RESEARCH ARTICLE

Screening of Sarjoo 52 Derived M2 Population for Drought Stress by Polyethylene Glycol (PEG) at Seedling Stage in Rice

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Abstract

The global population, currently at 8.2 billion, is projected to reach 9.7 billion by 2050, intensifying the demand for staple foods like rice. Rice cultivation, a critical component of Asian diets, requires 2000 to 5000 liters of water per kilogram, making it highly vulnerable to water scarcity. Factors such as climate change and soil conditions significantly affect rice yield. To overcome the yield loss of rice due to drought stress, we developed a mutagenized population of population of Sarjoo 52 rice. Whose screening for drought tolerance was done at the seedling stage through polyethylene glycol 6000. These lines were subjected to varying concentrations of Polyethylene Glycol (PEG) 6000 5, 10, 15, 20% (0.5, 1.0, 1.5, and 2.0 bar) to simulate drought stress. Observations of germination percentage, shoot length, root length, and root-to-shoot length ratio were assessed using a two-factorial, completely randomized design (CRD). Among the tested lines, ten mutants (SDTC-66, SDTC-68, SDTC-20, SDTC-28, SDTC-36, SDTC-35, SDTC-65, SDTB-1, SDTB-11, and SDTB-12) exhibited superior performance, with SDTC-66 demonstrating the most robust drought tolerance across all traits. These lines showed the potential of mutant rice lines in mitigating the impact of drought on rice production and ensuring food security. These can also be used in future breeding programs after further screening.

Keywords: Drought tolerance, Polyethylene glycol, Mutation, M_2 population.

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INTRODUCTION

Rice is the most important food crop for a large portion of the global population, particularly in densely populated regions of Asia (Sahebi *et al.,* 2018; Panda *et al.,* (2021). To meet the demand of growing population, rice production must be doubled by 2050, but various biotic and abiotic stresses reduce the production of rice. Among abiotic stresses, drought stress affects half of the world's arable land (Singhal *et al.,* 2016; Panda *et al.,* 2021). Rice is a water-intensive crop, requiring significant amounts of water for optimal growth. On average, rice needs about 3,000 to 5,000 liters of water to produce 1 kilogram of rice, water requirement varies depending on the variety, climate, and method of cultivation. In traditional flood irrigation, fields are kept submerged in water for a major part of the growing season, consuming large amounts of water (Sun *et al.,*2024; Kumar *et al.,*2024; Jyothsna *et al.,* 2024). Rice is most affected by the drought. Drought stress can result in a 100% reduction in rice output, depending on the plant's growth and development stages (Polania *et al.,* 2017; Panda *et al.,* 2021).

Nearly 68% of India's sown area is drought-prone, with 33% chronically affected, receiving less than 750 mm of rainfall annually. The resilience of rice to drought mainly lies in the ability of rice to survive under drought stress; therefore, screening of rice genotypes for drought tolerance is the primary requirement for the development of drought-tolerant varieties. Various methods are used for the development of drought-tolerant varieties. Despite notable advancements in rice breeding, the development of drought-tolerant rice varieties is still a major challenge due to the complex nature of drought. However, conventional breeding methods have identified various drought-tolerant genotypes, but they are not adapted to different environmental conditions and their yield potential is low. Additionally, genetic diversity within the cultivated rice is limited, which limits the potential for improvement. In mutation breeding, genetic variation is developed either through chemical and physical mutagen and then desirable traits are selected (Udage, 2021). Mutation breeding emerged as a potential tool for crop improvement, particularly for the improvement of drought tolerance (Majid *et al.,* 2020). Through mutation breeding, new genetic variability is developed, which is crucial in developing crops with enhanced tolerance to abiotic stresses such as drought (Saif, 2023). Physical and chemical mutagens can quickly activate the changes in the structural and nucleotide changes in the genome, which may lead to random mutation, which can result in phenotypic gain. Such mutant lines can have a broad range of useful traits, such as enhanced yield and resistance to biotic and abiotic stress. Therefore mutation breeding method has become an essential

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method in modern agriculture (Bhoi *et al.,* 2022; Ruengphayak *et al.,* 2015; Kamolsukyeunyong *et al.,* 2019). CNM 6 (Lakshmi) is an important rice drought-tolerant variety developed through mutation breeding.

Multiple techniques are employed to assess drought tolerance in rice, with PEG (Polyethylene glycol) treatment being the most prevalent, as it exhibits effects comparable to natural drought conditions. PEG treatment causes plants to experience osmotic stress, which mimics drought stress and eventually aids in identifying the genotypes that have the capacity to tolerate drought levels. PEG-induced drought stress has gained popularity because it is a reliable approach that can be used to ensure the effectiveness of tolerance and verify the genotype's genetic potential. It is also a reproducible method for screening rice germplasm for drought resistance. Through increased water use efficiency and normalized osmotic adjustment, PEG treatment has also been utilized to uncover critical traits that may play a substantial role in drought resistance (Hartyanto *et al.,* 2024; Samad, 2023). PEG's elevated molecular weight inhibits cellular infiltration, facilitating regulated water stress induction without damaging plants. It modifies drought-responsive gene expression and the accumulation of stress proteins in Arabidopsis (Pope *et al.,* 2024). Siddique *et al.,* (2023) also screened the coarse rice seedling through PEG-6000 on different concentrations, i.e., 5%, 10%, 15 and 20% and identified four drought tolerant lines (NRPC-9, NRPC-7, NRPC-6, NRPC-1). In the present study, mutant lines of Sarjoo 52 rice are developed through sodium azide and the M_2 population was screened for drought tolerance at the seedling stage with the help of polyethylene glycol (PEG).

MATERIAL AND METHODS

The experiment was conducted during 2022–23 at the Institute of Agricultural Science and Technology, Integral University, Lucknow, employing a factorial Completely Randomized Design (CRD) for precise analysis. The study was systematically structured into three distinct phases: **-** 1. Material Collection, 2. Treatment Application, 3. Data Collection and Analysis.

Material collection

Sixty-four Sarjoo-52 M2 mutant lines (details in Table 1), along with additional research materials, were obtained from the Department of Agriculture, IIAST, Integral University, Lucknow.

Treatment Application

The treatment application is divided into the steps given below. Experimental photographs are in the Fig. 1.

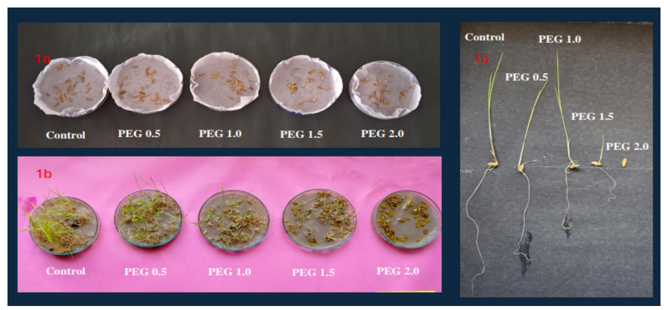

Fig. 1: Experiment photographs – a. seed treatment with various PEG concentration, b. Germination, c. Root Shoot Growth after 14 days.

Table 1: Mutant population information

S. No.	*Mutant lines*	*S. No.*	*Mutant lines*	*S. No.*	*Mutant lines*	*S. No.*	*Mutant lines*
1	SDTC10	17	SDTC46	33	SDTB1	49	SDTB39
2	SDTC20	18	SDTC47	34	SDTB3	50	SDTB41
3	SDTC21	19	SDTC52	35	SDTB5	51	SDTB43
4	SDTC24	20	SDTC56	36	SDTB7	52	SDTB44
5	SDTC26	21	SDTC57	37	SDTB9	53	SDTB49
6	SDTC27	22	SDTC58	38	SDTB11	54	SDTB50
7	SDTC28	23	SDTC65	39	SDTB12	55	SDTB53
8	SDTC29	24	SDTC66	40	SDTB19	56	SDTB54
9	SDTC31	25	SDTC68	41	SDTB21	57	SDTB58
10	SDTC32	26	SDTC69	42	SDTB24	58	SDTB60
11	SDTC34	27	SDTC70	43	SDTB25	59	SDTB61
12	SDTC35	28	SDTC71	44	SDTB27	60	SDTB62
13	SDTC36	29	SDTC73	45	SDTB29	61	SDTB63
14	SDTC37	30	SDTC75	46	SDTB31	62	SDTB64
15	SDTC39	31	SDTC77	47	SDTB33	63	SDTB65
16	SDTC40	32	SDTC78	48	SDTB35	64	SDTB68

Step 1: Seed Preparation

Uniform, high-quality rice seeds were sterilized in 1% sodium hypochlorite for 10 minutes to remove fungal contaminants and then rinsed thoroughly with distilled water.

Step 2: Preparation of PEG Solutions

PEG 6000 solutions (5, 10, 15, 20% w/v) were prepared by dissolving PEG in distilled water. Fresh solutions were used for each replication to ensure consistent osmotic stress levels.

Step 3: Seed Soaking

Sterilized seeds were grouped by PEG concentration (5, 10, 15, 20%) and control (distilled water) and soaked for 24 to 48 hours (Fig. 1a).

Step 4: Germination Setup

Soaked seeds were placed on damp filter paper in Petri dishes or a growth medium (e.g., sand/vermiculite) saturated with PEG solutions. Germination was conducted at 25°C with a 12-hour light/dark cycle (Fig. 1b).

Data collection and analysis

Data collection-

After the 14^{th} day (Fig. 1c), data collection was taken based on the observation are -

- *Germination percentage*

Observations are recorded after a treatment period of 7 to 14 days. The germination percentage is determined using the formula:

Germination % = (No. of germinated seeds / Total No. of tested seeds) x 100

- *Root length*

Root length is measured 14 days after treatment in millimeters or centimeters using a ruler.

- *Shoot length*

Shoot length is measured 14 days after treatment in millimeters or centimeters using a ruler.

- *Root-shoot ratio*

root shoot length to me measured with the formula –

Root-Shoot ratio = root length/shoot length.

Analysis

Factorial CRD design used for ANOVA table to check the significance level, and PCA heat map for better understanding trait performance.

RESULTS

ANOVA

Based on the factorial CRD - The ANOVA results indicate significant effects of Mutant line, concentration, and their interaction on all four response variables, as given in Table 2. A detailed discussion of each component is provided below**:**

Germination%

The germination percentage is significantly influenced by both the mutant line and concentration, with F values of 95.43 ($p < 0.001$) and 87273.78 ($p < 0.001$), respectively (Table 2). The significant impact of concentration underscores the crucial importance of water availability in the context of PEG-induced drought conditions. The notable interaction between mutant lines and concentration ($F = 10.33$, $p < 0.001$) highlights the

Table 2: Combine factorial completely randomized block design, analysis of variance evaluation of Sarjoo-52 M_2 population for drought tolerance with PEG 6000 concentrations at seedling stage

Response variable	*Source of variation*	*Sum of Sq.*	*df*	*F*	*PR(>F)*
Germination (%)	C(Genotype)	16450.36744	63	95.43029634	1.47E-283***
	C(Concentration)	955196.1504	4	87273.78094	0***
	C(Genotype):C(Concentration)	7126.105163	252	10.33481972	8.19E-125***
	Residual	1751.1718	640		
Shoot length (cm)	C(Genotype)	2428.227297	63	1189.519769	0***
	C(Concentration)	11291.71655	4	87121.00029	0***
	C(Genotype):C(Concentration)	969.7581592	252	118.7642631	0***
	Residual	20.73753333	640		
Root length (cm)	C(Genotype)	4276.26319	63	788.0212278	0***
	C(Concentration)	18232.15426	4	52916.61252	0***
	C(Genotype):C(Concentration)	1467.138498	252	67.59033701	0***
	Residual	55.1272	640		
Root-shoot ratio	C(Genotype)	50.73781625	63	15.68088648	3.47E-93***
	C(Concentration)	322.844159	4	1571.492567	0***
	C(Genotype):C(Concentration)	50.79750771	252	3.92483364	3.77E-44**
	Residual	32.87006667	640		

differing responses of mutant lines to various levels of water stress, implying that variations in germination among mutant lines play a vital role in evaluating drought tolerance.

Shoot length

In case of shoot length, concentration demonstrate a remarkable significance ($F = 87121.00$, $p < 0.001$), highlighting the profound effect of water stress on vegetative growth. The notable influence of the mutant line ($F = 1189.52$, $p < 0.001$) suggests that genetic elements play a crucial role in the variability of shoot growth when exposed to drought conditions (Table 2). Moreover, the interaction between the mutant line and concentration ($F = 118.76$, $p < 0.001$) highlights the varying responses of different mutant lines, emphasizing the importance of conducting mutant line-specific assessments when analyzing shoot length resilience in the context of drought stress.

Root length

The concentration effect is predominant for root length ($F = 52916.61$, $p < 0.001$), indicating that root development is significantly affected by drought stress caused by PEG. The notable influence of mutant line ($F = 788.02$, $p < 0.001$) indicates a fundamental genetic diversity in root length among different mutant lines. The interaction between mutant line and concentration ($F = 67.59$, $p < 0.001$) indicates that different mutant lines show unique responses in root elongation when faced with water stress (Table 2), highlighting the necessity of choosing mutant lines that possess enhanced root characteristics in drought scenarios.

Root-Shoot ratio

The root-shoot ratio shows a significant response to concentration ($F = 1571.49$, $p < 0.001$), suggesting that drought stress affects how resources are distributed between root and shoot systems. The mutant line effect is noteworthy ($F = 15.68$, $p < 0.001$, yet it is less pronounced than the other traits, indicating reduced variability among mutant lines for this particular trait. Nonetheless, the notable interaction between mutant line and concentration ($F = 3.92$, $p < 0.001$) indicates that different mutant lines exhibit varying capacities to modify root-to-shoot allocation when faced with water stress (Table 2).

Effect of PEG concentrations on Sarjoo-52 mutants

Effect of PEG concentrations on Sarjoo -52 mutant population Shown in boxplot diagram in Fig. 2

Germination %

Indicated a significant decrease as treatment concentrations increased (Fig. 2a). The control recorded the highest germination rate at 86.98 ± 2.12%, establishing the baseline for comparison. At concentrations of 5 and 10%, the germination rates were observed to decline to 80.56 ± 3.45% and 75.45 ± 3.11%, respectively. At a concentration of 20%, germination was almost completely hindered, resulting in only 0.58 ± 0.21% of seeds successfully germinating. The marked suppression of germination at levels exceeding 15% ($p < 0.001$) suggests a strong phytotoxic effect, likely resulting from interference with hormone signaling pathways crucial for starting the germination process.

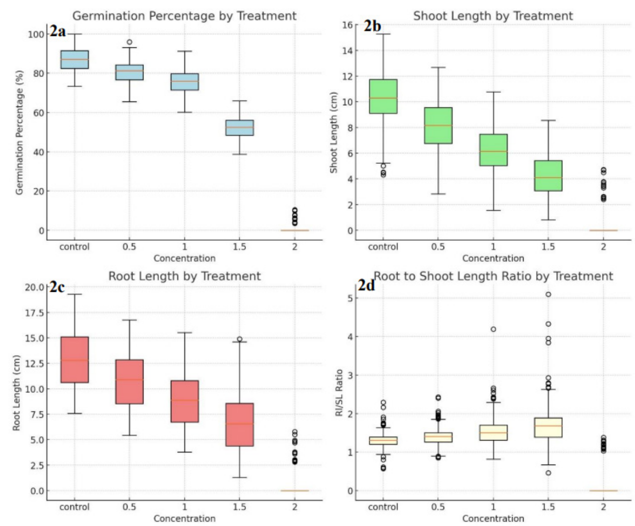

Fig. 2: Effect of PEG concentrations on observations- 2a. Effect of PEG concentrations on germination, 2b. Effect of concentrations on shoot length, 2c. Effect of PEG concentrations on root length, 2d. Effect of PEG concentrations on root-shoot ratio.

Shoot length

Shoot length indicated a notably significant effect (Fig. 2b.). The control showed the highest shoot elongation, achieving a mean SL of 10.30 ± 1.12 cm. Conversely, shoots subjected to a 20% concentration exhibited significantly stunted growth (0.26 ± 0.07 cm), indicating a 97.5% decrease in shoot length relative to the control. Moderate reductions in shoot length were observed at intermediate concentrations of 5 and 10%, resulting in measurements of 8.10 ± 0.85 and 6.14 ± 1.09 cm, respectively. The inhibition of shoot elongation indicates a disruption in auxin-driven cell elongation and division, which are essential processes for promoting shoot growth.

Root Length (RL)

Indicated significant effect of PEG concentration on root length (Fig 2c.). The control seedlings showed the greatest root length at 12.94 ± 1.47 cm, whereas the higher concentrations of 15 and 20% resulted in notably shorter roots measuring 6.26 ± 0.95 and 0.28 ± 0.09 cm, respectively. The treatment group at 20% showed nearly total suppression of root growth, aligning with significant disturbances in root development.

Root-Shoot Ratio

Indicated significant effect of PEG concentration on root-shoot ratio. Concentration 5% and 1.0%, the RL/SL ratio increased compared to the control (1.55 ± 0.13 at 10% against 1.30 ± 0.09 at 0%), indicating an adaptive reallocation of biomass towards root development under mild stress. At 20%, the RL/SL ratio decreased to 0.09 ± 0.02, signifying an almost complete suppression of root and shoot development. The noted decrease in the RL/SL ratio at elevated doses may indicate a breakdown in adaptive stress responses, possibly caused by systemic toxicity and impaired cellular function.

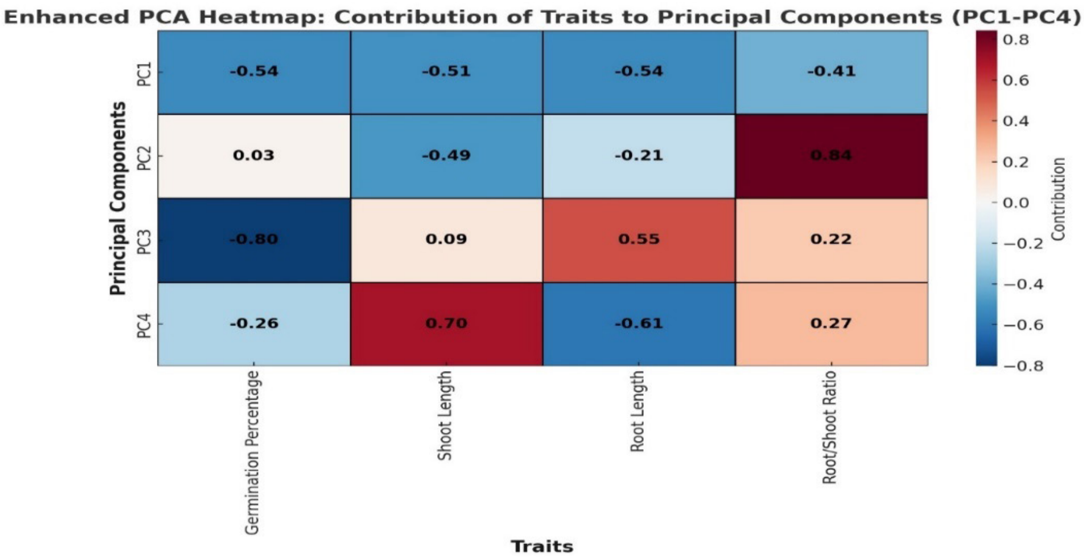

Fig. 3: Principal component analysis enhanced heat map PC1-PC4

Principal component analysis

Principal Component Analysis (PCA) was conducted to assess the contribution of different traits (Germination Percentage, Shoot Length, Root Length, and Root/Shoot Ratio) to the principal components. The loading matrix (Fig. 4) shows the correlations between the traits and the principal components, with values closer to 1 or -1 indicating a stronger contribution.

From Fig 3. PC1 exhibits a negative correlation with germination percentage (-0.54), shoot length (-0.51), root length (-0.54), and root/shoot ratio (-0.41), signifying that these qualities substantially detract from this component. This indicates that PC1 predominantly encapsulates a synthesis of these four features, with no one trait prevailing in the explained variance. PC2 exhibits a robust positive connection with the root/shoot ratio (0.84), signifying that this component is predominantly affected by the root/shoot ratio. Additional characteristics, including shoot length (-0.49) and root length (-0.21), have diminished negative effects on PC2. PC3 exhibits a high negative association with germination percentage (-0.80), alongside positive relationships with root length (0.55) and root/shoot ratio (0.22). This suggests that PC3 is shaped by a combination of features, with germination percentage exerting a negative impact and root length providing a positive contribution. PC4 is predominantly linked to shoot length (0.70) and exhibits a negative correlation with root length (-0.61), indicating that this component chiefly accounts for the variation in these two characteristics.

Top 10 Mutant Lines Exhibiting Performance Above 1.5 Bar (15%) PEG Concentration

Fig. 4. highlights the performance of the top 10 mutant lines at concentrations exceeding 15%, focusing on germination percentage, shoot length, and root length.

Germination percentage

According to Fig. 4, the mutant lines that exhibited the highest performance at concentrations greater than 1.5 for germination percentage are as follows: SDTC66 at 10.23%, SDTC68 at 7.68%, SDTC20 at 6.14%, SDTC28 at 5.63%, and SDTC36 at 4.09%. SDTC66 stood out as the top performer, demonstrating the highest germination rate even under the increased concentration conditions. This suggests that SDTC66 possesses genetic traits that enhance metabolic functions during stress, potentially incorporating osmotic adjustment mechanisms that allow seeds to take up water and commence germination activities. SDTC68 and SDTC20 later showed decreased rates, suggesting a level of moderate tolerance. The lower germination rates observed in mutant lines such as SDTC36 and SDTC28 indicate their relative susceptibility to increased osmotic stress during the germination phase, thereby limiting their ability to establish quickly in challenging field conditions.

Shoot length

According to Fig. 4, the mutant lines that performed best with a concentration greater than 15% for germination percentage

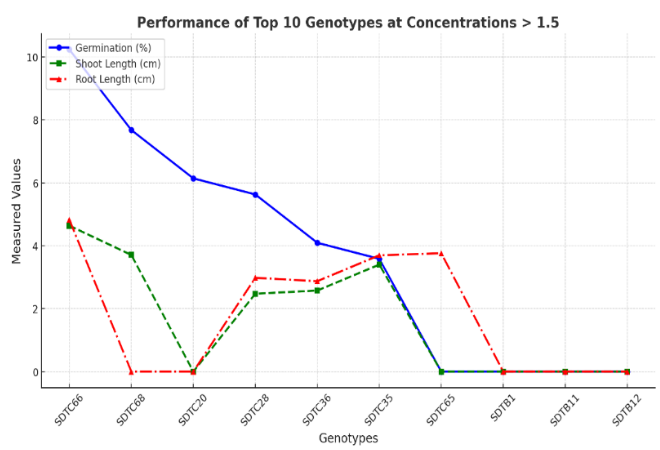

Fig. 4: Ten best genotypes perform best > 1.5 bar (<15%) PEG concentrations

are as follows: SDTC66 – 4.64 cm, SDTC68 – 3.71 cm, SDTC28 – 2.47 cm, SDTC36 – 2.58 cm, and SDTC59 – 2.25 cm. SDTC66 and SDTC68 show impressive durability, showcasing strong shoot development while efficiently balancing resource allocation between their root and shoot systems, even under challenging conditions. SDTC28 and SDTC36 show a moderate level of shoot growth; however, their ability to produce above-ground biomass might not be as robust. Extended shoots concentrate on improving photosynthesis and encouraging vegetative growth, making them well-suited for settings that facilitate early plant establishment.

Root length

According to Fig. 4, the mutant lines that performed best with a concentration greater than 15% for germination percentage are as follows: SDTC66 at 4.83 cm, SDTC28 at 2.98 cm, SDTC36 at 2.87 cm, and SDTC59 at 2.01 cm. SDTC66 showed exceptional performance, featuring a significantly longer root system in comparison to the other mutant lines. The significant root development observed in SDTC66 suggests that this mutant line is particularly suited for environments with restricted water supply, as its extended root system allows it to access moisture from deeper layers of soil. SDTC28 and SDTC36 showed a fair amount of root growth, suggesting some degree of drought resistance, although they might not achieve the resilience levels seen in SDTC66.

Multi-trait correlation

The correlation among germination percentage, shoot length, and root length indicates that SDTC66 is the most balanced mutant line, exhibiting the highest values in both germination and shoot/root length. Conversely, mutant lines like SDTC68 and SDTC20 exhibit trade-offs, wherein increased shoot growth correlates with diminished root growth or lowered germination rates. This indicates a genetic divergence in resource distribution during early developmental stages under stress.

Discussion

The results from the analysis of variance (ANOVA) indicate notable influences of mutant line, PEG concentration, and their interaction on all assessed traits, highlighting the intricate nature of plant responses to osmotic stress (Bhattacharjee *et al.,* 2023). The results are essential for comprehending the mechanisms of drought tolerance that vary by mutant line and can guide breeding approaches focused on enhancing performance in water-limited environments (Rosero *et al.,* 2020).

The significant decrease in germination percentage as PEG concentration rises indicates a robust osmotic barrier to the initial stages of seedling growth, which is essential for plant establishment in stressful conditions (Zhang & Shi, 2018). The notable interaction between mutant line and concentration indicates that mutant line SDTC-66 exhibit elevated germination rates in response to high PEG levels, probably due to intrinsic genetic factors, including better osmotic adjustment or superior hormonal regulation (Zivcak *et al.,* 2016). These mutant lines represent excellent options for breeding initiatives aimed at improving drought resistance during the seedling phase.

The concentration of PEG showed a significant influence on both shoot and root length, highlighting a considerable reduction in vegetative growth due to osmotic stress (Sagar *et al.,* 2020). e mutant line effect on both traits suggests the existence of genetic diversity in drought response, a finding that supports previous research. The relationship between mutant lines and PEG concentration highlights the varying abilities of mutant lines to influence growth when water is limited. Mutant lines such as SDTC66, demonstrating continuous root and shoot elongation, probably have enhanced characteristics for water absorption and resource distribution, positioning them as excellent candidates for future breeding initiatives focused on enhancing drought resilience during vegetative growth (Ye *et al.,* 2018).

The root-shoot ratio, indicative of the plant's resource allocation strategy during drought conditions, exhibited notable variation in response to PEG concentration (Mude *et al.,* 2023). The impact of the mutant line was not as strong, yet it remained significant, indicating a restricted genetic diversity for this trait compared to others. Nonetheless, the notable interaction between mutant lines and concentration suggests that different mutant lines exhibit varying capacities to adjust their biomass allocation strategies when faced with drought stress (Seleiman *et al.,* 2021).

The findings from the Principal Component Analysis (PCA) provide deeper insights into the connections among traits in drought conditions. The mutant line SDTC66 demonstrated outstanding performance in germination percentage, shoot length, and root length, highlighting its strong mechanisms for drought tolerance (Mohi-Ud-Din *et al.,* 2021). In the meantime, mutant lines like SDTC68 and SDTC28 showed a moderate level of tolerance, performing well under mild to moderate drought stress but facing growth limitations under harsher conditions, consistent with other studies in the field of drought tolerance (Shah *et al.,* 2024).

Conclusion

The study assessed various mutant lines regarding their stress responses, revealing that Mutant line SDTC66 had the most advantageous results in germination percentage, shoot length, and root length. The mutant line demonstrated remarkable endurance under high concentration stress, presumably owing to its efficient water utilization and osmotic control mechanisms. Mutant line SDTC68 demonstrated remarkable shoot length; nonetheless, it faced challenges in developing strong root systems. This indicates that it may be more appropriate for environments where water availability is not a critical issue or where rapid shoot growth is prioritized. The mutant lines SDTC20 and SDTC36 exhibited reasonable performance; however, they do not possess the adaptations in root or shoot development that are apparent in mutant line SDTC66.

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