



Physiological and Biochemical Responses of Drought-Tolerant and Drought-Susceptible Sunflower (*Helianthus annuus* L.) Hybrids under Different Soil-Water Regimes

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Abstract

Drought stress is a major abiotic constraint affecting the growth, physiological processes, and productivity of sunflower (*Helianthus annuus* L.). The present study evaluated the physiological and biochemical responses of two sunflower hybrids, drought-tolerant DRSH-1 and drought-susceptible KBSH-44, under three soil-water regimes corresponding to 100%, 50%, and 25% of field capacity (FC). The experiment was conducted for 21 days, from 35 to 56 days after sowing. The parameters assessed included relative water content (RWC), leaf chlorophyll content, net photosynthetic rate (Pn), stomatal conductance (gs), free proline, malondialdehyde (MDA), and the activities of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Increasing drought severity was associated with reductions in RWC, chlorophyll content, photosynthetic rate, and stomatal conductance, accompanied by increased proline accumulation, MDA content, and antioxidant enzyme activities. Under severe drought (25% FC), the reported pooled mean values for RWC, total chlorophyll, Pn, and gs were 55.00%, 1.21 mg g⁻¹ fresh weight, 9.90 μmol CO₂ m⁻² s⁻¹, and 0.14 mol H₂O m⁻² s⁻¹, respectively. Compared with KBSH-44, DRSH-1 maintained higher leaf water status and photosynthetic performance and exhibited lower MDA content and proline accumulation. The increased antioxidant enzyme activities under drought stress suggest the involvement of antioxidant defence mechanisms in the response to water deficit. Overall, the findings indicate that physiological traits, gas-exchange parameters, and biochemical stress markers can provide useful criteria for comparative screening of sunflower hybrids for drought tolerance. However, the results should be interpreted cautiously because of numerical inconsistencies and incomplete enzyme-activity data in the source manuscript.

Keywords: *Helianthus annuus* L.; drought stress; drought tolerance; relative water content; chlorophyll; photosynthesis; proline; malondialdehyde; antioxidant enzymes.

1. Introduction

Sunflower (*Helianthus annuus* L.), belonging to the family Asteraceae, is an important oilseed crop cultivated for its edible oil, nutritional value, and industrial applications. Its productivity is strongly influenced by environmental factors, particularly water availability, temperature, and soil conditions. Among the various abiotic stresses, drought is one of the major constraints limiting crop growth, development, and yield worldwide. Water deficit can affect plant growth at several levels, including tissue hydration, nutrient uptake, photosynthetic activity, membrane stability, and metabolic processes (Boyer, 1982; Passioura, 2007; Farooq et al., 2009). Although sunflower exhibits certain adaptive mechanisms that enable it to withstand temporary water shortages, prolonged or severe drought can substantially reduce its physiological efficiency and productivity. The extent of damage depends on genotype, developmental stage, stress intensity, duration of water deficit, and environmental conditions.

Water plays a fundamental role in maintaining cell turgor, facilitating nutrient transport, regulating stomatal movements, and supporting photosynthesis. Under drought conditions, reduced soil-water availability limits water uptake by roots and decreases leaf water potential and relative water content. Plants commonly respond by closing their stomata to reduce transpiration and conserve water. However, stomatal closure also restricts carbon dioxide entry into leaves, thereby reducing photosynthetic carbon assimilation and plant growth (Chaves et al., 2003; Flexas et al., 2004; Chaves et al., 2009). When water deficit becomes severe, photosynthesis may be further impaired by changes in chlorophyll content, photosynthetic electron transport, and the biochemical processes involved in carbon fixation. Consequently, the measurement of relative water content (RWC), chlorophyll concentration, net photosynthetic rate (P_n), and stomatal conductance (g_s) provides useful information for evaluating plant responses to drought stress.

Drought stress can also disturb cellular redox homeostasis and promote the excessive generation of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. Although ROS participate in normal cellular signalling, their excessive accumulation can damage proteins, nucleic acids, pigments, and membrane lipids. Lipid peroxidation, commonly assessed through malondialdehyde (MDA) content, is frequently used as an indicator of oxidative membrane damage under environmental stress (Apel & Hirt, 2004; Gill & Tuteja, 2010). Plants counteract oxidative stress through enzymatic and non-enzymatic antioxidant defence systems. Important antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which contribute to the detoxification of ROS and the maintenance of cellular homeostasis. Changes in the activities of these enzymes can therefore provide insights into the capacity of plants to respond to drought-induced oxidative stress (Mittler, 2002; Foyer & Noctor, 2005).

In addition to antioxidant defence, plants employ osmotic adjustment and other physiological mechanisms to maintain cellular function under water deficit. Proline is one of the compatible solutes that can accumulate in plant tissues exposed to drought and other abiotic stresses. It has been associated with osmotic adjustment, protection of cellular structures, redox regulation, and stress signalling. However, the degree of proline accumulation does not necessarily indicate greater drought tolerance, because it may also reflect the intensity of stress-induced injury (Ashraf & Foolad, 2007; Szabados & Saviouré, 2010). Therefore, proline concentration should be interpreted together with water-status measurements, photosynthetic performance, membrane damage, and antioxidant enzyme activities to obtain a more comprehensive understanding of drought responses.

Sunflower genotypes can differ considerably in their ability to maintain water relations, photosynthetic activity, and cellular protection

under limited water availability. Identifying these differences is important for the comparative evaluation of drought responses and the selection of promising genotypes for water-limited environments. Previous research has demonstrated that drought responses in sunflower are associated with changes in leaf water status, stomatal regulation, photosynthesis, and antioxidant metabolism (Chimenti et al., 2002; Rauf, 2008). Evaluating multiple physiological and biochemical parameters together is therefore more informative than relying on a single indicator of stress tolerance.

In this context, the present study investigated the physiological and biochemical responses of two sunflower hybrids, DRSH-1, described as drought-tolerant, and KBSH-44, described as drought-susceptible, under three soil-water regimes representing 100%, 50%, and 25% of field capacity (FC). The experiment was conducted over a 21-day period, from 35 to 56 days after sowing, to examine responses during the vegetative growth stage. The study focused on relative water content, leaf chlorophyll content, net photosynthetic rate, stomatal conductance, free proline accumulation, MDA content, and the activities of SOD, CAT, and POD. The specific objectives were to (i) determine the effects of different soil-water regimes on leaf water status, photosynthetic pigments, and gas-exchange characteristics; (ii) evaluate changes in proline accumulation and lipid peroxidation in response to drought stress; (iii) compare antioxidant enzyme responses between the two hybrids; and (iv) identify physiological and biochemical traits that may be useful for comparative screening of sunflower hybrids for drought responses.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The experiment was conducted during the 2025–2026 rabi season at the botanical research facilities of Government Degree College (Autonomous), Medak, Telangana, India. Seeds of two sunflower (*Helianthus annuus* L.) hybrids,

DRSH-1 and KBSH-44, were obtained from Professor Jayashankar Telangana State Agricultural University (JTSAU), Hyderabad, Telangana, India. DRSH-1 was considered drought-tolerant, whereas KBSH-44 was considered drought-susceptible, as described in the source manuscript.

2.2. Experimental Design and Drought Treatments

Plants were grown in earthen pots containing 10 kg of clay-loam soil. Three soil-water regimes were maintained gravimetrically: T₀, 100% field capacity (FC; control); T₁, 50% FC (moderate drought); and T₂, 25% FC (severe drought). The treatments were initiated at 35 days after sowing (DAS) and continued for 21 days, until 56 DAS. Five replications were used for each treatment. The experiment was described as a factorial randomized block design.

2.3. Physiological and Biochemical Measurements

Relative water content (RWC) was determined according to Barrs and Weatherley (1962). Chlorophyll pigments were extracted using 80% acetone, and chlorophyll content was calculated using the equations described by Arnon (1949).

Net photosynthetic rate (P_n) and stomatal conductance (g_s) were measured on fully expanded upper leaves using a LI-COR LI-6400XT infrared gas analyser.

Free proline content was estimated using the acid-ninhydrin method of Bates et al. (1973). Lipid peroxidation was assessed by estimating thiobarbituric-acid-reactive substances (TBARS), following Heath and Packer (1968), with absorbance measured at 532 and 600 nm.

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of nitro blue tetrazolium photoreduction according to Beauchamp and Fridovich (1971). Catalase (CAT) activity was measured by monitoring hydrogen peroxide decomposition at 240 nm,

following Aebi (1984). Peroxidase (POD) activity was assessed using guaiacol oxidation, as attributed to Castillo et al. (1984) in the source manuscript.

2.4. Statistical Analysis

The experimental data were analysed using two-way analysis of variance (ANOVA) in OPSTAT. Differences were considered statistically significant at $p < 0.05$. The analysis was intended to evaluate the effects of sunflower hybrid and drought treatment, including their interaction. The post-hoc comparison procedure and relevant statistical details should be specified according to the analysis actually performed.

3. Results

3.1. Relative Water Content and Photosynthetic Pigments

Relative water content (RWC) decreased with increasing drought severity. The pooled treatment means declined from 88.35% under control conditions (100% FC) to 68.70% at 50% FC and 55.00% at 25% FC. Under severe drought, RWC was 61.4% in DRSH-1 and 48.6% in KBSH-44. Total chlorophyll content also decreased from 2.84 mg g⁻¹ fresh weight under control conditions to 1.95 mg g⁻¹ at 50% FC and 1.21 mg g⁻¹ at 25% FC. The reported trends indicate that DRSH-1 maintained higher leaf water content and better chlorophyll retention than KBSH-44 under severe drought.

3.2. Leaf Gas Exchange

Net photosynthetic rate (Pn) decreased progressively with increasing drought stress, from 22.10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under control conditions to 15.40 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 50% FC and 9.90 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 25% FC. Under severe

drought, Pn was 11.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in DRSH-1 and 8.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in KBSH-44. Similarly, stomatal conductance (gs) declined from 0.42 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ under control conditions to 0.26 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ at 50% FC and 0.14 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ at 25% FC. At 25% FC, gs was 0.18 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in DRSH-1 and 0.11 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in KBSH-44. These results indicate that drought stress reduced photosynthetic performance and stomatal conductance, with DRSH-1 maintaining comparatively higher gas-exchange rates.

3.3. Proline Accumulation and Lipid Peroxidation

Under severe drought conditions (25% FC), free proline content was reported as 11.2 $\mu\text{mol g}^{-1}$ fresh weight in DRSH-1 and 14.6 $\mu\text{mol g}^{-1}$ fresh weight in KBSH-44. Malondialdehyde (MDA) content increased from 4.2 to 12.8 nmol g⁻¹ fresh weight in DRSH-1 and from 4.8 to 18.7 nmol g⁻¹ fresh weight in KBSH-44 between control and severe drought conditions. The greater increase in MDA content in KBSH-44 suggests higher membrane lipid peroxidation under severe drought. The higher proline accumulation in this hybrid may also reflect a stronger stress response.

3.4. Antioxidant Enzyme Responses

The source manuscript reported increases in superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities of 2.8-fold, 2.5-fold, and 3.8-fold, respectively, under severe drought (25% FC) relative to the control. It also reported higher antioxidant enzyme activities in DRSH-1 than in KBSH-44. However, absolute enzyme activities, measurement units, genotype-specific values, and statistical results were not available. Therefore, these findings should be interpreted as preliminary reported trends pending verification against the original experimental data.

Table 1. Pooled effects of drought treatment on leaf physiological traits in sunflower

Treatment	Soil moisture	RWC (%)	Total chlorophyll (mg g ⁻¹ FW)	Pn (μmol CO ₂ m ⁻² s ⁻¹)	gs (mol H ₂ O m ⁻² s ⁻¹)
T ₀ – Control	100% FC	88.35	2.84	22.1	0.42
T ₁ – Moderate drought	50% FC	68.7	1.95	15.4	0.26
T ₂ – Severe drought	25% FC	55	1.21	9.9	0.14

Note: Values are reproduced from the supplied draft and are described as means of five replications. No measures of dispersion or inferential statistics were supplied for this table.

4. Discussion

The decline in relative water content (RWC) with increasing drought severity indicates a reduction in leaf hydration under limited soil-water availability. The higher RWC maintained by DRSH-1 than KBSH-44 under severe drought suggests a comparatively greater ability to maintain leaf water status. Relative water content is an important physiological indicator of plant water balance and drought response (Barrs & Weatherley, 1962; Schonfeld et al., 1988). Differences in water uptake, water conservation, and osmotic adjustment may contribute to variation in drought responses among genotypes (Chaves et al., 2003; Farooq et al., 2009). However, the magnitude and statistical significance of the differences observed between the two hybrids should be confirmed using replicate-level data.

The reductions in chlorophyll content, net photosynthetic rate (Pn), and stomatal conductance (gs) demonstrate the adverse effects of increasing drought stress on photosynthetic performance. Stomatal closure under water deficit restricts CO₂ diffusion into leaves, thereby reducing photosynthetic carbon assimilation. Under prolonged or severe drought, photosynthesis may also be limited by biochemical and photochemical disturbances (Flexas et al., 2004; Chaves et al., 2009). The comparatively higher Pn and gs values reported for DRSH-1 under severe drought suggest that this hybrid maintained better gas-exchange performance than KBSH-44. Previous research has also demonstrated that drought-induced

changes in photosynthesis depend on stress intensity, duration, and plant genotype (Lawlor & Cornic, 2002; Rauf, 2008).

The decrease in total chlorophyll content under drought may reflect changes in pigment synthesis, degradation, and chloroplast function. Drought-induced oxidative stress can further affect photosynthetic membranes and pigment stability (Smirnoff, 1993; Farooq et al., 2009). The reported reduction in chlorophyll content was accompanied by lower photosynthetic rates, suggesting an association between pigment loss and declining photosynthetic performance. Nevertheless, chlorophyll degradation alone cannot fully explain the reduction in photosynthesis because stomatal and non-stomatal limitations may operate simultaneously (Flexas et al., 2004; Chaves et al., 2009).

Proline accumulation is a common biochemical response to drought and other abiotic stresses. Proline may contribute to osmotic adjustment, cellular protection, redox regulation, and stress signalling (Ashraf & Foolad, 2007; Szabados & Savaure, 2010). In the present study, KBSH-44 exhibited higher proline accumulation than DRSH-1 under severe drought. However, higher proline content does not necessarily indicate greater drought tolerance, as its accumulation may also reflect stronger stress exposure or a greater physiological response to injury. The higher proline content in KBSH-44, together with its lower RWC and higher MDA content, suggests that the susceptible hybrid experienced a comparatively greater stress response under the experimental conditions.

Malondialdehyde (MDA), commonly used as an indicator of membrane lipid peroxidation, increased in both hybrids under severe drought, with a greater increase reported in KBSH-44. This pattern suggests greater oxidative membrane damage in the susceptible hybrid. Drought-induced reactive oxygen species (ROS) can cause lipid peroxidation and damage cellular components when ROS production exceeds the capacity of antioxidant defence systems (Apel & Hirt, 2004; Gill & Tuteja, 2010). The comparatively lower MDA content in DRSH-1 may indicate better maintenance of membrane integrity under water deficit. However, MDA measurements should be interpreted alongside other indicators of oxidative stress because the thiobarbituric-acid-reactive substances assay is not entirely specific to MDA (Hodges et al., 1999).

The reported increases in superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities under severe drought are consistent with the activation of enzymatic antioxidant defence mechanisms. SOD converts superoxide radicals into hydrogen peroxide, whereas CAT and various peroxidases contribute to hydrogen peroxide removal. These enzymes play complementary roles in maintaining cellular redox balance under environmental stress (Mittler, 2002; Foyer & Noctor, 2005). Although the source manuscript reports higher antioxidant enzyme activities in DRSH-1, this interpretation requires confirmation using absolute enzyme activities, clearly defined units, replicate-level observations, and statistical comparisons. Enzyme activity alone does not establish greater drought tolerance without considering the extent of oxidative damage and other physiological responses.

Overall, the reported findings indicate contrasting physiological and biochemical responses between DRSH-1 and KBSH-44 under different soil-water regimes. DRSH-1 maintained higher RWC, chlorophyll-associated performance, and gas-exchange rates, while exhibiting lower MDA and proline accumulation under severe drought. In contrast, KBSH-44 showed greater proline

accumulation and lipid peroxidation, suggesting a stronger biochemical stress response. These observations support the use of multiple physiological and biochemical traits for comparative screening of sunflower hybrids for drought responses. However, because the experiment was conducted in pots over a 21-day period during vegetative growth, further validation under field conditions and across developmental stages is necessary before drawing conclusions about yield stability or recommending either hybrid for drought-prone regions.

5. Conclusion

The present study demonstrated that increasing drought severity adversely affected the physiological and biochemical responses of sunflower (*Helianthus annuus* L.). Severe drought (25% field capacity) was associated with reductions in relative water content (RWC), chlorophyll content, net photosynthetic rate, and stomatal conductance, accompanied by increased proline accumulation, malondialdehyde (MDA) content, and antioxidant enzyme activities. Compared with KBSH-44, DRSH-1 maintained higher leaf water status and photosynthetic performance, together with lower MDA accumulation, indicating a comparatively favourable response to water deficit under the experimental conditions. These findings suggest that RWC, photosynthetic traits, MDA content, and antioxidant enzyme activities can serve as complementary parameters for screening sunflower hybrids for drought responses. However, further validation using complete experimental data, appropriate statistical analyses, and field trials assessing growth and yield is necessary before confirming drought tolerance and recommending these hybrids for drought-prone environments.

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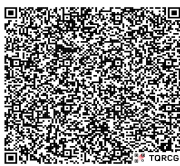
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