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Differential Drought Tolerance among Sorghum Genotypes under Polyethylene Glycol-Induced Osmotic Stress at the Seedling Stage

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ABSTRACT

This study evaluated drought tolerance in ten sorghum genotypes using polyethylene glycol (PEG-6000) to simulate osmotic stress. Seeds were germinated for 10 days and a completely randomized design with two moisture regimes 0% PEG and 30% PEG, seedling height, root length, plant height, fresh weight, seedling vigor and drought tolerance indices were assessed. Responses to PEG-induced drought was clearly genotype-dependent. Germination was severely inhibited in sensitive genotypes, declining in V2 (90±1.53% to 70±3.00%), V3 (80±0.10% to 50±2.65%), V4 (100± 0.58% to 70±2.00%) and V5 (100±0.58% to 70±1.00%), whereas V6 and V10 improved under stress (70±2.65% to 90±3.00%). V7 remained stable 100±1.00% in both conditions. Shoot height was markedly reduced in drought-sensitive genotypes, particularly V4 (14.5±8.00 cm to 6.5±3.78 cm), while V10 showed stability (4.6±2.51 cm to 4.8±1.76 cm). V3 maintained best root length (15.7±3.42 cm to 9.2±5.63 cm), indicating superior early root resilience. Fresh biomass was highest in V4 under drought (0.15±0.02 g), whereas V7 declined sharply (0.14±0.01 g to 0.01±0.03 g). V6 and V10 were the most resilient with a DTI of 129 ± 0.00%, while V3 and V4 also demonstrated consistent structural stability. Overall, V3 (Samsorg 46), V4 (Samsorg 48), V6 (Samsorg 52), V7 (Samsorg 53) and V10 (SRN39-2) are promising drought-tolerant genotypes for early-stage screening, which are recommended for further enzymatic and molecular studies on improving photosynthetic efficiency.

Keywords: Sorghum, Genotype, PEG-induced drought, Drought Tolerance Index, Photosynthetic efficiency

INTRODUCTION

Drought is one of the most limiting abiotic stresses affecting crop productivity globally (Abdulmalik et al., 2018), particularly in arid and semi-arid regions where rainfall variability is high and irrigation resources are limited. Its impact extends beyond reduced soil moisture, influencing physiological, biochemical and metabolic processes that determine plant survival and yield stability (Oshunsanya et al., 2019; Hussain et al., 2019; Nadeem et al., 2024). Prolonged water deficit disrupts cellular homeostasis, alters osmotic balance, and restricts nutrient uptake, thereby compounding stress severity during critical growth stages (Bhattacharya, 2021; Oguz et al., 2022). In many staple crops, even short periods of drought coincide with germination or early seedling development, leading to poor establishment and compromised stand density (Anjum et al., 2017; Dietz et al., 2021).

Sorghum (*Sorghum bicolor* L. Moench) is an important C4 cereal crop cultivated widely across Africa and Asia (Chaturvedi et al., 2022; Hossain et al., 2022). It is inherently tolerant to water deficit but however, significant genotypic variation exists in its ability to withstand early-stage drought stress (Mutava et al., 2011). Seedling establishment represents a critical developmental window during which water scarcity can severely impair germination, radicle emergence, seedling vigor and biomass accumulation, ultimately determining stand density and field performance (Wang et al., 2025). Therefore, early identification of drought-tolerant genotypes therefore provides a strategic advantage for breeding programs aimed at improving resilience and productivity under climate-induced stress conditions.

Polyethylene glycol (PEG) also known as PEG-6000 is a widely adopted osmotic agent used to simulate drought in controlled environments. PEG is chemically inert, non-toxic and due to its high molecular weight, does not penetrate seed tissues thereby creating a purely osmotic gradient that reduces water availability while avoiding ion toxicity (Reyes et al., 2023; Licaj et al., 2024). PEG-induced drought stress has been proven to offer reliable and reproducible approach for screening large sets of genotypes rapidly thereby enabling researchers to evaluate traits such as germination percentage, root elongation, shoot development and seedling vigor under controlled osmotic potentials (Chunyan et al., 2022; Licaj et al., 2024). These morphological parameters are tightly linked with physiological processes including water-uptake efficiency, membrane stability, and cellular metabolism which are key determinants of plant survival in water-limited environments (Faisal et al., 2019).

Photosynthetic efficiency is particularly sensitive to water deficit, as drought rapidly disrupts stomatal conductance, chlorophyll synthesis, photochemical activity and carbohydrate partitioning (Zahra et al., 2023; Qiao et al., 2024). Although this study focuses primarily on morphological and early growth responses, these parameters show underlying physiological mechanisms that influence photosynthetic performance and stress adaptation.

With the increasing climatic instability across major sorghum-producing regions, robust screening strategies for drought-tolerant germplasm are urgently needed. Although

extensive researches have been conducted on sorghum drought physiology, fewer studies have systematically compared multiple improved and local genotypes under standardized PEG-induced osmotic stress conditions at the seedling stage. Genotypic differences expressed during early growth present valuable insight into which lines are more capable of withstanding water deficit. These early responses not only highlight drought-tolerant genotypes but also help clarify the morphological traits that support stronger adaptation under limited moisture conditions (Blum, 2010).
Therefore, this study is aimed at investigating drought tolerance in ten sorghum genotypes using PEG-6000 to impose controlled water stress. Measurements of germination, root and shoot growth, plant height, biomass, seedling vigor, and several drought indices were used to compare their performance under reduced moisture. The evaluation highlights genotypes that cope more effectively with stress and clarified the physiological traits that shape their responses. These insights support ongoing breeding efforts aimed at developing sorghum varieties better suited to water-limited environments.

MATERIALS AND METHODS

Plant Material

Ten sorghum (*Sorghum bicolor* L. Moench) genotypes were collected from the Institute of Agricultural Research (IAR) Zaria and used for this study, and the experimental process was carried out in Department of Molecular Biology, Biotechnology Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria. All seed lots were visually inspected to ensure they are uniform and devoid of any physical damage prior to experimentation. Seeds were surface-sterilized in 2% (v/v) sodium hypochlorite for 5 minutes to eliminate surface microbial contaminants and subsequently rinsed thoroughly with sterile distilled water to remove residual disinfectant. The ten sorghum genotypes used in this study were coded as follows: V1 = Samsorg 40, V2 = Samsorg 45, V3 = Samsorg 46, V4 = Samsorg 48, V5 = Samsorg 49, V6 = Samsorg 52, V7 = Samsorg 53, V8 = Samsorg 54, V9 = NR71168-2 and V10 = SRN39-2.

Drought Stress Induction using PEG-6000

Drought stress was induced using polyethylene glycol 6000 (PEG-6000), a non-penetrating osmotic compound widely employed to simulate water deficit conditions during germination and early seedling development. Two osmotic treatments were prepared; a control solution containing 0% PEG and a 30% (w/v) PEG solution, which produces an osmotic potential of approximately –6.7 bars, in accordance with established methodologies for inducing controlled osmotic stress (de Queiroz et al., 2023; Rajashekhara et al., 2025). For each genotype, three replicates of 50 seeds were evenly placed between two sheets of germination paper previously moistened with the respective PEG solutions at a volume equivalent to 2.5 × the dry mass of the paper. The sheets were rolled tightly and placed upright in sealed containers to minimize moisture loss and maintain near-saturated humidity (>95%). Germination was carried out in a growth chamber at 25±1°C under constant conditions for 10 days (As shown in Figure 1). Seeds were considered germinated once radicle emergence exceeded 5mm.

Experimental Design

The study was arranged in a completely randomized design (CRD) with factorial treatment structure, comprising genotype and PEG level as factors, with a control treatment (0% PEG) and Drought stress treatment (30% PEG-6000) after 10 days of germination. Each treatment combination was replicated three times, following the guidelines of Gomez and Gomez (1984).

Measurement of Germination and Seedling Growth Parameters

Germination Percentage

Germination was assessed on the 10th day after planting. Germination percentage (GP) was calculated using the standard formula:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100 \quad (1)$$

Normal seedlings were identified according to international seed testing criteria, based on complete structural development and adequate elongation.

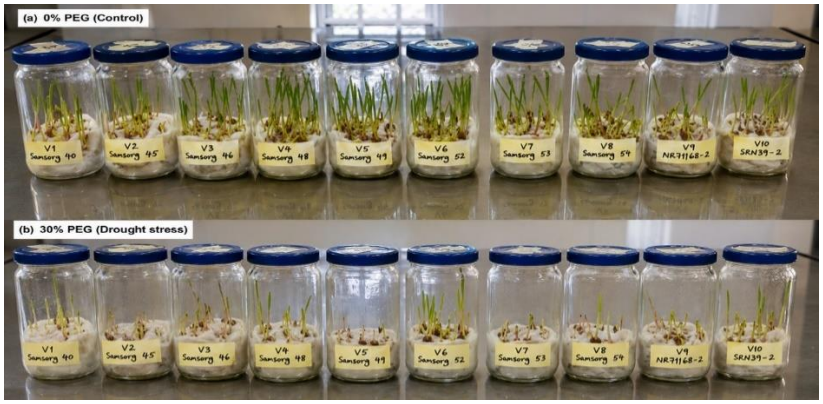


Figure 1: Experimental setup for drought stress induction in ten sorghum genotypes using polyethylene glycol (PEG-6000). (a) Control treatment (0% PEG). (b) Drought stress treatment (30% PEG-6000) after 10 days of germination

Root Length

On the 14th day after germination, ten normal seedlings from each replication and treatment were selected randomly. Primary root length was measured from the radicle tip to the hypocotyl junction using a calibrated ruler, and mean values were expressed in centimeters (cm).

Shoot Length

Shoot length was determined on the same seedlings used for root measurements. Length was recorded from the tip of the first true leaf to the base of the hypocotyl and expressed in centimeters.

Plant Height
Plant height was measured from the base of the seedling to the tip of the tallest emerging leaf. Measurements were recorded on the 9th day after planting.

Fresh Weight
Fresh weight was obtained by harvesting the shoots and roots of each seedling sample, blotting gently to remove surface moisture and weighing immediately using an analytical balance. Total fresh biomass per seedling was recorded.

Seedling Vigor Index
Seedling vigor was calculated according to Beedi et al. (2018) and Barik et al. 2022:
SVI = Germination (%) × Mean seedling length (cm) (2)
Where seedling length = shoot length + root length.

Drought Tolerance Indices
Three drought stress indices were computed to quantify genotypic response:
Germination Drought Tolerance Index (DTI)
$DTI\ (\%) = G_{control} \div G_{drought} \times 100$ (3)

Plant Height Stress Index (PHSI)
$PHSI\ (\%) = \text{Plant height}_{control} \div \text{Plant height}_{drought} \times 100$ (4)

Root Length Stress Index (RLSI)
$RLSI\ (\%) = \text{Root length}_{control} \div \text{Root length}_{drought} \times 100$ (5)
These indices were used to compare relative performance under drought and control conditions.

Statistical Analysis
All data analyses were performed using a two-way analysis of variance with a factorial model appropriate for a completely randomized design. The factors are: Variety (7 levels) and Treatment (2 levels). When treatment effects were significant ($p \leq 0.05$), means were separated using Tukey's Honest Significant Difference (HSD) test at the 5% probability level. Grouping notation used is Mean ± SD lowercase uppercase. Lowercase letters compare varieties within the same treatment column while uppercase letters compare Control vs Drought within the same variety row. Results were presented as arithmetic mean ± standard deviation (SD) of three replicates.

RESULTS AND DISCUSSION
The germination results summarized in Table 1 and depicted in Figure 2 showed clear genotype-dependent responses of sorghum to drought-induced osmotic stress. Complete failure of germination by V1 (Samsorg 40), V8 (Samsorg 54) and V9 (NR71168-2) under PEG treatment indicates a high level of sensitivity at the earliest developmental stage, as reflected by their absence from the mean plots. Among the germinating entries, drought markedly reduced emergence in V2 (Samsorg 45) from 90±1.53% to 70±3.00%, V3 (Samsorg 46) from 80±1.00% to 50±2.65%, V4 (Samsorg 48) from 100±0.58% to 70±2.00% and V5 (Samsorg 49) from 100±0.58% to 70 ±1.00%, demonstrating a 20–30% decline in establishment capacity under moisture limitation. These are clearly illustrated by the shorter drought bars in Figure 2 which is consistent with the documented effects of osmotic stress on seed water uptake, enzymatic activation and early cellular expansion in cereals (Farooq et al., 2009; Tari et al., 2013). This result provided invaluable insights into the early drought tolerance evaluation of the tested genotypes as GP is a critical indicator of seed resilience. Samples V4, V5, and V7 exhibited strong seed vigor and consistent emergence. This was followed by sample V2, while sample V3 showed moderate germination. Samples V6 (Samsorg 52) and V10 (SRN39-2) exhibited enhanced performance under stress, increasing from 70±2.65% to 90±2.00%, while V7 (Samsorg 53) maintained full germination at 100±1.00% under both control and drought conditions. The stability and improvement of these genotypes, evident in Figure 2 where drought bars match or exceed control values, suggest superior osmotic adjustment, membrane stability and conservation of metabolic activity during imbibition, traits widely associated with drought resilience in sorghum (Blum, 2010; Wimalasekera, 2016; Farooq et al., 2019). The statistical analysis showed that the three factors, variety, treatment, and their interaction, all had significant effects on the germination percentage at $p < 0.0001$. The significant effect of variety ($F = 135.50$), treatment ($F = 235.31$) and their interaction ($F = 173.03$) demonstrated considerable genetic variation in drought tolerance at the germination stage.

Table 1a: Germination Percentage of Sorghum Varieties under Control and Drought Stress (Mean ± SD)

Varieties	Control	Drought
V2	90 ± 1.53abA	70 ± 3.00cB
V3	80 ± 1.00bcA	50 ± 2.65dB
V4	100 ± 0.58aA	70 ± 2.00cB
V5	100 ± 0.58aA	70 ± 1.00cB
V6	70 ± 2.65cdB	90 ± 3.00bA
V7	100 ± 0.58aA	100 ± 1.00aA
V10	70 ± 4.16dB	90 ± 2.00bA

N =3. Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

Table 1b: Two-way ANOVA for Germination Percentage

Source of Variation	SS	df	MS	F-value	P-value
Variety	3697.24	6	616.21	135.50	< 0.0001
Treatment	1070.10	1	1070.10	235.31	< 0.0001
Variety × Treatment	4721.24	6	786.87	173.03	< 0.0001

Keys: SS= sum of squares, df= degree of freedom, MS = Mean square

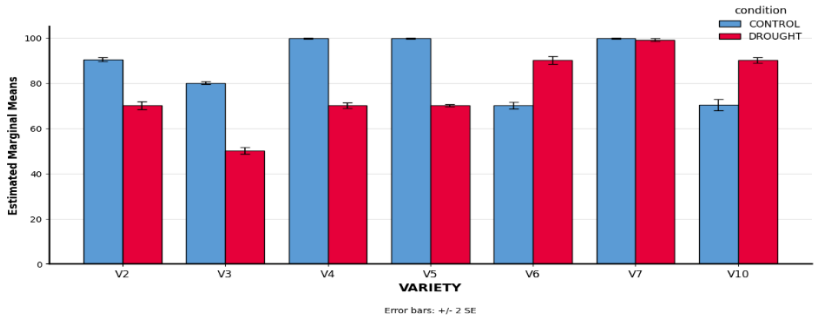


Figure 2: Mean Germination Percentage (GP) of Sorghum Varieties under Control and Drought Conditions

The result evaluates the germination potential (GP parameters) of the varieties under control and drought conditions. A pattern of genotypic variation was observed with some varieties showing higher percentage values than others under both conditions. This result provides invaluable insights into the early drought tolerance evaluation of the tested genotypes as GP is a critical indicator of seed resilience. This depicts strong genetic influence of germination in response to drought treatment. Sample V7 is the most stable and promising drought tolerant variety while samples V6 and V10 showed some drought-specific adaptations. Samples V2, V4, and V5 exhibited intermediate germination percentages under drought which indicates moderate drought tolerance whereas sample V3 showed the least response. These findings confirm germination percentage as a reliable early-stage indicator of drought tolerance in sorghum.

The shoot height results presented in Table 2 and illustrated in Figure 4 showed that PEG-induced drought substantially restricted early shoot growth in most sorghum genotypes, with the strongest reductions observed in V2 (Samsorg 45), V3 (Samsorg 46), V4 (Samsorg 48), V5 (Samsorg 49) and V6 (Samsorg 52). In these lines, mean shoot height decreased from 6.6 ± 5.81 cm to 3.2±2.6 cm in V2, 9.23±4.04 cm to 4.70±1.25 cm in V3, 14.43±8.01 cm to 6.50±3.78 cm in V4, 12.03±5.89cm to 5.23±4.52 cm in V5 and 9.7±5.11 cm to 4.53±4.05 cm in V6, demonstrating a clear downward shift under osmotic stress. These are evident in the shorter drought bars in Figure 4, which is consistent with the well-documented effects of polyethylene glycol in reducing external water potential, limiting turgor-driven cell expansion and restricting internode elongation in cereals (Saini, 2025). The magnitude of suppression in these genotypes points to limited osmotic adjustment capacity during early seedling growth, aligning with responses reported for drought-susceptible sorghum under simulated water stress (Tari et al., 2013; Tsago et al., 2014). By contrast, V7 (Samsorg 53) and V10 (SRN39-2) showed comparatively stable shoot development, with V7 declining only slightly from 7.33 ± 3.69 cm to 6.0 ± 2.78 cm and V10 increasing marginally from 4.6 ± 2.51 cm to 4.83 ± 1.76 cm. The relative stability of these two genotypes, as reflected in Figure 3 where drought bars remain close to their control values, suggests more effective maintenance of cellular hydration and growing tissue protection under water-limited conditions, mechanisms widely recognized as key contributors to early-stage drought tolerance (Blum, 2011; Hao et al., 2022). The sustained shoot growth displayed by V7 and V10 supports their potential relevance in breeding strategies targeting improved seedling vigor and adaptation to moisture-limited environments, in agreement with established concepts of drought resilience in cereals (Wimalasekera, 2016). The two-way ANOVA showed the statistically significant effect of drought on the germination percentage ($F = 9.628$, $p = 0.0043$). It also depicted that both effects of variety alone ($F = 1.295$, $p = 0.2917$) and the interaction between variety and treatment were not significant ($F = 0.669$, $p = 0.6757$). These suggest that while drought stress principally influenced germination percentage, genotypic differences played a minor role, implying a comparable reduction in germination rate under water dearth condition at this early developmental stage.

Table 2a: Shoot Height (cm) of Sorghum Varieties under Control and PEG-Induced Drought Stress (Mean ± SD)

Varieties	Control	Drought
V2	6.63±5.81abA	3.23±2.6aB
V3	9.23±4.04abA	4.70±1.25aB
V4	14.43±8.01aA	6.50±3.78aB
V5	12.03±5.89abA	5.23±4.52aB
V6	9.70±5.11abA	4.53±4.05aB
V7	7.33±3.89abA	6.00±2.78aB
V10	4.6 ± 2.51bA	4.83±1.76aB

N= 3. Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

Table 2b: Two-way ANOVA for Shoot Height

Source	SS	df	MS	F	P-value
Variety	145.093	6	24.182	1.295	0.2917
Treatment	179.800	1	179.800	9.628	0.0043
Variety × Treatment	74.901	6	12.484	0.669	0.6757

Keys: SS= sum of squares, df= degree of freedom, MS = Mean square

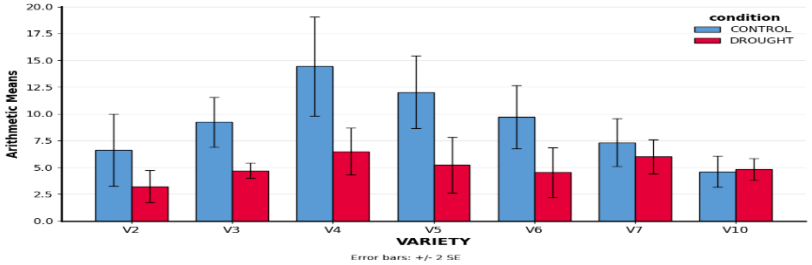


Figure 3: Mean Shoot Height (SH) for Sorghum Varieties under Control and Drought Conditions

The root length responses presented in Table 3 and illustrated in Figure 6 showed clear genotypic differences in early root development under PEG-induced drought stress among the sorghum varieties. Under control conditions, V2 (Samsorg 45), V3 (Samsorg 46), V4 (Samsorg 48) and V10 (SRN39-2) recorded the highest root lengths, measuring 16.4±2.37 cm, 15.70±3.43 cm, 15.6±10.41 cm and 15.67±0.76 cm, respectively, while V5 (Samsorg 49), V6 (Samsorg 52) and V7 (Samsorg 53) displayed moderate baseline values of 8.4±2.57 cm, 10.43±6.91 cm and 9.67±3.94 cm. Drought stress reduced root length across all genotypes, but with variable intensity. V3 (Samsorg 46) showed the highest length retention under stress at 9.20 ± 5.63 cm, followed by V5 (Samsorg 49) at 7.4±6.26 cm and V7 (Samsorg 53) and V10 (SRN39-2) at 6.67±2.53 cm and 6.67±1.26 cm. In contrast, V2 (Samsorg 45), V4 (Samsorg 48) and V6 (Samsorg 52) exhibited stronger suppression, declining to 6.37±4.38 cm, 6.63±2.25 cm and 4.03±2.35 cm respectively, with the largest absolute decreases observed in V2 (16.4 to 6.3 cm) and V6 (10.4 to 4.0 cm). These observed trends, captured in Figures 6 are consistent with reports that sorghum genotypes capable of sustaining root extension under limited water potential possess superior adaptive capacity, whereas those showing steep reductions exhibit diminished water-acquisition efficiency (Tsago et al., 2014; Abreha et al., 2022; Begna et al., 2022). The comparatively stable performance of V3 (Samsorg 46), together with the moderate retention displayed by V5 (Samsorg 49) and V7 (Samsorg 53), highlights their stronger early-stage drought adaptability, while the pronounced inhibition observed in V2 (Samsorg 45), V4 (Samsorg 48) and V6 (Samsorg 52) reflects a limited capacity for root system maintenance under osmotic stress.

Table 3a: Root Length Responses of Sorghum Genotypes under Control and PEG-Induced Drought Stress (Mean ± SD)

Varieties	Control	Drought
V2	16.47±2.37aA	6.37±4.38aB
V3	15.70±3.43aA	9.20±5.63aB
V4	15.63±10.41aA	6.63±2.25aB
V5	8.43±2.57aA	7.40±6.26aB
V6	10.43±6.91aA	4.03±2.35aB
V7	9.67±3.94aA	6.67±2.53aB
V10	15.67±0.76aA	6.67±1.26aB

Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

Table 3b: Two-way ANOVA for Root Length

Source	SS	df	MS	F	P-value
Variety	155.836	6	25.973	1.144	0.3636
Treatment	434.572	1	434.572	19.137	0.000153
Variety × Treatment	101.360	6	16.893	0.744	0.6191

Keys: SS= sum of squares, df= degree of freedom, MS = Mean square

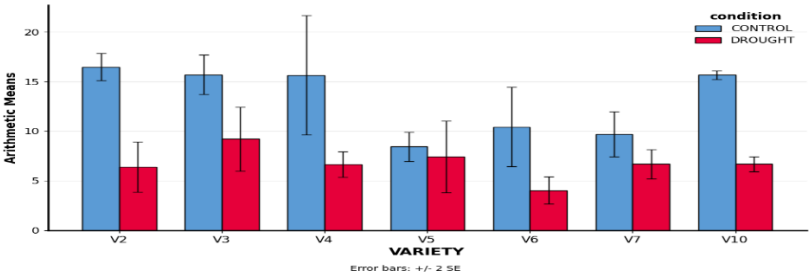


Figure 4: Mean Root Length (RL) for Sorghum Varieties under Control and Drought Conditions

The results in Table 4a and Figure 5 show that drought stress consistently reduced sorghum plant height, with clear differences among varieties in their ability to maintain growth under limited moisture conditions. Under non-stress conditions, substantial variation in plant height was observed among the varieties, with Samsorg 48 (V4) attaining the highest mean height (30.1±10.64 cm), followed by Samsorg 46 (V3) (24.9±1.86 cm) and Samsorg 45 (V2) (23.1±4.80 cm), while Samsorg 53 (V7) recorded the lowest baseline height (17.1±1.09 cm), reflecting intrinsic genetic diversity in vegetative growth potential. Exposure to drought stress resulted in marked reductions in plant height across all varieties, as illustrated in Figure 8, with drought-stressed mean heights declining to 9.6±6.68 cm in V2, 13.9±5.90 cm in V3, 13.1±5.59 cm in V4, 12.6±10.78 cm in V5, 8.6±6.37 cm in V6, 13.3±5.32 cm in V7 and 11.5±3.00 cm in V10, confirming the strong inhibitory effect of water deficit on canopy development. The proportional reductions, depicted in Figure 9, revealed that Samsorg 52 (V6) and Samsorg 45 (V2) exhibited the most pronounced height suppression, whereas Samsorg 53 (V7) and Samsorg 46 (V3) maintained comparatively higher structural integrity under stress, suggesting superior drought tolerance. These responses are consistent with established physiological mechanisms of drought-induced growth limitation, including reduced cell expansion, impaired turgor maintenance, inhibition of photosynthesis and disturbance of hormonal balance, as widely described by Farooq et al. (2009), Abreha et al. (2022) and Prasad et al. (2021). Furthermore, the relatively large standard deviations observed under drought conditions, particularly in V5 (±10.78 cm) and V6 (±6.37 cm), indicate heterogeneous stress responsiveness within genotypes, which may be associated with variability in root penetration depth, stomatal conductance and osmotic adjustment capacity, traits reported to support drought adaptation in sorghum. Therefore, the quantitative trends in Table 4 and the visual patterns in Figures 8 and Figure 9 provide evidence that drought stress significantly constrains vegetative growth in sorghum, while also identifying Samsorg 53 (V7) and Samsorg 46 (V3) as comparatively more stable performers.

Table 4a: Effect of Drought Stress on Plant Height (Cm) of Selected Sorghum Varieties (Mean ± SD)

Varieties	Control	Drought
V2	23.10±4.83abA	9.60±6.68aB
V3	24.93±1.87aA	13.90±5.90aB
V4	30.07±10.65abA	13.13±5.59aB
V5	20.47±8.08abA	12.63±10.76aB
V6	20.13±10.53bA	8.60±6.37aB
V7	17.07±1.10bA	13.33±5.33aB
V10	20.27±2.46abA	11.50±3.00aB

N= 3. Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

Table 4b: Two-Way ANOVA for Plant Height

Source	SS	df	MS	F	P-value
Variety	234.165	6	39.027	0.863	0.5342
Treatment	1152.381	1	1152.381	25.468	0.000024
Variety × Treatment	161.459	6	26.910	0.595	0.7319

Keys: SS= sum of squares, df= degree of freedom, MS = Mean square

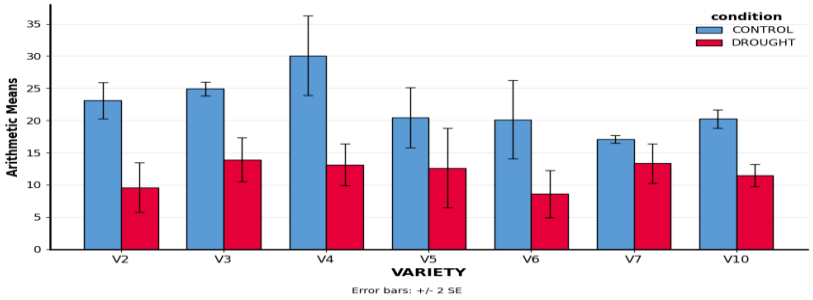


Figure 5: Comparative Plant Height (PH) of Sorghum Varieties under Control and Drought Conditions

As shown in Table 5, and further illustrated in Figure 6, the fresh weight profiles of the sorghum samples displayed clear genotype-specific responses to moisture availability. Under control conditions, varieties V4 (Samsorg 48) and V2, V3, V7 (Samsorg 45, 46, and 53) maintained the highest fresh weights (0.17±0.06g and approximately 0.14g each), indicating superior biomass accumulation, whereas V5 (Samsorg 49) recorded the lowest value (0.06 ±0.06g). When subjected to drought, most varieties exhibited reductions consistent with the expected decline in turgor-driven growth under water limitation, supporting earlier reports on sorghum's differential physiological responses to drought stress (Qadir et al., 2015; Saddam et al., 2014). V4 retained relatively high biomass under drought (0.15±0.02g), marking it as the most stable genotype in Table 5 and aligning with the drought-responsive traits described by Amelework et al. (2015) and Ali et al. (2009). In contrast, V7 (Samsorg 53) showed a drastic decrease from 0.14±0.01g to 0.01±0.03g, confirming its susceptibility. Varieties V6 (Samsorg 52) and V10 (SRN39-2) also demonstrated notable reduction,

matching patterns of reduced cellular water retention in sensitive lines as described by Ali et al. (2009). A slight increase observed in V5 under drought (0.06 → 0.09g) likely reflects compensatory early-stage physiological adjustments in genotypes with low baseline biomass, a phenomenon previously noted by Qadir et al. (2015). The distribution trends in Figures 10 and Figure 11 visually reiterates the separation between tolerant and susceptible genotypes, with V4 consistently outperforming others under both conditions. These findings collectively position V4 (Samsorg 48) and V3 (Samsorg 46) as promising varieties for drought-prone cultivation, in line with breeding priorities highlighted by Hao et al. (2022), while performance deficits in V7 and others emphasize the need for targeted genetic improvement within the sorghum breeding pipeline.

Table 5. Fresh Weight (FW) Responses of Sorghum Varieties under Control and Drought Conditions (Mean ± SD)

Varieties	Control	Drought
V2	0.137±0.031abA	0.089±0.025abB
V3	0.138±0.028abA	0.103±0.033abA
V4	0.172±0.061aA	0.133±0.027aB
V5	0.061±0.025cA	0.070±0.030abA
V6	0.132±0.047abA	0.054±0.014bB
V7	0.139±0.013abA	0.096±0.031abB
V10	0.090±0.018bcA	0.006±0.010abB

N= 3. Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

Two-Way ANOVA

Source	SS	df	MS	F	P-value
Variety	0.030560	6	0.005093	7.617	0.000065
Treatment	0.014709	1	0.014709	21.999	0.000065
Variety × Treatment	0.005903	6	0.000984	1.471	0.2240

Keys: SS= sum of squares, df= degree of freedom, MS = Mean square

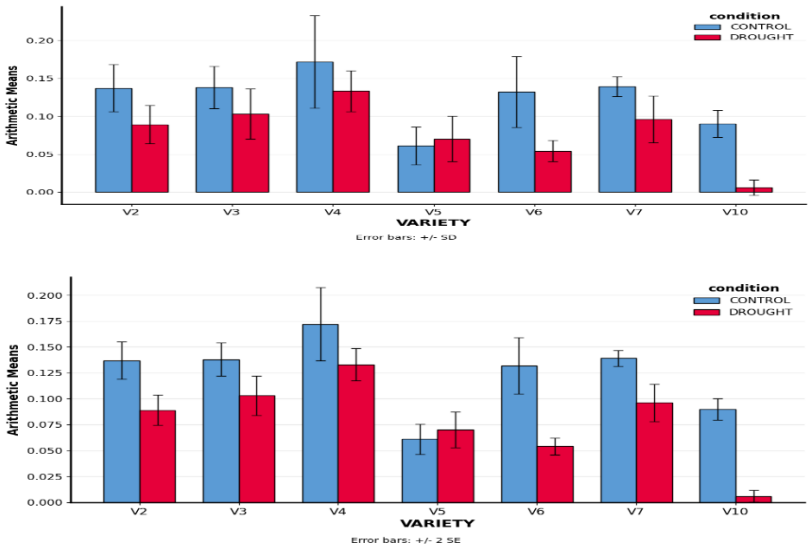


Figure 6: Comparative Fresh Weight (FW) of Sorghum Varieties under Control and Drought Conditions

Table 6 clearly show that drought stress markedly reduced seed vigor (SV) across all evaluated sorghum varieties. Under well-watered (control) conditions, variety V4 (Samsorg 48) exhibited the highest vigor (3006.7), while under drought stress its SV dropped to 919.3 which is a considerable decline but still among the higher values under stress. In contrast, some varieties especially V2 and V3) had relatively low vigor under drought, indicating drought-sensitivity. The differential performance suggests substantial genotypic variation for early-stage drought tolerance, implicating that certain varieties (notably V4 and V10) may possess intrinsic physiological or biochemical traits conferring resilience during seed germination or early growth despite moisture deficit. This is in line with previous findings that drought generally suppresses germination attributes and seedling vigor in sorghum, with genotypic variation determining the extent of reduction (Bibi et al., 2012; Wang et al., 2025).

Table 6: Seed Vigor (SV) Index of Sorghum Varieties under Control and Drought Conditions

Varieties	Control	Drought
V2	2079.0000	672.0000
V3	1994.6667	695.0000
V4	3006.6667	919.3333
V5	2046.6667	884.3333
V6	1409.3333	774.0000
V7	1706.6667	1166.6667
V10	1418.6667	1215.0000

N= 3. Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

The drought tolerance performance of the evaluated sorghum varieties, as indicated by DTI (As shown on Table 7), showed substantial variation, reflecting differential resilience to water-deficit stress. Varieties V6 and V10 exhibited the highest overall DTI values (129±0.00%), indicating superior drought tolerance, whereas V3 recorded the lowest overall DTI (63±0.00%). When individual morphological traits were considered. For shoot height (SH), V7 recorded the highest SH-DTI (72±33.40%), while V10 showed the lowest (24±16.09%), suggesting that shoot growth under drought is highly variable among genotypes. Root length (RL) exhibited pronounced variability, with V5 achieving the highest RL-DTI (83±73.11%) and V2 the lowest (37±25.69%), highlighting the importance of root development in accessing residual soil moisture and maintaining plant growth under stress. Similarly, plant length (PL) varied, with V7 attaining the highest PL-DTI (88±17.52%) and V2 the lowest (38±13.58%). These observations underscore that while overall DTI provides a useful summary of drought tolerance, trait-specific responses are critical for identifying genotypes with adaptive advantages under water-limited conditions. The high standard deviations observed in root and plant length DTIs for some varieties (in which RL-DTI of V5 = 83±73.11%) suggest significant within-variety variation or genotype and environment interactions. Consistent with previous studies, root traits such as length and architecture are reliable indicators of drought resilience, often more so than aboveground growth alone (Borrell et al., 2014; Yahaya and Shimelis, 2022).

Table 7: Drought Tolerance Index (DTI) of Sorghum Varieties Based on Shoot Height (SH), Root Length (RL) and Plant Length (PL) under Water-Deficit Conditions (Mean ± SD)

Varieties	(DTI) %	SH (DTI) %	RL (DTI)%	PL (DTI)%
V2	78 ± 0.00	49 ± 8.08	37 ± 25.69	38 ± 13.58
V3	63 ± 0.00	53 ± 9.50	63 ± 42.33	72 ± 19.80
V4	70 ± 0.00	44 ± 5.50	63 ± 45.83	46 ± 11.02
V5	70 ± 0.00	35 ±28.69	83 ± 73.11	52 ± 25.40
V6	129± 0.00	40 ±17.24	51 ± 28.54	42 ± 7.81
V7	100± 0.00	72 ±33.40	69 ± 6.92	88 ± 17.52
V10	129± 0.00	24 ±16.09	43 ± 9.50	56± 6.506

N= 3. Means followed by the same lowercase letters within a column are not significantly different, whereas means followed by the same uppercase letters within a row are not significantly different according to Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

The varying responses observed from the studied sorghum genotypes under PEG-induced drought stress condition suggest that intrinsic physiological adaptability contributes more to seedling stage drought tolerance than just the baseline vigor under optimal conditions. Early germinating genotypes that sustained growth at the initial stage under drought stress are attributed to their robust osmotic adjustment capacity, aiding their adaptative potential to external water dearth conditions. It also indicates stress-induced metabolic framework activation, which triggered adaptive responses due to osmotic stress exposure, leading to early growth enhancement. On the other hand, genotypes that struggled with germination or that showed rapid decline after germination and growth parameters can be due to limited enzymatic activation. It could also be due to low cellular expansion as a result of insufficient hydration which significantly hindered their early establishment. The differences in root length retention capacities observed from the samples reinforces the key aspect of 'below-the-ground' role in plants early adaptation to osmotic imbalance. Genotypes with good root elongation potential especially under stress demonstrate better drought resilience than the ones with poor elongation abilities. This is in agreement with findings of (Navyashree et al., 2024) and (Abreha et al., 2021) which established the critical role of root system plasticity in drought resilience, particularly at seedlings developmental stages.

Furthermore, the observed variations across the tested drought tolerance indices highlighted the multifaceted nature of drought tolerance in line with (Blum, 2010) and (Yahaya and Shimels, 2022) revolving mostly between growth maintenance and stress survival balance (Wimalasekera, 2016). Some genotypes adjust to drought by limiting shoot growth while maintaining root functions which is likely applicable to genotype V3 (Samsorg 46), whereas others may attain drought adaptation via effective physiological regulation without conserving their shoot growth as likely applicable to genotype V10 (SRN 39-2). Again, the differential stress-responsive traits among the genotypes reflected their underlying genetic heterogeneity, presenting large standard deviations within certain genotypes.

Conclusion

This study demonstrated clear genotypic variation in the response of sorghum to PEG-induced drought stress during germination and early seedling development. Drought markedly reduced germination percentage, shoot height, root length, plant height, fresh weight and seed vigor across most varieties, confirming the strong inhibitory effect of reduced water potential on early physiological processes though, the extent of reduction differed widely among genotypes. V1 (Samsorg 40), V8 (Samsorg 54) and V9 (NR71168-2) failed to germinate under stress, indicating high sensitivity, whereas V6 (Samsorg 52), V10 (SRN39-2) and V7 (Samsorg 53) maintained comparatively stable germination and growth and were also ranked as superior performers by the drought tolerance indices. V4 (Samsorg 48) and V3 (Samsorg 46) likewise performed relatively well across several traits. Variety × treatment interactions were not significant, except for germination potential, indicating a generally similar response pattern across varieties in the early developmental parameters. The consistent reductions in susceptible varieties, alongside the stability of tolerant genotypes, highlight early root maintenance, osmotic adjustment and biomass conservation as key adaptive traits. Accordingly, V4 (Samsorg 48), V3 (Samsorg 46), V6 (Samsorg 52), V7 (Samsorg 53) and V10 (SRN39-2) are suitable candidates for downstream enzymatic and molecular analyses aimed at improving photosynthetic efficiency, and they may provide useful baseline information for breeding programmes targeting sorghum establishment and seedling vigor in drought-prone environments.

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