopted with 2 factors and two replications. First factor was 10 GSR lines while second factor was four (0, 10, 15 and 20%) level of drought stress. The 5% sodium hypochlorite solution was used to immerse the seeds for 5 min followed by washing thrice with distilled water. Petri plates containing filter paper were arranging in CRD design, 5 seeds of each entry (rice line) were transferred into petri plates and treated with respective treatments. Distilled water was used in treatment 1 whereas for other treatments first two days 5ml of PEG solution was used twice a day and then on later days 5 mL PEG solution was used once a day. Each entry treated with respective treatment was allowed to germinate at 25 °C. The data on germination was noted on daily basis until full germination. In tenth day germinated seeds were taken out for data collection.

Parameters studied

The parameters examined in the study were as follows: Seedling fresh and dry weight, stress tolerance index, germination rate and percentage with stress tolerance index (GSTI) and vigor index.

Germination percentage: After 10 days of seed sowing germination percentage was calculated by using following formula (Yari *et al*., 2010).

$$\text{Germination (\%)} = (\text{germinated seed/ total number of seeds}) * 100$$

Germination stress tolerance index: The germination stress tolerance index (GSTI) was determined as a percentage and for the calculation of GSTI promptness index was required. Promptness index was calculated by using the following formula (Ashraf *et al*., 2008).

$$PI= (nd1*1.0) + (nd2*0.75) + (nd3*0.50) + (nd4*0.25)$$

Whereas nd1 is the number of seeds that germinated on day 1, nd2, nd3, and nd4 accordingly, and PI denotes the promptness index.

$$GSTI = \text{PI of stressed /PI of Control}$$

Seedling height, fresh and dry weight: Seedling length was measured in centimeter by using scale

Table 3: *Mean square of the analysis of variance of measured traits in rice genotypes under drought stress.*

S.O.V	GR	GE	VI	SH	SFW	SDW
G	18182	2534.1**	4572	1.241*	0.000107	1.5E-06
T	294694***	23040***	412740***	42.025***	0.215502***	0.005243***
G*T	9104	174.5	8226	1.514**	0.012224	0.000135
Residuals	8511	214.5	2144	0.199	0.010468	0.000192

*GR: Germinate rate; GE: Germination emergence (%); VI: Vigor index; SH: Seedling height(cm); SFW: Seedling fresh weight(g); SDW: Seedling dry weight(g), *=significant at 5% alpha, **= significant at 1% alpha, ***= significant at 10% alpha.*

Table 4: *Correlation matrix of the variables.*

	SH	SDW	SFW	GR	GE	VI
SH	1.0000000					
SDW	0.5656065	1.0000000				
SFW	0.4983885	0.87391593	1.0000000			
GR	-0.5006166	- 0.35930451	-0.3535502	1.0000000		
GE	0.6695678	0.40561925	0.4071369	-0.8845300	1.0000000	
VI	0.9811822	0.54470360	0.5071867	-0.5021269	0.7081684	1.0000000

GR: Germinate rate; GE: Germination emergence (%); VI: Vigor index; SH: Seedling height (cm); SFW: Seedling fresh weight(g); SDW: Seedling dry weight(g).

whereas seedling fresh weight was measured in (g) by using digital weighing balance and seedlings were dried 24 hours at 70°C then by using digital weighing balance seedling dry weight was measured.

Seedling fresh and dry weight stress tolerance index: The following formula was used to compute the stress tolerance index for seedling fresh weight and dry weight.

$$SFWSTI= (SFWS/SFWC) *100$$
$$SDWSTI= (SDWS/SDWC) *100$$

Where SFWS = seedling fresh weight stressed, SFWC = seedling fresh weight control, SDWS = seedling dry weight stressed, and SDWC = seedling dry weight control; SFWSTI = seedling fresh weight stress tolerance index and SDWSTI = seedling dry weight stress tolerance index.

Vigor index: According to Sagar (2017), the vigor index (VI) was determined using the method below:

$$VI= SL/GE$$

Where SL= length of the seedling and GE= germination rate (germination percentage).

Statistical analysis

Data collected was analyzed statistically for analysis of variance (ANOVA) by using R studio agricolae package) and (least significant differences (LSD) was applied at 0.05% for comparisons of means among treatments. Correlation and principal component analysis (PCA) were done by using R Studio 4.2.1 (Facto extra package).

Results and Discussion

To evaluate the importance of specific traits (variables), a two-step process was followed. First, an analysis of variance was conducted, and then a correlation matrix was generated for all the traits. The results, shown in Table 4, revealed that several variables exhibited significant positive correlations. These correlations could be explored further using principal component analysis (PCA). The Eigen values in Table 5 explain the partitioning of total variance that account into each component (principal component) and explained the variance percentage and cumulative proportion for all components (Raza *et al*., 2017). For the number of principal components in Table 5, eigenvalues greater than 1 were taken into consideration Figure 9 scree plot, in this regard PC1 and PC2 were retained Table 6. Principal component analysis was used to evaluate the relationship between the attributes of 10 elite lines of rice. PCA classified 6 traits into two components, PC1 and PC2 contributed 84.5% of total variance, PC 1 with highest variance of 65.7% and PC2 with 18.8% as observed in Figure 10.

Table 5: *Eigen analysis of the variables.*

Variables	Eigen value	Variance percentage	Cumulative percentage
SH	3.9	39	39
SDW	1.1	11	50
SFW	0.7	7	57
GR	0.1	1	58
GE	0.1	1	59
VI	0	0	59

GR: Germinate rate; GE: Germination emergence; VI: Vigor index; SH: Seedling height; SFW: Seedling fresh weight; SDW: Seedling dry weight.

Table 6: *Structure of first four principal components.*

Variable	PC1	PC2
SH	-0.4453294	-0.06262574
SDW	-0.3822033	0.54525627
SFW	-0.3695610	0.55456305
GR	0.3709988	0.45148173
GE	-0.4246018	-0.42468171
VI	-0.4484419	-0.08392336

GR: Germinate rate; GE: Germination emergence; VI: Vigor index; SH: Seedling height; SFW: Seedling fresh weight; SDW: Seedling dry weight.

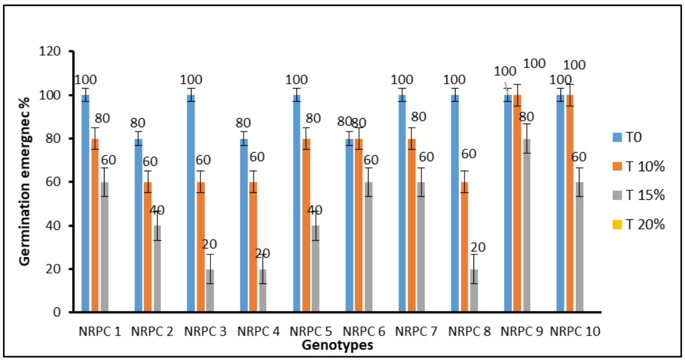

Figure 1: *Effect of Drought stress on germination emergence percentage (GE %).*

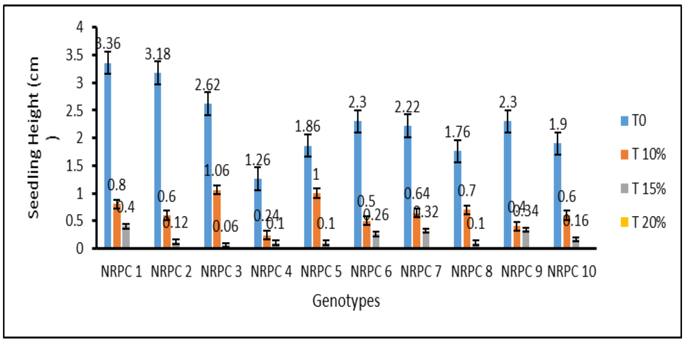

Figure 2: *Effect of drought stress on seedling height (cm).*

Based on treatment PC1 and PC2 divide into 3 groups in Figure 10, the traits SDW and SFW depicted positive and close association with each other and with treatments in PC2 whereas only one trait GR showed positive association with treatment in PC 1. Based on treatment PC1 and PC2 divide into 3 groups in Figure 10, in first group all traits lie except GR, SFW and SDW have positive association whereas VI and SH have positive association with each other but negative association with treatments. Correlation between SDW and SFW was highly significant Table 4. The graph Figure 10 shows that objects are displayed as points. The observations in three groups are represented with respect to PC1 and PC2.The variables are depicted as vectors. The average value of each variable and the performance of each object against a said variable can be predicted by the origin. Just at the center, value refers to 0 while moving away arrows indicate the variable vectors and predict loading scores of each variable towards overall variability. The variable vector indicates how well the variables are explained by the graph as in Figure 10. The angle between the arrows indicates relationship between the variables. For instance, the variables SFW and SDW are strongly and positively correlated because of the close angle between them. Similarly, GE and SH are positively associated. GR and SFW are negatively correlated and in correlation table we can validated this they have a negative value. Similarly, GR and SFW are negatively correlated. As most of the variation is explained by GE, SH, and GR due to the greater length of variable vectors. The variables GE and SH are positively correlated and fall under PC1 whereas GR fall under PC2.

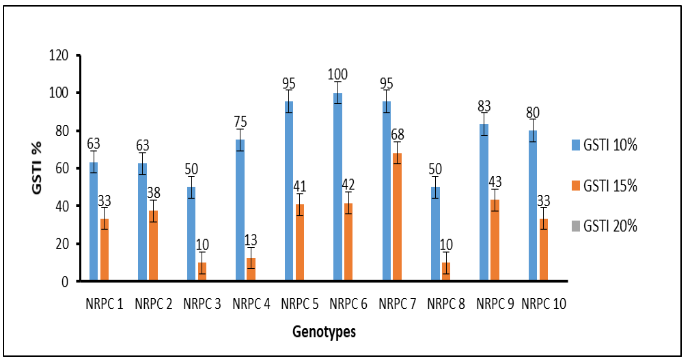

Figure 3: *Germination stress tolerance index (GSTI).*

The plant life cycle's most crucial stage is germination. To ascertain the tolerance level of rice germplasm under moisture stress (water deficit) circumstances, the effects of increased moisture stress generated by PEG on seed germination and the response of elite coarse GSR lines by increasing the PEG concentrations were examined. The mean square analysis of variance (Table 3) showed that for germination emergence

(GE) percentage and for germination rate was highly significant among treatments. The results obtained from the study showed that maximum percentage germination was found in control (T0) whereas zero percent germination (seeds were not germinated) was observed at 20% PEG concentration.

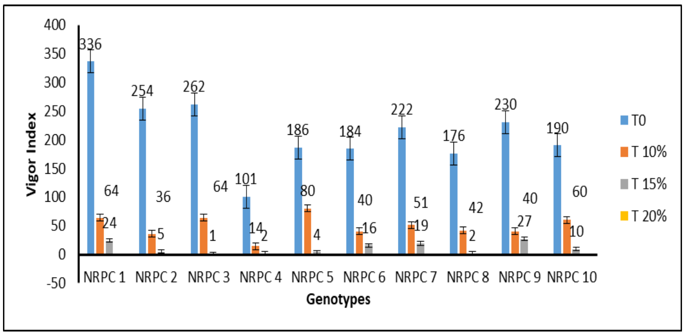

Figure 4: *Effect of drought stress on the vigor index (VI).*

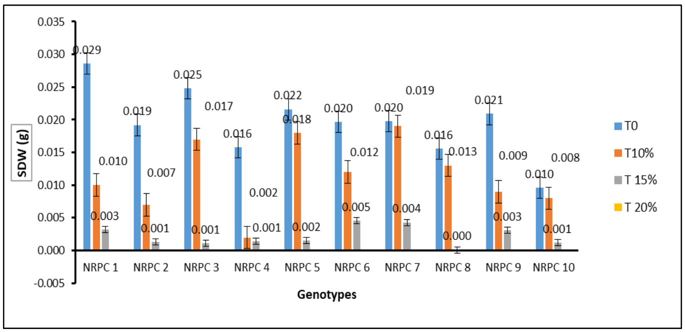

Figure 5: *Seedling dry weight under different level of drought stress (SDW).*

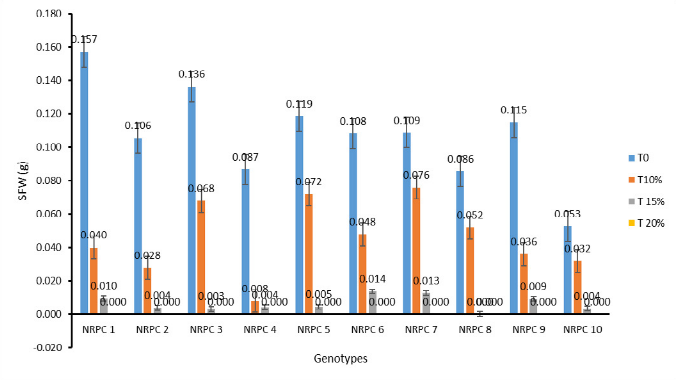

Figure 6: *Seedling fresh weight under different level of drought stress (SFW).*

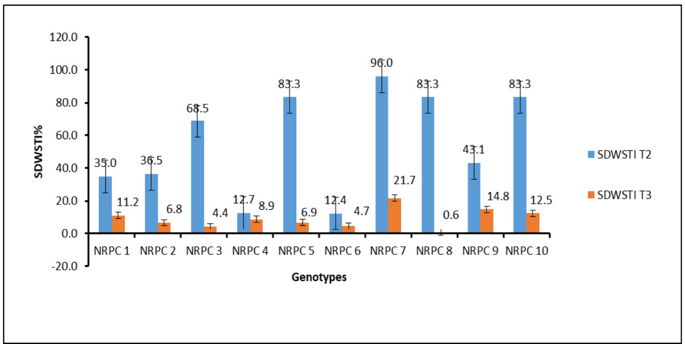

Figure 7: *Seedling dry weight stress tolerance index (SDWSTI).*

In Figure 1 graph showed that increased in concentration of PEG result in decreased germination percentage. Under control (T0) condition all genotypes showed 100% germination whereas in treatment two at 10% PEG maximum germination (100%) was observed in NRPC 9 and NRPC 10 followed by NRPC 1, 5, 6 and NRPC 7 with 80% germination whereas minimum GE (60%) was found in NRPC 2, 3, 4 and NRPC 8. In treatment three at 15% PEG concentration NRPC 9 showed maximum GE (80%) and minimum GE (20%) was observed in NRPC 3, 4 and 8. Table 3 shows vigor index among treatments was highly significant. In Figure 4 graph showed the vigor index of all rice lines highest index was found in control (T0) condition (336) in NRPC 1 and the lowest vigor index was observed at 15% PEG induced moisture stress (1) in NRPC 3. Under treatment two 10% PEG induced water stress highest index was found in NRPC 3 (64). At 15% PEG imposed moisture stress highest vigor index was observed in NRPC 9 (27).

The GSTI (germination stress tolerance index) showed similar pattern in Figure 3, declining as PEG concentration increased. Figure 3 graph demonstrated that NRPC 6 at 10% PEG-induced stress had the greatest GSTI (100), while NRPC 3 and NRPC 4 had the lowest (50). The highest GSTI was found in NRPC 7 (68) and the lowest (10) in NRPC 3 and NRPC 8 at 15% PEG concentration. The water potential in the osmotic solution decreases as PEG concentrations rise because there is less oxygen available for the seed to germinate (Purbajanti *et al*., 2019; Ashaduzzaman *et al*., 2020).

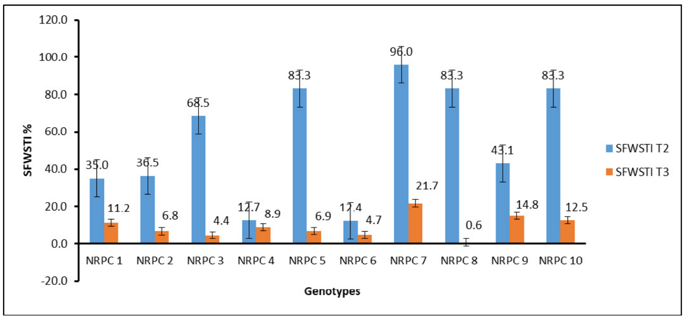

Figure 8: *Seedling fresh weight stress tolerance index (SFWSTI).*

ANOVA showed (Table 3) significant result among treatments for seedling fresh weight, within treatment significant and between genotypes non-significant. Figure 6 revealed that maximum fresh weight was observed in control (T0) condition (0.1573 g) in NRPC 1 followed by T1 (10% PEG) with fresh weight of (0.1 g). At 15 % PEG (T2) highest seedling fresh weight was observed in (0.014g) in NRPC 6. In all the green super rice lines seedling fresh weight decreased by increasing PEG concentrations. For seedling dry weight, the analysis of variance (Table 3) revealed significant results within treatments and non-significant results within genotypes and for genotype x treatment interaction was not significant. The results of the Seedling Fresh and Dry Weight Stress Tolerance Index (SFWSTI and SDWSTI) showed that as the level of drought rose, seedling fresh weight stress tolerance decreased. The SWFSTI of rice seedlings subjected to higher PEG concentrations showed a discernible decline (Figure 8). In NRPC 7, the greatest SWFSTI value (96.0) was found at 10% PEG concentration, and the smallest value (21.7) was found at 15% PEG concentration. Similar outcomes were seen in Figure 7, where SDWSTI decreased as the level of drought stress increased. Several studies revealed consistent trend of decreased seedling fresh and dry weight under more severe drought stress conditions (Ji *et al*., 2012; Mostajeran and Rahimi-Eichi, 2009). The low availability of water to plants is due to osmotic stress which causes a reduction in cell elongation and division process therefore by lowering cell growth and the turgor pressure as a result, there is a corresponding decrease in dry mass and biomass. (Farooq *et al*., 2015; Sagar, 2017; Roy *et al*., 2018; Ashaduzzaman *et al*., 2020; Islam *et al*., 2018).

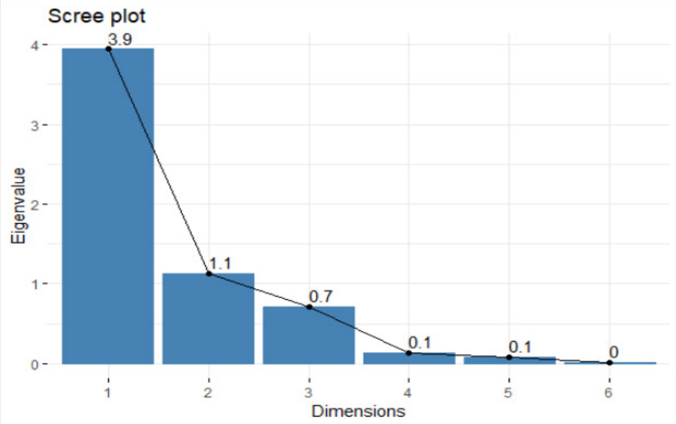

Figure 9: *Scree plot showing variance of each principal component.*

The ANOVA showed (Table 3) significant variance among treatments whereas non-significant among genotypes for seedling height. The interaction among genotype and treatment showed significant difference at 1% alpha. The graph in Figure 2 showed maximum seedling height was observed in NRPC1 (3.2800 cm) in control condition (T0). Seedling height decreased with increasing PEG concentrations at 10% PEG maximum seedling height was found in NRPC 3 (1.0050 cm) and at 15%PEG concentration maximum height was found at NRPC 1 (0.3250 cm). The water stress condition inhibits the emergence of radicle is due to decreased water potential gradient between seed and the external environment, therefore impairs height of seedling (Murillo-Amador *et al*., 2002; Sokoto and Muhammad, 2014). This could be a result of the negative impacts of water stress on the growth of root cells, which would impede nutrient uptake and negatively impact photosynthesis, which is necessary for biomass buildup and, consequently, shoot and root elongation. Therefore, it appears that under water stress, plants' ability to absorb and use water is reduced to the point where their drought-tolerance mechanisms cannot support continued normal development (Ashaduzzaman *et al*., 2020; Islam *et al*., 2018; Magar *et al*., 2019).

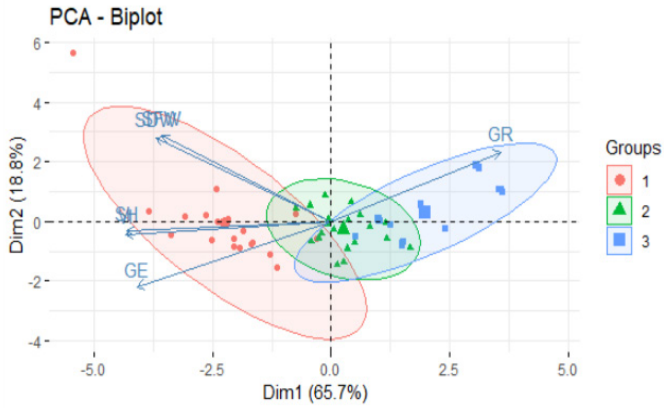

Figure 10: *Biplot for Treatment Based on PC1 And PC2.*

Conclusions and Recommendations

The results of this experiment led to the conclusion that the drought has a distinct impact on the seed germination and seedling growth, as PEG concentration increases the percentage of seeds that germinate decrease. Selection may be based on these traits at an early growth stage for the purpose of screening large populations against drought stress. Screening the germplasm at an early growing stage would be more productive, cost effective and labor intensive. The study also showed that variance among genotypes for germination stress tolerance index (GSTI) was proven to be a valid indicator of drought tolerance in rice. Germination was not seen at 20% PEG concentration, all lines survived at 10% PEG, but at 15% PEG under conditions of drought stress, resistant lines could be exploited further in breeding initiatives to create drought-tolerant cultivars.

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From the bottom of my heart, I sincerely appreciate each author who helped with this experiment's execution, data analysis, or manuscript writing.

Novelty Statement

In-vitro screening of rice germplasm for induced drought stress is a rapid technique by creating artificial drought stress condition by using polyethylene glycol (PEG) is a unique idea. A flux of rice germplasm can be screened for drought stress in short period of time with less space in Lab.

Author's Contribution

This study was conceptualized and planned by Abid Majeed.
Data entry, writing up, and manuscript formatting were assisted by Faiza Siddique.
Muhammad Shahzad: Assisted with data collection.
Data analysis was aided by Irum Raza.
The document was examined by Muhammad Arshad.
Alvina Hanif: Assisted with graph preparation.
Rana Arsalan Javaid: Assisted with data analytic cross-checking.
Maria Rabnawaz: Assisted with structuring the document and writing references.

Conflict of interest

The authors have declared no conflict of interest.

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