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Evaluating Drought Tolerance in Rice through *In-Vitro* PEG Screening and Analysing the Effectiveness of drought Tolerant Traits through Correlation

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ABSTRACT: Rice is considered a staple food for half of the world's population, is notably vulnerable to a spectrum of abiotic stresses, with drought ranking among the most formidable challenges due to its detrimental impact on yield. Evaluating drought tolerance in field conditions is a labour intensive and time consuming process. Consequently, an alternative approach involves the use of polyethylene glycol to artificially induce drought. The main aim of this study was to identify rice genotypes that exhibit early-stage drought tolerance from a pool of 95 genotypes. To accomplish this, polyethylene glycol (PEG-6000) was harnessed as an artificial inducer of drought stress, facilitating the examination of a diverse range of rice genotypes. The experimental protocol was executed under a Completely Randomised Design (CRD) with four distinct PEG concentrations and three replications. As PEG solution concentrations escalated from the control (0%) to 15%, 20%, and 25%, the examined traits encompassing germination percentage, shoot length, root length, seedling fresh weight, seedling dry weight, total seedling length, relative water content and seedling vigour index experienced significant reductions. Subsequent correlation analysis illuminated strong positive relationships among these traits under the imposed stress conditions. This research not only enhances our understanding of trait interactions during drought stress but also provides valuable insights for the potential refinement of drought-tolerant rice cultivars.

Keywords: Rice, Drought, Polyethylene glycol (PEG), Correlation.

INTRODUCTION

Rice belongs to the *Oryza* genus and the *Poaceae* family. Rice is considered as an essential need for more than half of the global population and serves as a fundamental dietary resource for the majority of people. In India, rice is consumed by 65% of the population. Rice cultivation occupies a wide range of area since it is considered as a significant crop among the cereals. For the cultivation of the rice crop, water plays a significant role for the yield of the crop. Most of the area under cultivation is irrigated and requires artificial sources of irrigation for the growth of the crop (Verbeeck *et al*., 2023). Due to the uneven distribution of rainfall, farmers are dependent on irrigation for the water supply (Kumar *et al*., 2022).

A major challenge for agriculture, especially in developing countries, is climate change, which affects rainfall levels and causes various abiotic stresses on plants. Drought is recognized as the primary restricting abiotic element affecting the growth and production of the rice crop and around half of the area under rice is affected by drought (Maurya *et al*., 2021; Panda *et al*., 2021). Drought is more severe in South and South East Asian and African countries (Swapna *et al*., 2017). In Asia 131 million hectares of rice land is affected by drought and lead to decrease in rice production (Kumar *et al*., 2022).

Rice is one of the most crucial crops and is highly vulnerable to water stress, especially during critical stages such as seed germination, early seedling growth, panicle initiation, anthesis, and grain filling. The consequences of drought stress on rice differ depending on the timing, length, water accessibility, rice variety, and growth phase (Sagar *et al*., 2020). In response to environmental challenges, plants have evolved a robust protective strategy that employs a combination of cellular and molecular responses to enhance their resilience. The serious symptoms of drought become visible after 7-10 days after the cessation of irrigation in the uplands, similarly they become visible after 18-21 days in the case of the lowlands (Swapna *et al*., 2017). The drought condition to be used for drought screening must be similar to that prevailing in the area (Swapna *et al*., 2017). Adequate drought screening is necessary for the successful identification of high-yielding rice varieties in drought prone conditions and to accurately distinguish between drought tolerant and drought susceptible lines (Anik *et al*., 2021).

In an effort to enhance plant productivity in regions susceptible to drought, scientists are exploring plants' reactions to water stress conditions and assessing the effectiveness of novel genotypes in these circumstances. The initial growth of plants in stress environments heavily relies on the successful germination of seeds and the subsequent development of seedlings. It is a well-known fact that a plant's tolerance in its mature state is determined by its tolerance in its early stages (Sagar *et al*., 2020). The germination stage drought tolerance is the major characteristic for a rice variety to adapt in areas with less water resource (Wang *et al*., 2019).

Plant behaviour under environmental stress is complicated and involves multiple genes, but the function of many genes is unknown. Because of this complex nature, breeding drought-tolerant cultivars is difficult (Swapna *et al*., 2017). Plant selection under field conditions can be cumbersome due to factors such as limited heritability, the considerable time involved, and the complicated interplay between environmental influences and genotypic traits (Rahim *et al*., 2020). The conventional breeding method is widely used to evaluate breeding stocks by testing them under different field conditions such as irrigated and drought conditions. In addition to these, several *in vitro* studies are being conducted to identify the traits of drought tolerance by artificially inducing drought using polyethylene glycol, and varieties exhibiting superior performance for these traits have also been identified (Altaf *et al*., 2021). To facilitate the easy identification of rice genotypes that can thrive in drought-prone conditions, conducting a comprehensive drought screening process is critical. This process should aptly differentiate between genotypes that are susceptible to drought and those that show the ability to withstand such challenging circumstances (Islam *et al*., 2018).

Application of *in vitro* conditions with polyethylene glycol (PEG) is an alternative technique to field experiments that uses moisture stress (Sahu *et al*., 2023). Polyethylene glycol, particularly PEG 6000, serves as a high molecular weight osmotic agent and does not penetrate cell membranes. This feature leads to a reduction in water potential and allows the creation of an artificial soil drying environment (Majid *et al*., 2020; Ahmad *et al*., 2020). PEG can be used for *in vitro* drought study of the rice seedlings and mass screening can be done because it is easy and fast (Anik *et al*., 2021). In the study conducted by Sampath *et al*. (2022), the researchers investigated the physiological and molecular reactions of rice genotypes when subjected to drought stress during the seedling phase. The primary aim was to identify drought resistant rice varieties, an investigation carried out in a controlled laboratory setting employing PEG 6000. In a study conducted by Fatimah *et al*. (2023), the screening of drought tolerance in early-stage red rice (*Oryza sativa* L.) landraces was undertaken using two levels of PEG 6000. The application of PEG led to decreased growth parameters across all variables due to limited water availability within the medium, in contrast to the control. This process aided in the identification of ten drought-tolerant genotypes during the germination stage. Siddique *et al*. (2023) conducted research on screening of elite coarse rice lines for drought stress at the seedling stage using polyethylene glycol (PEG) simulation. The study revealed the marked effects of drought on seed germination and seedling growth, with a decrease in germination percentage as the PEG concentration increased. The results of the study identified four promising drought-tolerant lines suitable for future breeding efforts. The innovative approach to induce drought stress by *in vitro* screening of rice germplasm using PEG provides a fast and efficient technique. This method enables the rapid screening of a diverse range of rice germplasm for drought stress within a compact lab space and a short time frame. Evamoni *et al.* (2023) discovered that increased stress impairs plant water absorption, hindering their growth tolerance mechanisms. Osmotic stress under water deficiency lowers seed water uptake, enzyme activity, and nutrient supply, leading to reduced germination and growth. PEG 6000 affects rice germination and early growth, with a concentration-dependent impact on seedlings. The highest PEG 6000 concentration significantly suppresses germination and growth attributes. The study recognized eight drought tolerant genotypes. Manonmani *et al*. (2020) assessed drought tolerance in rice mutants using PEG. Drought affects all rice growth stages; germination and growth impair seedling establishment, impacting later stages. PEG aids in efficient *in vitro* screening of mutants, offering potential candidates for field evaluation under natural drought stress. In the study two mutants showed exceptional characteristics and could be further tested under natural drought conditions for reliable outcomes.

The rice genotypes were then scored for drought tolerance based on a set of germination related traits. Therefore, drought tolerant cultivars can be identified by analysing the germination and seedling growth traits from the *in vitro* study, using PEG to screen the genotypes. The present study was conducted with the aim of examining the rice genotypes based on the early stage drought tolerance ability.

MATERIALS AND METHOD

The experiment was conducted under laboratory conditions at the Genetics and Plant Breeding laboratory, school of Agriculture and Bioscience in Karunya Institute of Technology and Sciences during summer, 2023. A total of 95 genotypes collected from various parts of Tamil Nadu and Kerala were used for this study. The design used was Completely Randomised Design (CRD) with three replications. The rice cultivar seeds were exposed to four distinct PEG 6000 stress levels, specifically 0%, 15%, 20%, and 25%. Distilled water was used for control. To create a 15% PEG solution, 15 g of PEG were dissolved in 100 ml of water. In the similar way 20% and 25% PEG solutions were also prepared.

Rice seeds were sterilized using sodium hypochlorite solution, rinsed in distilled water, and placed on filter paper in Petri dishes. The dishes were covered to retain moisture and kept in the lab for germination. To identify the genotypes having drought tolerance potential, several morpho-physiological indices were used, those include germination percentage, shoot length, root length, seedling length vigour index, total seedling height, dry weight, fresh weight and relative water content. The number of germinated seeds was recorded every 24 hours. Germination was confirmed when the radicle and plumule each exceeded 2 mm in length. On the last day, seedling height and dry weight were measured

Germination percentage: The germination percentage was calculated using the formula by Scott *et al*. (1984).

Shoot length and root length: The length of the root and shoot were calculated by using the procedure followed by Sagar *et al*. (2020).

Seedling fresh and dry weight: The dry weight and fresh weight were taken by utilizing the methodology followed by Siddique *et al*. (2023).

Vigour index: The seedling vigour index by using the formula by Abdul-Baki and Anderson (1973).

Relative water content: The relative water content was estimated by following the method by Weatherley and Slatyer (1957).

Statistical analysis: The observations were recorded in the respective time after the procedure was completed and the data were subjected to statistical analysis. The R Studio software version 4.2.2 was utilized for performing the statistical analysis.

RESULT AND DISCUSSION

Drought stress poses a significant obstacle to crop growth and development. Its primary impact is the inhibition of plant growth, hindering the overall progress and maturity of the crops (Sampath *et al*., 2022). Assessing the drought tolerance of rice accessions under field conditions proves challenging due to the inability to create controlled and consistent drought stress conditions in the field. Through the utilization of drought screening techniques under controlled conditions, involving the application of a standardized PEG solution, it becomes feasible to create consistent environments for inducing water stress (Swain *et al*., 2020).

The approach of genotype selection using stress screening under *in vitro* conditions and employing diverse concentrations of polyethylene glycol (PEG) has effectively demonstrated its success in identifying genotypes displaying improved drought tolerance (Anupriya *et al*., 2020). One way to maximize crop productivity while ensuring resilience to drought is by applying selective pressure on relative water content, root length, and root shoot length. Relative water content is suggested as a crucial measure of water condition compared to other factors during periods of drought (Anupriya *et al*., 2020).

Table 1: Analysis of variance for the studied traits among 95 rice genotypes during *in vitro* drought stress, induced by four concentrations (0 to 25%) of polyethylene glycol 6000.

Source of Variance	Treatment (T)	Genotype (G)	T × G	Residuals
RL	2258.1***	148.78***	24.05***	5.23
SL	919.77***	25.58***	5.14***	0.88
GP	79804***	5891***	605***	36
TSL	6348.6***	248.2***	39.6***	7
DW	13262.5***	814.9***	188.9***	5.8
FW	333323***	7907***	4187***	250
RWC	5784.2***	2682.8***	1437.5***	103.7
SVI	68157994***	2266361***	248027***	48965

(*** - Significant at 0.1% level of significance, RL; Root Length, SL; Shoot Length, GP; Germination Percentage, TSL; Total Seedling Length, DW; Dry Weight, FW; Fresh Weight, RWC; Relative Water Content, SVI; Seedling Vigour Index)

The result of analysis of variance (Table 1) showed that there is significant genetic variation among the genotypes for the characters under study. The analysis revealed that the genotypes exhibited significant differences in the mean sum of squares for all the examined traits, signifying that the selected genotypes showed genetic heterogeneity and displayed a substantial level of variability among them. This suggests a wide range of possibilities for the selection of diverse qualitative traits in rice improvement. Similar results were observed for the studies done in rice by Gampala *et al.* (2015). In the present experiment employing Polyethylene glycol among the pool of 95 rice genotypes, it was observed that 84 genotypes exhibited successful germination, while 11 genotypes failed to germinate.

Root Length: The assessment of root length encompassed both non-stress (control) conditions and varying stress levels (15%, 20%, and 25%). In non-stress conditions, root length ranged from 2.6 cm (ASD 16) to 27.27 cm (TCR4281), with an average of 11.85 cm. Stress conditions led to root lengths spanning 3.8 cm (TCR4254) to 26.3 cm (TCR4281) (mean: 9.97 cm), 1.8 cm (Seeragasamba) to 19.27 cm (TCR6860) (mean: 7.17 cm), and 1.47 cm (TRY 4) to 17.8 cm (TCR6860) (mean: 5.08 cm), for 15%, 20%, and 25% stress levels, respectively.

Shoot Length: The analysis of shoot length unveiled diverse responses to different stress intensities. In non-stress scenarios, shoot length ranged from 1.37 cm (ASD 16) to 11.37 cm (TCR4789), with an average of 6.67 cm. Stress levels of 15%, 20%, and 25% resulted in shoot lengths varying between 2.5 cm (TCR6283) and 10.27 cm (TCR4789) (mean: 5.22 cm), 1.07 cm (ASD 16) and 7.3 cm (Ezhome 3) (mean: 3.80 cm), and 0.93 cm (TRY 3) and 6.17 cm (Karunguruvai) (mean: 2.22 cm), respectively.

Germination Percentage: Examining germination percentage showcased distinct patterns under non-stress and stress conditions. Non-stress values ranged from

30% (TCR4281) to 96.67% (TRY 4), with a mean of 83.37%. Stress intensities of 15%, 20%, and 25% led to germination percentages spanning from 13.33% (CO 51) to 93.33% (TKM 9) (mean: 67.54%), 16.67% (Kullakar) to 93.33% (TCR6284) (mean: 57.18%), and 16.67% (Kullakar) to 83.33% (TCR6284) (mean: 41.03%), respectively.

Total Seedling Length: The investigation of total seedling length revealed varying responses across stress levels. In non-stress settings, seedling lengths oscillated from 3.97 cm (ASD 16) to 33.3 cm (TCR4281), averaging at 18.53 cm. Stress conditions of 15%, 20%, and 25% led to total seedling lengths ranging from 9.03 cm (Seeraga Samba) to 31.57 cm (TCR4281) (mean: 15.20 cm), 2.97 cm (ASD 16) to 25.73 cm (TCR6860) (mean: 10.98 cm), and 1.67 cm (TRY 4) to 23.03 cm (TCR6860) (mean: 6.99 cm), respectively.

Dry Weight: Exploring dry weight showcased notable variations between non-stress and stress scenarios. Non-stress values spanned from 16.78 g (Ezhome 4) to 64 g (TCR6245), with an average of 36.87 g. Stress levels of 15%, 20%, and 25% yielded dry weights ranging from 13.44 g (Ezhome 4) to 55.47 g (ANNA 4) (mean: 29.97 g), 14.4 g (TKM 15) to 43.4 g (Ezhome 3) (mean: 24.67 g), and 6.4 g (TCR6291) to 42.58 g (Ezhome 3) (mean: 19.98 g), respectively.

Fresh Weight: The evaluation of fresh weight unveiled distinct trends in both non-stress and stress conditions. Non-stress values ranged from 66.67 g (ASD 16) to 270g (TCR4281), with a mean of 144.91 g. Stress intensities of 15%, 20%, and 25% resulted in fresh weights varying from 55.55 g (TCR4935) to 243.33 g (TCR6056) (mean: 102.76 g), 39.33 g (TCR4935) to 236.67 g (ADT 37) (mean: 78.44 g), and 35 g (TCR4935) to 110.67 g (TCR6287) (mean: 59.82 g), respectively.

Relative Water Content: An analysis of relative water content exhibited noteworthy shifts across non-stress and stress levels. Non-stress values spanned from 24.32% (TCR6291) to 95.75% (TCR4413), with a mean of 78.88%. Stress scenarios of 15%, 20%, and 25% yielded relative water content ranging from 39.25% (TCR4361) to 94.82% (Chithiraikar) (mean: 72.98%), 40.63% (TCR5731) to 93.91% (TCR6860) (mean: 71.27%), and 46.16% (TCR7161) to 92.32% (CO 52) (mean: 67.03%), respectively.

Seedling Vigour Index: The seedling vigour index was assessed by measuring both seedling length and germination percentage. Scrutinizing the seedling vigour index revealed significant variations across stress levels. Non-stress values oscillated from 131.33 (ASD 16) to 2962.33 (TCR5979), with a mean of 1562.29. Stress intensities of 15%, 20%, and 25% led to seedling vigour index values spanning from 163.67 (CO 51) to 2468.67 (TCR6219) (mean: 1120.29), 66.67 (ASD 16) to 1989 (TCR6860) (mean: 731.27), and 119 (TCR6287) to 1231.33 (TCR6860) (mean: 350.01), respectively.

Table 2: Descriptive statistics of drought tolerance traits among 95 rice genotypes in laboratory condition.

TRAIT	CONC	MIN	MAX	MEAN	STD DEV	CV
Root Length	CONTROL	2.60	27.27	11.85	4.58	12.08
	15%	3.80	26.30	9.97	5.00	26.96
	20%	1.80	19.27	7.17	4.00	37.29
	25%	1.47	17.80	5.08	3.42	41.63
Shoot Length	CONTROL	1.37	11.37	6.67	1.89	14.59
	15%	2.50	10.27	5.22	2.15	19.85
	20%	1.07	7.30	3.80	1.85	27.21
	25%	0.93	6.17	2.22	1.43	29.34
Germination Percentage	CONTROL	30.00	96.67	83.37	19.59	7.33
	15%	13.33	93.33	67.54	29.59	8.70
	20%	16.67	93.33	57.18	27.93	10.10
	25%	16.67	83.33	41.03	23.00	15.12
Total Seedling Length	CONTROL	3.97	33.30	18.53	5.48	10.53
	15%	9.03	31.57	15.20	6.59	19.77
	20%	2.97	25.73	10.98	5.39	27.77
	25%	1.67	23.03	6.99	4.46	34.96
Dry Weight	CONTROL	16.78	64.00	36.87	9.85	9.23
	15%	13.44	55.47	29.97	12.55	6.96
	20%	14.40	43.40	24.67	10.56	7.67
	25%	6.40	42.58	19.98	9.71	9.60
Fresh Weight	CONTROL	66.67	270.00	144.91	48.77	14.52
	15%	55.55	243.33	102.76	46.83	15.04
	20%	39.33	236.67	78.44	37.70	17.85
	25%	35.00	110.67	59.82	28.81	18.87
Relative Water Content	CONTROL	24.32	95.75	78.88	12.68	12.26
	15%	39.25	94.82	72.98	25.00	13.07
	20%	40.63	93.91	71.27	27.17	14.82
	25%	46.16	92.32	67.03	28.42	16.32
Seedling Vigour Index	CONTROL	131.33	2962.33	1562.29	600.56	13.67
	15%	163.67	2468.67	1120.29	595.05	24.64
	20%	66.67	1989.00	731.27	464.81	30.59
	25%	119.00	1231.33	350.01	269.58	44.28

(SD; Standard Deviation, CV; Coefficient of Variation)

These detailed findings (Table 2) illuminate the intricate responses of various traits in rice seedlings to different stress levels. There were similar reduction in the mean performance of the traits such as germination percentage, root length, shoot length, fresh weight, dry weight, seedling vigour index relative water content and total seedling height by Biswas *et al*. (2018); similar for root and shoot length was observed by Fatimah *et al*. (2023); Anik *et al*. (2021). Mishra *et al*. (2019) also got reduction in performance of traits as concentration increased on shoot length, root length, fresh weight, dry weight, seedling vigour index and relative water content. Priya *et al*. (2022) also reported reduction in mean performance as the concentration increased in root length. Mahreen *et al*. (2022) also observed the same trend in root length, shoot length, fresh weight and dry weight. Evamoni *et al.* (2023) also obtained a similar reduction pattern in germination percentage, fresh weight, dry weight, seedling vigour index and total seedling length. Similar pattern of reduction in germination percentage, root length and shoot length was observed by Herawati *et al*. (2020). Sampath *et al*. (2022) reported a reduction in the mean value as the concentration of the PEG increased for the traits germination percentage, shoot length, root length, seedling vigour index and total seedling length. In a similar way Anupriya *et al*. (2020) recorded reduction in mean value of root length, shoot length, seedling vigour index and relative water content as the PEG concentration increased. Akte *et al*. (2016) observed a decrease in the mean value of germination percentage, shoot length, root length, fresh weight, dry weight and relative water content as the rise in PEG concentration. Similar findings were obtained by Gampala *et al*. (2015) excluding relative water content. Ali *et al*. (2021) reported that the seedling vigour index decreased as the concentration of the PEG increased. Shatpathy *et al*. (2018) also obtained a similar finding on the traits germination percentage and seedling vigour index.

The comprehensive findings of this study shed light on the intricate responses of various traits in rice seedlings when subjected to varying stress levels. These insights are highly valuable as they provide a deeper understanding of how these seedlings adapt and display resilience under different stress conditions. By utilizing mean performance as a criterion, it becomes feasible to identify and eliminate undesirable plants from the selection process. This approach not only streamlines the selection process but also allows for the inclusion of variability, which is often a crucial factor in breeding programs aimed at enhancing drought tolerance and overall crop resilience.

The quality of seed or seedling traits played a crucial role in enhancing crop yield, a correlation inherently linked to the genetic makeup of the specific cultivar. Exceptional seeds demonstrated their commendable potential by showcasing remarkable performance in seedling traits that can be quantified within controlled laboratory settings. The systematic tactics employed in various research efforts, particularly those focused on cultivation techniques and breeding strategies, relied significantly on the different specifications of seeds or seedlings. This dependence on the diverse traits of crop genotypes served as a wellspring of valuable insights (Biswas *et al*., 2018).

Control Condition Correlation: Under the control condition (Table3.), a significant disparity emerged among the studied traits. Root length exhibited a remarkably robust and positive correlation with total seedling length (0.94**) and seedling vigour index (0.704**), along with a notable positive correlation with shoot length (0.309**). Moreover, a positive and significant correlation was found between root length and fresh weight (0.13*). Notably, the shoot length displayed a strong positive correlation with total seedling length (0.616**), seedling vigour index (0.548**), germination percentage (0.202**), and fresh weight (0.183**), accompanied by a significant and positive correlation with relative water content (0.142*). A robust and positive correlation between the seedling vigour index and total seedling length was evident (0.78**). Additionally, the germination percentage exhibited a high and positive significant correlation with seedling vigour index (0.695**), total seedling length (0.155*), and dry weight (0.143*), while conversely displaying a negative and significant correlation with fresh weight (-0.248**). The fresh weight indicated a positive and significant correlation with total seedling length (0.173**), dry weight (0.129*), and relative water content (0.372**). However, the interaction between other traits showcased comparatively lesser significance or absence of correlation.

Similarly, under control condition, Olawamide *et al*. (2018) observed seedling vigour index had positive high significant correlation with root length, shoot length and germination percentage.

Table 3: Estimates of Correlation among 95 rice genotypes for the drought traits under different concentrations of PEG (A. Control (0%) B. 15% C. 20% D. 25%).

A. Correlation at 0% (Control) PEG concentration.

	RL	SL	GP	TSL	DW	FW	RWC	SVI
RL	1.000							
SL	0.309**	1.000						
GP	0.099	0.202**	1.000					
TSL	0.94**	0.616**	0.155*	1.000				
DW	-0.098	0.038	0.143*	-0.067	1.000			
FW	0.13*	0.183**	-0.248**	0.173**	0.129*	1.000		
RWC	-0.063	0.142*	-0.009	-0.001	-0.001	0.372**	1.000	
SVI	0.704**	0.548**	0.695**	0.78**	-0.013	-0.071	-0.036	1.000

B. Correlation at 15% PEG concentration.

	RL	SL	GP	TSL	DW	FW	RWC	SVI
RL	1.000							
SL	0.564**	1.000						
GP	0.364**	0.508**	1.000					
TSL	0.963**	0.767**	0.45**	1.000				
DW	0.329**	0.506**	0.602**	0.422**	1.000			
FW	0.48**	0.496**	0.221**	0.536**	0.484**	1.000		
RWC	0.51**	0.559**	0.57**	0.579**	0.54**	0.655**	1.000	
SVI	0.74**	0.636**	0.789**	0.784**	0.417**	0.227**	0.459**	1.000

C. Correlation at 20% PEG concentration.

	RL	SL	GP	TSL	DW	FW	RWC	SVI
RL	1.000							
SL	0.555**	1.000						
GP	0.535**	0.618**	1.000					
TSL	0.959**	0.769**	0.623**	1.000				
DW	0.426**	0.519**	0.628**	0.505**	1.000			
FW	0.429**	0.43**	0.39**	0.477**	0.653**	1.000		
RWC	0.468**	0.599**	0.713**	0.565**	0.693**	0.731**	1.000	
SVI	0.846**	0.685**	0.803**	0.885**	0.426**	0.298**	0.514**	1.000

D. Correlation at 25% PEG concentration.

	RL	SL	GP	TSL	DW	FW	RWC	SVI
RL	1.000							
SL	0.564**	1.000						
GP	0.373**	0.478**	1.000					
TSL	0.956**	0.757**	0.437**	1.000				
DW	0.402**	0.515**	0.606**	0.464**	1.000			
FW	0.52**	0.523**	0.456**	0.568**	0.72**	1.000		
RWC	0.509**	0.572**	0.677**	0.574**	0.728**	0.843**	1.000	
SVI	0.786**	0.673**	0.723**	0.835**	0.407**	0.398**	0.51**	1.000

(* - Significant at 5% Level of significance, ** - Significant at 1% Level of significance, RL; Root Length, SL; Shoot Length, GP; Germination Percentage, TSL; Total Seedling Length, DW; Dry Weight, FW; Fresh Weight, RWC; Relative Water Content, SVI; Seedling Vigour Index)

Stress-Induced Condition Correlations: Conversely, under stress-induced conditions (15%, 20%, and 25% PEG concentration) (Table 3, a remarkable shift was observed as all traits demonstrated positive, significant, and strong correlations with each other. Specifically, at 15% PEG concentration, a very high, strong, and positive correlation emerged between total seedling length and root length (0.963**) and shoot length (0.767**). The seedling vigour index exhibited strong and positive correlations with root length (0.74**), total seedling length (0.784**), and germination percentage (0.789**). Root length displayed positive correlations with shoot length (0.564**), germination percentage (0.364**), dry weight (0.329**), fresh weight (0.48**), and relative water content (0.51**). Furthermore, shoot length depicted a strong positive correlation with germination percentage (0.508**), dry weight (0.506**), fresh weight (0.496**), relative water content (0.559**), and seedling vigour index (0.636**). Notably, germination percentage established a robust positive correlation with total seedling length (0.45**), dry weight (0.602**), fresh weight (0.221**), and relative water content (0.57**). Moreover, a pronounced positive correlation between dry weight and total seedling length was evident (0.422**), while fresh weight exhibited strong positive correlations with total seedling length (0.536**) and dry weight (0.484**). The relative water content was significantly positively correlated with total seedling length (0.579**), dry weight (0.54**), and fresh weight (0.655**). Remarkably, seedling vigour index showcased strong and positive correlations with dry weight (0.417**), fresh weight (0.227**) and relative water content (0.459**).

Similar patterns emerged under 20% PEG concentration, where total seedling length displayed very high, strong, and positive correlations with root length (0.959**) and shoot length (0.769**). The relative water content demonstrated very high, strong, and positive correlations with germination percentage (0.713**) and fresh weight (0.731**). Moreover, the seedling vigour index exhibited very high, strong, and positive correlations with root length (0.846**), germination percentage (0.803**), and total seedling length (0.885**). Interestingly, root length established strong positive correlations with shoot length (0.555**), germination percentage (0.535**), dry weight (0.426**), fresh weight (0.429**), and relative water content (0.468**). Additionally, shoot length showcased strong positive correlations with germination percentage (0.618**), dry weight (0.519**), fresh weight (0.43**), relative water content (0.599**), and seedling vigour index (0.685**). Furthermore, germination percentage displayed strong positive correlations with total seedling length (0.623**), dry weight (0.628**), and fresh weight (0.39**). Remarkably, total seedling length exhibited strong positive correlations with dry weight (0.505**), fresh weight (0.477**), and relative water content (0.565**). Notably, dry weight demonstrated strong positive correlations with fresh weight (0.653**), relative water content (0.693**), and seedling vigour index (0.426**), while seedling vigour index established strong positive correlations with fresh weight (0.298**) and relative water content (0.514**).

Lastly, at 25% PEG concentration, a similar trend persisted as total seedling length exhibited very high, strong, and positive correlations with root length (0.956**) and shoot length (0.757**). A noteworthy finding emerged with a very high, strong, and positive correlation between fresh weight and dry weight (0.72**). Equally significant were the very high, positive, and strong correlations between the relative water content and dry weight (0.728**) and fresh weight (0.843**). The seedling vigour index demonstrated very high, strong, and positive correlations with root length (0.786**), germination percentage (0.723**), and total seedling length (0.835**). Root length displayed strong positive correlations with shoot length (0.564**), germination percentage (0.373**), dry weight (0.402**), fresh weight (0.52**), and relative water content (0.509**). Similarly, shoot length exhibited strong positive correlations with germination percentage (0.478**), dry weight (0.515**), fresh weight (0.523**), relative water content (0.572**), and seedling vigour index (0.673**). The germination percentage itself showcased strong positive correlations with total seedling length (0.437**), dry weight (0.606**), fresh weight (0.456**), and relative water content (0.677**). Total seedling length, as before, established strong positive correlations with dry weight (0.464**), fresh weight (0.568**), and relative water content (0.574**). Meanwhile, seedling vigour index demonstrated strong positive correlations with dry weight (0.407**), fresh weight (0.398**), and relative water content (0.51**).

In a study conducted, Fatimah *et al*. (2023) reported the root length had a strong positive significant correlation with the shoot length in stress conditions. Mishra *et al*. (2019) observed that seedling vigour index, root length, shoot length and relative water content had positive significant correlation, the shoot length had significant positive correlation with fresh weight and dry weight. In a study by Nulit (2018), the germination percentage, fresh weight, dry weight, shoot length and root length had positive significant correlation. Eslami *et al*. (2018) reported significant positive correlation for root and shoot length at various concentrations of PEG.

In the present study, a thorough analysis of the collected data revealed significant correlations among the various traits that were investigated. These observed correlations provide valuable insights into the potential of drought tolerant traits that were the focus of our study. The significance of these correlations lies in their implications for rice cultivation, especially in drought prone regions. When traits related to drought tolerance, such as root length, shoot length, total seedling length, seedling vigour index, relative water content, seedling fresh weight, seedling dry weight, and germination percentage, show strong and positive correlations, it suggests that these traits tend to co-occur or influence each other positively in response to drought conditions. This knowledge is essential for plant breeders and agricultural scientists because it helps in identifying key traits that can be targeted for improvement in rice varieties.

CONCLUSIONS

In the present study, the traits responsible for early stage drought screening have been identified. These intricate correlations among the traits unveil the complex interplay between different traits under varying conditions, providing valuable insights into the rice seedlings' adaptability to stress. The traits under study have strong positive correlation among them and showed significant results. Thus, these traits have the potential to serve as a valuable tool in the selection process within breeding programs and to aid in the identification of early stage drought tolerant genotypes. Identifying lines displaying greater variability among the traits, particularly those strongly correlated with drought resistance, can serve as valuable parent candidates in breeding programs aimed at developing varieties capable of withstanding drought conditions.

FUTURE SCOPE

Future research in enhancing drought tolerance in rice using PEG (Polyethylene Glycol) as a tool holds immense potential as these require less time and space. The present research knowledge about the traits studied can be applied in breeding programs to identify rice varieties that are more resilient to water scarcity, ultimately contributing to global food security in the face of changing climate conditions. The varieties identified can be used for further field evaluation and the best performing varieties which have significant yield as well as economic benefit can be released as variety, improving the livelihoods of farmers in the face of uncertain climate changes.

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Conflict of Interest. None.

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