Evaluating the Effect of Seed Priming of Sunflower (Helianthus Annuus L.) with Salicylic Acid to Mitigate Drought Stress

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Abstract:- Plant normal growth and development may be restricted due to water deficit; Therefore, many chemicals have been used to reduce the harmful effects of drought. Salicylic acid can increase the plant's tolerance to drought. Sunflower (Helianthus annuus L.) is a flower that has many benefits, as it produces the largest vegetable oil in the world. The study examined how normal circumstances and three levels of drought stress (75%, 50%, and 25% FC) and salicylic acid (SA) at 20 mg/L affected sunflowers. This investigation demonstrated that the pH value of the soil considerably dropped to levels lower than the controls, and soil EC increased considerably under each studied water stress level. The value of plants' fresh and dry weight rose after SA treatments. While the SA treatment enhanced the leaf RWC, it did not affect the shoot or root length. Under all examined water levels, SA treatments enhanced the amount of plant pigment in comparison with the samples that were not treated. These results show the importance of (SA) in increasing plant tolerance to drought stress.

Keywords: *Plant Growth, Salicylic Acid (SA), Sunflower, Soil PH.*

I. INTRODUCTION

Water covers two-thirds of the Earth's Surface, making water one of the most plentiful resources on the planet. However, water scarcity is common in many places around the world, and it restricts the production of agricultural goods. Drought stress is a significant abiotic factor impacting the quality and production of plants vital for agriculture and the economy. Plants are more vulnerable to stress induced by frequent climate changes, which results in enormous losses (Jeena *et al.*, 2017). Various biomolecules include carbohydrates, proteins, nucleic acids, fatty acids, hormones, ions, and nutrients, chemical–physical phenomenon of drought (Dhanda *et al.*, 2004; Malak *et al.*, 2017).

Environmental stress frequently causes physiological and biochemical changes in plants that have an impact on their physiology and growth. Drought affects all aspects of plant growth and is mostly to blame for lower agricultural yields *(Golbashy et al.,* 2010). Growth is reduced under mild water stress, and cell hyperplasia is especially sensitive to this effect (Fernandez *et al*., 2006).

The decrease in photosynthesis activity is associated with such changes in the state of water. Moreover, the mechanistic basis for this inhibition of photosynthesis is not well understood (Nogués & Baker, 2000). Some plants can modulate different biochemical and physiological responses, to be able to withstand the harsh effects of drought stress (Hsieh *et al*., 2002).

Salicylic Acid (SA) stands for an endogenous phenolic regulator that controls several physiological processes in plants (Shakirova *et al*., 2003).This enhances stomatal control, photosynthesis, and transpiration rates (Khan *et al*., 2003; Khodary, 2004 and Metwally *et al*., 2003). The transport and absorption of iron (Gunes *et al.*, 2005). Due to its capacity to have a protective impact on stressed plants, SA has garnered substantial study because of its critical role in abiotic stress tolerance (El Tayeb & Ahmed, 2010).

According to studies, SA causes an increase in wheat's resistance to osmotic stress (Singh & Usha, 2003), okra (Amin *et al*., 2009), and salinity stress; Shakirova *et al.*, 2003), as well as in resistance to salt stress in maize (Gunes *et al.*, 2007), rice under heavy metal stress (Mishra). Additionally, (SA) has been shown to have several physiological and biochemical impacts on the plant system in barley plants (El-Tayeb *et al*., 2006; El-Tayeb, 2005; Raskin, 1992).

Sunflower *(Helianthus annuus* L.) is One of the most significant oilseed crops, which has a high content of unsaturated fatty acids (Darvishzadeh *et al*., 2010; Razi and Assad, 1998). Sunflowers came second only to soybean as a source of vegetable oil (Tahir *et al*., 2002). Sunflower is one of the 67 species of *Helianthus* (from Greek helios: the sun; Anthus: flower). Of these, only two spp. *H. annuus* and *H. tuberosum* are cultivated for food and the spp. *remain.* They are ornamental grasses and wild plants (Azania *et al*., 2003). It has many uses: they serve as a rich source of proteins and ornamental and medicinal plants that are used to produce oils for, salad, and margarine. They also act as replacements for diesel oil in industry and power generation (Azania *et al*., 2003). However, other sunflower parts have some applications. For example, farmers grind the remaining sunflower stalks and spread them as organic fertilizer (Mirzabe *et al*., 2018). Furthermore, ruminants can consume whole sunflowers; thus, these parts of sunflowers are not toxic (Mirzabe *et al*., 2018).

II. METHODOLOGY

- *Experimental Design:*

The experiment was conducted at King Abdul-Aziz University (January to March), a period characterized by moderate temperatures and low humidity. The average daily maximum temperature during the experiment was 29.8 °C, while the average daily minimum temperature was 20.2°C. The experiment was conducted without any rain.

Sunflower *(Helianthus annuus L.)* seeds were divided into two groups, the first group was grown in the soil without being treated with salicylic acid (SA), and the second group, the seeds were soaked in (20 mg/L) salicylic acid (SA) for one hour, then left to dry for half an hour. Then they were shown in pots, filled with 5 Kg. Homogenously mixed soil consists of sand, field soil, and compost (1:1:1), and using the field capacity it was irrigated with tap water. After the third true leaf appeared, six homogenous plants were left in each pot.

For drought stress conditions, each group is subdivided into four groups. These subsets were irrigated with tap water at 100% (control, water field capacity), 75%, 50%, and 25% FC, respectively. The experiment was carried out in a Complete Randomized Design (CRD) with three replicates. Plant samples were collected after two months for vegetative growth analysis and agronomy parameters analysis.

- *Salicylic Acid Stock Solution Procedure:*

To prepare Salicylic Acid (SA) at a concentration of 20 mg/L, due to the difficulty of dissolving salicylic acid in the water, 20 mg of salicylic acid was dissolved in 8 ml of sodium hydroxide, NaOH. Then, distilled water was added to complete the volume to one liter.

- *Soil Analysis:*
- ✓ *Soil pH*

Using a pH meter The pH of the soil solution was determined (Mettler Toledo AG) (Conklin, 2005).

- ✓ *Soil Electrical Conductivity (EC)*

Following the procedure of (Page *et al*., 1982) an EC meter was used to measure the electrical conductivity of soil extracts.

- *Plant Analysis:*
- ✓ *Fresh and Dry Biomass of Plants' Shoots and Roots*

The samples were gently dried using tissue paper after being rinsed with distilled water. Three replicates of freshly picked shoots and roots from each experiment were weighed and recorded. The samples were then dried using a convection oven for 48 hours at 70 °C. allowing the dry weight to be calculated for each sample (Shanker *et al*., 2005).

- *Shoot and Root Length (cm)*

The heights of three random plants were measured from each treatment via a metric ruler (Shanker *et al*., 2005).

- *Leaf Relative Water Content:*

(Cornic, 1994) method was used to measure the Relative Water Contents of leaves. Fresh Weight (FW) was recorded by using three leaves for each treatment before being submerged in test tubes filled with distilled water for 24 hours. Following the removal of the leaves and tissue wiping, the fully turgid weight (TW) was recorded. And to obtain the dry weight (DW), then the samples were dried at 60 ° C for 72 hours, and using the following law, the calculations were made RWC = [(FW-DW) / (TW-DW)] x 100.

- *Photosynthetic Pigments:*

By spectrophotometrically in acetone extracts. The contents of chlorophyll a, chlorophyll b, and total carotenoids were estimated (Metzner *et al*., 1965).

- *Soluble Proteins:*

The Lowry method for protein quantification was used to determine the amount of soluble protein in the extracts from the shoots and roots (Lowery & Newman, 1951). Protein was gauged via a spectrophotometer at a wavelength of 750 nm.

- *Statistical Analysis:*

All the data from the one-way variety analysis (ANOVA) for different water levels were provided by the SPSS Statistical program. To compare the means, multiple range tests by Duncan ($P < 0.05$) were applied. At each water level, treated and untreated SA plants were compared using T-student tests.

III. RESULTS

- *Soil Properties:*

(Figure 1) shows the effect of different levels of water stress on the soil pH value and (EC). the soil pH significantly decreased to be lower than that of the control group under all drought stress levels (Figure 1-A). While soil EC significantly increased under all studied water stress levels compared to that of the control group, which reach its maximum EC at the moderate (50% FC) water stress conditions (Figure 1-B).

- *Plant Growth:*

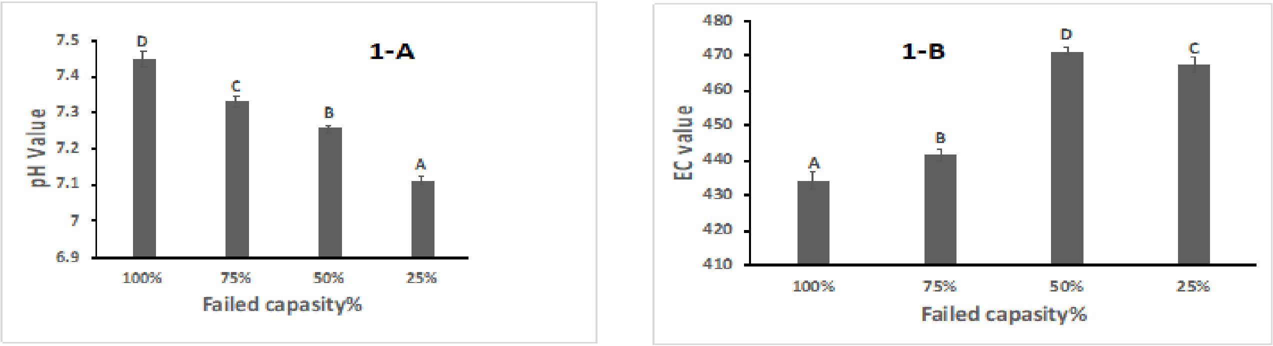

Fig 1 Soil pH value and EC value of Soil under different Drought Levels, Each Column Shows the Mean value and the Vertical Bars Show the Standard Deviation. The Letters (A-D) Show the Statistical Significance between all Water Levels at $P < 0.05$

- *Biomass (Fresh and Dry Weight):*

The biomass of the Sunflower *(Helianthus annuus L.)* was influenced by the application of SA in 100%FC conditions. Sunflower growth was significantly reduced when exposed to drought stress, While SA treatment significantly increased the fresh and dry weight of plants under all drought stress levels. According to (Figure 2-A), after plant treatment using SA, the plant shoot FW (g) increased by 8 %, 18 %, and 52 % in cases of 75, 50, and 25%FC respectively compared to their corresponding SA-untreated drought levels. Likewise, plant Root FW (g) increased by about 70 %, 39.9 %, and 36.3 % after treatment with SA in cases of 75, 50, and 25% FC respectively higher than their corresponding SA-untreated plant (Figure 2-B).

Moreover, the dry weight of roots and shoots gradually decreased with increasing water shortage conditions. (Figure 3-A) shows that DW (g) of plant shoots increased after treatment using SA by 11.5 %, 14.1 %, and 38.5 % in cases of 75%, 50%, and 25%FC respectively compared to their corresponding SA untreated drought levels. The plant root also showed the same pattern, since the DW(g) of the plant root increased by 24.4 %, and 18.2 % after treatment using SA in case of 75, and 50%FC respectively higher than their corresponding SA-untreated plant. While SA treatment in the 25 FC% didn't influence the root DW as shown in (Figure3-B).

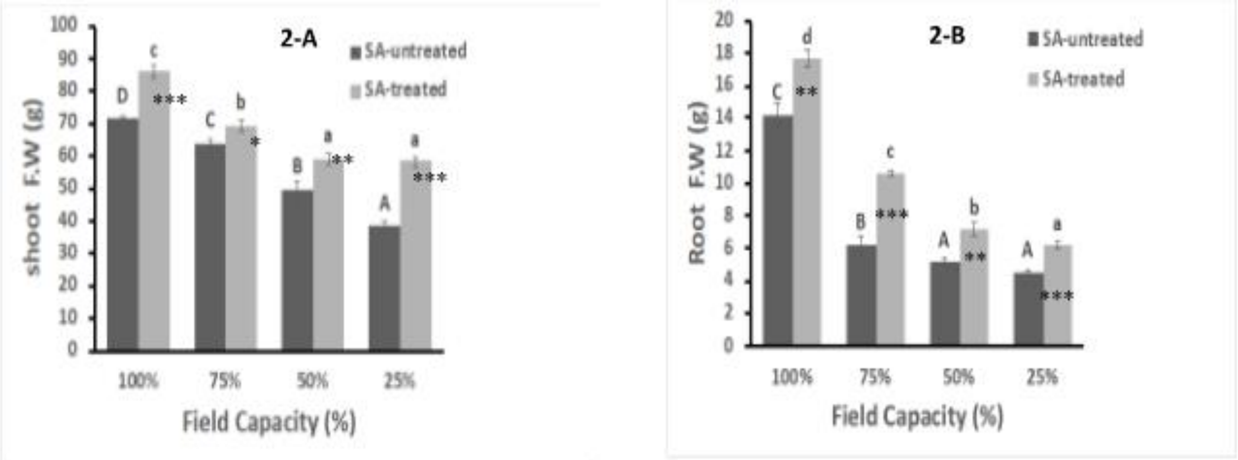

Fig 2 Fresh Weight (FW) of Shoot and Root (g) of SA-untreated and treated Sunflower (Helianthus annuus L.) plant under different levels of drought stress. Each column show the mean value and the vertical bars shows standard deviation. Different letters represent the statistically significant differences between plant with (a-d) or without (A-D) SA treatment at $p \leq 0.05$. Asterisks represent significant differences between plant with or without SA treatment at each drought level using t-student test.

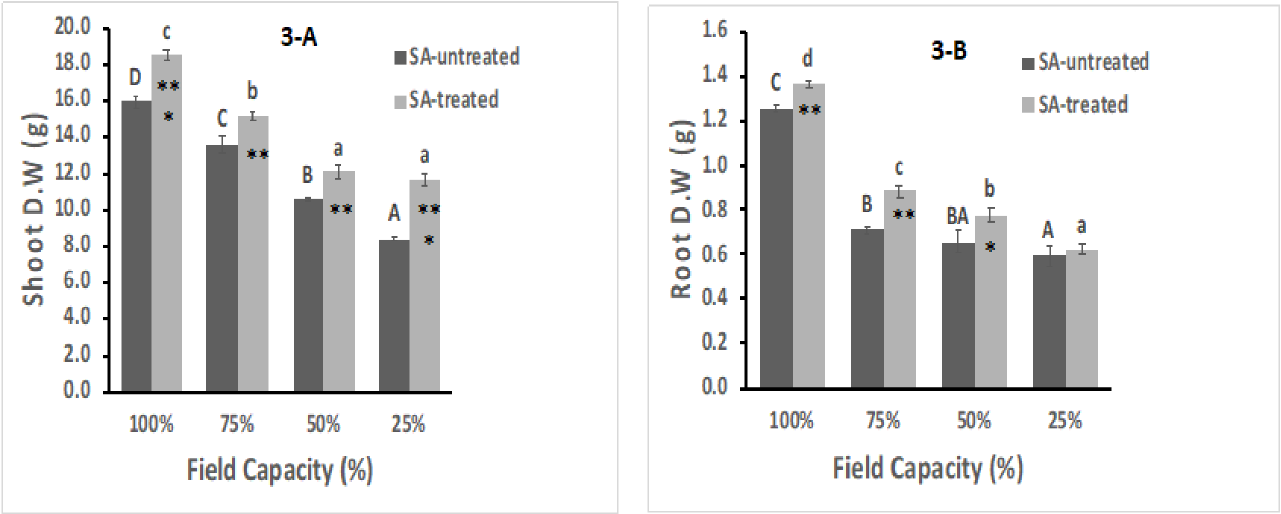

Fig 3 Dry Weight (DW) of Shoot and Root (g) of SA-untreated and treated Sunflower *(Helianthus annuus L.)* plant under different levels of drought stress. Each column show the mean value and the vertical bars shows standard deviation. Different letters represent the statistically significant differences between plant with (a-d) or without (A-D) SA treatment at p≤0.05. Asterisks represent significant differences between plant with or without SA treatment at each drought level using t-student test.

➢ *Shoot and Root Lengths:*

Information about the influence of drought stress on Sunflower *(Helianthus annuus L.)* shoot and root length (cm) were shown in (figure 4). According to (Figure 4-A), increasing water stress level decreased shoot length compared to unstressed plants. In case of 100%FC, the application of SA significantly influences the shoot length. While in cases of 75%, 50%, and 25%FC, there was not significantly increase in shoot length after SA application. In contrast (Figure 4-B) shows that the length of plant root significantly increased compared to unstressed plant, while SA application led to a significant increase in plant root length only in case of 100% FC and at severe drought stress by 11.9% compared to their corresponding SA untreated drought levels.

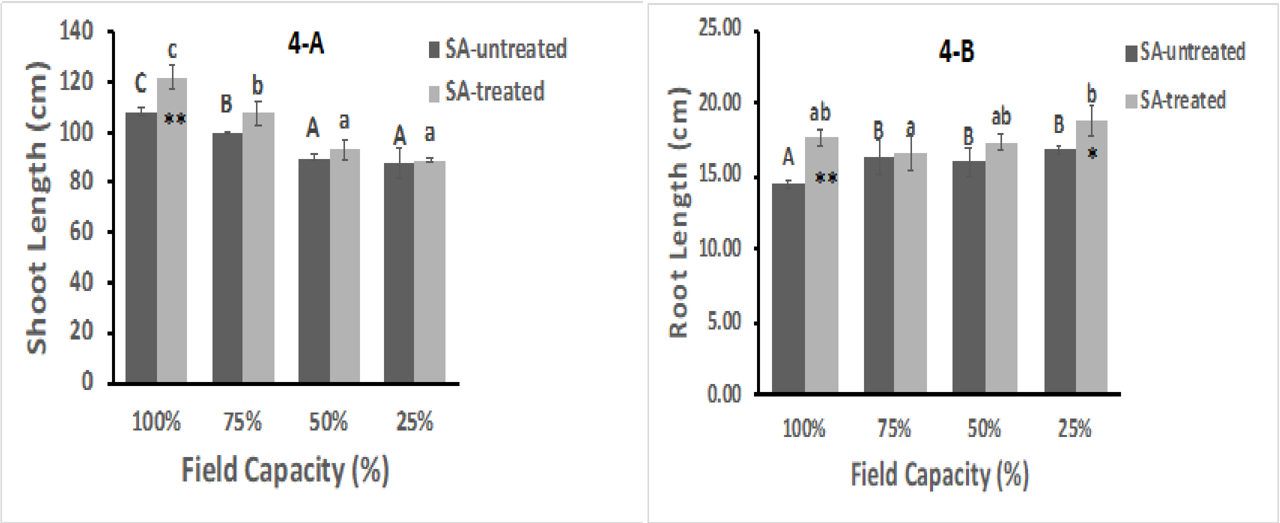

Fig 4 Length of Shoot and Root (cm) of SA-untreated and treated Sunflower *(Helianthus annuus L.)* plant under different levels of drought stress. Each column show the mean value and the vertical bars shows standard deviation. Different letters represent the statistically significant differences between plant with (a-d) or without (A-D) SA treatment at p≤0.05. Asterisks represent significant differences between plant with or without SA treatment at each drought level using t-student test.

➢ *Leaf Relative Water Content:*

The effect of drought stress on leaf RWC of Sunflower *(Helianthus annuus L.)* is shown in (Figure 5), leaf RWC significantly decreased upon expose to drought stress under all drought stress levels. But SA application significantly increased plant leaf RWC, after SA treatment the plant leaf RWC increased as following; 4.7%, 10.8%, and 6.3% in the conditions of 75%, 50%, and 25%FC respectively compared to their corresponding SA-untreated drought levels.

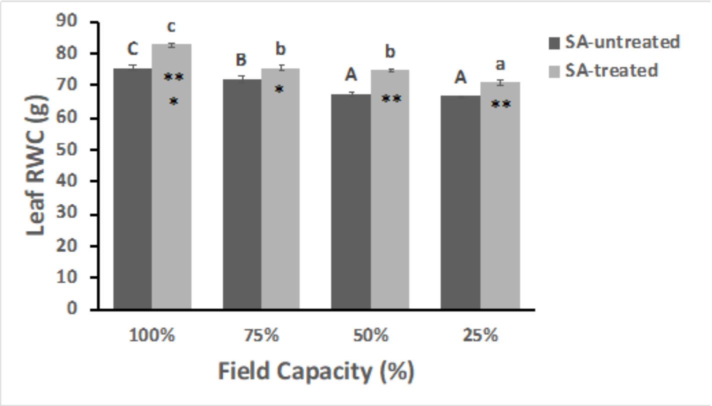

Fig 5 Leaf Relative Water Content (RWC) (g) of SA-untreated and treated Sunflower *(Helianthus annuus L.)* plant under different levels of drought stress. Each column shows the mean value and the vertical bars show the standard deviation. Different letters represent the statistically significant differences between plants with (a-d) or without (A-D) SA treatment at p≤0.05. Asterisks represent significant differences between plants with or without SA treatment at each drought level using t-student test

- *Plan Pigments:*

(Figure 6) shows that drought stress significantly reduced the content of Chlorophyll A, B and carotenoids under all studied water levels, these contents reached their lowest at 25% FC. Under all drought condition, the application of SA increased Chlorophyll A content. Since the concentration of Chlorophyll A increased by about 28 %,35 %, and 97 % higher than SA-untreated plant at 75%, 50%, and 25% FC respectively (Figure 6-A). In contrast, the application of SA increased Chlorophyll B by 24.9% in conditions of 75%, 35.29% in conditions of 50%, and 35.69% in conditions of 25%FC compared to their corresponding SA-untreated drought levels (Figure 6-B). SA application resulted in increased carotenoid content also by 11.5 %, 14.1 %, and 38.5 % in conditions of 75%, 50%, and 25%FC respectively compared to their corresponding SA-untreated drought levels (Figure 6-C).

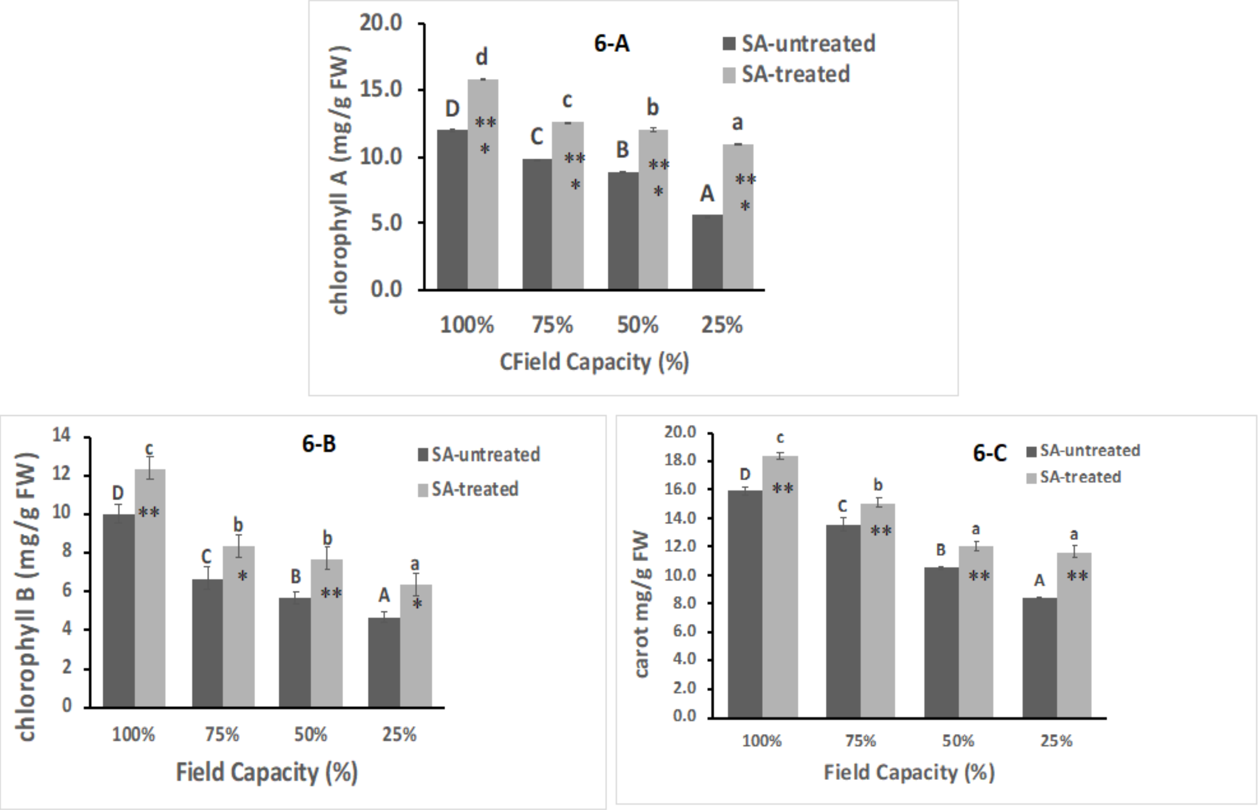

Fig 6 Pigment content, Chlorophyll A, B, and carotenoids content of SA-untreated and treated Sunflower (Helianthus annuus L.) plant under different levels of drought stress. Each column shows the mean value, and the vertical bars show the standard deviation. Different letters represent the statistically significant differences between plants with (a-d) or without (A-D) SA treatment at p≤0.05. Asterisks represent significant differences between plants with or without SA treatment at each drought level using the t-student test.

- *Soluble Proteins:*

The influence of drought stress on Soluble proteins in Sunflower *(Helianthus annuus L.)* shoots and root was displayed in (Figure 7). Gradually increasing of drought conditions led to gradually decrease of the shoot and root soluble proteins, in all drought stress levels. After SA treatment, the shoot soluble proteins increased by 72.1 %, 57.1 %, 47.2 %, in 75%, 50%, and 25%FC conditions respectively compared to their corresponding SA-untreated drought levels as shown in (Figure 7-A). While root Soluble proteins increased after SA treatment by 27.1 %, 26.7 %, 28.6 %, in 75%, 50%, and 25%FC conditions respectively compared to their corresponding SA-untreated drought levels as shown in (Figure 7-B).

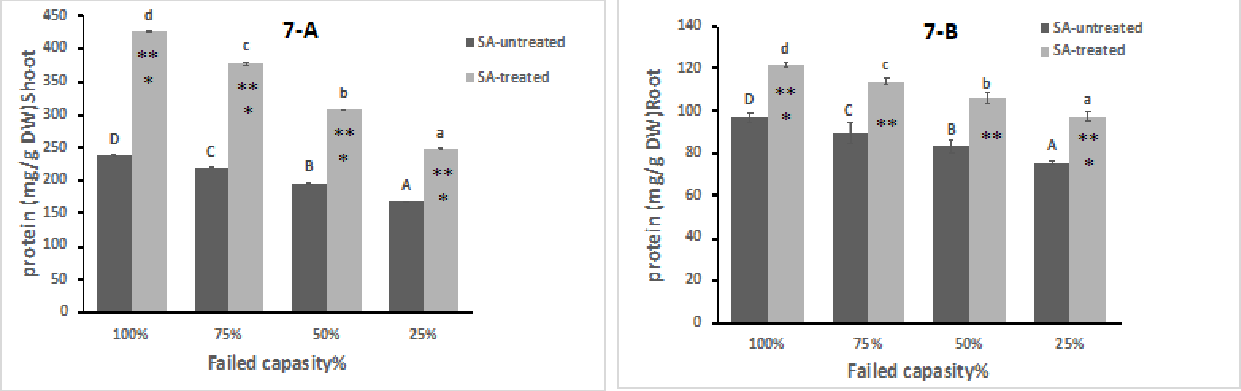

Fig 7 Protein content, Soluble protein Shoot and Root of SA-untreated and treated Sunflower *(Helianthus annuus L.)* plant under different levels of drought stress. Each column show the mean value and the vertical bars shows standard deviation. Different letters represent the statistically significant differences between plant with (a-d) or without (A-D) SA treatment at p≤0.05 Asterisks represent significant differences between plant with or without SA treatment at each drought level using t-student test

IV. DISCUSSION

One of the primary causes limiting plant development and physiological activities is drought (Shen *et al.*, 2014). Drought has an impact on soil pH as well. (Zhang *et al.*, 2020) showed that under water stress circumstances, soil pH drastically reduced, which was related to inadequate phosphate compound solubilization. In this investigation, increasing drought stress steadily decreased soil pH, whereas 100% FC–watered soils maintained a higher PH. by (Siebielec *et al.*, 2020) reported similar outcomes. In this study, soil EC increased significantly under all studied water stress levels reaching its highest at the moderate 50% FC water stress conditions. Similar results were found by (Patel & Lakdawala, 2014) that soil EC increased during water stress.

Cell development, differentiation, and division all contribute to plant growth. Reduced cellular turgor, disturbed cell development mechanisms, and poor plant growth are all effects of drought stress. Under drought conditions, plants can use plant growth regulators to maintain a good water balance (Fahad *et al.*, 2017). In this study, drought stress in sunflower *(Helianthus annuus L.)* showed negative impacts on nearly all measured growth parameters, and under all analyzed drought levels, the fresh and dry weight of the roots and shoots dramatically decreased to be less than that of the control values. The length of the shoots and root all fell below the values of plants that had received adequate water. These findings concur with Bideshki and Arvin, (2010) who discovered that, compared with well-watered plants, drought lowered shoot height, root fresh weight, root dry weight, leaf area, and whole plant fresh weight. Less cell division and elongation restriction are undeniably the main causes of the decrease in root and shoot length as well as dry weights (Danish *et al.*, 2020; Paul *et al.*, 2018). As a result, this study concluded that a common sign of drought stress is a decline in sunflower growth.

According to a study by Fariduddin *et al.*, (2003), SA application accelerated Brassica juncea's growth rate, which is similar to our observations on sunflowers. SA acts as a growth signal in cell resistance and controls the oxidative effects of stress that result in cell death (Shirasu *et al.*, 1997).

In this study, SA treatment significantly increased the sunflower (*Helianthus annuus* L.) plant dry weight. Also, SA treatment increased growth parameters under drought stress. The result of the SA application alleviated the drought stress in respective growth variables. Other studies which have similar results were SA has increased the dry weight in the stressed, maize, and other plants reported by (Bideshki and Arvin, 2010 ; El-Tayeb, 2005 and Khodary, 2004).

To evaluate the physiological condition of water in stressed plants, relative water content is an appropriate factor (Kadioglu *et al.*, 2011). This study demonstrated that RWC significantly dropped Whereas, SA increased plant leaf RWC under all levels of drought stress. These findings are in-line with those of Estaji, (2020) and Ying *et al.*, (2013) who found that SA treatment improved RWC in plants under drought-stress conditions.

In the current study, the results showed that drought had no significant effect on root length, while SA application increased root length only under severe 25% FC drought stress levels. Our results concur with Safari *et al.*, (2022), who showed that the drought stress did not affect root length. However, it still differs in stating that salicylic acid did not affect root length.

The findings of this investigation demonstrate that drought stress was greatly decreased the chlorophyll A, B, and carotenoids and lowest quantities were found at 25% FC across all examined water levels. Photosynthetic pigments responded favorably to SA treatment and showed increased pigment content levels than untreated samples. These results concur with (Doganlar *et al*., 2010; El-Tayeb, 2005 and Siddiqi *et al*., 2009), who reported an increase in photosynthetic pigments under abiotic stress conditions upon SA application.

According to the study's findings, rising drought conditions steadily reduced soluble proteins in the shoots and roots, whereas SA treatment markedly enhanced these proteins. These findings concur with those of Kabiri *et al*., 2014 and Singh & Usha, 2003, who found that protein content dropped during drought circumstances and increased during SA pretreatment during drought conditions.

V. CONCLUSION

Based on current findings it could be concluded that both irrigation and salicylic acid significantly affected the growth of sunflower. Irrigation at 100%FC and 75%FC enhanced the vegetative growth. However, 50%FC and 25%FC drought conditions adversely affect sunflower growth. soaking the seeds in (20 mg/L) salicylic acid showed an increased in plant growth rate and its tolerance to different levels of drought. Where The study showed that 100%FC and 25%FC levels of drought in addition to SA were optimal to increase the plant growth.

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