***Original Research Article***

**Functional characterization of water saving traits to enhance drought adaptation in Sorghum**

**Abstract**

Sorghum (*Sorghum bicolor* [L.] Moench), a key staple in South Asia and sub-Saharan Africa, is increasingly challenged by climate variability. In India, post-rainy sorghum remains a vital food and fodder crop. In alignment with the ICRISAT post-rainy sorghum product profile, this study characterized sorghum parental lines for key traits associated with drought adaptation. Atmospheric drought experiments revealed significant genotypic variation in transpiration rate (TR) under high vapor pressure deficit (VPD), with K359W showing restricted TR and R16 exhibiting high TR. In soil drought trials, transpiration decline began at an FTSW of 0.49 in K359W and 0.56 in R16. K359W consistently outperformed R16 in plant growth, water use, biomass, and showed higher transpiration efficiency (TE). Genotypes with greater vigor, reduced TR under high VPD, and higher TE offer strong potential for improving post-rainy sorghum drought resilience.

**Keywords:** Sorghum,Stay-green, Drought stress, Dry-down, VPD, Nodal roots,

**1 Introduction**

Sorghum [Sorghum bicolor (L.) Moench] ranks as the fifth most important cereal crop worldwide, following wheat, maize, rice, and barley (Khaskheli et al., 2025). Sorghum is a staple food for the world’s poorest and most food-insecure populations, primarily in the semiarid tropics (Gomashe et al., 2025). As of 2021, global sorghum production reached 60.10 million tonnes, cultivated on 45.90 million ha with an average yield of 1309 kg/ha (Charyulu et al., 2024), of this, about 90% was produced in developing countries, mainly in the semi-arid regions of Africa and Asia (Mwamahonje et a., 2025). In peninsular India, 5.5 million hectares of sorghum are grown on residual moisture, producing quality grain and stover but facing increasing moisture stress (Charyulu et al., 2024). The most severe drought stress occurs during the post-flowering stage (terminal drought) (Pooja et al., 2025). Genotypes susceptible to terminal drought show early leaf and plant aging, stem breakage, lodging, charcoal rot, and a decrease in grain quantity and size (Ali et al., 2025). In sorghum, the most well-known form of drought tolerance during this growth stage is the "stay-green" trait, which enables the plant to resist premature senescence (maintain green leaf area), prevent lodging, and fill grain normally (Kamal et al., 2025). When water is scarce during grain filling, sorghum genotypes with the stay-green trait maintain photosynthetically active leaf area more effectively than those without it (Danquah et al., 2025). Delayed leaf senescence in sorghum has been associated with higher grain yields, especially in environments where water availability during grain filling is insufficient to meet potential transpiration needs (Otwani et al., 2025). Breeding efforts to develop improved varieties and top-cross hybrids for water-limited environments have progressed slowly, especially in developing countries, mainly due to the unpredictable nature of drought conditions (Kaliamoorthy et al., 2024). Recent focus on water use traits highlights two key drought adaptation strategies: limiting transpiration under high evaporative demand and reducing water use early during stress, even when soil moisture (FTSW) is still adequate (Loftus et al., 2025; Raymundo et al., 2024). These water-saving strategies help preserve moisture for the critical grain-filling phase (Vadez et al., 2014). Genotypic variation in traits that limit transpiration rate (TR) has been documented in sorghum by several studies (Somu et al., 2024). Limiting maximum transpiration rate under high evaporative demand generally improves grain yield in regions prone to post-anthesis drought stress (Kaliamoorthy et al., 2024). However, reduced stomatal conductance may limit CO₂ uptake and photosynthesis, leading to yield penalties under well-watered conditions (Kholova et al., 2013). Improving post-rainy sorghum requires a detailed understanding of physiological traits that enable drought adaptation. This study aimed to evaluate water-use traits in sorghum parental lines, specifically, assessed for plant transpiration response to atmospheric and progressive soil drought.

**2 Material and Methods**

This study involved two sorghum (*Sorghum bicolor* [L.] Moench) genotypes: K359W, an introgression line carrying the stay-green QTL STG3B developed at ICRISAT, and R16, a high-yielding but senescent post-rainy (rabi) season variety. Experiments were conducted from November to December 2023 at ICRISAT-India under controlled glasshouse and outdoor conditions.

**2.1 Plant Growth Conditions**

Plants were grown in 10-inch plastic pots filled with 7 kg of an Alfisol-sand mix (3:2 ratio). Soil was amended with di-ammonium phosphate (0.3 g/kg) and treated with carbofuran (0.3 g/pot) one day before sowing to control soil-borne pests. Four hills per pot were sown, with two seeds per hill. Thinning was done one week after emergence to retain four plants per pot, followed by a final thinning at three weeks to maintain two plants per pot. A completely randomized design was used with ten replications per treatment (well-watered [WW] and water-stressed [WS]).

**2.2 Soil Dry-Down Experiment**

To assess genotypic differences in transpiration response to progressive soil drying, dry-down experiments were performed in a glasshouse following Kholová et al. (2010). Pots were irrigated to field capacity and covered with plastic sheeting and a 2 cm layer of plastic beads to reduce evaporation. Transpiration was calculated gravimetrically from daily pot weight loss. For WW conditions, soil moisture was maintained at ~80% field capacity. In the WS treatment, daily water loss was limited to 70 g, and excess transpiration was replenished. The experiment ended when WS plant transpiration dropped below 50% and 25% of WW levels. Fraction of Transpirable Soil Water (FTSW) was calculated as: (Daily weight – Final weight)/(Initial weight − Daily weight).Normalized transpiration rate (NTR) was plotted against FTSW, and a two-segment linear regression was used to determine the FTSW threshold and slope for each genotype.

**2.3 Vapor Pressure Deficit (VPD) Experiment**

To evaluate transpiration under atmospheric drought, plants were assessed outdoors under clear sky conditions, following Kholová et al. (2010). Thirty-day-old plants were maintained at ~90% field capacity and covered with plastic sheets and beads (Karthika et al., 2019) to minimize evaporation. Pot weights were recorded hourly from 6:00 a.m. to 6:00 p.m. using a precision scale (KERN 24100, Kern & Sohn GmbH). Transpiration rate (TR) was calculated as water loss per unit leaf area (mg H₂O cm⁻² min⁻¹). Leaf area was measured post-harvest, and plants were oven-dried at 60°C for 72 hours to determine dry biomass.

**2.4 Statistical Analysis**

Genotypic variation in response to soil and atmospheric drought was analyzed using one-way ANOVA followed by the Tukey–Kramer post-hoc test (CoStat v6.204, Cohort Software). Graphs and regression analyses were performed using Microsoft Excel 2017 and GraphPad Prism v6 (GraphPad Software Inc.).

**3. Results and discussion**

In this study, R16 (drought susceptible) and K359W (drought tolerant), were characterized for various traits such as leaf area, biomass, root and shoot weights, and other growth-related metrics under drought stress conditions.

**3.1 Traits characterized under soil-drought (dry-down)**

**3.1.1 Leaf related traits**

Significant differences in leaf area were observed between the two parental lines. R16 had a total leaf area of 1954.8 cm², whereas K359W exhibited a significantly larger leaf area of 2104.8 cm² (Table 1; Fig 2). This increased leaf area in K359W may contribute to its enhanced drought tolerance, as a larger leaf surface area can improve photosynthesis. Additionally, K359W showed a higher leaf fresh weight (17.45 g) compared to R16 (16.57 g) (Table 1), suggesting a better ability for water retention or improved leaf hydration under drought conditions. However, no significant differences were found in leaf dry weight between the two parents, with R16 and K359W showing almost identical values (8.43 g vs. 8.38 g) (Table 1), indicating that leaf dry weight may not be a critical factor in K359W’s drought tolerance. Finally, K359W exhibited a lower senescence score (8.5) compared to R16 (9.3) (Table 1), suggesting that K359W experiences delayed senescence and retains better physiological health under drought stress.

**3.1.2 Stem related traits**

K359W exhibited superior performance in stem fresh weight (42.18 g) compared to R16 (39.54 g). Similarly, K359W surpassed R16 in stem dry weight, with values of 14.89 g and 13.52 g, respectively (Table 1).

**3.1.3 Root related traits**

K359W outperformed R16 in both root fresh weight (34.18 g vs. 31.26 g) and root dry weight (2.66 g vs. 1.78 g), indicating that K359W, the drought-tolerant variety, possesses more robust root development, a key factor for water uptake during water-scarce periods (Table 1). Additionally, K359W demonstrated a significantly larger root surface area (2470.3 cm² vs. 1754.8 cm²), which may contribute to the observed differences in drought-related traits between the two genotypes (Table 1). K359W also exhibited a significantly greater root volume (25.15 cm³ vs. 15.74 cm³), suggesting a more developed and efficient root system (Table 1). These enhanced root traits in K359W are indicative of its capacity to better access water and nutrients under conditions of limited water availability, further supporting its drought tolerance.

**3.1.4 Plant height (cm)**

K359W exhibited a slightly greater plant height (142 cm vs. 137.3 cm), although the difference was relatively minor (Table 1). This increased height may reflect improved overall growth and more efficient water management under drought conditions. This result is consistent with the findings of Kailamoorthy et al. (2024), where significant differences in plant height were observed between B and R lines, with B lines averaging 115 cm and R lines averaging 145 cm.

**3.2 Traits characterized under VPD**

**3.2.1 Leaf related traits**

The difference in total plant leaf area between R16 and K359W was statistically significant. R16 had a leaf area of 4355.97 cm², while K359W exhibited a slightly larger leaf area of 4453.34 cm² (Table 2; Fig. 1), suggesting superior leaf expansion, which may enhance photosynthesis and drought resilience. K359W also demonstrated a higher leaf fresh weight (98.45 g) compared to R16 (87.15 g) (Table 2; Fig. 3), indicating its improved water retention capacity in leaves, contributing to better drought tolerance. Additionally, K359W showed a higher leaf dry weight (21.87 g) than R16 (19.04 g) (Table 2), suggesting more efficient biomass accumulation and enhanced metabolic adaptation under drought stress. No significant difference in senescence was observed between the two genotypes, with R16 scoring 6.3 and K359W scoring 6.5 (Table 2), indicating similar aging patterns under drought conditions. However, K359W exhibited a significantly higher number of developed leaves (14.71) compared to R16 (13.75) (Table 2), suggesting better leaf development and physiological adaptation, which may enhance photosynthetic capacity and resource use under water-limited conditions.

**3.2.2 Stem related traits**

A highly significant difference was observed in stem fresh weight, with K359W outperforming R16 (193.33 g vs. 152.09 g) (Table 2; Fig. 5). This suggests that K359W is better equipped to support growth under drought stress, likely due to its increased stem biomass. Similarly, a highly significant difference was noted in stem dry weight, with K359W again outperforming R16 (82.36 g vs. 55.18 g) (Table 2; Fig. 6).

**3.2.3 Total biomass (g)**

K359W exhibited significantly higher total biomass (111.47 g) compared to R16 (81.62 g) (Table 2; Fig. 9), reflecting its superior performance under drought stress and its ability to efficiently accumulate biomass despite water limitations. These findings are consistent with Kailamoorthy et al. (2024), who linked enhanced photosynthetic assimilate production and increased biomass to higher grain yields in post-rainy sorghum. Similar patterns have been observed in other crops, such as pearl millet (Kholová et al., 2010) and chickpea (Kailamoorthy et al., 2024), where high-vigor genotypes exhibited improved growth and biomass under water-limited conditions.

**3.2.4. Root related traits**

K359W outperformed R16 in root fresh weight (74.62 g vs. 63.85 g) (Table 2; Fig. 7), indicating superior root growth and development. It also showed higher root dry weight (9.15 g vs. 6.74 g) (Table 2), a key trait for drought tolerance, as it enhances water and nutrient uptake under moisture-limited conditions. Additionally, K359W exhibited a larger root surface area (3025.38 cm² vs. 2627.80 cm²) (Table 2; Fig. 8), suggesting greater soil exploration capacity. K359W also had a higher root volume (46.66 cm³ vs. 23.18 cm³) (Table 2), indicating a more efficient root system capable of supporting improved water uptake during drought. Furthermore, K359W displayed a greater number of nodal roots (32.16 vs. 26.25), reflecting enhanced root architecture that likely facilitates better resource acquisition (Table 2; Fig. 14 & 15).

**3.2.5 Plant height (cm)**

A significant variation in plant height was observed, with K359W exhibiting greater height (185.1 cm) compared to R16 (173.57 cm). This increased stature in K359W may reflect enhanced overall growth and a superior ability to utilize available resources under drought stress (Table 2).

**3.2.6. Transpiration & Transpiration rate**

K359W demonstrated significantly higher transpiration rates compared to R16 under both moderate and high vapor pressure deficit (VPD) conditions. At moderate VPD (1.6 kPa), K359W had a transpiration rate of 65.12 mg H₂O/hr, which was significantly greater than R16's 58.62 mg H₂O/hr (Table 2; Fig. 10). Similarly, at high VPD (3.0 kPa), K359W exhibited a transpiration rate of 77.87 mg H₂O/hr, whereas R16 showed 69.25 mg H₂O/hr (Table 2; Fig. 10). The mean transpiration rate of K359W (55.16 mg H₂O/hr) was also significantly higher than R16 (49.95 mg H₂O/hr) (Table 2; Fig. 10), further supporting K359W's ability to manage water loss while maintaining growth during drought stress.

Additionally, K359W outperformed R16 in terms of transpiration rates per unit leaf area (mg H₂O cm-2 min-1) at both moderate and high VPD. At moderate VPD (1.6 kPa), K359W exhibited a transpiration rate of 0.237 mg H₂O cm-2 min-1, while R16 had a lower rate of 0.224 mg H₂O cm-2 min-1 (Table 2; Fig. 11). Under high VPD (3.0 kPa), K359W's transpiration rate was 0.284 mg H₂O cm-2 min-1 compared to R16’s 0.264 mg H₂O cm-2 min-1 (Table 2; Fig. 11). The higher transpiration rates of K359W under varying VPD conditions demonstrate its efficient water regulation system, contributing to its improved drought tolerance. These findings align with Sinclair et al. (2005), who linked reduced transpiration rate (TR) to higher sorghum yields in arid regions, improving water conservation and transpiration efficiency (TE). Limiting TR under high VPD may enhance water conservation during grain filling (Vadez et al., 2013).

**3.3. Impact of water-stress (WW & WS) on R16 and K359W**

This study evaluated the effects of water stress on various physiological traits in two sorghum genotypes, R16 and K359W, under two treatment conditions: well-watered (WW) and water-stressed (WS). The following key findings were observed based on statistical analysis of the data.

**3.3.1 Leaf dry weight (g) at** **the** **5th leaf stage under WW**

A significant difference in leaf dry weight was observed between the two treatments. Under the well-watered (WW) condition, K359W exhibited a higher leaf dry weight (0.972 g) compared to R16 (0.62 g) (Table 3). This indicates that K359W allocates more resources to leaf structure, likely as an adaptive mechanism to enhance photosynthetic capacity and improve overall plant growth.

**3.3.2 Stem dry weight (g) under WW**

A significant difference in stem dry weight was observed between the parental genotypes. Under the well-watered (WW) treatment, K359W exhibited a higher stem dry weight (0.5 g) compared to R16 (0.35 g) (Table 3). This increase in K359W suggests an adaptive strategy where the plant allocates more resources to stem development, potentially to enhance water storage capacity or provide better structural support under optimal conditions.

**3.3.3 Total plant leaf area (cm²) under WW and WS**

A significant difference in total plant leaf area (TPLA) was observed between the parental genotypes. Under the well-watered (WW) treatment, K359W exhibited a higher TPLA (2436.4 cm²) compared to R16 (1999.02 cm²) (Table 3). Similarly, under water-stress (WS) conditions, K359W maintained a larger TPLA (2111.8 cm²) compared to R16 (1939.14 cm²) (Table 3;). This suggests that K359W may have an enhanced ability to maintain leaf area under both optimal and stressed conditions. These findings are consistent with those reported by (Kholova et al., 2010) where, R lines demonstrated greater leaf area, a trait strongly associated with high transpiration in pearl millet and chickpea.

**3.3.4 Plant height (cm) at 5th to 11th leaf stage under WW & WS**

Plant height (PH) measurements from the 5th to the 11th leaf stage under well-watered (WW) conditions revealed a consistent and progressive increase in both genotypes, R16 and K359W, with K359W showing a significant growth advantage. At the 5th leaf stage, K359W had a height of 81.33 cm, notably taller than R16, which recorded 63.16 cm. This difference in plant height persisted through all subsequent stages, with K359W continuing to outperform R16 at the 6th leaf (93 cm vs. 78.8 cm), 7th leaf (109.1 cm vs. 90.6 cm), and 8th leaf stages (132.6 cm vs. 119.2 cm). The height difference widened further at the 9th leaf (148.3 cm vs. 135.3 cm), 10th leaf (165.1 cm vs. 152.5 cm), and 11th leaf stages (172.8 cm vs. 162 cm), highlighting a more vigorous vertical growth in K359W under WW conditions (Table 3). Notably, the rate of height increase was most pronounced between the 6th and 8th leaf stages for both genotypes, with K359W exhibiting accelerated growth during this period, indicating higher growth efficiency or responsiveness to water availability during this critical vegetative phase.

Under water-stressed (WS) conditions, both genotypes exhibited progressive increases in plant height from the 5th to the 10th leaf stage. At the 5th leaf stage, K359W had a greater plant height (80.14 cm) compared to R16 (64.71 cm), indicating an early growth advantage. This trend continued at the 6th leaf (92.3 cm vs. 77.7 cm) and 7th leaf stages (108.6 cm vs. 92.5 cm). However, the difference between the genotypes slightly narrowed at the 8th leaf stage, with K359W measuring 124.4 cm and R16 reaching 115.1 cm. At the 9th leaf stage, the difference was marginal, with K359W at 139.1 cm and R16 at 133.5 cm. By the 10th leaf stage, the height difference was minimal, with K359W at 141.5 cm and R16 at 139.4 cm, suggesting a convergence in plant height under prolonged water stress (Table 3). This indicates that while K359W initially maintains a height advantage under water-limited conditions, the growth gap between the two genotypes narrows over time, likely due to a reduction in the growth rate as stress intensifies or physiological adjustments are made in both genotypes.

**3.3.5 Leaf senescence at 5th to 11th leaf stage under WW & WS**

Leaf senescence remained minimal during the early vegetative stages (5th to 8th leaf stage) in both genotypes, R16 and K359W, under well-watered (WW) conditions. At the 5th leaf stage, both genotypes exhibited identical senescence values (0.2). Between the 6th and 8th leaf stages, R16 showed a slightly higher but still low level of senescence (0.4) compared to K359W (0.2), indicating a marginally earlier onset of leaf aging in R16. A noticeable increase in leaf senescence was observed starting from the 9th leaf stage. At this point, R16 displayed a higher level of senescence (3.4) compared to K359W (3.2). This trend continued at the 10th leaf stage, where R16 reached a senescence value of 3.8, while K359W exhibited a senescence score of 3.6. By the 11th leaf stage, R16 recorded a senescence value of 5.4, compared to K359W’s 5.0. These findings suggest that K359W exhibited delayed leaf senescence compared to R16 under WW conditions, implying that K359W maintains healthier leaves for a longer period during the vegetative growth phase (Table 3). Under water-stressed (WS) conditions, both genotypes exhibited low levels of leaf senescence during the early vegetative stages. At the 5th leaf stage, senescence was minimal and identical in both genotypes (0.2). From the 6th to the 8th leaf stages, R16 consistently showed slightly higher senescence values (0.6) compared to K359W (0.4), indicating a modestly earlier onset of stress-induced leaf aging in R16. A significant increase in leaf senescence was noted from the 9th leaf stage onwards. At this stage, R16 exhibited a senescence value of 3.8, while K359W showed a slightly lower value of 3.4. The gap between the two genotypes widened as stress progressed; by the 10th leaf stage, R16 had reached a senescence score of 5.2, while K359W was slightly lower at 4.8. By the 11th leaf stage, R16 peaked at 6.0, while K359W remained comparatively lower at 5.2. This consistent trend indicates that K359W displayed delayed and reduced leaf senescence compared to R16, even under water-limited conditions, suggesting that K359W is better at maintaining leaf integrity under drought stress (Table 3).

**3.3.6 Number of developing leaves at 5th to 11th leaf stage under WW & WS**

The number of developing leaves in both R16 and K359W remained comparable throughout all observed leaf stages under well-watered (WW) conditions. At the 5th and 6th leaf stages, both genotypes exhibited 2.6 developing leaves, indicating synchronized early vegetative development. A slight increase occurred at the 7th leaf stage, where both genotypes recorded 3.4 developing leaves. By the 8th leaf stage, K359W showed a minor increase to 3.6 developing leaves, compared to 3.4 in R16, suggesting a slightly more active meristematic growth in K359W. However, this advantage was short-lived, as both genotypes aligned at 3.8 developing leaves from the 9th leaf stage onward, maintaining this number consistently through the 10th and 11th leaf stages (Table 3). These results indicate a parallel pattern of leaf development in both genotypes, with minimal divergence. The temporary increase in K359W at the 8th leaf stage reflects a subtle difference in growth dynamics, but this difference did not persist into later stages. Under water-stressed (WS) conditions, both R16 and K359W began similarly at the 5th and 6th leaf stages, each maintaining 2.6 developing leaves. Divergence between the two genotypes began at the 7th leaf stage, where K359W maintained 2.8 developing leaves, while R16 slightly dropped to 2.4. This suggests an early impact of water stress on R16's leaf development. The difference became more pronounced at the 9th leaf stage, with K359W maintaining 2.8 developing leaves, while R16 further declined to 2.2. This pattern continued through the 10th and 11th leaf stages, where K359W sustained a modest lead with 2.4 developing leaves compared to 2.2 in R16 (Table 3). This consistent advantage in K359W indicates better resilience in maintaining leaf initiation and development processes under drought stress. The ability of K359W to sustain a higher number of developing leaves under water-limited conditions likely reflects superior meristem activity and resource allocation, contributing to prolonged vegetative growth and delayed senescence.

**3.3.7. NTR vs FTSW**

The sensitivity of plants to soil moisture deficit can be effectively assessed through the soil moisture threshold, referred to as the Fraction of Total Soluble Water (FTSW), at which plant transpiration significantly decreases compared to well-watered (WW) plants. A notable range of variation was observed for both R16 and K359W under WW and water-stressed (WS) conditions across a decreasing gradient of FTSW values from 0.90 to 0.03, measured between November 29 and December 26, 2023 (Table 4; Fig. 12 & 13). Under WW conditions, both genotypes, K359W and R16, maintained consistently high NTR (ranging from ~1.00 to 1.14), indicating stable transpiration regardless of water availability. In contrast, under WS conditions, K359W demonstrated superior drought tolerance by consistently showing higher NTR than R16 across almost all FTSW levels, particularly under moderate to severe stress (FTSW < 0.5). As FTSW decreased, NTR in both genotypes declined, but the decline was more gradual in K359W, suggesting better water use efficiency and stress adaptation. At severe water stress (FTSW ≤ 0.10), K359W maintained higher NTR values (~0.03–0.05) compared to R16 (~0.04–0.06), further reinforcing its resilience under drought conditions. The observed variability in FTSW thresholds among the two sorghum genotypes highlights the genetic diversity in their response to soil moisture deficit and underscores the potential of K359W as a more drought-tolerant genotype. This trend is consistent with findings in sorghum (Gholipoor et al., 2010) and pearl millet (Kholová et al., 2010), where slower transpiration decline in certain R lines suggests a water conservation strategy. This helps these varieties endure drought, especially during grain-filling. Genetic variability in NTR-FTSW thresholds is crucial for breeding programs (Karthika et al., 2019).

**Table 1: Dry-down one-way ANOVA for various plant traits**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNO** | **Trait** | **GMS** | **EMS** | **P-value** | **CV** | **R16** | **SD** | **SE** | **K359W** | **SD** | **SE** | **LSD** |
| 1 | Total plat leaf area (cm2) | 67456.50 | 1803.20 | .0001 \*\*\* | 2.09 | 1954.8 b | 7.91 | 3.54 | 2104.8 a | 4.87 | 2.18 | 54.60 |
| 2 | Leaf fresh weight (g) | 1.96 | 0.03 | .0000 \*\*\* | 0.01 | 16.57 b | 0.21 | 0.09 | 17.45 a | 0.13 | 0.06 | 0.30 |
| 3 | Leaf dry weight (g) | 0.01 | 0.06 | .7525 ns | 0.03 | 8.43 a | 0.33 | 0.13 | 8.38 a | 0.13 | 0.05 | 0.32 |
| 4 | Senescence | 3.20 | 0.25 | .0023 \*\* | 0.06 | 9.3 a | 0.48 | 0.15 | 8.5 b | 0.53 | 0.17 | 0.47 |
| 5 | No of developed leaves | 1.71 | 0.24 | .0205 \* | 0.04 | 10.75 b | 0.46 | 0.16 | 11.42 a | 0.53 | 0.20 | 0.57 |
| 6 | No of developing leaves | 0.80 | 0.40 | .2069 ns | 0.85 | 0.6 a | 0.52 | 0.17 | 1 a | 0.82 | 0.08 | 0.64 |
| 7 | Stem fresh weight (g) | 17.42 | 0.40 | .0002 \*\*\* | 0.02 | 39.54 b | 0.58 | 0.26 | 42.18 a | 0.58 | 0.26 | 0.92 |
| 8 | Stem dry weight (g) | 3.76 | 0.09 | .0007 \*\*\* | 0.02 | 13.52 b | 0.12 | 0.06 | 14.89 a | 0.41 | 0.20 | 0.52 |
| 9 | Shoot biomass (g) | 1.04 | 0.25 | .0872 ns | 2.21 | 22.21 a | 0.14 | 0.07 | 22.94 a | 0.69 | 0.35 | 0.86 |
| 10 | Total biomass (g) | 8.17 | 0.18 | .0003 \*\*\* | 0.02 | 23.68 b | 0.31 | 0.14 | 25.59 a | 0.55 | 0.27 | 0.71 |
| 11 | Diameter (mm) | 0.44 | 0.02 | .0018 \*\* | 0.03 | 5.01 b | 0.13 | 0.05 | 5.4 a | 0.18 | 0.07 | 0.20 |
| 12 | Root fresh weight (g) | 23.30 | 1.20 | .0017 \*\* | 0.03 | 31.26 b | 0.42 | 0.19 | 34.18 a | 1.43 | 0.64 | 1.57 |
| 13 | Root dry weight (g) | 1.74 | 0.01 | .0000 \*\*\* | 0.05 | 1.78 b | 0.11 | 0.05 | 2.66 a | 0.12 | 0.05 | 0.18 |
| 14 | Root to shoot ratio | 0.00 | 8.48 | .0001 \*\*\* | 0.09 | 0.082 b | 0.01 | 0.00 | 0.118 a | 0.01 | 0.00 | 0.01 |
| 15 | Root surface area (cm2) | 1137605.00 | 170.39 | .0000 \*\*\* | 0.01 | 1754.8 b | 11.28 | 5.05 | 2470.3 a | 15.09 | 7.55 | 21.80 |
| 16 | Root volume (cm3) | 241.34 | 0.93 | .0000 \*\*\* | 0.05 | 15.74 b | 0.33 | 0.15 | 25.15 a | 1.26 | 0.52 | 1.37 |
| 17 | Nodal roots | 14.29 | 0.26 | .0000 \*\*\* | 0.04 | 13.3 b | 0.52 | 0.21 | 15.37 a | 0.52 | 0.18 | 0.65 |
| 18 | Plant height (cm) | 59.39 | 7.70 | .0215 \*  | 0.02 | 137.3 b | 3.67 | 1.50 | 142 a | 0.71 | 0.32 | 3.97 |

**Table 2: VPD ANOVA Table**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNO** | **Trait** | **GMS** | **EMS** | **P value sign** | **CV** | **R16** | **SD** | **SE** | **K359W** | **SD** | **SE** | **LSD** |
| 1 | Total plant leaf area (cm2) | 23706.2 | 58.82 | .0000 \*\*\* | 0.17 | 4355.97 b | 9.16 | 4.10 | 4453.34 a | 5.81 | 2.60 | 11.18 |
| 2 | Leaf fresh weight (g) | 319.3 | 3.05 | .0000 \*\*\* | 1.88 | 87.15 b | 2.01 | 0.90 | 98.454 a | 1.44 | 0.64 | 2.55 |
| 3 | Leaf dry weight (g) | 21.9 | 0.58 | .0002 \*\*\* | 3.70 | 19.04 b | 0.74 | 0.33 | 21.87 a | 0.78 | 0.32 | 1.09 |
| 4 | Senescence | 0.1 | 0.26 | .4903 ns | 0.08 | 6.3 a | 0.50 | 0.17 | 6.5 a | 0.53 | 0.17 | 0.51 |
| 5 | No of developed leaves | 3.5 | 0.22 | .0017 \*\* | 0.03 | 13.75 b | 0.46 | 0.16 | 14.71 a | 0.49 | 0.18 | 0.54 |
| 6 | No of developing leaves | 0.6 | 0.15 | .0769 ns | 0.12 | 3a | 0.00 | 0.00 | 3.42 a | 0.53 | 0.20 | 0.50 |
| 7 | Stem fresh weight (g) | 4638.4 | 5.61 | .0000 \*\*\* | 0.01 | 152.09 b | 2.71 | 1.21 | 193.33 a | 2.05 | 0.92 | 3.39 |
| 8 | Stem dry weight (g) | 1846.9 | 1.02 | .0000 \*\*\* | 0.01 | 55.18 b | 0.64 | 0.29 | 82.36 a | 1.28 | 0.57 | 1.47 |
| 9 | Shoot biomass (g) | 1829.3 | 0.41 | .0000 \*\*\* | 0.01 | 75.37 b | 0.41 | 0.18 | 102.42 a | 0.81 | 0.36 | 0.93 |
| 10 | Total biomass (g) | 2227.5 | 0.56 | .0000 \*\*\* | 0.01 | 81.62 b | 0.45 | 0.20 | 111.47a | 0.97 | 0.43 | 1.10 |
| 11 | Diameter (mm) | 3.1 | 0.08 | .0000 \*\*\* | 0.05 | 5.43 b | 0.12 | 0.05 | 6.38 a | 0.36 | 0.13 | 0.35 |
| 12 | Root fresh weight (g) | 290.2 | 5.72 | .0001 \*\*\* | 0.03 | 63.85 b | 2.18 | 0.97 | 74.62 a | 2.59 | 1.16 | 3.48 |
| 13 | Root dry weight (g) | 13.9 | 0.05 | .0000 \*\*\* | 0.03 | 6.74 b | 0.20 | 0.09 | 9.15 a | 0.25 | 0.10 | 0.38 |
| 14 | R/S ratio | 5.6 | 2.63 | .65ns | 0.06 | 0.086a | 0.01 | 0.00 | 0.085a | 0.00 | 0.00 | 0.01 |
| 15 | Root surface area (cm2) | 395166.7 | 237.76 | .0000 \*\*\* | 0.01 | 2627.80b | 14.41 | 6.45 | 3025.38 a | 16.36 | 7.32 | 22.48 |
| 16 | Root volume (cm3) | 1225.1 | 1.13 | .0000 \*\*\* | 0.03 | 23.18 b | 0.61 | 0.27 | 46.66 a | 1.47 | 0.73 | 1.78 |
| 17 | Nodal roots | 120.0 | 0.86 | .0000 \*\*\* | 0.03 | 26.25 b | 0.71 | 0.25 | 32.16 a | 1.17 | 0.48 | 1.16 |
| 18 | Plant height (cm) | 468.6 | 1.38 | .0000 \*\*\* | 0.01 | 173.57b | 1.27 | 0.48 | 185.1 a | 1.07 | 0.62 | 1.36 |
| 19 | Low VPD (0.6kPa) Transpiration (mg H2O per hr) | 0.5 | 0.42 | .3153 ns | 2.90 | 22 a | 0.816 | 0.408 | 22.5 a | 0.408 | 0.204 | 1.11 |
| 20 | Moderate VPD (1.6kPa) Transpiration (mg H2O per hr) | 84.5 | 1.14 | .0001 \*\*\* | 1.72 | 58.62 b | 1.109 | 0.554 | 65.12 a | 1.031 | 0.515 | 1.85 |
| 21 | High VPD (3.0kPa) Transpiration (mg H2O per hr) | 148.8 | 3.57 | .0007 \*\*\* | 2.56 | 69.25 b | 1.555 | 0.777 | 77.87 a | 2.175 | 1.087 | 3.27 |
| 22 | Mean transpiration | 54.6 | 0.55 | .0001 \*\*\* | 1.42 | 49.95 b | 0.160 | 0.080 | 55.16 a | 1.009 | 0.505 | 1.29 |
| 23 | Low VPD 0.6kPa) Transpiration Rate (mg H2O cm-2 min-1) | 0.0 | 4.66 | .2383 ns | 2.60 | 0.084 a | 0.003 | 0.001 | 0.082 a | 0.001 | 0.001 | 0.00 |
| 24 | Moderate VPD 1.6kPa) Transpiration Rate (mg H2O cm-2 min-1) | 0.0 | 0.00 | .0133 \* | 2.38 | 0.224 b | 0.007 | 0.004 | 0.237 a | 0.003 | 0.001 | 0.01 |
| 25 | High VPD (3.0kPa) Transpiration Rate (mg H2O cm-2 min-1) | 0.0 | 0.00 | .0115 \* | 2.76 | 0.264 b | 0.006 | 0.003 | 0.284 a | 0.009 | 0.004 | 0.01 |
| 26 | Mean transpiration rate | 2.10E-04 | 1.08E-05 | .0045 \*\* | 1.67 | 0.191 | 0.003 | 0.002 | 0.201 | 0.004 | 0.002 | 0.01 |

**Table 3: Dry down ANOVA for leaf traits under WW and WS**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Trait** | **Trt** | **GMS** | **EMS** | **P value sign** | **CV** | **R16** | **SD** | **SE** | **K359W** | **SD** | **SE** | **LSD** |
| LDW 5th | WW | 0.309 | 1.6 | .0000 \*\*\* | 1.58% | 0.62 b | 0.016 | 0.007 | 0.972 a | 0.008 | 0.004 | 0.018 |
| SDW 5th | WW | 0.05 | 1.9 | .0000 \*\*\* | 3.20% | 0.35 b | 0.011 | 0.005 | 0.5 a | 0.016 | 0.006 | 0.02 |
| TPLA | WW | 287030.6 | 545.2 | .0000 \*\*\* | 1.05% | 1999.02 b | 5.72 | 3.30 | 2436.4 a | 32.52 | 18.78 | 52.93 |
| TPLA | WS | 44736.2 | 53.3 | .0000 \*\*\* | 0.36% | 1939.14 b | 8.41 | 4.86 | 2111.8 a | 6.00 | 3.46 | 16.55 |
| PH at 5 lstg | WW | 990.08 | 1.81 | .0000 \*\*\* | 1.86% | 63.16 b | 0.98 | 0.40 | 81.33 a | 1.63 | 0.67 | 1.73 |
| PH at 6 lstg | WW | 547.3 | 1.2 | .0000 \*\*\* | 1.28% | 78.8 b | 0.98 | 0.40 | 93 a | 1.10 | 0.49 | 1.56 |
| PH at 7 lstg | WW | 940.1 | 1.55 | .0000 \*\*\* | 1.23% | 90.6 b | 1.52 | 0.68 | 109.1 a | 0.98 | 0.40 | 1.78 |
| PH at 8 lstg | WW | 448.9 | 1 | .0000 \*\*\* | 0.79% | 119.2 b | 0.84 | 0.37 | 132.6 a | 1.14 | 0.51 | 1.45 |
| PH at 9 lstg | WW | 507 | 1.46 | .0000 \*\*\* | 0.85% | 135.3 b | 1.03 | 0.42 | 148.3 a | 1.37 | 0.56 | 1.55 |
| PH at 10 lstg | WW | 481.3 | 0.83 | .0000 \*\*\* | 0.57% | 152.5 b | 0.55 | 0.22 | 165.1 a | 1.17 | 0.48 | 1.17 |
| PH at 11 lstg | WW | 352.08 | 1.48 | .0000 \*\*\* | 0.72% | 162 b | 0.89 | 0.37 | 172.8 a | 1.47 | 0.60 | 1.56 |
| PH at 5 lstg | WS | 833.14 | 1.02 | .0000 \*\*\* | 1.39% | 64.71 b | 1.11 | 0.42 | 80.14 a | 0.90 | 0.34 | 1.17 |
| PH at 6 lstg | WS | 690.46 | 1.34 | .0000 \*\*\* | 1.37% | 77.7 b | 1.11 | 0.42 | 92.3 | 1.21 | 0.49 | 1.47 |
| PH at 7 lstg | WS | 1040.06 | 1.84 | .0000 \*\*\* | 1.35% | 92.5 b | 1.51 | 0.53 | 108.6 a | 1.19 | 0.42 | 1.45 |
| PH at 8 lstg | WS | 301.7 | 2.38 | .0000 \*\*\* | 1.28% | 115.1 b | 1.46 | 0.55 | 124.4 a | 1.62 | 0.61 | 1.79 |
| PH at 9 lstg | WS | 96.3 | 1.83 | .0000 \*\*\* | 0.99% | 133.5 b | 1.38 | 0.56 | 139.1 a | 1.33 | 0.54 | 1.74 |
| PH at 10 lstg | WS | 16.07 | 1.28 | .0041 \*\* | 0.80% | 139.4 b | 1.27 | 0.48 | 141.5 b | 0.98 | 0.37 | 1.32 |
| LS at 5 lstg | WW | 0 | 0.2 | 1 ns | 223.6 | 0.2 a | 0.45 | 0.20 | 0.2 a | 0.45 | 0.20 | 0.65 |
| LS at 6 lstg | WW | 0.1 | 0.25 | 0.544 ns | 166.6 | 0.4 a | 0.55 | 0.24 | 0.2 a | 0.45 | 0.20 | 0.73 |
| LS at 7 lstg | WW | 0.1 | 0.25 | 0.544 ns | 166.6 | 0.4 a | 0.55 | 0.24 | 0.2 a | 0.45 | 0.20 | 0.73 |
| LS at 8 lstg | WW | 0.1 | 0.25 | 0.544 ns | 166.6 | 0.4 a | 0.55 | 0.24 | 0.2 a | 0.45 | 0.20 | 0.73 |
| LS at 9 lstg | WW | 0.1 | 0.25 | 0.544 ns | 15.15 | 3.4 a | 0.55 | 0.24 | 3.2 a | 0.45 | 0.20 | 0.73 |
| LS at 10 lstg | WW | 0.1 | 0.25 | 0.544 ns | 13.51 | 3.8 a | 0.45 | 0.20 | 3.6 a | 0.55 | 0.24 | 0.73 |
| LS at 11 lstg | WW | 0.4 | 0.15 | 0.141 ns | 7.44 | 5.4 a | 0.55 | 0.24 | 5.0 a | 0.00 | 0.00 | 0.56 |
| LS at 5 lstg | WS | 0 | 0.2 | 1 ns | 223.6 | 0.2 a | 0.45 | 0.20 | 0.2 a | 0.45 | 0.20 | 0.65 |
| LS at 6 lstg | WS | 0.1 | 0.3 | 0.579 ns | 109.54 | 0.6 a | 0.55 | 0.24 | 0.4 a | 0.55 | 0.24 | 0.79 |
| LS at 7 lstg | WS | 0.1 | 0.3 | 0.579 ns | 109.54 | 0.6 a | 0.55 | 0.24 | 0.4 a | 0.55 | 0.24 | 0.79 |
| LS at 8 lstg | WS | 0.1 | 0.3 | 0.579 ns | 109.54 | 0.6 a | 0.55 | 0.24 | 0.4 a | 0.55 | 0.24 | 0.79 |
| LS at 9 lstg | WS | 0.4 | 0.25 | 0.241 ns | 13.88 | 3.8 a | 0.45 | 0.20 | 3.4 a | 0.55 | 0.24 | 0.72 |
| LS at 10 lstg | WS | 0.4 | 0.2 | 0.195 ns | 8.94 | 5.2 a | 0.45 | 0.20 | 4.8 a | 0.45 | 0.20 | 0.65 |
| LS at 11 lstg | WS | 1.6 | 0.1 | 0.03 ns | 5.64 | 6.0 a | 0.00 | 0.00 | 5.2 b | 0.45 | 0.20 | 0.46 |
| Dping at 5 lstg | WW | 0 | 0.3 | 1 ns | 21.06 | 2.6 a | 0.55 | 0.24 | 2.6 a | 0.55 | 0.24 | 0.79 |
| Dpng at 6 lstg | WW | 0 | 0.3 | 1 ns | 21.06 | 2.6 a | 0.55 | 0.24 | 2.6 a | 0.55 | 0.24 | 0.79 |
| Dpng at 7 lstg | WW | 0 | 0.3 | 1 ns | 16.1 | 3.4 a | 0.55 | 0.24 | 3.4 a | 0.55 | 0.24 | 0.79 |
| Dpng at 8 lstg | WW | 0.1 | 0.3 | 0.58 ns | 15.64 | 3.4 a | 0.55 | 0.24 | 3.6 a | 0.55 | 0.24 | 0.79 |
| Dpng at 9 lstg | WW | 0 | 0.2 | 1 ns | 11.76 | 3.8 a | 0.45 | 0.20 | 3.8 a | 0.45 | 0.20 | 0.56 |
| Dpg at 10 lstg | WW | 0 | 0.2 | 1 ns | 11.76 | 3.8 a | 0.45 | 0.20 | 3.8 a | 0.45 | 0.20 | 0.56 |
| Dpng at 11 lstg | WW | 0 | 0.2 | 1 ns | 11.76 | 3.8 a | 0.45 | 0.20 | 3.8 a | 0.45 | 0.20 | 0.56 |
| Dpng at 5 lstg | WS | 0 | 0.3 | 1 ns | 21.06 | 2.6 a | 0.55 | 0.24 | 2.6 a | 0.55 | 0.24 | 0.79 |
| Dpng at 6 lstg | WS | 0 | 0.3 | 1 ns | 21.06 | 2.6 a | 0.55 | 0.24 | 2.6 a | 0.55 | 0.24 | 0.79 |
| Dpng at 7 lstg | WS | 0.4 | 0.25 | 0.24 ns | 19.23 | 2.4 a | 0.55 | 0.24 | 2.8 a | 0.45 | 0.20 | 0.72 |
| Dpng at 8 lstg | WS | 0.4 | 0.25 | 0.24 ns | 19.23 | 2.4 a | 0.55 | 0.24 | 2.8 a | 0.45 | 0.20 | 0.72 |
| Dpng at 9 lstg | WS | 0.9 | 0.2 | 0.66 ns | 17.88 | 2.2 a | 0.45 | 0.20 | 2.8 a | 0.45 | 0.20 | 0.65 |
| Dpng at 10 lstg | WS | 0.1 | 0.25 | 0.544 ns | 21.73 | 2.2 a | 0.45 | 0.20 | 2.4 a | 0.55 | 0.24 | 0.72 |
| Dpng at 11 lstg | WS | 0.1 | 0.25 | 0.544 ns | 21.73 | 2.2 a | 0.45 | 0.20 | 2.4 a | 0.55 | 0.24 | 0.72 |

(LDW-Leaf dry weight, SDW-Shoot dry weight; PH-Plant height; LS-Leaf senescence; lstg-leaf stage; Dpng-Developing leaves)

**Table 4: NTR-FTSW**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Dates** | **FTSW** | **NTR-K359W\_WW** | **NTR-R16\_WW** | **NTR-K359W\_WS** | **NTR-R16\_WS** |
| 11/29/2023 | 0.90 | 1.00 | 1.01 | 0.96 | 1.00 |
| 11/30/2023 | 0.90 | 1.00 | 1.01 | 0.98 | 0.99 |
| 12/1/2023 | 0.88 | 1.00 | 0.99 | 0.96 | 0.97 |
| 12/2/2023 | 0.87 | 0.95 | 0.99 | 0.97 | 0.97 |
| 12/3/2023 | 0.86 | 0.94 | 0.99 | 0.90 | 0.92 |
| 12/4/2023 | 0.85 | 0.94 | 0.97 | 0.88 | 0.90 |
| 12/5/2023 | 0.82 | 0.96 | 1.01 | 0.86 | 0.92 |
| 12/6/2023 | 0.80 | 0.91 | 0.97 | 0.85 | 0.88 |
| 12/7/2023 | 0.75 | 0.95 | 0.99 | 0.80 | 0.82 |
| 12/8/2023 | 0.70 | 0.94 | 0.99 | 0.80 | 0.83 |
| 12/9/2023 | 0.65 | 0.94 | 0.99 | 0.74 | 0.78 |
| 12/10/2023 | 0.60 | 0.96 | 1.00 | 0.68 | 0.76 |
| 12/11/2023 | 0.59 | 0.97 | 1.01 | 0.62 | 0.72 |
| 12/12/2023 | 0.55 | 1.03 | 0.99 | 0.56 | 0.66 |
| 12/13/2023 | 0.54 | 1.00 | 1.01 | 0.53 | 0.63 |
| 12/14/2023 | 0.52 | 1.02 | 0.99 | 0.49 | 0.56 |
| 12/15/2023 | 0.50 | 1.01 | 1.05 | 0.42 | 0.50 |
| 12/16/2023 | 0.45 | 0.99 | 1.05 | 0.32 | 0.45 |
| 12/17/2023 | 0.40 | 0.93 | 0.99 | 0.22 | 0.35 |
| 12/18/2023 | 0.30 | 1.02 | 0.98 | 0.15 | 0.23 |
| 12/19/2023 | 0.35 | 1.00 | 1.10 | 0.12 | 0.18 |
| 12/20/2023 | 0.30 | 1.14 | 1.12 | 0.13 | 0.17 |
| 12/21/2023 | 0.25 | 1.10 | 1.10 | 0.08 | 0.11 |
| 12/22/2023 | 0.20 | 1.12 | 1.12 | 0.07 | 0.11 |
| 12/23/2023 | 0.15 | 1.12 | 1.10 | 0.06 | 0.08 |
| 12/24/2023 | 0.10 | 1.10 | 1.10 | 0.04 | 0.06 |
| 12/25/2023 | 0.05 | 1.10 | 1.10 | 0.05 | 0.06 |
| 12/26/2023 | 0.03 | 1.14 | 1.12 | 0.03 | 0.04 |

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**Fig. 1: Total plant leaf area (cm2)**

**Fig. 2: Total plant leaf area (cm2)**

**Fig. 3: Leaf fresh weight (cm)**

**Fig. 4: Leaf dry weight (cm)**

**Fig. 5: Stem fresh weight (gm)**

**Fig. 6: Stem dry weight (gm)**

**Fig. 7: Root fresh weight (gm)**

**Fig. 8: Root surface area (cm3)**

**Fig. 9: Total biomass (gm)**

**Fig. 10: VPD transpiration (mg H2O per hr)**

**Fig. 11: VPD transpiration rate (mg H2O cm-2 min-1)**

**NTR-FTSW**



**Fig. 12: NTR vs FTSW under WS**

**Fig. 13: NTR vs FTSW under WW and WS**

|  |
| --- |
|  |
| **Fig. 14: Root image of R16\_WW vs K359\_WW**  |
|  |

**Fig. 15: Root image of R16\_WS vs K359\_WS**

**Conclusion**

In conclusion, this study provides valuable insights into the variations in sorghum plant growth, water use, and biomass traits between R16 (RP) and K359W (DP) under different water regimes. The significant genotypic variation observed in biomass production, leaf area, and plant height highlights the adaptive strategies employed by these parental lines in response to varying water availability. K359W demonstrated enhanced biomass accumulation, increased leaf area, and a delayed decline in transpiration under water-stressed (WS) conditions, suggesting its suitability for post-rainy sorghum breeding and its potential to thrive in water-limited environments.Additionally, the study emphasizes the importance of limited transpiration (TR) as a key factor in water conservation, particularly under high vapor pressure deficit (VPD) conditions. K359W exhibited reduced TR during the vegetative stage, indicating its potential for improved water-use efficiency. The observed genetic diversity in FTSW thresholds further underscores the significance of genotype-specific water conservation strategies. These findings contribute to a better understanding of how different sorghum genotypes manage water during soil drying and can aid in selecting cultivars with enhanced drought tolerance and sustained productivity under water-limited conditions.

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