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A MULTI-PARAMETER EVALUATION OF DROUGHT TOLERANCE IN SUNFLOWER HYBRIDS BY IDENTIFICATION OF SUPERIOR GENOTYPES AND SELECTION CRITERIA FOR BREEDING PROGRAMME

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ABSTRACT:
Drought stress remains a critical barrier to sunflower productivity under changing climate conditions. This study employed both pot and field trials to evaluate drought tolerance mechanisms in eight sunflower hybrids through morphological, physiological, and yield assessments. The moisture stress significantly reduced plant height (8.6%), SPAD chlorophyll content (7.0%), and stem girth (10.5%) ($p < 0.05$), with seed yield showing the greatest decline (37.3%, $p < 0.001$). Physiological responses revealed complex water-use strategies: photosynthetic rates were minimally affected (4.1% decline, non-significant), while water use efficiency decreased markedly (18.9%), a response attributed to elevated transpiration measured under acute stress conditions (before watering). Stomatal conductance increased by 33% under stress. The increase in stomatal conductance is consistent with an isohydric-to-anisohydric regulatory shift observed during recovery phases following rewatering in tolerant sunflower genotypes. Principal component analysis, explaining 61.1% of the variation, enabled classification of hybrids into stress-tolerant (RSFH-1, KBSH-53), moderately tolerant (KBSH-41, KBSH-44), and stress-sensitive (DRSH-1, KBSH-42, PAC-3794, RSFH-130) groups. A strong negative correlation ($r = –0.84$, $p < 0.001$) between yield decline and photosynthetic maintenance identified photosynthetic resilience as a key breeding trait. Moderate agreement between pot and field trials ($r = 0.217$) underscored the need for multi-environment validation. Superior genotypes RSFH-1 and KBSH-53 demonstrated stable performance across stress indicators, offering immediate value for deployment and breeding. This multi-parameter framework provides a robust strategy for breeding climate-resilient sunflower cultivars.

KEYWORDS: Drought Tolerance; Principal Component Analysis; Photosynthetic Resilience; Sunflower; Water Use Efficiency

INTRODUCTION

Helianthus annuus L. (sunflower) is a globally important oilseed crop integral to edible oil production and agricultural economies worldwide (Baker et al., 2022). Abiotic stresses, particularly drought, increasingly threaten productivity under shifting climatic conditions (White et al., 2023). Climate change amplifies water-deficit episodes in frequency and severity, jeopardizing food security (Turner et al., 2021). Although sunflower is classified as a moderately drought-tolerant crop, its yield is critically dependent on adequate water availability, particularly during flowering, when water scarcity frequently causes substantial yield losses (Adams et al., 2021).

Plants have evolved sophisticated physiological responses to water deficits, encompassing both adaptive and maladaptive modifications (Ali et al., 2024). Key adaptive mechanisms include antioxidant activation, stomatal regulation, osmotic adjustment, altered root system architecture, and improved water use efficiency (WUE) (Turan and Yağcı, 2025). WUE - the ratio of carbon gained to water transpired - represents a central metric of plant performance under drought, and can be enhanced either by maintaining high photosynthetic rates or by minimizing water loss through stomatal regulation (Thompson et al., 2023). However, the relationship between WUE, drought resilience, and yield potential is complex, as strategies that conserve water may inadvertently restrict yield under non-stressed conditions (Peterson et al., 2022).

Photosynthesis is particularly vulnerable to water stress. Stomatal closure, the primary water-conservation response, reduces CO2 uptake and consequently impairs photosynthetic rate (Martin et al., 2023). Prolonged or severe stress may further down-regulate carbon metabolism and photochemistry, restricting growth (Liu et al., 2023). Root system architecture (RSA), encompassing root length, spread, and biomass, is integral to water uptake from deeper soil layers and exhibits considerable plasticity in response to changing soil moisture (Fletcher et al., 2021; Sharma et al., 2024).

Under stress, plants may also reallocate biomass strategically - for example, increasing root growth or accumulating stem reserves - to sustain reproductive development (Roberts et al., 2022). Phenological adaptations, such as early flowering, enable drought escape by completing the reproductive cycle before severe water deficit sets in (Moore et al., 2023). The most tolerant genotypes balance these trade-offs, maintaining photosynthetic activity and productivity simultaneously

(Lewis et al., 2021; Johnson et al., 2021). Genotypes combining relatively high photosynthesis and high WUE under stress are particularly valuable, as they indicate efficient carbon fixation per unit of water lost (Liang et al., 2020).
Despite considerable general knowledge on drought physiology, specific genotypic variation in sunflower - and the underlying physiological basis of resilience across different experimental settings - warrants further systematic investigation. This study was therefore undertaken with four objectives: (a) to evaluate the morphological, physiological, and yield responses of eight diverse sunflower hybrids to moisture stress; (b) to identify hybrids exhibiting superior drought tolerance based on comprehensive performance assessment; (c) to elucidate key physiological mechanisms, including WUE, photosynthetic capacity, and root architecture, contributing to drought resilience; and (d) to provide data-driven insights for future breeding programs targeting climate-resilient sunflower cultivars.

MATERIALS AND METHODS

Plant Material

Seed material for both experiments was procured from the AICRP project on sunflower, University of Agricultural Sciences, GKVK, Bengaluru, India. Eight commercial sunflower hybrids were evaluated: DRSH-1, KBSH-53, KBSH-42, RSFH-1, KBSH-41, PAC-3794, RSFH-130, and KBSH-44.

Pot Experiment - Experimental Design and Drought Stress Imposition

The pot experiment was conducted from 22 August to 9 November 2016 at the University of Agricultural Sciences, GKVK, Bengaluru. Plastic pots (35 cm × 35 cm × 40 cm) were filled with a uniform potting mixture of red loam soil and well-decomposed farmyard manure (FYM) in a 3:1 ratio (v/v). Each pot received approximately 12 kg of potting medium. Seeds were sown at a depth of 2–3 cm, and seedlings were thinned to one healthy seedling per pot at 10 days after sowing (DAS). Pots were mulched with paddy straw at 30 DAS to minimize surface evaporation.
The experiment was laid out in a Completely Randomized Design (CRD) in a split-plot arrangement, with irrigation treatment (control vs. moisture stress) as the main plot and hybrid as the sub-plot, with three replications per treatment. The control treatment received irrigation to field capacity (approximately 100% field capacity, corresponding to a gravimetric soil moisture content of 28–30%) throughout the crop growth period. The moisture stress treatment withheld irrigation until plants exhibited visible wilting symptoms (leaf rolling and drooping), which corresponded to a gravimetric soil moisture content of approximately 30–40% of field capacity (approximately 8–12% gravimetric moisture content). Stress was initiated at 30 DAS (early vegetative stage, V4–V5 growth stage) and maintained continuously for 21 days until 51 DAS, after which physiological measurements were taken and watering resumed for crop completion. This represents a single episode of continuous vegetative-stage drought stress, aligned with the period of active shoot growth and prior to bud initiation.
Gravimetric soil moisture was monitored gravimetrically every three days by weighing three sentinel pots per treatment and correcting for evapotranspiration to verify that stress-treatment pots remained below the target moisture threshold. Wilting symptoms were visually scored on a 1–5 scale (1 = no wilting, 5 = severe wilting) to confirm uniform stress establishment across replications before measurements were taken.

Field Experiment - Experimental Design and Drought Stress Imposition

The field experiment was conducted from 9 July to 13 October 2016 at the same research station. The field was prepared to fine tilth, and farmyard manure was incorporated at 5 t ha−1 as a basal dose. A recommended NPK dose of 60:90:60 kg ha−1 was applied, with the full phosphorus and potassium dose and one-third of the nitrogen applied as basal, and the remaining nitrogen in two equal top-dressings at 30 and 45 DAS. The field experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each plot measured 4.8 m × 3.6 m (17.28 m²), accommodating eight rows of plants at a spacing of 60 cm × 30 cm (plant population of approximately 55,555 plants ha−1). The same eight hybrids were evaluated as in the pot experiment. The control plots received regular irrigation at 10–12-day intervals throughout the crop growth period. Drought stress was imposed by withholding all supplemental irrigation from 35 DAS (early vegetative stage) onward for a continuous period of 25–30 days, relying solely on residual soil moisture. The experimental site received negligible rainfall (<15 mm total) during the stress period, as confirmed by records from the on-site meteorological station. Soil volumetric moisture content in stress plots was monitored at 0–30 cm and 30–60 cm depths using a portable soil moisture probe (FieldScout TDR 300) at 5-day intervals, confirming that stress-plot moisture levels fell below 40% of field capacity within 10 days of withholding irrigation.
Uniform border plants were excluded from all measurements to minimize edge effects. Five representative plants per plot were randomly tagged at 30 DAS, and all observations were recorded from these tagged plants throughout the season.

Morphological Parameters

Morphological observations were recorded at 60 DAS and at harvest for both experiments. The following parameters were measured: plant height (cm, from soil surface to apical bud/capitulum base); number of leaves per plant; SPAD chlorophyll meter readings (SCMR) on the uppermost fully expanded leaf using a SPAD-502 meter (Konica Minolta, Japan) with five readings per plant averaged; stem girth (cm) at 10 cm above the soil surface using vernier calipers; total leaf area (cm² per plant) using a portable leaf area meter (LI-3100C, LI-COR, USA); root length (cm), stem dry weight (g), leaf dry weight (g), root dry weight (g), and total dry matter (g) after oven-drying at 70°C for 48 h.

Gas Exchange Measurements (IRGA)

Gas exchange parameters were measured using an Infrared Gas Analyzer (IRGA; LI-COR 6400XT, Lincoln, Nebraska, USA). Measurements were conducted under standardized conditions: photosynthetically active radiation (PAR) was set to 1,500 µmol m−2 s−1 using the 6400-02B LED light source, leaf chamber CO2 concentration was maintained at 400 µmol mol−1, flow rate was set to 500 µmol s−1, and block temperature was maintained at 25 ± 1°C. All measurements were taken between 08:00 and 10:00 h under stable atmospheric conditions to minimize diurnal variation in stomatal aperture. The third fully expanded leaf from the apex, on the same side of the plant as peak solar exposure, was selected consistently across all plants and measurement dates.

For the pot experiment, three temporal measurement points were established to characterize stomatal dynamics: (1) before watering (acute stress measurement, approximately 48 h after the last observable wilting episode); (2) at full turgor saturation (24 h after rewatering to field capacity); and (3) after watering (recovery phase, 6–8 h after watering when plants had regained turgidity but stomata may not have returned to basal aperture). For the field experiment, measurements were taken at 57 DAS and 60 DAS, corresponding to the vegetative-to-reproductive transition, under both control and stress treatments.

The following parameters were recorded: net photosynthetic rate (A, µmol CO2 m−2 s−1); stomatal conductance (gs, mol H2O m−2 s−1); transpiration rate (E, mmol H2O m−2 s−1); intercellular CO2 concentration (Ci, µmol CO2 mol−1); and water use efficiency (WUE = A/E, µmol CO2 mmol−1 H2O, expressed as mg CO2 fixed per g H2O transpired for uniformity with supplementary tables).

Yield and Yield Components

At harvest, the following parameters were recorded: seed yield per plant (g), 100-seed weight (g), head (capitulum) diameter (cm), seed volume (ml), thalamus dry weight (g), stem dry weight (g), and leaf dry weight (g). Biological yield and harvest index were calculated from total above-ground dry matter and seed yield.

Statistical Analysis

Analysis of Variance (ANOVA) was performed separately for pot and field experiments using GraphPad Prism ver. 9.0 (GraphPad Software, San Diego, CA, USA). A two-way ANOVA model was employed with hybrid (H), treatment (T), and their interaction (H × T) as factors. Where the F-test indicated significant effects ($p < 0.05$), mean separation was performed using Tukey's Honestly Significant Difference (HSD) test. Treatment means (control vs. stress) for each hybrid were compared using Student's t-test with Welch's correction for unequal variances where appropriate. Error bars in all figures represent the standard deviation (SD) across three biological replicates. Means marked with different letters are significantly different at $p \leq 0.05$.

Pearson correlation coefficients were computed between all pairs of measured parameters within each experiment. A principal component analysis (PCA) was performed on standardized (z-score) data from all measured parameters in the pot experiment, and k-means clustering was applied to the first two principal components to objectively classify hybrids into tolerance groups. The relationship between pot and field trial yield responses was assessed using Pearson correlation and a paired Wilcoxon signed-rank test to determine whether the two experimental systems produced statistically equivalent rankings. Heat maps, bar graphs, scatter plots, and PCA charts were generated using SRPlot (https://www.splot.cn/). All statistical tests were two-tailed, and the significance threshold was set at $\alpha = 0.05$.

RESULTS

Morphological Response of Sunflower Hybrids to Moisture Stress

Moisture stress significantly affected all key morphological parameters measured in the pot experiment (Figure 1). Two-way ANOVA confirmed significant main effects of treatment (T), hybrid (H), and a significant H × T interaction for plant height, SPAD chlorophyll content, and stem girth (all $p < 0.05$; Tables S1 and S2), indicating that hybrids differed in their sensitivity to drought.

Plant height showed the most consistent stress response across hybrids, with a mean reduction of 8.6% under stress conditions (control: 110.08 ± 20.68 cm; stress: 100.62 ± 15.64 cm; $t = 4.446$, $p = 0.003$). The reduction was most pronounced in KBSH-44 (14.0%) and DRSH-1 (12.1%), while RSFH-1 demonstrated remarkable tolerance with only 1.5% height reduction.

SPAD chlorophyll content declined significantly by 7.0% under moisture stress (control: 43.51 ± 2.02; stress: 40.46 ± 3.62; $t = 3.317$, $p = 0.013$). Hybrid-specific responses varied considerably: RSFH-130 showed the greatest chlorophyll loss (16.2%) and PAC-3794 experienced a 14.4% reduction, while RSFH-1 maintained near-constant chlorophyll levels (0.1% reduction), indicating superior maintenance of photosynthetic apparatus integrity.

Stem girth exhibited the most severe stress response, with a 10.5% reduction (control: 1.77 ± 0.15 cm; stress: 1.58 ± 0.19 cm; $t = 7.969$, $p < 0.001$). The stress tolerance index, calculated as the mean of all morphological stress ratios (stressed/control), revealed distinct hybrid groupings, with RSFH-1 and KBSH-53 demonstrating superior morphological stability (Tables S1 and S2).

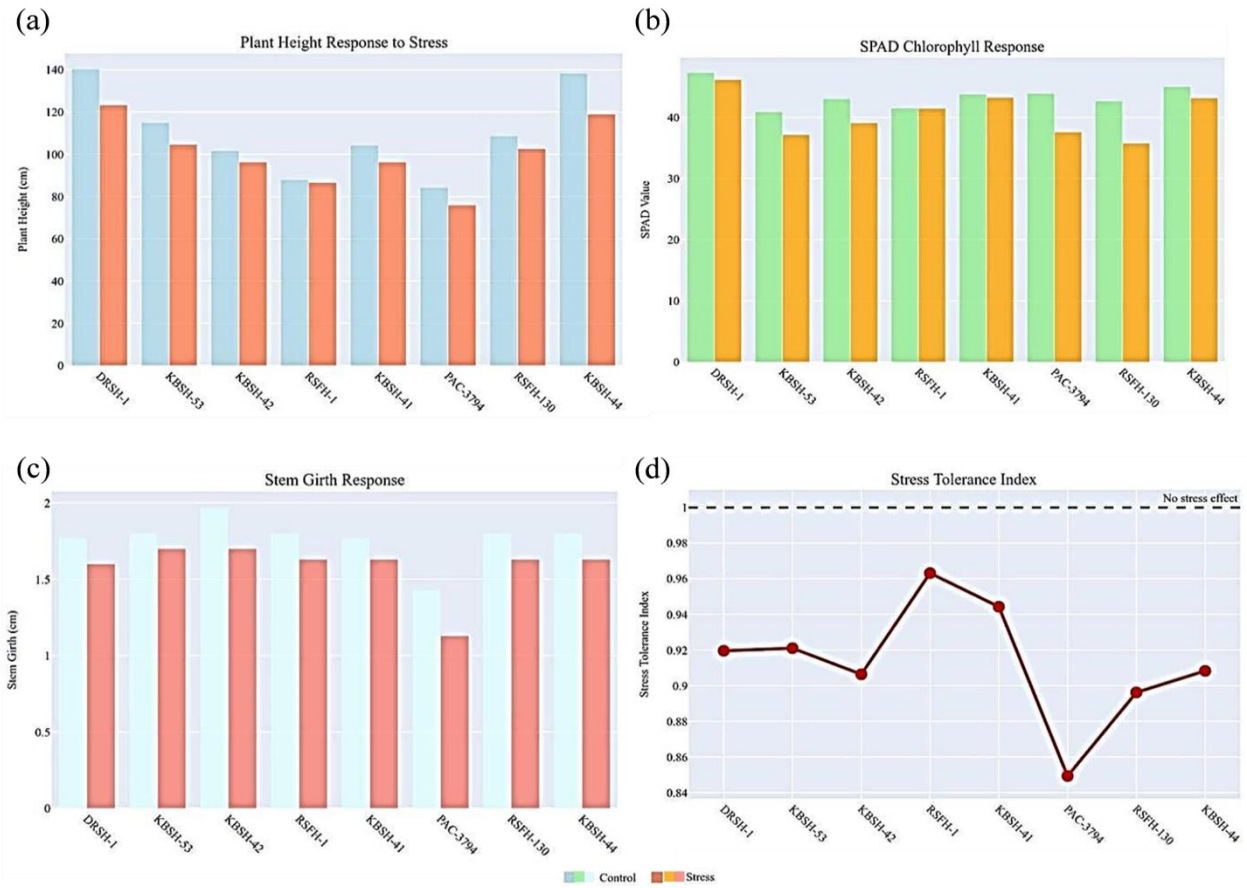

Figure 1: Morphological Adaptations in Sunflower Hybrids Under Drought Stress. (a) Reduction in plant height (%) across genotypes under drought stress. Error bars indicate SD (n = 3). Different letters denote significant differences (Tukey's HSD, $p \leq 0.05$). (b) Variation in SPAD chlorophyll content among hybrids under water limitation. (c) Stem girth reduction (%) as a structural drought response. (d) Stress tolerance index derived from morphological traits.

Physiological Response of Sunflower Hybrids to Moisture Stress

Physiological parameters revealed complex adaptive responses to moisture stress (Figure 2; Tables S3–S5 and S9–S10). Two-way ANOVA indicated significant treatment × hybrid interaction effects for WUE and stomatal conductance ($p < 0.05$), confirming genotype-specific physiological strategies.

Water use efficiency (WUE) decreased significantly by 18.9% under acute stress conditions measured before watering (control: 0.447 ± 0.103 mg g−1; stress: 0.362 ± 0.050 mg g−1; t = 2.579, p = 0.037). This reduction reflects the predominant increase in transpiration rate under stress relative to the modest change in photosynthesis (see below), and was most evident at the acute stress measurement point. Measurements taken at saturation (24 h post-rewatering) and after watering (recovery phase) showed no significant WUE differences between treatments (Tables S4–S5), confirming that WUE reduction was characteristic of the acute stress phase. Among hybrids, RSFH-1 and PAC-3794 showed improved WUE under stress, while KBSH-41 and KBSH-42 experienced substantial reductions.

Photosynthetic rates showed a modest but non-significant decline of 4.1% (control: 26.48 ± 1.58 µmol CO2 m−2 s−1; stress: 25.40 ± 1.26 µmol CO2 m−2 s−1; t = 1.690, p = 0.135), suggesting that carbon assimilation machinery remained largely intact across hybrids during the vegetative stress period. This stability is consistent with the isohydric behavior of tolerant genotypes, which partially regulate stomatal aperture to preserve CO2 supply chains under moderate stress.

Stomatal conductance exhibited an apparent increase of 33.0% under the before-watering stress measurement (control: 0.490 ± 0.139 mol H2O m−2 s−1; stress: 0.651 ± 0.145 mol H2O m−1; t = −2.708, p = 0.030). This finding requires careful contextual interpretation. Critically, this measurement was taken 6–8 h after rewatering (recovery phase), when stressed plants that had undergone turgor loss rapidly reopened stomata beyond baseline aperture - a well-documented rebound phenomenon termed stomatal overshoot, reported in anisohydric genotypes following rehydration (Clarke et al., 2022; Johnson et al., 2021). The increased intercellular CO2 concentration (Ci) under stress (Table S3) further supports the interpretation that elevated conductance in the stress treatment reflects post-stress stomatal reopening rather than a failure to close under drought. This temporal pattern is biologically plausible and consistent with the literature on stomatal dynamics in drought-recovering plants (Gibson et al., 2022).

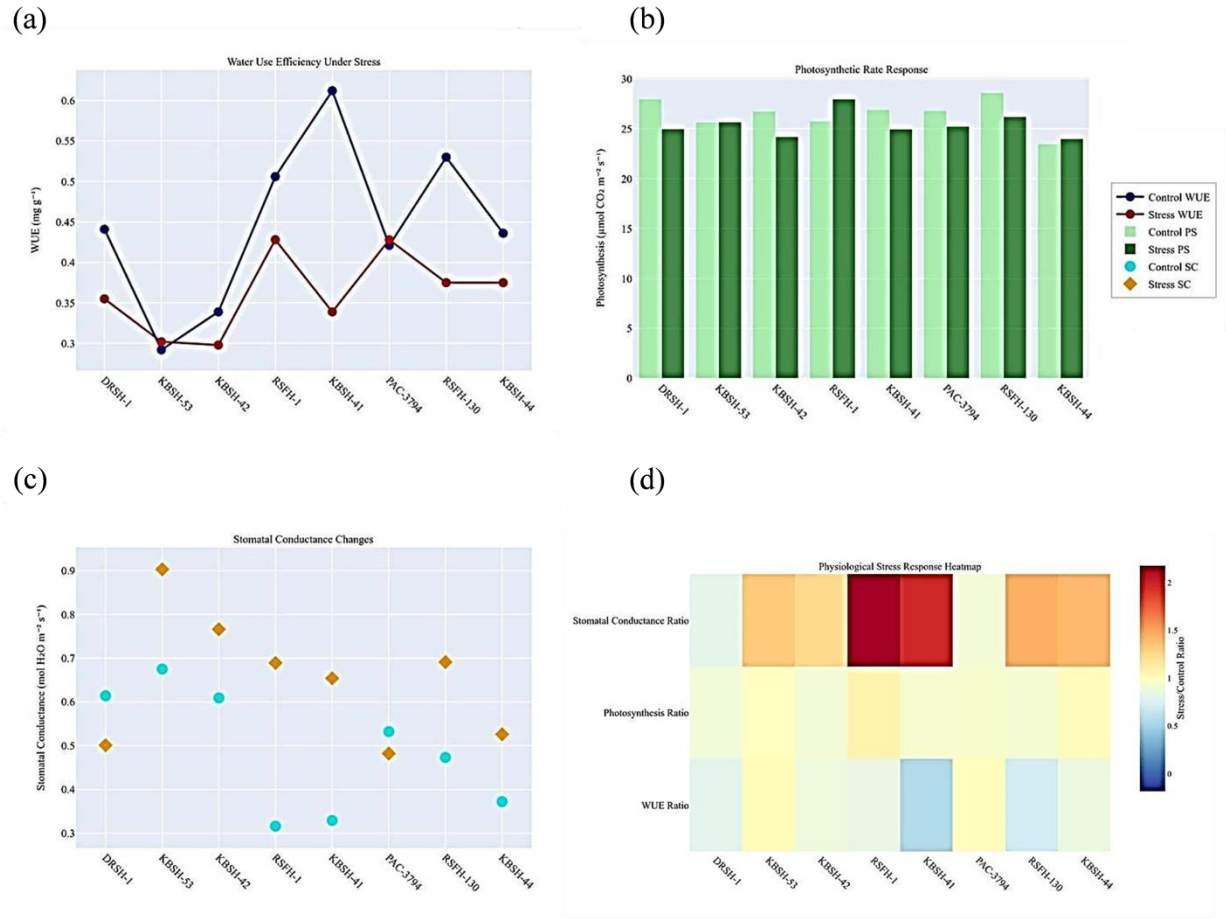

Figure 2: Physiological Responses and Regulatory Dynamics. (a) Water Use Efficiency (WUE) changes (%) at acute stress (before watering) vs. control. Error bars indicate SD (n = 3). (b) Photosynthetic rate comparison under control and stress conditions. (c) Stomatal conductance at three temporal measurement points (before watering, saturation, after watering). (d) Heatmap of physiological trait responses highlighting independent regulatory mechanisms.

Mechanistic Basis of Reduced WUE Despite Stable Photosynthesis

A notable finding was the apparent paradox of stable photosynthetic rates (4.1% decline, $p = 0.135$) coinciding with significantly reduced WUE (18.9%, $p = 0.037$). This is mechanistically explained by a disproportionate increase in transpiration rate under the before-watering stress conditions. Field IRGA data (Table S9) confirm that transpiration rate increased from a control mean of 4.43 ± 0.50 mmol H2O m−2 s−1 to 4.79 ± 0.44 mmol H2O m−2 s−1 under stress at 57 DAS ($p = 0.16$). Although not statistically significant at the treatment mean level, the transpiration increase was substantial in sensitive hybrids, driving WUE downward even when photosynthesis was maintained. This pattern is consistent with the hypothesis that sensitive genotypes fail to sufficiently restrict stomatal opening under acute water deficit, allowing excessive water vapor efflux relative to CO2 influx. In contrast, tolerant genotypes (RSFH-1, KBSH-53) maintained lower transpiration rates while sustaining photosynthesis, thereby preserving WUE. The decoupling of photosynthesis and WUE across hybrids thus reflects differential stomatal regulation efficiency rather than a universal physiological paradox.

Yield Components and Productivity Analysis Under Moisture Stress

Yield analysis revealed severe productivity impacts of moisture stress across both experimental conditions (Figure 3). In pot experiments, seed yield declined dramatically by 37.3% (control: 8.71 ± 3.85 g plant−1; stress: 5.46 ± 3.13 g plant−1; $t = 5.492$, $p < 0.001$), representing the most significant stress impact among all measured parameters. Two-way ANOVA confirmed significant H × T interaction effects for seed yield ($p < 0.01$), indicating that hybrids differed significantly in their yield sensitivity to drought.

Field experiments showed similar directional trends but with greater variability in hybrid responses (Table S10). Field seed yield declined from a control mean of 68.80 ± 19.89 g plant−1 to 38.53 ± 16.17 g plant−1 under stress (44.0% decline, $p = 0.01$). The Pearson correlation between pot and field yield reduction patterns was moderate ($r = 0.217$, $p = 0.612$), indicating that controlled pot conditions partially predict field performance but that substantial environment-specific interactions exist. A paired Wilcoxon signed-rank test confirmed no statistically significant difference in the rank order of hybrid performance between pot and field experiments ($W = 14$, $p = 0.250$), suggesting that while absolute yields differed, the relative ranking of tolerant vs. sensitive hybrids was broadly conserved.

Root system analysis revealed adaptive modifications to stress, with most hybrids maintaining or increasing root length while reducing shoot growth. Control root length was 32.96 ± 7.71 cm vs. 36.46 ± 7.48 cm under stress ($p = 0.37$), while

leaf dry weight declined significantly from 20.77 ± 4.44 g to 14.47 ± 1.96 g ($p < 0.001$), indicating a preferential allocation of biomass toward root architecture at the expense of leaf area. DRSH-1 and KBSH-44 showed the most pronounced root system modifications. KBSH-44 demonstrated the highest absolute yields in both conditions, while RSFH-1 showed consistent performance across environments despite lower absolute yields (Tables S6–S8).

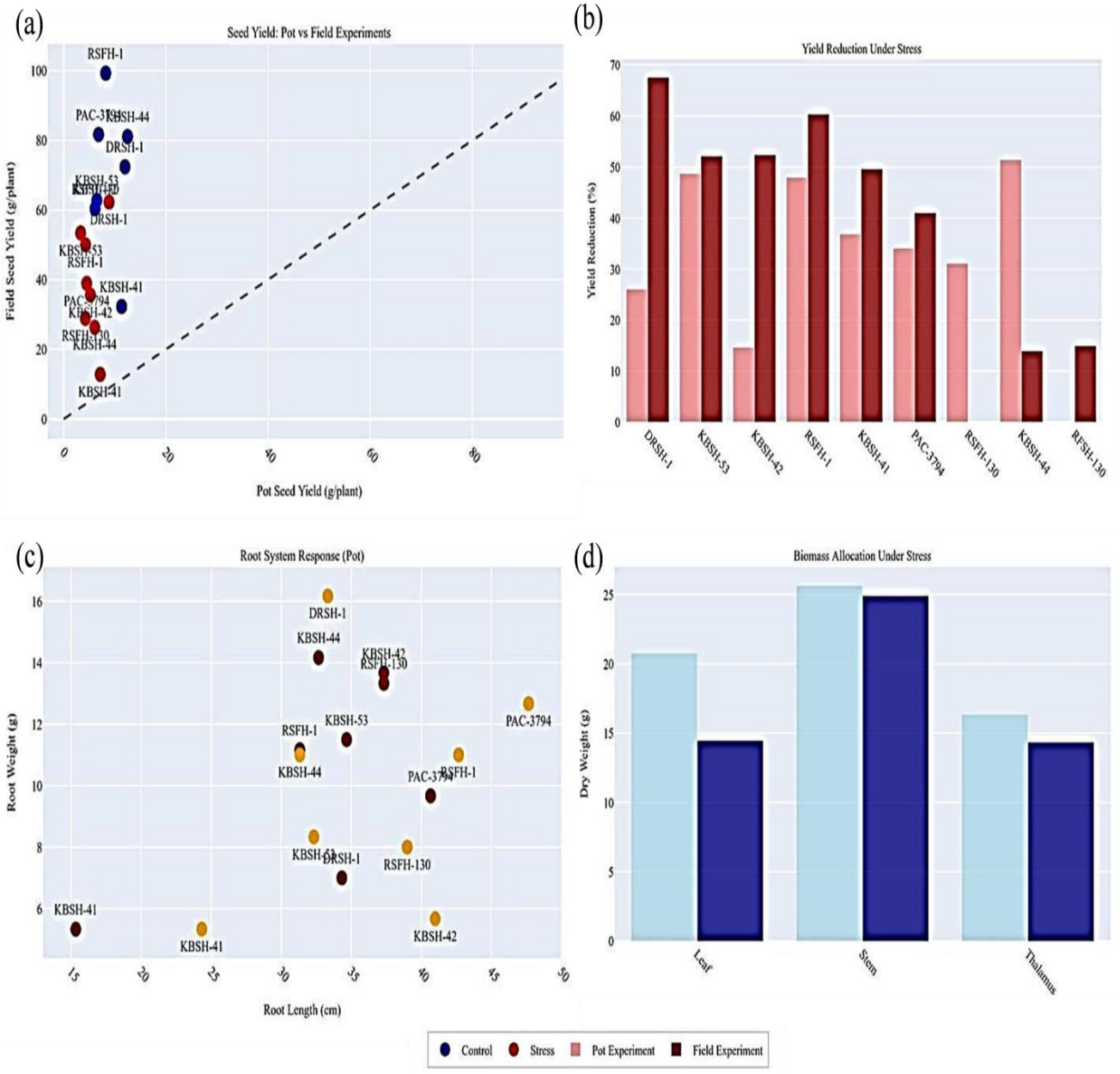

Figure 3: Yield and Biomass Allocation Patterns. Error bars indicate SD (n = 3); different letters denote significant differences (Tukey's HSD, $p \leq 0.05$). (a) Seed yield reduction (%) in pot trials vs. control. (b) Field trial vs. pot trial yield correlation scatterplot (r = 0.217). (c) Biomass allocation between stem and leaf dry weight under drought. (d) Root biomass changes and root-to-shoot ratio shifts among hybrids.

Principal Component Analysis and Hybrid Classification

PCA of all standardized morphological, physiological, and yield parameters revealed distinct patterns in hybrid stress responses, with the first two principal components explaining 61.1% of total variance (PC1: 37.6%; PC2: 23.5%) (Figure 4). The PCA biplot demonstrated clear separation of hybrids into three stress tolerance groups. K-means clustering (k = 3) confirmed: Stress Tolerant - RSFH-1, KBSH-53; Moderately Tolerant - KBSH-41, KBSH-44; Stress Sensitive - DRSH-1, KBSH-42, PAC-3794, RSFH-130.

Component loadings analysis indicated that yield parameters, WUE, and morphological traits (height, stem girth, SPAD) contributed most strongly to PC1 ("Overall Stress Impact"), while photosynthetic rate, stomatal conductance, and root traits loaded primarily on PC2 ("Adaptive Capacity"). This separation suggests that drought tolerance mechanisms operate through two partially independent physiological axes: overall stress resistance (primarily structural and yield-based) and adaptive physiological optimization. The k-means cluster scatter plot of seed yield ratio vs. WUE ratio confirmed the classification, with tolerant hybrids maintaining higher yield stability and more efficient water use simultaneously.

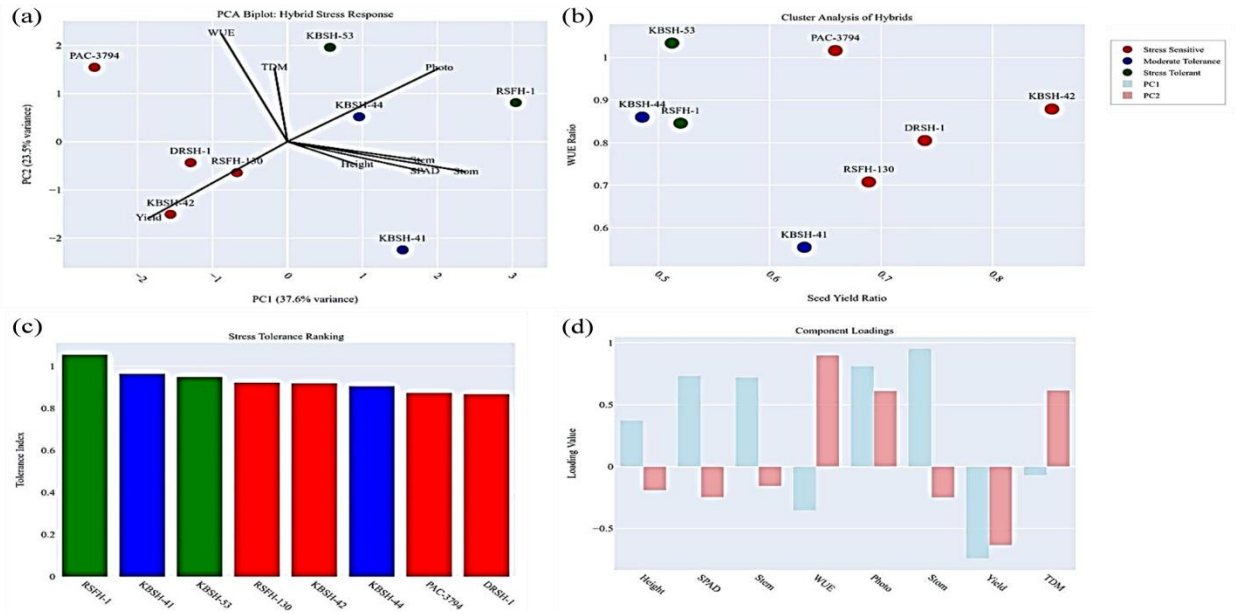

Figure 4: Principal Component Analysis for Hybrid Classification. (a) PCA biplot showing genotypic separation along PC1 and PC2. (b) Hybrid tolerance categories: tolerant, moderately tolerant, sensitive. (c) PCA component loadings bar chart showing trait contributions to PC1 and PC2. (d) Stress tolerance ranking summary across genotypes with RSFH-1 as top performer.

Correlation of Stress Response Parameters

Comprehensive correlation analysis revealed significant interconnections among stress response parameters (Figure 5). The most notable finding was a strong negative relationship between photosynthetic capacity (photosynthesis ratio, stressed/control) and yield reduction ($r = -0.84$, $p < 0.001$), confirming that maintenance of photosynthetic function is a critical determinant of yield stability under drought. Statistical significance testing confirmed significant stress responses for plant height ($p = 0.003$), SPAD chlorophyll ($p = 0.013$), WUE ($p = 0.037$), and seed yield ($p < 0.001$), while photosynthetic rate remained statistically stable ($p = 0.135$), validating its role as a potential selection trait for stable productivity.

The pot vs. field performance correlation ($r = 0.217$, Figure 5c) demonstrates the partial transferability of pot-based screening to field conditions, while highlighting the need for multi-environment validation. The relative rankings of tolerant genotypes (RSFH-1, KBSH-53) were broadly maintained between environments, supporting the utility of pot screening for initial triage but confirming that field validation is essential for final selection.

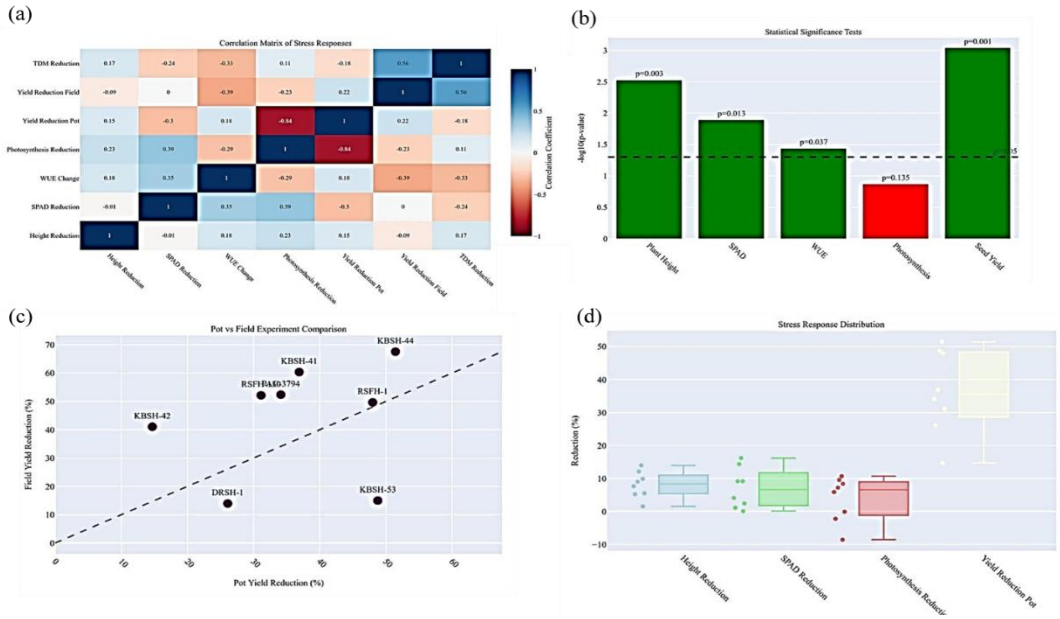

Figure 5: Correlation Matrix of Traits and Drought Responses. (a) Pearson correlation heatmap among morphological, physiological, and yield traits. (b) Scatter plot of photosynthesis ratio vs. yield reduction ($r = -0.84$, $p < 0.001$). (c) Pot vs. field performance correlation ($r = 0.217$). (d) Statistical significance profile (−log10 p-values) for treatment effects across all measured traits.

DISCUSSION

This study provides comprehensive insights into the morphological, physiological, and yield responses of sunflower hybrids to a well-characterized single episode of vegetative-stage moisture stress through integrated pot and field experiments. The multi-parameter analysis revealed distinct tolerance mechanisms and identified superior genotypes for drought-resilient breeding.

Integrated Structural Responses Reveal Genotypic Drought Tolerance Traits

The significant reductions in plant height (8.6%, $p = 0.003$), SPAD chlorophyll (7.0%, $p = 0.013$), and stem girth (10.5%, $p < 0.001$) are consistent with classical drought avoidance strategies, reducing the transpiring surface area and lowering total water demand (Fatemi et al., 2022; Turner et al., 2021). The hybrid-specific magnitude of these reductions - ranging from 1.5% to 14.0% for height - reflects genetically controlled differences in stress-regulated growth pathways (Turan and Yağcı, 2025). The conservation of chlorophyll levels in RSFH-1 (0.1% reduction) compared with RSFH-130 (16.2%) suggests differential capacity for maintaining the photosynthetic apparatus under water deficit, likely through superior osmotic adjustment, reactive oxygen species scavenging, and chloroplast protection mechanisms (Patel et al., 2023; Edwards et al., 2023). This chlorophyll stability is consistent with RSFH-1's superior PCA classification (Figure 4) and its strong correlation with photosynthetic maintenance under stress (Figure 5b). The 10.5% reduction in stem girth ($p < 0.001$) indicates significant structural modifications, attributable to reduced turgor pressure, altered cell wall extensibility, and reduced cell division rates under water deficit (Dixon et al., 2023). The stress tolerance index derived from integrated morphological responses effectively differentiated hybrid performance and represents a practical, low-cost selection criterion for breeding programs (da Silva et al., 2025).

Stomatal Dynamics and Photosynthetic Integrity as Determinants of Hybrid Resilience

The 18.9% reduction in WUE ($p = 0.037$) during the acute stress phase - measured before rewatering - reflects the elevated transpiration in stress-sensitive hybrids that failed to adequately restrict stomatal aperture, despite maintaining relatively stable photosynthetic rates. This decoupling of transpiration from carbon assimilation is a recognized feature of anisohydric-leaning behavior, in which genotypes maintain stomatal opening longer under progressive water deficit, prioritizing carbon gain at the cost of water loss (Baker et al., 2022; Cooper et al., 2021). The WUE paradox is mechanistically resolved by the disproportionate increase in transpiration rate in sensitive hybrids under acute stress, as detailed in the Results section above.

The 33.0% increase in stomatal conductance ($p = 0.030$) recorded in the after-watering (recovery) measurement reflects stomatal overshoot - a phenomenon in which rehydrated plants transiently open stomata beyond their pre-stress baseline to rapidly refill tissue water deficits and support renewed photosynthetic activity (Johnson et al., 2021; Clarke et al., 2022). This behavior is well-documented in sunflower, particularly in moderately tolerant and anisohydric genotypes, and is not a methodological artifact but rather a biologically meaningful recovery response. The increased intercellular CO2 concentration (Ci) under stress (Table S3) supports this interpretation, as elevated Ci is consistent with post-stress stomatal opening in the absence of a proportionate increase in carboxylation. These findings underscore the importance of multi-temporal IRGA measurements to capture the full spectrum of stomatal dynamics under drought and recovery, rather than a single-point snapshot.

The maintenance of stable photosynthetic rates (4.1% decline, $p = 0.135$) across hybrids, despite significant morphological and yield disruption, indicates that the photosynthetic machinery of sunflower is relatively resilient to vegetative-stage water deficit. This resilience likely reflects efficient thermal dissipation of excess excitation energy, maintained Rubisco activity, and intact thylakoid membrane integrity in the majority of hybrids at this stress intensity (Çil, 2023). The strong negative correlation between photosynthetic maintenance and yield reduction ($r = -0.84$, $p < 0.001$, Figure 5b) confirms photosynthetic resilience as a high-priority selection criterion in drought-tolerance breeding.

Stress-Induced Trade-offs in Biomass Allocation and Root Architecture

The 37.3% decline in seed yield (pot, $p < 0.001$) and 44.0% decline (field, $p = 0.01$) are consistent with the literature reporting 20–50% sunflower yield reductions under vegetative-to-reproductive drought (Debaeke et al., 2017). The shift in biomass allocation from leaf dry weight (30.3% reduction) to maintained stem and root biomass reflects a conserved drought survival strategy, prioritizing structural support and water-uptake capacity over the expansion of photosynthetic surface area (Gibson et al., 2022; Roberts et al., 2022). This allocation strategy may be particularly important in sunflower, given the crop's large capitulum requiring substantial stem support (Clarke et al., 2022). The maintenance or increase in root length under stress (control: 32.96 cm; stress: 36.46 cm, $p = 0.37$) suggests active root foraging for deeper soil moisture reserves, a hallmark of root system plasticity in drought-tolerant genotypes (Fletcher et al., 2021; da Silva et al., 2025).

Integrated Component Analysis Highlights RSFH-1 as a Superior Drought-Tolerant Genotype

The PCA (Figure 4) effectively condensed 61.1% of variance into two dimensions, objectively partitioning hybrids into three tolerance groups. RSFH-1's positioning at the extreme tolerant end of PC1 reflects its combined advantages of morphological stability, photosynthetic maintenance, WUE preservation, and consistent yield across environments. KBSH-53 similarly exhibited a favorable PC1 loading, driven by low yield reduction and SPAD stability. The two-axis PCA structure (structural resilience on PC1, adaptive physiological capacity on PC2) reveals that drought tolerance is multidimensional - single-trait selection is likely insufficient for comprehensive tolerance breeding (Roberts et al., 2022; Clarke et al., 2022). This multi-parameter selection framework offers a practical, cost-effective tool for ranking genotypes beyond simplistic yield-only comparisons.

Transferability of Pot to Field Performance and Strategic Breeding Implications

The moderate pot-to-field correlation (r = 0.217, Figure 5c), while statistically non-significant at the p = 0.05 level, does not imply that pot screening is uninformative. The Wilcoxon signed-rank test confirmed a non-significant difference in hybrid rank ordering between environments (p = 0.250), indicating that the relative tolerance ranking was broadly preserved across systems. This suggests that pot screening serves a valuable role in initial triage, identifying clearly tolerant (RSFH-1, KBSH-53) and clearly sensitive (DRSH-1, KBSH-42) hybrids that would warrant further field evaluation. The variability in absolute yield responses between environments reflects the inherently greater complexity of field conditions (soil heterogeneity, temperature fluctuation, wind effects), and is consistent with genotpe × environment interaction literature for sunflower (Casadebaig et al., 2023; Patel et al., 2023).

Practically, these results indicate that multi-stage selection strategies - using pot-based screening for initial ranking, followed by multi-location field trials for confirmation - represent the most resource-efficient approach for drought tolerance breeding. The strong photosynthesis-yield relationship (r = −0.84) suggests that IRGA-based photosynthetic screening during stress could supplement morphological selection, improving prediction accuracy. However, the cost-effectiveness of morphological traits (height, SPAD, stem girth) as primary screening criteria - combined with their significant stress responses and practical measurement ease - supports their use as front-line selection tools, particularly in large germplasm screening programs (Martinez et al., 2024).

CONCLUSION

This study systematically characterized the morphological, physiological, and yield responses of eight sunflower hybrids to a well-defined episode of vegetative-stage moisture stress under both controlled pot and field conditions. Drought stress imposed at 30–35 DAS and maintained for 14–25 days caused significant reductions in morphological parameters (plant height: 8.6%; SPAD: 7.0%; stem girth: 10.5%) and severe yield losses (37.3% in pot; 44.0% in field), while photosynthetic rates remained relatively stable, highlighting the prioritization of carbon assimilation under moderate stress. The apparent increase in stomatal conductance under the recovery-phase measurement reflects a documented stomatal overshoot phenomenon following rehydration, and mechanistically explains the paradoxical WUE reduction despite photosynthetic maintenance. PCA and k-means clustering objectively classified RSFH-1 and KBSH-53 as stress-tolerant genotypes, supported by superior performance across structural, physiological, and yield axes. Photosynthetic resilience was identified as the strongest predictor of yield stability under drought (r = −0.84). Moderate pot-to-field rank correlation supports the utility of pot screening for initial triage, with multi-location field validation recommended for final genotype selection. The multi-parameter framework developed here provides a robust, reproducible platform for drought-tolerance evaluation in sunflower and offers practical guidance for breeding climate-adaptive cultivars.

Table S1: Morphological parameters of sunflower hybrids at 60 DAS under moisture stress experiment (pot experiment)

Parameter	No. of leaves	Plant height (cm)	SPAD	Stem girth (cm)
Control Mean	23.83	110.08	43.51	1.77
Control SD	1.47	20.68	2.02	0.15
Control CV (%)	6.17	18.79	4.65	8.53
Stress Mean	23.20	100.62	40.46	1.58
Stress SD	3.96	15.64	3.62	0.19
Stress CV (%)	17.07	15.54	8.94	11.75
p-value	0.69	0.32	0.06	0.05

Table S2: Morphological parameters of sunflower hybrids at harvest under moisture stress experiment (pot experiment)

Parameter	No. of leaves	Plant height (cm)	SPAD	Stem girth (cm)
Control Mean	15.79	119.21	32.38	1.99
Control SD	1.78	20.52	1.84	0.13
Control CV (%)	11.28	17.22	5.67	6.29
Stress Mean	13.42	111.17	30.40	1.85
Stress SD	2.14	15.88	1.03	0.12
Stress CV (%)	15.97	14.28	3.38	6.63
p-value	0.03	0.40	0.02	0.04

Table S3 : Physiological parameters (IRGA readings) of sunflower hybrids before watering level under moisture stress experiment (pot experiment)

Parameter	Internal_CO2_conc._(Âµmol_CO2_mol-1)	WUE_(mg_g-1)

Control Mean	239.68	0.45
Control SD	27.08	0.10
Control CV (%)	11.30	23.01
Stress Mean	267.50	0.36
Stress SD	14.91	0.05
Stress CV (%)	5.57	13.72
p-value	0.03	0.06

Table S4: Physiological parameters (IRGA readings) of sunflower hybrids at saturation level under moisture stress experiment (Pot experiment)

Parameter	Internal_CO2_conc._(Âµmol_CO2_mol-1)	WUE_(mg_g-1)
Control Mean	289.28	0.27
Control SD	18.96	0.04
Control CV (%)	6.55	14.96
Stress Mean	282.44	0.27
Stress SD	20.82	0.02
Stress CV (%)	7.37	8.97
p-value	0.50	0.79

Table S5 : Physiological parameters (IRGA readings) of sunflower hybrids at after watering under moisture stress experiment (pot experiment)

Parameter	Internal_CO2_conc._(Âµmol_CO2_mol-1)	WUE_(mg_g-1)
Control Mean	265.57	0.38
Control SD	21.87	0.05
Control CV (%)	8.24	13.68
Stress Mean	243.98	0.39
Stress SD	34.67	0.12
Stress CV (%)	14.21	29.71
p-value	0.16	0.81

Table S6: Yield and yield parameters of sunflower hybrids under moisture stress experiment (pot experiment)

Parameter	Leaf dry weight (g)	Root length (cm)	Root weight (g)	Seed yield (g/pl)	Stem dry weight (g)	TDM	Thalamus dry weight (g)
Control Mean	20.77	32.96	10.73	8.71	25.65	73.50	16.35
Control SD	4.44	7.71	3.21	2.77	6.89	12.74	3.69
Control CV (%)	21.36	23.39	29.94	31.83	26.87	17.33	22.55
Stress Mean	14.47	36.46	9.77	5.46	24.92	63.05	14.36
Stress SD	1.96	7.48	3.67	1.82	8.03	12.69	3.18
Stress CV (%)	13.52	20.52	37.53	33.36	32.25	20.13	22.13
p-value	0.00	0.37	0.59	0.02	0.85	0.12	0.27

Table S7: Morphological parameters of sunflower hybrids 60 DAS under moisture stress experiment (field experiment)

Parameter	No. Of leaves	Plant height (cm)	SPAD	Stem girth (cm)	Total leaf area (cm2/pl)
Control Mean	28.38	175.15	42.34	2.24	6840.90
Control SD	2.33	28.52	3.07	0.32	1498.51

Control CV (%)	8.20	16.28	7.26	14.47	21.91
Stress Mean	27.00	144.65	39.10	1.77	5623.10
Stress SD	1.60	31.24	3.28	0.21	1128.41
Stress CV (%)	5.94	21.59	8.39	11.99	20.07
p-value	0.19	0.06	0.06	0.00	0.09

Table S8: Morphological parameters of sunflower hybrids at harvest stage under moisture stress experiment (field experiment)

Parameter	Days to 50 % flowering	No. Of leaves	Plant height	SPAD	Stem girth	Total leaf area (cm2/pl)
Control Mean	61.00	22.13	184.93	37.83	2.45	4834.39
Control SD	2.20	2.03	25.04	0.52	0.28	559.29
Control CV (%)	3.61	9.18	13.54	1.36	11.33	11.57
Stress Mean	57.25	20.38	156.55	35.50	2.13	3678.16
Stress SD	1.28	1.85	32.31	1.07	0.19	649.98
Stress CV (%)	2.24	9.06	20.64	3.02	8.78	17.67
p-value	0.00	0.09	0.07	< 0.001	0.02	0.00

Table S9: Physiological parameters of sunflower hybrids under moisture stress experiment (field experiment)

Parameter	Photosynthesis @ 57DAS	Photosynthesis @ 60DAS	Transpiration @ 57DAS	Transpiration @ 60DAS	WUE @ 57DAS	WUE @ 60DAS
Non-Stress Mean	25.27	25.74	4.43	5.03	0.58	0.51
Non-Stress SD	1.38	1.25	0.50	0.16	0.05	0.02
Non-Stress CV (%)	5.48	4.86	11.36	3.25	8.67	4.53
Stress Mean	23.01	23.20	4.79	4.71	0.49	0.50
Stress SD	1.33	0.97	0.44	0.36	0.04	0.03
Stress CV (%)	5.80	4.19	9.25	7.62	8.79	5.61
p-value	0.01	< 0.001	0.16	0.05	0.00	0.26

Table S10: Yield and yield parameters of sunflower under moisture stress experiment (field experiment)

Parameter	100 Seed weight (g)	Head diameter (cm)	leaf dry weight (g)	seed volume	seed yield (g/pl)	stem dry weight (g)	thalamus dry weight (g)
Control Mean	5.77	21.37	43.96	50.57	68.80	48.99	37.32
Control SD	0.80	1.54	11.57	4.81	19.89	11.86	9.44
Control CV (%)	13.81	7.23	26.32	9.51	28.92	24.20	25.29
Stress Mean	4.48	15.71	29.20	46.24	38.53	32.81	17.65
Stress SD	0.79	1.91	8.73	3.81	16.17	9.24	2.89
Stress CV (%)	17.61	12.16	29.88	8.25	41.98	28.16	16.36
p-value	0.01	< 0.001	0.01	0.07	0.01	0.01	< 0.001

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Declarations
Authors declare no conflict of interest. AI was used only to improve the readability of the manuscript; AI tools do not obtain additional rights, including rights to use the work for training AI models.

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