



Enumeration of Genetic Variability Parameters and Association among Early Seedling Growth Parameters under Polyethylene Glycol (PEG) induced Osmotic Stress in *rabi* Sorghum (*Sorghum bicolor* (L.) Moench)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The research aims to explore the variability within diverse rabi sorghum germplasms and identify the relationships among different early seedling growth traits. This will provide valuable insights for future breeding programs, aiding in the development of resilient sorghum varieties that can contribute to improved food security and agricultural sustainability.

Study Design: The study was conducted by employing factorial complete randomized design (FCRD) with two replications. Where, two factors was considered, genotype as first factor and PEG concentration as second.

Place and Duration of Study: The experiment was conducted during *rabi* season of 2023 at University of Agricultural Sciences Dharwad, Karnataka.

Methodology: A total of 156 germplasm lines of rabi sorghum were assessed in vitro for early seedling growth characteristics, including germination percentage, shoot length, root length, seedling dry weight, and seedling vigor indices I and II, under both control and osmotic stress conditions induced by polyethylene glycol (PEG). Osmotic stress was induced by dissolving PEG 6000 in distilled water to achieve the desired concentration. The germplasm lines were then subjected to germination and early growth under these conditions using the paper towel method. The genetic variability parameters for each trait were quantified to examine the degree of variability in the material used, and correlation analysis was performed to understand the relationships among the traits studied.

Results: The variance analysis showed highly significant differences among genotypes, treatments, and the genotype-by-environment (G×E) interactions. Genetic variability parameters revealed considerable variability across all traits studied, which tended to increase with higher stress levels. Moderate to high GCV and PCV was observed for all the traits studied. Correlation analysis demonstrated a strong, significant positive relationship among all seedling parameters, except between root length and shoot length, as well as between shoot length and germination percentage under control conditions.

Conclusion: The study emphasizes the effect of osmotic stress on the early vigor and growth of sorghum seedlings. The findings reveal significant variability in seedling traits, suggesting that selection can be employed for genetic enhancement of these traits. Correlation analysis identified specific traits or combinations of traits that should be considered when selecting genotypes tolerant to osmotic stress conditions. These findings offer great potential to guide and improve selection and breeding programs focused on enhancing drought tolerant sorghum varieties.

Keywords: Osmotic stress; genetic variability; heritability; rabi Sorghum; polyethylene glycol.

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the world's most significant crops, often referred to as "King of Millets" or "Great Millet" due to its large grain size compared to other millets. It is known by various names, including Great Millet and Guinea Corn in West Africa, Kafir Corn in South Africa, Dura in Sudan, and Jowar in India, with its origins tracing back to Africa. The greatest diversity of both cultivated and wild sorghum is found in Africa (Aruna 2018). Globally, it ranks as the fifth most important cereal crop, following wheat, corn, rice, and barley. Sorghum is often called the "camel plant" because of its resilience to drought. It also shows tolerance to waterlogging, saline and alkaline conditions. As a C₄ plant, it is highly efficient in photosynthesis and performs better in carbon assimilation under high temperatures and drought conditions compared to C₃ plants.

Millions of people in Africa and Asia rely on sorghum as a staple food. In addition to being used as fodder and livestock feed in the form of hay and pasture, it also serves as poultry feed.

In India, sorghum is predominantly cultivated in Maharashtra, Karnataka, and Andhra Pradesh, which together contribute 80% of the country's total production. India accounts for approximately 16% of the global sorghum output. The crop is grown during both the *kharif* (rainy) and *rabi* (post-rainy) seasons. Sorghum is cultivated across 4.38 million hectares in India, producing 1.99 million tonnes in the *kharif* season and 2.83 million tonnes in the *rabi* season. However, *kharif* sorghum has a higher productivity of 1210 kg/ha compared to 1033 kg/ha for *rabi* (Indiastat, 2022). India ranks third globally in terms of sorghum acreage and fifth in production. Karnataka is the top producer, contributing 26% of India's sorghum production, followed by

Maharashtra (25%) and Rajasthan (14%), while Andhra Pradesh contributes 7%. In Karnataka, sorghum is grown on 9.43 lakh hectares, with a production of 8.92 lakh tonnes and an average yield of 1475 kg/hectare (Agricultural Market Intelligence Centre, ANGRAU, 2022).

Rabi sorghum cultivation is primarily concentrated in the semi-arid Deccan Plateau, covering the states of Maharashtra, Karnataka, and Andhra Pradesh. A decrease in *rabi* sorghum productivity has been observed in regions with unpredictable and limited rainfall, leading to moisture stress during crucial growth stages of the plant, which causes substantial yield losses (Kumar et al. 2022). Therefore, maintaining sufficient soil moisture is essential for successful crop production in arid regions. Screening genotypes for drought tolerance in field conditions can be challenging and resource-intensive, making the process cumbersome and difficult.

An alternative to conducting field experiments for moisture stress is to simulate stress *in vitro* using polyethylene glycol (PEG). Water stress impacts nearly every stage of plant development, but its harmful effects are most evident during key growth phases like germination, shoot length, and root length (Khayatnezhad et al. 2010). Among these critical stages, stress applied during the seedling phase has been used to screen germplasm in various crops, including wheat (Bukhari et al. 2021), sorghum (Tsago et al. 2014), maize (Raj et al. 2020), rice (Sathyabharathi et al. 2022), Soybean (Vijay et al. 2018) and sunflower (Ahmad et al. 2009).

Polyethylene glycol (PEG) with a molecular mass of 6000 or higher is a non-toxic, impermeable osmotic agent that can reduce water potential and mimic drought stress in plant tissues. As a polymer, PEG is considered more effective than other chemicals for inducing water stress (Li et al. 2017). Increasing PEG concentration has been shown to reduce germination and seedling vigor in certain crops (Sani and Boureima 2014). Screening genotypes at the seedling stage offers several benefits, including low cost, ease of handling, reduced labor, and early elimination of susceptible genotypes. Against this background, the objective of this experiment is to examine the variability in sorghum germplasm for shoot and root traits under osmotic stress conditions.

2. MATERIALS AND METHODS

The experiment was conducted at the University of Agricultural Sciences, Dharwad in 2023,

utilizing 156 sorghum germplasm lines which includes the elite breeding lines from Institutes all over India collected from Indian Institute of Millet Research, Hyderabad and AICRP on Sorghum, MARS, UAS Dharwad. Experiment was designed using a factorial complete randomized design (FCRD) with two replications, where the genotypes and PEG levels were the factors. The maximum concentration of PEG was determined by evaluating its effects on four randomly selected genotypes. These genotypes were subjected to germination tests under various PEG concentrations (5%, 10%, 15%, and 20%). The concentration at which 50% mortality (LD50) occurred was recorded and used as the upper threshold for screening the entire germplasm collection. And PEG concentration of 10% caused 50% mortality (LD50) hence this concentration was considered as upper threshold for screening entire germplasm set. Seeds in the control group were germinated under normal conditions using distilled water (T1), while stress conditions were simulated by dissolving polyethylene glycol (PEG-6000) in distilled water to prepare 5% (T2) and 10% (T3) PEG solutions.

Sorghum seeds were surface sterilised with sodium hypochlorite solution (2%, v/v) for 5 minutes. After that, different concentrations of polyethylene glycol 6000 (PEG 6000) were applied to the seedlings. In order to maintain a control, distilled water was used. Two replicates of 50 seeds from each genotype are evenly distributed across two sheets of germination paper (Germitest®), which have been moistened with various PEG solutions in a volume equal to 2.5 times the paper's dried mass and rolled. The rolls are then sealed in plastic containers to prevent evaporation and maintain a humidity level close to 100 percent. 14 days of germination were conducted in a germinator at a constant temperature of 25 °C (24-26 °C) in the light. When the radicle length exceeds 5.0 mm, seeds are considered to have germinated.

Germination (%) =

$$\frac{\text{Number of normal seedlings}}{\text{Total number of seeds kept for germination}} \times 100$$

On the fourteenth day after the germination test, ten normal seedlings were chosen at random from all replications in each treatment. The root length was measured with a scale from the tip of the primary root to the base of the hypocotyl, and the mean root length was expressed in centimetres (cm). Shoot length was measured from the tip of the primary leaf to the base of the hypocotyl and represented in centimetres (cm).

The seedlings' dry weight was measured using seedlings that were used to measure the root and shoot lengths by keeping in butter paper bag and drying in hot air oven at 70°C for 24 hrs.

The seedling vigour index I was determined using the approach proposed by (Abdul-Baki and Anderson 1973) and expressed numerically using the formula below:

$$\text{Seedling vigour index (I)} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

The seedling vigour index II was calculated by multiplying the germination % by the dry weight of the seedlings and expressing the result as a whole number.

$$\text{Seedling vigour index (II)} = \text{Germination (\%)} \times \text{Seedling dry weight (g)}$$

2.1 Statistical Analysis

Differences between genotypes for different characters were tested for significance by using analysis of variance technique (Burton and Devane 1953). The mean, range and variance values of each character were calculated for each genotype. The variability present in the germplasms was estimated by phenotypic and genotypic coefficient of variations using the procedure suggested by (Burton 1952). Phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) were estimated using the formula suggested by (Sivasubramanian and Madhavamenon 1973) and expressed in percentage. The estimates of PCV and GCV were categorized based on the scale given by (Johnson et al. 1955). Heritability in the broad sense (H^2_b) was computed using the formula given by (Burton 1952) and expressed in percentage. The range of heritability, expected genetic gain or genetic advance as percent of mean (GAM) under selection were calculated as suggested by (Searle et al. 1961). The correlation coefficients were calculated based on the analysis of variance and covariance, as recommended by

(Maranna et al. 2021). All the genetic parameters analysis, correlation analysis and visual illustrations were computed using R statistical Package.

3. RESULTS AND DISCUSSION

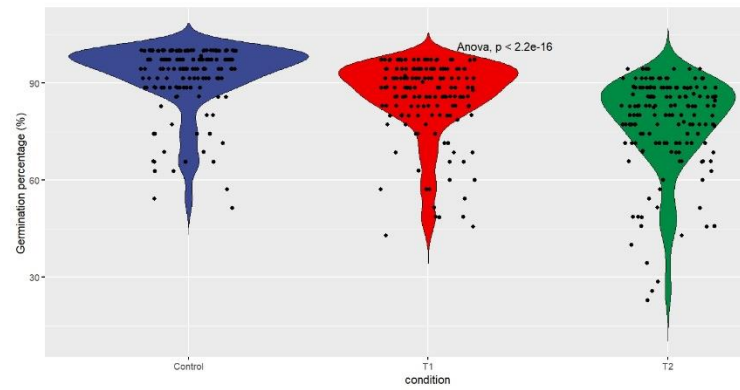
3.1 Anova and Genetic Variability Parameters

The analysis of variance revealed highly significant differences (at 0.1 percent probability level) among genotypes, treatments, and the genotype-by-environment ($G \times E$) component, indicating substantial variation in the germplasm for the traits examined. Table 1 shows the mean sum of squares for all characters. Table 2 provides a comparison of mean values for various traits, including the range, PCV, GCV, broad sense heritability estimates, and genetic advance as percent of mean, both under osmotic stress and control conditions.

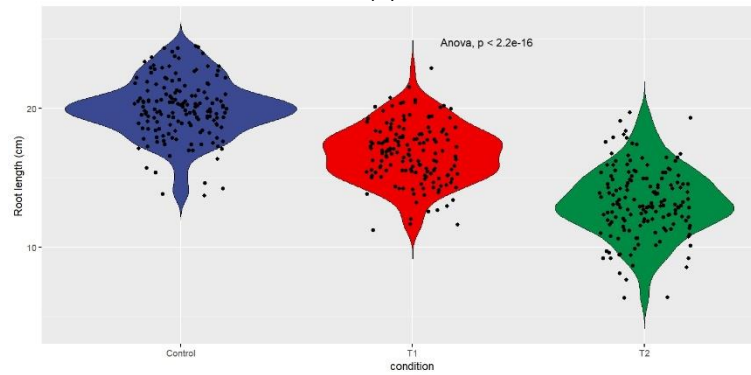
The extent of variability of all seedling parameters were illustrated using violin plots (Fig. 1). In the past, numerous studies have utilized violin plots to depict the phenotypic variability and diversity in different crops (Anwar et al. 2022, Brdar-Jokanović et al. 2020). Under control condition, the germination percentage averaged 92.19%, with a range from 51.43% to 100%. Under T2 and T3 conditions, the ranges were 42.86-97.14% and 22.86-94.29%, with means of 85.38% and 77.55%, respectively. The germination percentage displayed moderate levels of both genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) across all conditions (T1, T2 & T3). The narrow variation in GCV and PCV values suggests that this trait is minimally influenced by environmental factors. Additionally, germination percentage exhibited high heritability and genetic advance over mean (GAM) in both control and stress conditions, indicating that it is a reliable parameter for selecting sorghum lines with considerable drought tolerance.

Table 1. Analysis of variance (ANOVA) for various seedling parameters among diverse sorghum germplasm lines

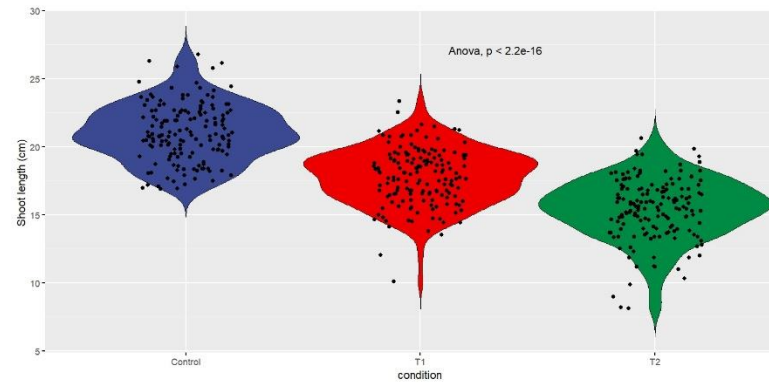
Source of variation	Genotypes (G)	Treatment (T)	G × T	Residuals
df	155	2	310	468
Traits				
Germination (%)	854.0***	15686.7***	48.1***	2.4
Shoot length (cm)	21.71***	2510.43***	3.05***	0.11
Root length (cm)	22.5***	3538.6***	4.0***	0.4
Seedling dry weight (mg)	8836.0***	491903.0***	308.0***	26
Seedling vigour index I	1753827.0***	183372692.0***	124568.0***	11164
Seedling vigour index II	105.3***	6333.4***	3.9***	0.4



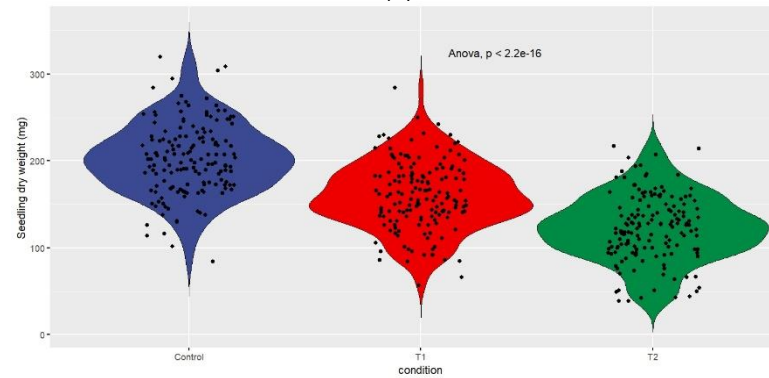
(a)



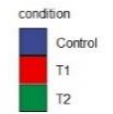
(c)



(b)



(d)



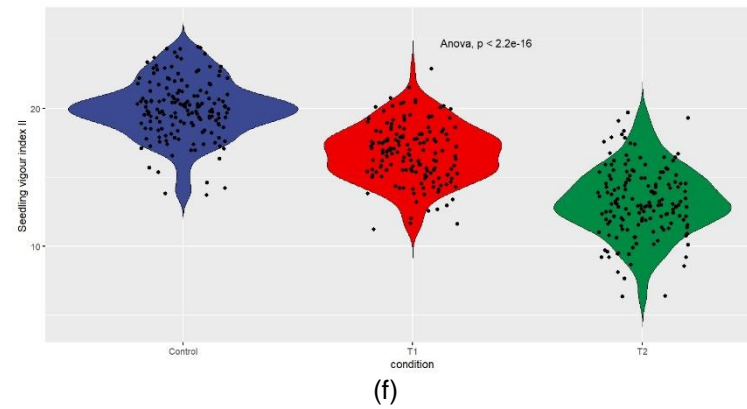
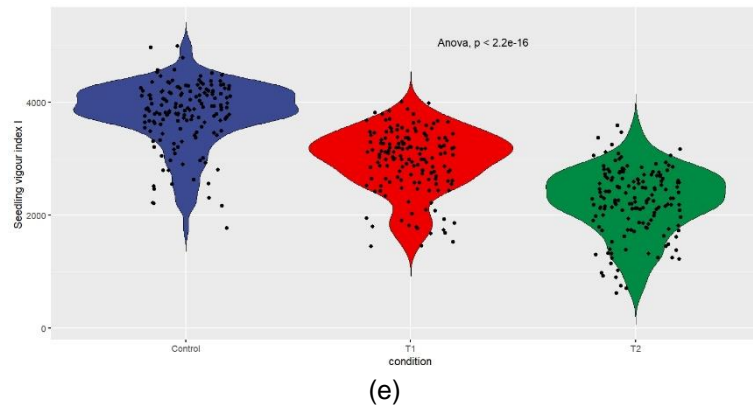


Fig. 1. Range of variability in (a) Germination percentage, (b) Shoot length, (c) Root length, (d) Seedling dry weight, (e) Seedling vigour index I and (f) Seedling vigour index II as represented using violin plots. T1- 5% PEG treatment and T2- 10% PEG treatment

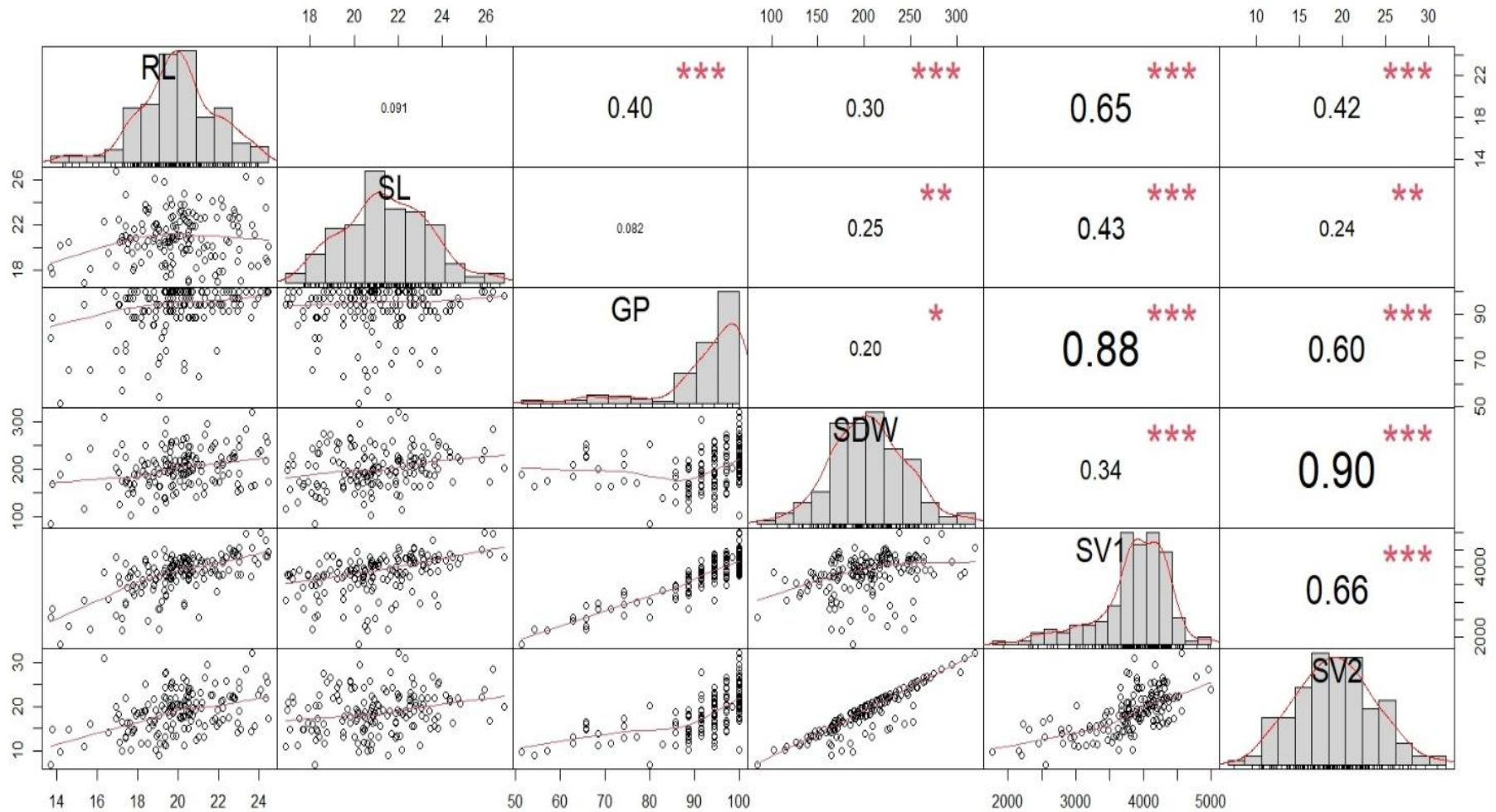


Fig. 2. Correlation among various seedling parameters of sorghum germplasm lines under control condition. RL: Root Length (cm), SL: Shoot Length (cm), GP: Germination Percentage (%), SDW: Seedling Dry Weight (mg), SVI: Seedling Vigour Index I, SVII: Seedling Vigour Index II

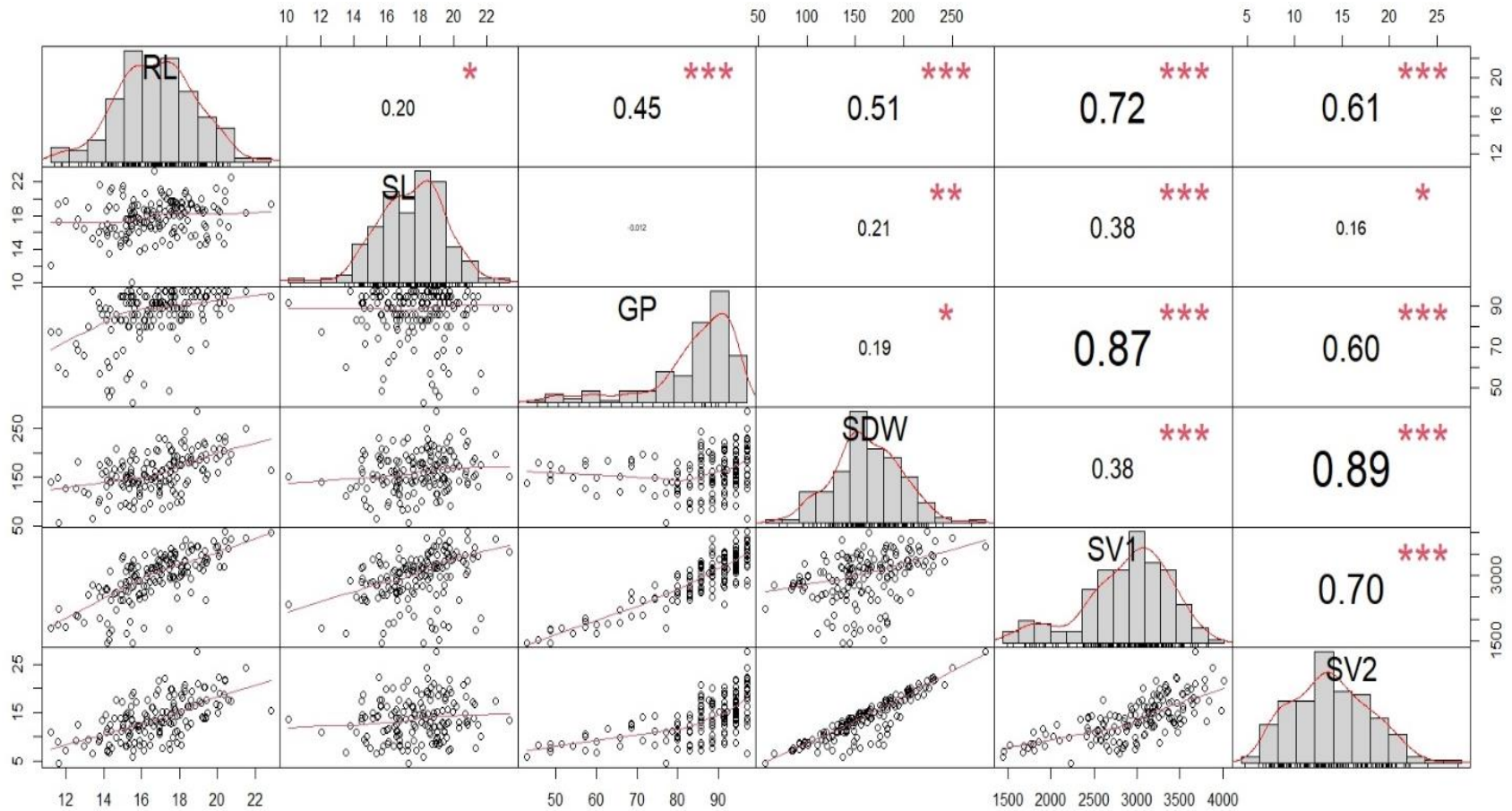


Fig. 3. Correlation among various seedling parameters of sorghum germplasm lines under 5% PEG (T2) treatment. RL: Root Length (cm), SL: Shoot Length (cm), GP: Germination Percentage (%), SDW: Seedling Dry Weight (mg), SVI: Seedling Vigour Index I, SVII: Seedling Vigour Index II

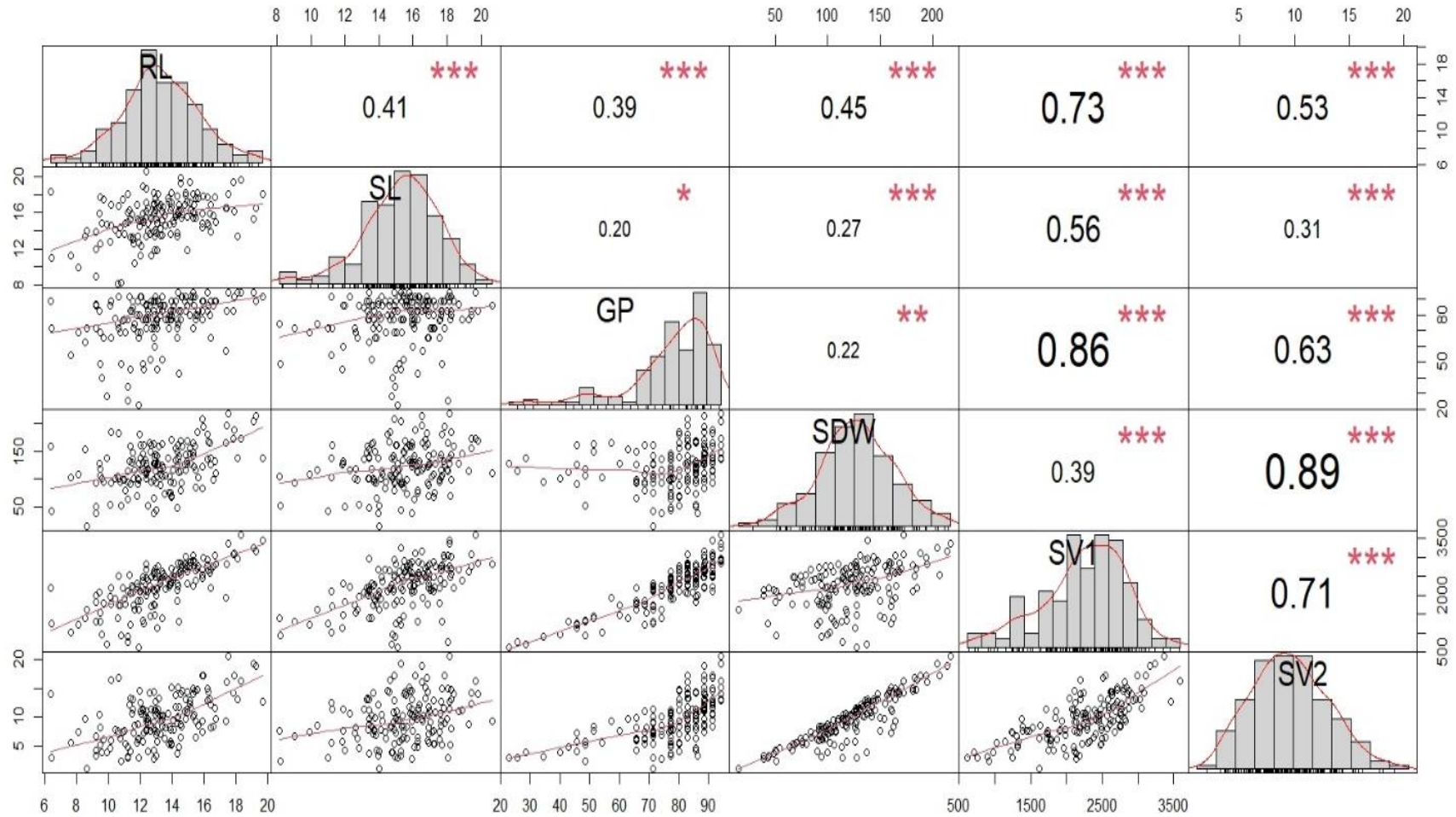


Fig. 4. Correlation among various seedling parameters of sorghum germplasm lines under 10% PEG (T3) treatment. RL: Root Length (cm), SL: Shoot Length (cm), GP: Germination Percentage (%), SDW: Seedling Dry Weight (mg), SVI: Seedling Vigour Index I, SVII: Seedling Vigour Index II

Table 2. Comparative estimates of genetic parameters in sorghum germplasm set for various traits under control (T1) and osmotic stress (T2 & T3)

Traits	Mean	Range		GCV	PCV	h ²	GAM	CV	CD @ 5%	
		min	max							
Control (T1)										
Germination (%)	92.19	51.43	100.00	11.98	12.00	99.70	24.65	0.64	1.17	
Shoot length (cm)	21.06	16.90	26.80	10.13	10.13	99.90	20.86	0.20	0.05	
Root length (cm)	19.97	13.70	24.45	10.51	10.99	91.42	20.71	3.22	1.27	
Seedling dry weight (mg)	202.53	84.00	320.00	20.46	20.46	99.90	42.14	0.34	1.15	
Seedling vigour index I	3793.09	1769.33	4995.00	15.53	15.63	98.60	31.80	1.79	133.83	
Seedling vigour index II	18.76	6.72	32.00	25.72	25.73	99.90	52.98	0.71	0.26	
5% PEG Treatment (T2)										
Germination (%)	85.38	42.86	97.14	14.15	14.19	99.30	29.05	1.12	1.89	
Shoot length (cm)	17.81	10.10	23.30	11.62	11.68	98.80	23.80	1.23	0.43	
Root length (cm)	16.73	11.20	22.85	12.38	12.85	92.88	24.59	3.42	1.13	
Seedling dry weight (mg)	159.93	57.00	284.00	24.55	24.69	98.90	50.31	2.58	8.17	
Seedling vigour index I	2960.58	1446.59	4007.57	18.72	18.85	98.57	38.28	2.25	132.16	
Seedling vigour index II	13.75	4.40	27.59	31.09	31.30	98.60	63.63	3.58	0.97	
10% PEG Treatment (T3)										
Germination (%)	77.55	22.86	94.29	18.48	18.57	99.03	37.88	1.82	2.80	
Shoot length (cm)	15.44	8.15	20.65	14.48	14.63	97.90	29.53	2.08	0.63	
Root length (cm)	13.24	6.35	19.70	18.71	18.71	99.90	38.52	0.53	0.14	
Seedling dry weight (mg)	122.85	39.00	217.00	30.82	31.18	97.70	62.75	4.70	11.50	
Seedling vigour index I	2244.12	617.21	3587.97	26.11	26.14	99.79	53.74	1.18	52.63	
Seedling vigour index II	9.65	2.48	20.46	39.77	40.20	97.80	81.05	5.86	1.12	

Under control conditions, shoot length ranged from 16.90 to 26.80 cm among the genotypes, with an average of 21.06 cm. In contrast, the average shoot length dropped to 17.81 cm and 15.44 cm under T2 and T3 treatments, respectively, indicating a significant impact of osmotic stress on shoot length. Root length had mean values of 19.97 cm, 16.73 cm, and 13.24 cm under T1, T2, and T3 treatments, respectively. The range of root length was 13.70 to 24.45 cm under control conditions, 11.20 to 22.85 cm under T2, and 6.35 to 19.70 cm under T3 conditions. Both shoot and root lengths showed moderate GCV and PCV values along with higher heritability and genetic advance over the mean, suggesting that these traits are influenced by additive gene action and that there is potential for selecting genotypes based on these traits for drought tolerance.

The mean seedling dry weight under control conditions was 202.53 mg, with a range from 84 to 320 mg. Under T2 treatment, the mean dry weight was 159.93 mg, ranging from 57 to 284 mg. With T3 treatment, the mean value decreased to 122.85 mg, with a range of 39 to 217 mg. Seedling dry weight exhibited high genotypic and phenotypic coefficients of variability, along with high heritability and genetic advance over the mean in both control and stress conditions. This suggests minimal environmental influence and indicates that selection for drought tolerance based on this trait can be effective.

Under control conditions, the seedling vigor index I ranged from 1769.33 to 4995, with a mean of 3793.09. In contrast, the mean seedling vigor index I under T2 treatment was 2960.58, with values ranging from 1446.59 to 4007.57. For the T3 treatment, the mean seedling vigor index I was 2244.12, with a minimum of 617.21 and a maximum of 3587.97. The seedling vigor index I showed moderate genotypic and phenotypic coefficients of variation under control and T2 treatment, and high GCV and PCV under T3 treatment. This indicates that different genotypes respond variably to increasing drought stress, leading to higher variability among them. The index also exhibited high heritability and genetic advance over the mean, suggesting minimal environmental influence.

Under control conditions, the seedling vigor index II ranged from 6.72 to 32, with a mean of 18.76. For the T2 treatment, the mean seedling vigor index II was 13.75, with values ranging from 4.40

to 27.59. Under the T3 treatment, the mean was 9.65, with a range of 2.48 to 20.46. The seedling vigor index II displayed high genotypic and phenotypic coefficients of variation across all treatments and also showed high heritability and genetic advance over the mean. Reduction of these seedling parameters under PEG treatments were also reported by several workers (Avcı et al. 2017, Baldaniya et al. 2022, Navyashree 2024, Babu and Revanappa 2018). Increment of the variability parameters such as GCV and PCV as increase in the osmotic stress level for the traits such as root length, shoot length, seedling length and germination was also reported by (Rasheed 2022) in tomato and (Baloch et al. 2012) in Groundnut. Since all the traits studied showed high heritability and a significant genetic advance as a percentage of the mean, environmental influence on these traits is minimal. Therefore, a simple selection method can be effectively used to identify drought-tolerant genotypes. This approach is highly efficient for screening a large number of genotypes compared to field conditions, which require more time and labor.

3.2 Correlation Analysis

Correlation coefficients among the vigour and seedling parameters under treatments T1, T2 and T3 were presented in Fig. 2, Fig. 3 & Fig. 4 respectively. Under control conditions, shoot length did not show any significant association with root length or germination percentage. However, root length had a significant positive correlation with germination percentage ($r=0.40$), seedling dry weight ($r=0.30$), seedling vigor index I ($r=0.65$), and seedling vigor index II ($r=0.42$). The strongest correlation was observed between seedling dry weight and seedling vigor index II ($r=0.90$), followed by germination percentage and seedling vigor index I ($r=0.88$). Germination percentage also had a significant positive correlation with seedling vigor index II ($r=0.60$).

Under treatment T2, root length and shoot length showed a significant positive correlation with each other and with other traits, though shoot length had no significant association with germination percentage. Germination percentage had a significant positive association with seedling dry weight ($r=0.19$), seedling vigor index I ($r=0.87$), and seedling vigor index II ($r=0.60$). Under treatment T3, root length and shoot length exhibited a highly significant positive correlation ($r=0.41$). and, all other seedling traits were significantly and positively correlated, indicating

that these parameters are strongly connected in relation to the overall plant water dynamics. Positive correlation of shoot length with root length and seedling vigour index was also reported by (Kunasekarn et al. 2017) in wheat. Highly significant positive association among shoot length, root length, root fresh weight and root dry weight was also reported by (Rauf et al. 2007) and (Shivhare and Lata 2019). (Tsago et al. 2014) also reported positive association between coleoptile length and root length. In contrary (Sandhu et al. 1998) reported non-significant association between root length and shoot length, however they reported positive significant association of shoot length, root length, root weight and shoot weight with seedling vigour index while working with bajra.

This association study explores the connections between shoot and root parameters with the plant's internal water status at various developmental stages. The findings may aid in predicting drought tolerance in genotypes at early stages, providing a foundation for selection (Ali et al. 2011). These seedling parameters have been linked to yield under moisture stress (Blum 2011). Therefore, the correlation coefficient estimates could be used for indirect selection of drought-tolerant sorghum genotypes at the seedling stage to achieve high yields under moisture stress conditions. Additionally, root length, seedling dry weight, and seed vigor index showed significant positive correlations with other seedling traits, suggesting that improvements in one attribute are likely to positively affect the others. This implies that selecting a reliable trait under osmotic stress can enhance other seedling traits, facilitating the development of better varieties suited to stress environments. Identifying the most reliable drought tolerance parameters can assist in efficiently recognizing drought-tolerant genotypes. Evaluating genetic variability and correlation coefficients for these traits at the seedling stage will be highly valuable for effective breeding programs focused on improving drought tolerance.

4. CONCLUSION

The study assessed the impact of osmotic stress, induced by polyethylene glycol (PEG 6000), on germination, seedling growth, and vigor in 156 *rabi* sorghum genotypes. The results showed significant interactions between genotypes and stress, reflecting variability in drought tolerance. Early screening of these genotypes could

improve future breeding programs by allowing for efficient evaluation of large populations and reducing time and costs. PEG-induced osmotic stress is an effective technique for identifying drought-tolerant sorghum genotypes, offering valuable insights for developing varieties that thrive in water-scarce conditions. The study also demonstrates significant genetic variability and high heritability for vigor and seedling parameters among the genotypes. Therefore, genotypes that perform well under induced osmotic stress can be selected from the germplasm set, with further field evaluations conducted to validate the results. This enables the identification of genotypes with superior drought resistance, which can be utilized as donor parents in hybridization programs or released as varieties suited for moisture-stress conditions. After assessing their yield potential through multilocation trials, these genotypes can play a crucial role in enhancing food security in drought-affected areas.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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