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Research Article

MITIGATING THE ADVERSE EFFECTS OF DROUGHT STRESS ON SUNFLOWER PLANTS VIA FOLIAR APPLICATION OF SALICYLIC ACID

[a]Muhammad Nosherwan, [a]Ali Raza, [a]Muhammad Haider Ali, [b]Hafiz Muhammad Atif Noor, [a]Joher Abbas, [a]Iman Shakeel Qureshi, [a]Abdul Rehman, [c]Muatter Rana, [d]Anam Moosa, [d]Hafiz Muhammad Ayub, [d]Tahir Mahmood

[a] Department of Plant Breeding and Genetics, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan.
[b] Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan.
[c] National Agricultural Research Centre, Islamabad, Pakistan.
[d] Department of Plant Pathology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan.

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ABSTRACT

Sunflower is the primary oilseed crop of Pakistan, valued for its edible oil and high yield potential under favorable conditions. However, drought stress is a major limiting factor affecting sunflower productivity, causing significant yield reductions. The present study aimed to evaluate the potential role of salicylic acid in enhancing the growth and physiological performance of sunflowers under drought stress. Drought stress was applied 20 days after sowing, with three experimental treatments conducted in five replications: (a) control (normal conditions), (b) drought stress, and (c) drought stress supplemented with 250 mg L^{-1} salicylic acid. Key findings revealed that the foliar application of 250 mg L^{-1} salicylic acid significantly improved various growth parameters, including shoot length (24.92%), root length (18.97%), fresh shoot weight (15.78%), dry shoot weight (24.15%), fresh root weight (21.32%), and dry root weight (26.72%). Moreover, chlorophyll content increased by 32.90%, stomatal conductance by 19.52%, relative water content by 20.84%, and membrane stability index by 41.79%. Moreover, salicylic acid markedly mitigated oxidative damage, reducing malondialdehyde levels by 32.81% and hydrogen peroxide levels by 17.80% compared to untreated plants. Furthermore, the treatment enhanced the activity of antioxidant enzymes, with superoxide dismutase activity increasing by 17.25%, catalase by 40.05%, and peroxidase by 10.41%. In conclusion, the foliar application of 250 mg L^{-1} salicylic acid effectively enhances the growth and physiological parameters of sunflower under drought stress, offering a sustainable and reliable strategy to mitigate the adverse effects of water scarcity on crop production.

Corresponding Author: Muhammad Nosherwan
Email: nosherwan320@gmail.com

INTRODUCTION

Sunflower (*Helianthus annuus* L.) oil ranks fourth among global vegetable oil sources, following soybean, palm, and rapeseed (Khurana and Singh, 2021). In Pakistan, during 2019-20, sunflower cultivation extends over 219,000 acres, yielding 105,000 tons of seed and producing 40,000 tons of oil. By 2022-23, the cultivated area decreased to 179,000 acres, but seed production increased to 124,000 tons, resulting in 47,000 tons of oil (Kandhro et al., 2024).

Edible oil is a crucial component of the Pakistani diet. However, the increasing demand far exceeds domestic production, compelling Pakistan to rely on imports to meet its needs (Khan et al., 2021). Consequently, the country spends a substantial portion of its foreign exchange reserves on purchasing edible oil due to insufficient domestic oilseed production (Raza et al., 2023). Of the total 3.255 million tons of oilseed required annually, Pakistan produces only 0.507 million tons domestically, while importing the remaining 2.748 million tons (Manzoor et al., 2024).

Sunflower is considered superior to other oilseed crops in Pakistan because of its high oil content and adaptability to local agronomic conditions (Javed et al., 2003). It is cultivated primarily for its high-quality edible oil (40-50%), dietary protein (17-20%), and use as animal feed (Tabassum et al., 2020). Moreover, sunflower seeds and oil offer various health benefits due to their rich content of unsaturated fats, vitamin B, dietary fiber, and easily digestible protein (Saudy et al., 2021).

Drought stress is a critical abiotic factor that significantly impacts plant growth and productivity, leading to a reduction of over 25% in global agricultural output (Bano et al., 2021). Approximately one-third of the world’s land area falls within arid and semi-arid regions, and by 2050, it is projected that more than 50% of global agricultural land will be affected by drought stress. Pakistan, ranked third among water-deficient countries, is expected to experience severe drought conditions until 2025 (Aryal et al., 2020).

Severe drought induces substantial physiological and developmental changes in plants (Hussein et al., 2022). In sunflowers, drought stress adversely affects growth and development by reducing root and shoot lengths, as well as decreasing leaf area (Shi et al., 2023). Furthermore, drought stress exacerbates oxidative damage by increasing levels of hydrogen peroxide, lipid peroxidation, and membrane leakage. It also disrupts the balance of antioxidant compounds, enzymes, and osmolytes (Hanafy and Sadak, 2023).

Sunflower, a vital oilseed crop, exhibits moderate drought tolerance (Ashraf et al., 2024). However, the increasing frequency and severity of drought under changing climate conditions are likely to make sunflower cultivation more vulnerable to water stress.

Salicylic acid (SA) is a naturally occurring phenolic compound known to mitigate the adverse effects of abiotic stress (Kaya et al., 2020). Studies have shown that SA significantly reduces lipid peroxidation and alleviates the negative impacts of drought stress (Kang et al., 2013). It has been reported that SA can mitigate drought stress in various crops, including pepper and rice (Urmi et al., 2023; Zhang et al., 2023).

Furthermore, the application of SA at specific growth stages has proven effective in improving drought tolerance. Spraying SA at concentrations of 10^{-4} and 10^{-5} mol L^{-1} on maize plants at the four-leaf stage significantly mitigated the deleterious effects of drought (Latif et al., 2016). Another study found that the application of 0.5 mM SA alleviated drought stress by enhancing proline synthesis through increased glutamate kinase activity and reduced proline dehydrogenase activity (Nazar et al., 2015). Similarly, spraying winter wheat plants with 0.5 mM SA effectively ameliorated the negative effects of drought stress under both normal and stress conditions (Khalvandi et al., 2021).

The objective of the present study was to investigate the efficacy of salicylic acid as a plant growth regulator in enhancing sunflower tolerance to drought, focusing on growth and physiological parameters.

MATERIALS AND METHODS

Experimental site and plant material

A pot experiment was conducted in the greenhouse of the Department of Plant Breeding and Genetics, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan (29°24'N latitude, 71°41'E longitude; 214 m above sea level). The hybrid sunflower variety Hysun-33 was sown in clay loam soil using plastic pots with a base diameter of 12 cm and a height of 14 cm, at a rate of three seeds per pot.

Research design

The experiment included three treatments:

T1 = Normal irrigation (100% proper irrigation),

T2 = Drought stress (50% irrigation),

T3 = Drought stress + foliar application of 250 mg L^{-1} salicylic acid.

Each treatment was replicated five times, resulting in 15 pots per treatment. The experiment followed a completely randomized design (CRD) with a factorial arrangement.

Drought imposition and foliar application

After germination, the seedlings were thinned to two plants per pot. Fertilization was applied uniformly to all pots following the recommendations of oilseed research institute. All pots were irrigated with tap water for the first 20 days after sowing.

Drought stress was imposed 20 days after sowing by withholding water for seven days. Following this period, SA (250 mg L^{-1}) was applied as a foliar spray to the leaves in the designated treatment group. Five days after the foliar application, plants were sampled to assess growth parameters and physiological traits.

Growth and physiological parameters

All plants were selected from pots to measure the studied attributes. Shoot length (SL) and root length (RL) were recorded using a measuring scale. Fresh shoot weight (FSW), dry shoot weight (DSW), fresh root weight (FRW), and dry root weight (DRW) were determined using an electronic weighing balance. The chlorophyll content (CC) of the leaves was measured with a SPAD meter, and stomatal conductance (SC) was recorded using a Leaf Porometer SC^{-1} (Mahmood et al., 2024).

Fresh weight (FW) of the leaves was immediately recorded, after which the leaves were immersed in distilled water at room temperature for 4 h under constant light and saturated humidity. The turgid weight (TW) was then measured. Subsequently, the samples were dried at 80°C for 24 h to determine the dry weight (DW). Relative water content (RWC) was calculated using the formula:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

The membrane stability index (MSI) was determined using two sets of 100 mg sunflower leaf samples, each placed in test tubes containing 10 ml of double-distilled water. One set of test tubes was incubated in a water bath at 40°C for 30 min, and the electrical conductivity (EC) of the solution (C1) was measured using a conductivity meter (HANA Instruments HI-99300 EC/TDS). The second set of test tubes was heated in boiling water at 100°C for 10 min, and the EC (C2) was then measured (Sajid et al., 2023).

The MSI was calculated as:

$$\mathrm{MSI}\ \% = 1 - \frac{\mathrm{C1}}{\mathrm{C2}} \times 100$$

Oxidative stress and antioxidant enzymes

The method described by Velikova et al. (2020) was used to determine hydrogen peroxide (H_2O_2) levels. The concentration of H_2O_2 was assessed by measuring the optical density at 390 nm and utilizing a standard curve for quantification.

To estimate malondialdehyde (MDA) content, a 200 mg leaf sample from a 40 day old sunflower plant was homogenized in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 1000 rpm for 10 min. After centrifugation, 1 mL of the supernatant was mixed with a 0.5% thiobarbituric acid (TBA) solution. The mixture was heated at 95°C for 30 m and subsequently cooled to room temperature. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (ANA-720W, Tokyo Photo-electric Company Limited, Japan). MDA content was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} (Zulfiqar et al., 2024).

Activity of antioxidative defense enzymes assays

The leaves of sunflower plants were utilized for estimating the activity of antioxidative defense enzymes. A sample of 2 g of leaves was ground with 10 mL of 0.1 M phosphate buffer (pH 6.5) and centrifuged at 20,000×g for 20 min at 2°C. The resulting supernatant was used as the enzyme extract.

Superoxide dismutase (SOD) enzyme assay

The reaction mixture for the SOD enzyme assay consisted of 50 mM potassium borate buffer (pH 7.5), 13 mM methionine, 0.1 mM EDTA, 2 µL riboflavin, 20 µL enzyme extract, and 75 µM nitroblue tetrazolium (NBT). Riboflavin was added last to initiate the reaction, and the tubes were placed under fluorescent lamps (18 W). After 10 min, the reaction was stopped by turning off the light. Blank treatments were prepared by keeping the tubes in the dark. The reaction mixture was transferred into 1.5 mL cuvettes, and the absorbance was recorded at 560 nm using a spectrophotometer. The photo-chemical reduction of NBT was calculated at a 50% inhibition rate following the method of Maalik et al. (2023).

Catalase (CAT) enzyme assay

The reaction mixture, with a total volume of 3 mL, comprised 50 mM phosphate buffer (pH 7.0), 40 mM H_2O_2, and 0.1 mL of enzyme extract. Catalase activity was determined by monitoring the decrease in absorbance at 240 nm using a spectrophotometer, following the method of Malik et al. (2024).

Peroxidase (POD) enzyme assay

The reaction mixture included 0.2 mL enzyme extract, 50 mM phosphate buffer (pH 7.0), 2 mL of 20 mM H_2O_2, and 2 mL of 20 mM pyrogallol. A buffer solution was used as a blank. The absorbance was measured at 470 nm to determine POD activity at 25°C (Abbas et al., 2015).

Statistical analysis

The experiments followed a CRD. Statistical analyses were conducted using the SPSS software package. Mean values for all treatments were compared using Tukey's HSD test at a significance level of $p \leq 0.05$ after performing ANOVA.

RESULTS

Plant growth and biomass parameters

The study aimed to evaluate the effects of foliar application of SA on mitigating the adverse impacts of drought stress on the growth and biomass parameters of sunflower plants. Different treatments showed significant variations in their effects on these parameters. The experimental results indicated that drought stress caused reductions of 30.91% in SL, 33.03% in RL, 28.57% in FSW, 35.10% in DSW, 25.13% in FRW, and 39.27% in DRW compared to plants grown under normal conditions.

However, the foliar application of SA significantly alleviated the negative effects of drought stress and improved growth and biomass parameters in sunflower plants. Following SA application, SL, RL, FSW, DSW, FRW, and DRW exhibited statistically significant improvements of 24.92%, 18.97%, 15.78%, 24.15%, 21.32%, and 26.72%, respectively (Figure 1).

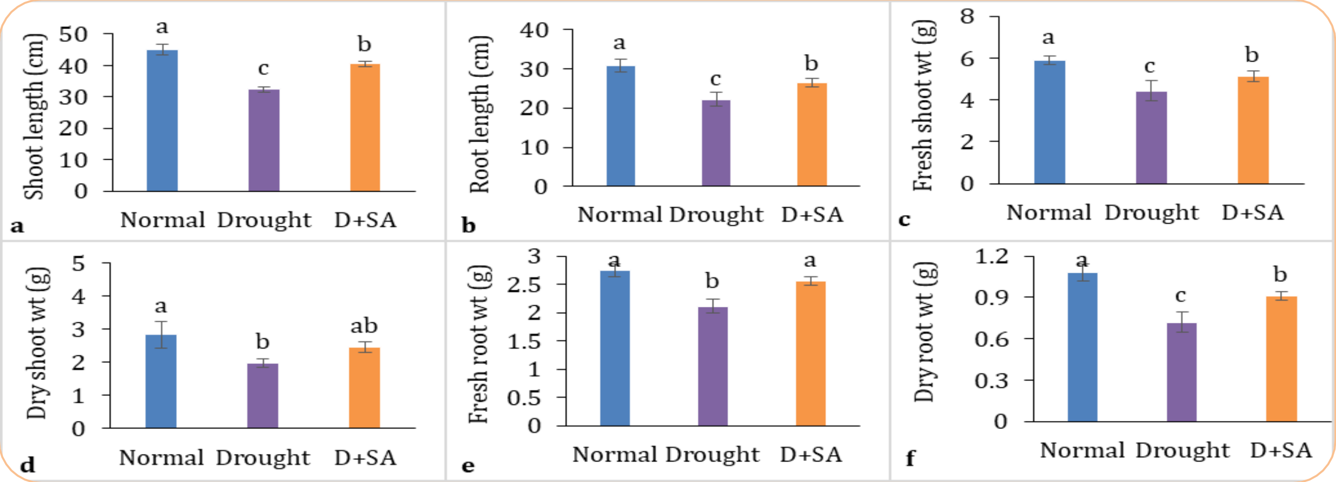

Figure 1. Effect of salicylic acid on growth attributes of sunflower plants under drought stress. (a) shoot length, (b) root length, (c) fresh shoot weight, (d) dry shoot weight, (e) fresh root weight, (f) dry root weight.

The data were assessed using the One-way ANOVA Tukey's HSD test, using a significance level of $P \leq 0.05$. The presence of distinct lowercase letters signifies significant differences between the treatments.

Physiological parameters

The statistical analysis of physiological parameters revealed significant differences among all treatments at the 5% probability level. The experimental results indicated reductions of 45.87% in chlorophyll content, 22.13% in stomatal conductance, 32.50% in relative water content, and 45.30% in MSI under drought conditions compared to normal conditions. Drought stress adversely affected chlorophyll content, stomatal conductance, and water relations, leading to an overall decline in plant growth and development. However, the foliar application of SA significantly mitigated the adverse effects of drought stress, improving physiological parameters in sunflowers. Chlorophyll content, stomatal conductance, relative water content, and MSI showed statistically significant improvements of 32.90%, 19.52%, 20.84%, and 41.79%, respectively (Figure 2).

Oxidative stress and antioxidants enzymes

The evaluation also included examining the effects of SA supplementation at a concentration of 250 mg L^{-1}. The findings revealed that drought stress significantly reduced the activity of antioxidant enzymes and increased oxidative stress in sunflower plants. Under drought conditions, there was a 126.91% increase in MDA content and a 97.85% rise in hydrogen peroxide (H_2O_2) levels, accompanied by reductions of 58.35% in SOD activity, 55.70% in CAT activity, and 24.12% in POD activity.

However, the foliar application of SA markedly mitigated oxidative damage, reducing MDA and H_2O_2 levels by 32.81% and 17.80%, respectively, compared to untreated plants. Furthermore, SA enhanced the activity of antioxidant enzymes, with increases of 17.25% in SOD, 40.05% in CAT, and 10.41% in POD (Figure 3).

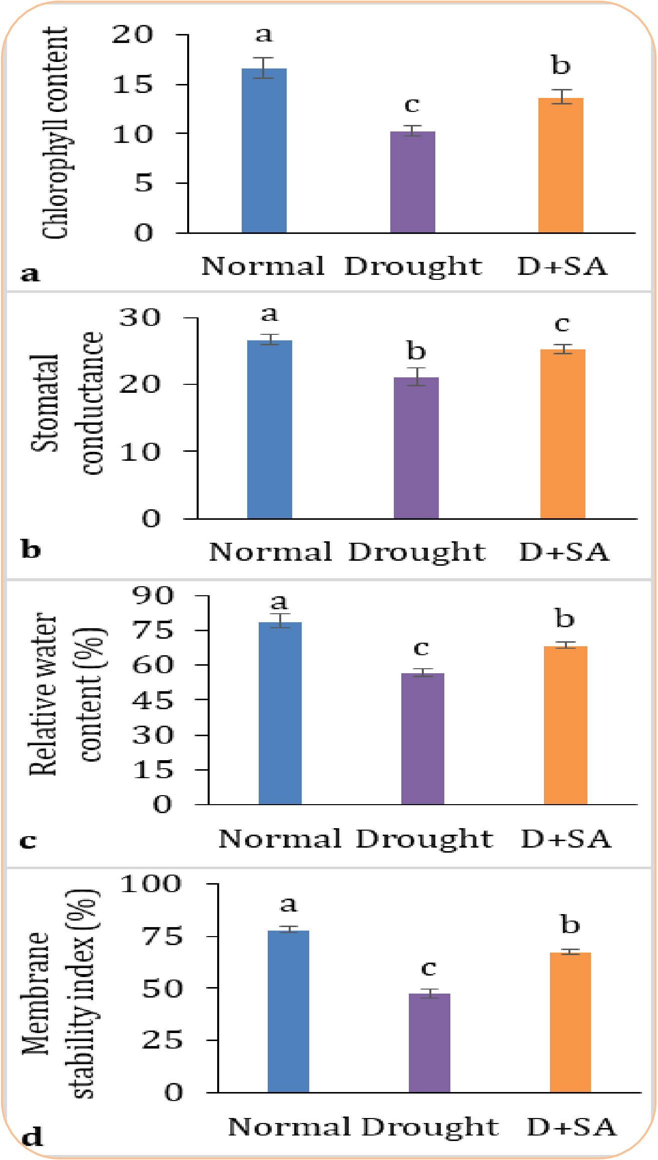

Figure 2. Effect of salicylic acid on physiological attributes of sunflower plants under drought stress. (a) chlorophyll content, (b) stomatal conductance, (c) relative water content, and (d) membrane stability. The data were assessed using the One-way ANOVA Tukey's HSD test, using a significance level of $P \leq 0.05$. The presence of distinct lowercase letters signifies significant differences between the treatments.

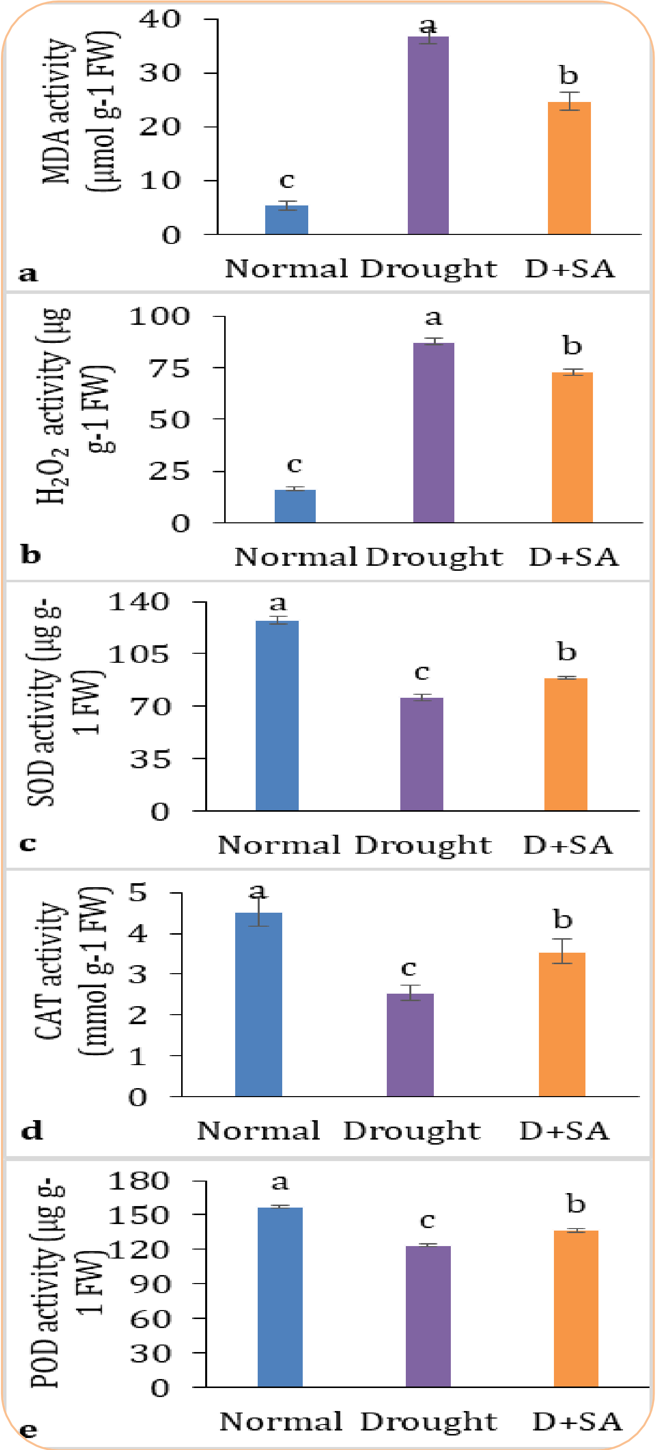

Figure 3. Effect of salicylic acid on oxidative stress and antioxidant enzymes of sunflower plants under drought stress. (a) MDA content, (b) H_2O_2 content, (c) SOD, (d) CAT, and (e) POD. The data were assessed using the One-way ANOVA Tukey's HSD test, using a significance level of $P \leq 0.05$. The presence of distinct lowercase letters signifies significant differences between the treatments.

DISCUSSION

Under climate change scenarios and the early onset of drought, crops are increasingly affected by drought stress (Debaeke et al., 2017). One of the most common adverse effects of drought stress on crops is the reduction in fresh and dry biomass production. This is primarily due to a decreased leaf area, which leads to reduced photosynthate production (Farooq et al., 2012; Fatima et al., 2023). Drought stress significantly impairs plant growth and biomass accumulation by inhibiting cell division and elongation (Vassilevska-Ivanova et al., 2016; Zaharieva et al., 2001).

Studies have shown that drought stress reduces stomatal conductance in various crops, including sesame and sunflower, due to its adverse physiological effects (Angadi, and Entz, 2002; Boureima et al., 2012). However, maintaining a high relative water content (RWC) under drought conditions, achieved through greater root growth relative to shoot growth and abscisic acid-induced stomatal closure, helps sustain cell turgidity, chlorophyll content, and photosynthesis (Keyvan, 2010).

Research has highlighted the beneficial effects of SA in mitigating drought stress. In maize, foliar application of SA at a concentration of 300 mg L^{-1} significantly improved growth and yield, enhancing stress tolerance and productivity (Muhammad et al., 2022). Similarly, foliar-applied SA improved growth, gas exchange, and pigment levels in wheat under drought stress, particularly in the Galaxy-2013 cultivar (Ahmad et al., 2021). SA has also been shown to reduce the adverse effects of salinity stress on the growth of barley and wheat. It likely enhances antioxidant activity, which protects plants from oxidative stress damage while promoting growth (El-Tayeb, 2005).

In the current investigation, foliar application of SA significantly improved growth parameters under drought stress, particularly at a concentration of 250 mg L^{-1}. This improvement aligns with previous studies that demonstrated the efficacy of exogenously applied SA in enhancing growth parameters in crops such as maize, rice, and soybean (Khan et al., 2003; Farooq et al., 2009; Latif et al., 2016).

Studies have shown that SA significantly enhances root growth in groundnut plants (Jadhav and Bhamburdekar, 2011). Similarly, another study reported that the application of SA and ascorbic acid resulted in a substantial increase in the fresh root weight of maize under drought stress (Loutfy et al., 2020). The ability of SA to improve plant dry mass and mitigate the adverse effects of water stress offers a promising solution to enhance plant growth and address yield limitations caused by water scarcity (Sayyari et al., 2013).

In the present study, foliar application of SA increased stomatal conductance and chlorophyll content. These improvements in physiological parameters align with previous findings, which reported that SA application enhanced stomatal conductance and chlorophyll content in wheat (Singh and Usha, 2003).

The study by Iqbal et al. (2022) found that the application of SA under drought stress reduced the levels of abscisic acid and ethylene, resulting in enhanced stomatal conductance and photosynthesis in mustard plants. In the present research, the application of SA significantly improved chlorophyll content and RWC under drought conditions. These findings align with previous studies of Rao et al. (2012), which reported that SA application at 100 ppm enhanced physiological parameters in maize, sustaining maximum RWC (79.37%) and chlorophyll content (63.62 μM) during drought stress. Furthermore, foliar application of SA has been shown to regulate stomatal behavior, thereby influencing the photosynthetic rate (Khan et al., 2003).

RWC is a critical physiological indicator reflecting a plant's ability to tolerate water stress (Ober et al., 2005). Previous studies have also highlighted the beneficial effects of SA on RWC in water-stressed crops such as canola and lettuce (Ullah et al., 2012; Sayyari et al., 2013).

In the present study, SA-induced growth amelioration in drought-stressed plants may be attributed to favorable changes in biochemical and physiological processes (Idrees et al., 2010; Yazdanpanah et al., 2011). Our results show that drought stress led to a significant increase in H_2O_2 content, indicating the presence of oxidative stress. However, spraying with SA prevented the accumulation of H_2O_2 in drought-stressed plants, likely due to the upregulation of H_2O_2-scavenging enzymes, such as CAT, SOD, and POD. Consistent with previous studies, foliar application of various compounds has been shown to increase antioxidant enzyme activities and reduce MDA content in different crops (Sedaghat et al., 2017; Ullah et al., 2019; Sohag et al., 2020). Our findings suggest that the prevention of membrane damage may result from the induction of antioxidant responses by SA, thus protecting the plant from oxidative damage. A similar mechanism has been reported in bean and tomato plants, where SA induces tolerance to various stresses (Senaratna et al., 2000). Furthermore, the positive effects of foliar applications of SA and chlormequat chloride (CCC) on antioxidant enzyme activities under moderate and severe drought stress have been documented, though these applications did not provide significant benefits to control plants with full irrigation (Anosheh et al., 2012). Previous studies have also shown that SA application enhanced POD activity in barley plants under drought conditions (Habibi, 2012).

CONCLUSION

The findings of the current study indicate that foliar application of salicylic acid has considerable potential to enhance the growth and physiological parameters of sunflower crops. The experimental investigation suggests that applying salicylic acid at a concentration of 250 mg L^{-1} to the leaves during drought conditions may be an effective strategy for improving sunflower production. Given the global issue of water scarcity and its severe impact on agriculture, foliar application of salicylic acid could potentially boost yield in regions where drought poses a threat to sunflower crops.

AUTHORS' CONTRIBUTIONS

MN and AR conducted the research, performed experiments, analyzed the results; TM, AM, and MHA planned and designed the research, analyzed the results, and performed formal analysis; HMAN, JA, ISQ, AR, and HMA wrote the manuscript, reviewed and edited the final draft.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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