

## Abscisic Acid Stimulates Drought Hardiness in Cotton Plants

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### ABSTRACT



Drought is a major environmental stress that can significantly impact the growth and productivity of agricultural crops, including cotton (*Gossypium* spp.). As a key cash crop, ensuring the resilience of cotton plants to drought conditions is crucial for maintaining sustainable agricultural production. One of the critical plant hormones involved in the drought response is abscisic acid (ABA). Abscisic acid, a vital plant hormone, plays a crucial role in the plant's adaptation to various abiotic stresses, including drought, by enhancing drought hardiness through regulating water loss, inducing stomatal closure, and controlling gene expression related to stress tolerance. Therefore, the present study was carried out to investigate the efficacy of exogenous abscisic acid (ABA) application on the growth and various physiological characteristics of drought-stressed and unstressed cotton plants. A foliar spray of abscisic acid (ABA) was applied as pretreatments to water-stressed cotton plants (cultivar Giza-90). Two concentrations of ABA were used: 40  $\mu\text{M}$  and 80  $\mu\text{M}$ . Water irrigation was applied as normal irrigation at 100% full field capacity (FC) and drought stress (50% FC). Growth attributes were measured, and the contents of leaf pigments (chlorophyll a, b, and carotenoids), amino acids, proline, sugars, flavonoids, and phenolic compounds were estimated. Additionally, antioxidant enzyme activities (catalase and peroxidase) and protein profiles were analyzed. The results showed that water shortage stress decreased cotton growth characteristics and leaf pigment contents, while enhancing the activities of antioxidant enzymes including catalase and peroxidase activities, free amino acids, proline, soluble sugars, total phenols, flavonoids, and lipid peroxidation. Drought stress induced the synthesis of the unique polypeptides 11, 20, and 100 KDa as drought-responsive proteins. Low concentration of ABA had minimal impact on plant growth; except for shoot-fresh weight in unstressed plants, but drought-stressed plants treated with 40  $\mu\text{M}$  significantly increased plant growth and plant drought tolerance. Pretreatment with ABA at 80  $\mu\text{M}$  significantly increased chlorophyll a and b, proline, phenols, peroxidase, catalase, and ascorbate peroxidase in stressed plants while decreasing lipid peroxidation, soluble sugars, and flavonoids stressed conditions. The interaction between drought stress and ABA induces a polymorphic polypeptide with 235 and 68 KDa. Pretreatment of cotton with ABA at optimal concentration can increase drought tolerance by enhancing physiological parameters, and photosynthetic pigments, regulating the protein metabolism and antioxidant capacity.

**Keywords:** Antioxidant enzymes; Cotton plants; Drought hardiness; Phenols; Proline; Plant hormone; Water deficit.

### INTRODUCTION

Drought, a persistent threat to agricultural productivity, has sparked interest within the scientific community to investigate the mechanisms behind the resilience of certain plant species. Cotton plants have shown to be resilient to prolonged periods of water scarcity, making them valuable resources in agricultural production. Cotton, a significant fiber crop, has played a crucial role in the economic and industrial development of many countries (Jabran and Chauhan, 2019). Despite facing tough competition from synthetic fibers, the high demand for cotton products has solidified its position as the most cultivated crop worldwide. Cotton, being a primary source of natural fibers, satisfies 35% of the global fiber demand (Mahmood *et al.*, 2022). *Gossypium* cultivars, known for their diverse reproductive and vegetative traits, are extensively grown for cotton production. The

development of high-yielding cultivars is essential for maximizing cotton yield.

Drought stress can affect crops at different phases of maturity during the growing season because warm, dry weather are a common feature of the key cotton-producing countries (Iqbal *et al.*, 2019). Agricultural production systems depend heavily on the availability of water, and drought can cause a variety of physiological and biochemical reactions that have an impact on agricultural plants' cellular functions (Hussein *et al.*, 2023). Cotton can withstand moderate levels of drought, but its growth is severely constrained by the lack of water (Mahmood *et al.*, 2022). According to Mekki *et al.* (2015), drought stress substantially inhibits cotton growth and development by altering the dry weights of the shoot system, leaf area, plant height, and yield-related qualities. The detrimental effects of drought on cotton plants can be attributed to various physiological and biochemical

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changes, including reduced photosynthesis, stomatal closure, and the accumulation of reactive oxygen species (ROS), which can lead to oxidative damage (Iqbal *et al.*, 2019; Hussein *et al.*, 2023). Understanding the mechanisms by which cotton plants respond to drought stress is crucial for developing strategies to improve their drought tolerance and ensure sustainable cotton production in water-limited environments. Additionally, drought stress disrupts osmotic balance, causing cotton plants to store various inorganic and organic compounds to lower their osmotic potential. Organic substances such as amino acids, proline, and sugars are involved in osmotic adjustment (Mekki *et al.*, 2015).

Drought stress also disrupts photosynthesis, development, and growth, leading to the generation of reactive oxygen species (ROS) that impact membrane integrity, membrane stability, and water relationships. Cotton plants have developed scavenging systems and regulatory pathways to regulate ROS redox equilibrium and inhibit ROS accumulation. Changes in the metabolism of antioxidant enzymes, including enzymatic antioxidants like catalase, superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), and polyphenol oxidase (PPO), as well as nonenzymatic antioxidants like flavonoids, phenol, carotenoids, and proline (Hussien *et al.*, 2015), can affect cotton drought tolerance. These antioxidant enzymes and compounds play crucial roles in maintaining the plant's redox balance and protecting it from oxidative damage caused by drought stress.

Abscisic acid (ABA), one of several phytohormones, is the primary promotor in plants' tolerance to withstand abiotic stress and coordinates a wide range of processes (Wani, 2015). Sesquiterpene ABA is produced by oxidative cleavage of carotenoids. ABA is produced in the roots and moved through the xylem to the leaves, which regulates stomatal closure to control water loss (He *et al.*, 2019). Catabolism of ABA is initiated by various modifications such as conjugation and oxidation. ABA is a mobile molecule, and its transport is organized by the efflux and influx of carrier protein (Nambara, 2016). The alteration in ABA is a fundamental mechanism of plant reactions to drought stress. In addition, it is crucial for a variety of cellular functions, such as seed formation, dormancy process, seed germination, vegetative growth, and root architecture regulation. After receiving drought stress signals, cotton plant plastids synthesize ABA in response to stomatal closure signals in guard cells (Sah *et al.*, 2016).

ABA, a naturally occurring compound, elicits two responses during drought: stomatal closure, that reduces the loss of water through transpiration, and increased hydraulic conductivity, promoting root cell elongation and osmotically active compounds (An *et al.*, 2014). It also regulates antioxidant responses and can promote drought tolerance. ABA improved the plant's ability to scavenge ROS by activating antioxidant enzymes (e.g., SOD, POD, and CAT). Exogenous foliage ABA effectively reduced heavy

metal translocation in strawberry leaves while increasing leaf antioxidant enzymes and phytohormones that reduced stress (Kocaman, 2023). However, its physiological role is based on species, stress level, genotype, application method, vegetative period, concentrations, and tissues, with both positive and negative effects (Brito *et al.*, 2020). ABA signaling balances growth programs against stress responses, allocating resources to face the adverse impact of environments. ABA treatment delays drought effects, improves physiological functions, and potentially impacts the growth of young potted olive trees (Brito *et al.*, 2020). During drought stress, the expression of stressor-responsive genes depends heavily on ABA receptors. The amount of ABA in cells is minimal under normal circumstances, but drought stress raises cellular ABA levels and causes physiological and molecular reactions (Ullah *et al.*, 2017).

Protein profile is a powerful tool for exploring drought-responsive proteins. Drought-response proteins are divided into protective proteins like antioxidants and osmotin family, and ion-adsorption and movement proteins. Water soluble stress protein induces cellular turgor and tolerance to stress (Zhou *et al.*, 2014). ABA application decreases electrolyte leakage and stomatal conductance, increasing growth and leaf water potential in heat sensitive and heat tolerant Lucerne (*Medicago sativa* L.) genotypes (An *et al.*, 2014). The exogenous ABA to soybean grown under water stress augmented ABA accumulation during soil drying, enhancing desiccant tolerance and leaf water relations, while does not promote crop yield (He *et al.*, 2019).

Based on the positive effects of ABA (abscisic acid) on enhancing drought hardiness of crops, this study aims to investigate the potential benefits of foliar ABA application as a pretreatment for improving the growth performance and drought tolerance of cotton plants. The study also aims to explore the role of antioxidant mechanisms in response to ABA and its relationship with plant growth. The findings from this study will provide valuable insights for cotton management and establishment under water deficit conditions.

## Materials and Methods

### Experimental design

The current study was performed during summer season of 2018-2019 in a greenhouse at the National Research Center, Dokki, Giza, Egypt. Uniform seeds of cotton (Giza- 90 cultivar; is a long staple cotton variety characterized by high yielding) were grown in plastic pots (40 cm in height and 40 cm in diameter) containing a clay soil obtained from a private farm in Kalubia governorate. Soil samples were analyzed according to the procedures described by Jackson (1969). The average values of the obtained data are as follows: field capacity (FC), 38%; electrical conductivity (EC), 0.96 dSm<sup>-1</sup>; pH 7.95; CaCO<sub>3</sub>%, 0.7; OM%, 2.9; N (155 ppm); potassium (K), 265 ppm, and phosphorus (P), 5.5 ppm.

The soil was amended with 3 g of potassium sulfate (48-50% K<sub>2</sub>O) and 6 g of calcium sulfate (15.5% P<sub>2</sub>O<sub>5</sub>) per pot prior to sowing. Plants were thinned 30 days after planting to maintain one plant per pot until the time of analysis. Additionally, nitrogen fertilizer in the form of ammonium nitrate (33.5% N) was applied at a rate of 0.6 g per pot at 30 and 60 days after planting.

#### Irrigation regime, ABA application and sample collection

Water irrigation was applied as normal irrigation at 100% full field capacity (FC) and drought stress at 50% FC. Levels of abscisic acid (ABA) were 40 μM (ABA1) and 80 μM (ABA2), with three replicates for each treatment. Twice-weekly foliar application for anti-stressor treatments was initiated 30 days after planting. Water deficit was initiated 45 days after planting (DAP). On day 90 after sowing, physiological traits were measured for treated plants to determine growth characteristics such as root length (cm), plant height (cm), number of leaves per plant, and fresh weights (g) of shoot and roots per plant. The third and fourth leaves from terminal buds were collected for estimating certain biochemical parameters.

#### Biochemical analyses

Different biochemical measurements were performed to evaluate the effect of abscisic acid (ABA) at various concentrations on drought stress at 50% FC, compared to normal irrigation.

##### Photosynthetic pigment

Photosynthetic pigments including chlorophyll a, chlorophyll b, and carotenoids were assayed in cotton leaf samples using 85% acetone and measured according to Metzner *et al.* (1965).

##### Proline content

Proline was determined as the described method by (Bates *et al.*, 1973). Total free amino acids were determined in ethanol extract of cotton leaves by the ninhydrin method according to (Rosen, 1957).

$$\text{Proline } (\mu\text{mol/g FW}) = \left( \frac{A_{520} \times V_t}{\text{Weight} \times S \times V_s} \right) \times \text{DF}$$

Where A<sub>520</sub> is absorbance at 520; V<sub>t</sub>, volume of toluene; TS, tissue sample; V<sub>s</sub>, volume of sample and DF, dilution factor.

##### Total phenolic and flavonoid contents

The total phenolics content was assayed using the method described by Pourmorad *et al.* (2006) and Mahdi *et al.* (2023). In this method, the Folin-Ciocalteu reagent is used, and the results are expressed in gallic acid equivalents (GAE). The total flavonoid content in the cotton plant was estimated using the method described by Adom and Liu (2002). This method involves the aluminum chloride colorimetric assay, and the results are expressed in milligrams of quercetin equivalents (mg QE) per gram of sample.

##### Total soluble sugars

Total soluble sugars were determined in the ethanol extract of cotton leaves using the anthrone method, as described by Cerning (1975). The total soluble sugar content was calculated using the following equation:

$$\text{Total Soluble Sugars (mg/g)} = (A_{620} - A_{620 \text{ blank}}) \times C / W$$

Where, A<sub>620</sub>, absorbance of the sample at 620 nm; A<sub>620 blank</sub>, absorbance of the blank at 620 nm; C, concentration of glucose standard (mg/mL); W, weight of the sample (g).

##### Antioxidant enzymes

Crude enzyme extracts were obtained to assay the activities of various enzymes (Mukherjee and Choudhuri, 1983). Catalase (CAT, EC 1.11.1.6) activity was measured according to the method described by Chen *et al.* (2000). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assessed using the method outlined by Koricheva *et al.* (1997). Peroxidase (POD, EC 1.11.1.7) activity was determined following the protocol of Bergmeyer (1974). Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) content, as described by Hodges *et al.* (1999).

##### Protein profile

Protein extracts were prepared by flash-freezing fresh cotton leaves (0.2 g), and the proteins of the samples were determined using sodium dodecyl sulfate-polyacrylamide gels (Laemmli, 1970). The molecular weights of the separated proteins were measured in comparison with conventional molecular weight markers (Marker, 15-180 kDa; Sigma, USA).

##### Statistical Analysis

The data were statistically analyzed using two-way analysis of variance (ANOVA) with Minitab<sup>®</sup> 18.1 Statistical Software (Minitab Inc., State College, PA, USA; Snedecor and Cochran, 1989). To compare the means, the Tukey's honest significant difference (HSD) test was performed at a 5% probability level. The results are presented as mean ± standard error of the mean (SEM), with n = 3 replicates.

## RESULTS

### Growth traits

Drought conditions portrayed a significant inhibition in the growth parameters of cotton (Giza-90) as shown in Table 1. Morphological attributes such as height of plant, length of root, number of leaves, fresh weight of shoot, and fresh weight of root of drought-stressed cotton plants were decreased than those of the normal irrigated plants by 18.2%, 27.8%, 27.3%, and 16.5% respectively while root fresh weight increased by 40.7%. Exogenous ABA at low concentrations had insignificant effects on the plant growth parameters except shoot fresh-weight cotton plants under normal irrigation. Shoot fresh weight (SFW) was increased by 45% at 40 μM ABA regarding the control plants. Oppositely, ABA at 80 μM decreased all growth characteristics such as height of plant, length of root, leaves no. and shoot fresh weight by 12%, 33.3%, 24.3%, and 16.6%, respectively while having no change in root fresh weight per plant compared to untreated plants. Under drought conditions, the cotton plants treated with ABA at 40 μM significantly impact height of plant, length of root, number of leaves, shoot

and root fresh weight of stressed plants with drought, plants were increased than those of the untreated stressed plants by 22.2%, 25.4%, 58.4%, 46.6%, 13.2%, respectively (Table 1). Meanwhile, ABA-treated plants observed non-significant changes in all growth characteristics with respect to untreated stressed cotton plants. The results suggested that the drought-stressed cotton (G-90) plants showed highly responsive to exogenous ABA. ABA at low concentrations can improve cotton plant growth and stimulate drought hardiness.

**Photosynthesis pigments:**

Data in (Figs. 1A-C) represented the effect of ABA, drought, and their combinations on certain leaf pigments (chlorophylls and carotenoids) of cotton. Drought stress decreased chlorophyll pigments and carotenoids. Drought stress decreased carotenoids by 13.4%, Chl-a by 30.5% and Chl-b by 22.4% in cotton plants in comparison with control. On the other side, pretreatment cotton plants with different concentrations (0.0, 40, and 80  $\mu\text{M}$ ) of ABA at 40  $\mu\text{M}$  increased Chl-b by 10.3% while showing no significant impact in chlorophyll and carotenoids of cotton plants grown under normal conditions. ABA at 80  $\mu\text{M}$  decreased chl-a by 10% associated with no significant change in chl-b and carotenoids regarding control plants under normal irrigation. Regarding the interaction treatments, exo-genous application of ABA at 80  $\mu\text{M}$  enhanced Chl-a by 22.7% and Chl-b by 15.9%, associated with an insignificant increase in carotenoids in cotton plants cultivated under water-deficit stress conditions, with respect to untreated plants.

**Proline and amino acids**

The results in (Fig. 2A and B) illustrated the influence of ABA, drought, and their combinations on cotton plants' proline and amino acids. Drought conditions led to significant enhancement in amino acids content (32.5%), and proline content (284%) compared with unstressed plants.

On the other side, pretreatment with ABA at 40, and 80  $\mu\text{M}$  enhanced amino acids contents by 11%, and 52% and proline by 109%, and 192%, respectively regarding control plants grown in normal irrigation. The most significant application for increasing the proline and amino acids was 80  $\mu\text{M}$  of ABA. Corresponding to the efficacy of ABA on cotton plants grown under drought conditions, the selected concentrations augmented proline levels at 40  $\mu\text{M}$  and 80  $\mu\text{M}$  ABA increased proline content by 28%, and 21.6% regarding the untreated stressed plants.

At the same time, foliar treatment of ABA showed no significant impact on the free amino acids.

**Phenols and flavonoids**

Drought conditions markedly accumulated the contents of phenols and flavonoids (Fl) by 25.9% and 68.0% compared with normal water treatment (Fig. 3A and B). The value of flavonoids increased from 0.11 to 0.18 (FL) under drought conditions (Fig. 3B). Regarding ABA treatments, under normal irrigation, foliar application of ABA at 80  $\mu\text{M}$  caused a marked enhancement in phenols and flavonoids content in cotton plants. The increment in phenols and flavonoids was 25% and 45%, respectively in plants treated with 80  $\mu\text{M}$  ABA under normal irrigation.

By viewing the interaction, exogenous ABA clarified a significant increase in FL under water-deficit conditions. ABA at 40  $\mu\text{M}$  and 80  $\mu\text{M}$  increased FL by 24% and 30.7% regarding the untreated stressed plants. On the opposite side, the foliar treatment of ABA observed a non-significant impact on total phenols in drought-stressed plants in comparison with stressed plants while portraying a high effect when compared with the control plants. Interestingly, the interaction treatments showed the highest values of total phenols.

**Total soluble sugars**

Drought stress conditions significantly reinforced the total soluble sugars (TSS) by 34% in stressed cotton plants compared with the control (Fig. 4). However, 40  $\mu\text{M}$  ABA-treated plants showed no significant effect on TSS under normal irrigation conditions. ABA at 80  $\mu\text{M}$  caused an increment (20%) in TSS in comparison with the control value. Under water shortage stress, ABA at 40 and 80  $\mu\text{M}$  decreased TSS value by 11% and 24.3% regarding the untreated stressed cotton plants.

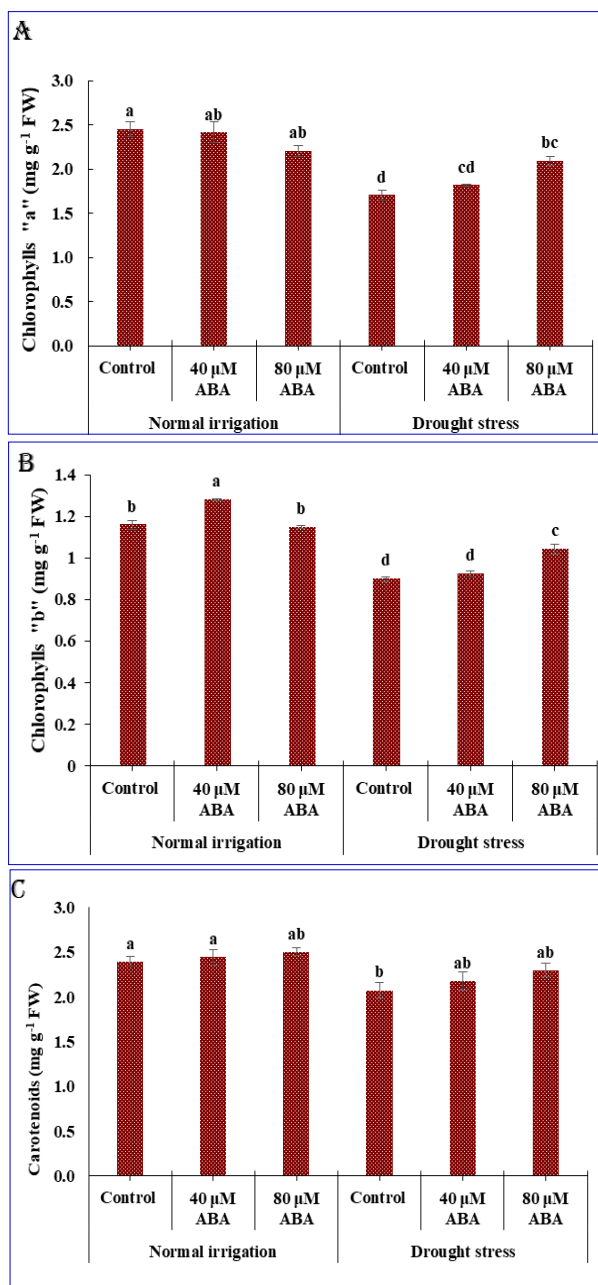
**Antioxidant enzymes**

Drought stress significantly increased CAT activity by 268% more than the control value (Fig. 5). Similarly, ABA at 40 and 80  $\mu\text{M}$  enhanced CAT activities by 96.5 and 179%, respectively in treating cotton plants. Regarding the interaction, the highest significant induction in CAT antioxidant enzyme activity was clarified in plants that were treated with ABA at 80  $\mu\text{M}$ , followed by ABA at 40  $\mu\text{M}$  compared to other treatments (Fig. 5A). The increments reached 21 and 27% compared to the untreated stressed plants. The activity of POD was significantly increased under shortage irrigation than in normal irrigation (Fig. 5B).

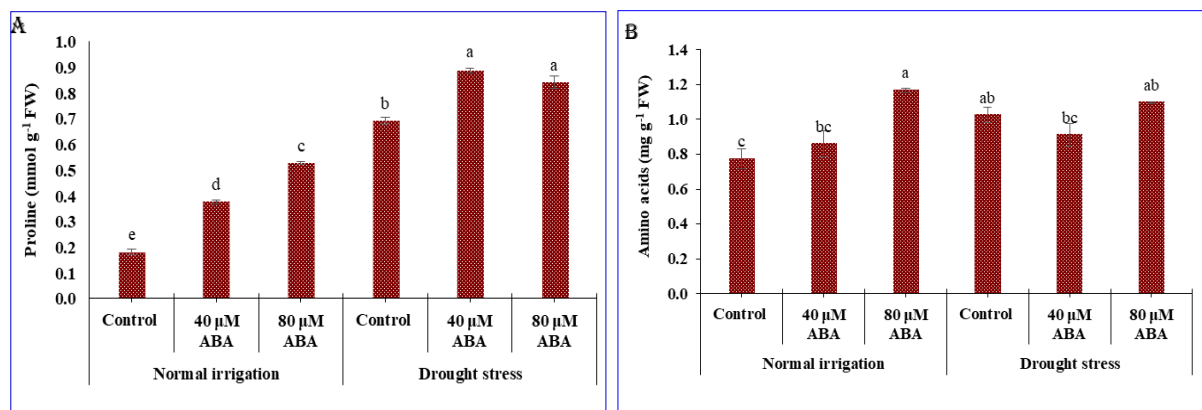
**Table (1):** Effect of abscisic Acid (ABA) on growth parameters of cotton plants under drought stress.

Treatments		Growth parameters				
Irrigation	ABA ( $\mu\text{M}$ )	Plant height cm plant <sup>-1</sup>	Root length cm plant <sup>-1</sup>	Leaves number plant <sup>-1</sup>	Shoot fresh weight g plant <sup>-1</sup>	Root fresh weight g plant <sup>-1</sup>
Control (100%FC)	0	44.0±1.2 <sup>a</sup>	18.0±0.6 <sup>a</sup>	11.00±0.1 <sup>ab</sup>	25.15±0.9 <sup>c</sup>	1.72±0.1 <sup>c</sup>
	40	45.0±1.5 <sup>a</sup>	16.3±0.3 <sup>a</sup>	9.00±0.6 <sup>bc</sup>	36.48±0.7 <sup>a</sup>	1.68±0.1 <sup>c</sup>
	80	38.7±1.3 <sup>b</sup>	12.0±0.1 <sup>c</sup>	8.33±.3 <sup>c</sup>	20.97±0.5 <sup>d</sup>	1.71±0.1 <sup>c</sup>
Drought (50% FC)	0	36.0±1.2 <sup>b</sup>	13.0±0.6 <sup>bc</sup>	8.00±0.6 <sup>c</sup>	20.99±0.5 <sup>d</sup>	2.42±0.1 <sup>ab</sup>
	40	44.0±0.6 <sup>a</sup>	16.3±0.7 <sup>a</sup>	12.67±0.7 <sup>a</sup>	30.78±0.4 <sup>b</sup>	2.74±0.04 <sup>a</sup>
	80	40.3±0.3 <sup>ab</sup>	16.0±1.2 <sup>ab</sup>	9.00±0.6 <sup>bc</sup>	20.74±0.9 <sup>d</sup>	2.28±0.09 <sup>b</sup>

Data represent mean ± standard error. Means with different superscript letters per column are statistically significant different at  $p \leq 0.05$ .



**Figure (1):** Effect of irrigation regimes and abscisic Acid (ABA) applications on photosynthetic pigments in cotton plants. The bars represent the mean  $\pm$  standard error. Bars with superscript different letters are significantly at different  $p \leq 0.05$ .



**Figure (2):** Effect of irrigation regimes and abscisic acid (ABA) applications on proline and amino acids contents. The bars represent the mean  $\pm$  standard error. Means with different letters are significantly at different  $p \leq 0.05$ .

The increment percent reached 38.5% in drought-stressed cotton plants. Exogenous treatment of ABA at 40 and 80  $\mu\text{M}$  increased POD activity by 84.6% and 157.7% in unstressed plants and by 83.5% and 102.8% in treated stressed cotton plants compared to the corresponding controls.

The APX activity was increased (12.5%) under drought stress than the corresponding control value in normal irrigation (Fig. 5C). ABA at 40  $\mu\text{M}$  and 80  $\mu\text{M}$  caused insignificant effects in unstressed plants while increasing APX activity about 30.6% and 43.5%, respectively, in stressed cotton plants regarding control with no significant changes between the two concentrations.

#### Lipid peroxidation

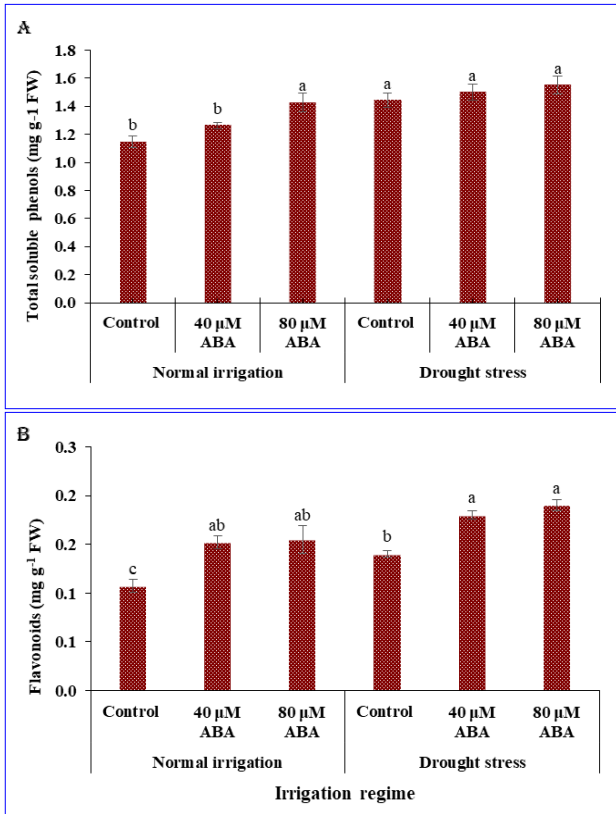
Malondialdehyde (MDA) is a significant biomarker for oxidative stress and lipid peroxidation in biological systems. MDA, the content of MDA increased in cotton plants by 29%, respectively under drought stress (Fig. 6). Exogenous ABA at 40  $\mu\text{M}$  and 80  $\mu\text{M}$  decreased MDA by 78.4 and 50% in unstressed plants with respect to control ones. Regarding combination treatments, ABA at 40  $\mu\text{M}$  increased the amounts of MDA by 11% while ABA at 80  $\mu\text{M}$  reduced MDA about 16.7% in treated stressed plants in comparison with the corresponding control.

#### Protein pattern

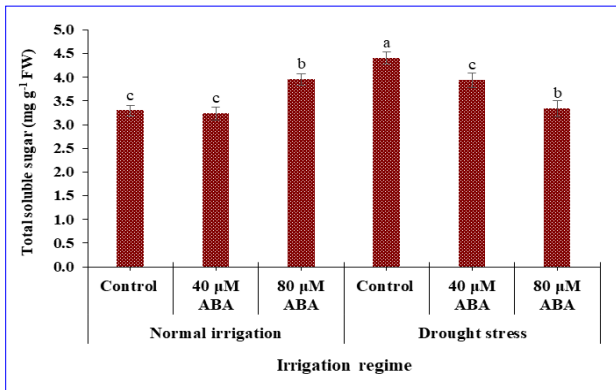
Protein pattern comparison in cotton plants demonstrated certain changes in some bands (Table 2 and Figure 7). These findings clarified 16 polypeptide bands having various molecular weights ranging between 9 and 235 kDa with a polymorphic percentage of about 25% and 25% unique bands. With respect to stabilization of protein, 8 bands (50%) have various molecular weights which set without changing in the tested treatments, known as monomorphic bands. Water-deficit conditions led to changes in the protein pattern by inducing various protein bands syntheses.

Also, water-deficit stress caused the appearance of some unique polypeptides, 11, 20, and 100 kDa (drought-responsive protein bands) in cotton plants at the vegetative stage. Under normal irrigation, only ABA at 40  $\mu\text{M}$  initiated the presence of the unique bands at 87 kDa (ABA-responsive protein) in treated plants





**Figure (3):** Effect of irrigation regimes and abscisic acid (ABA) applications on total soluble phenol and flavinoid contents. The bars represent the mean ± standard error. Means with different letters are significantly at different  $p \leq 0.05$ .

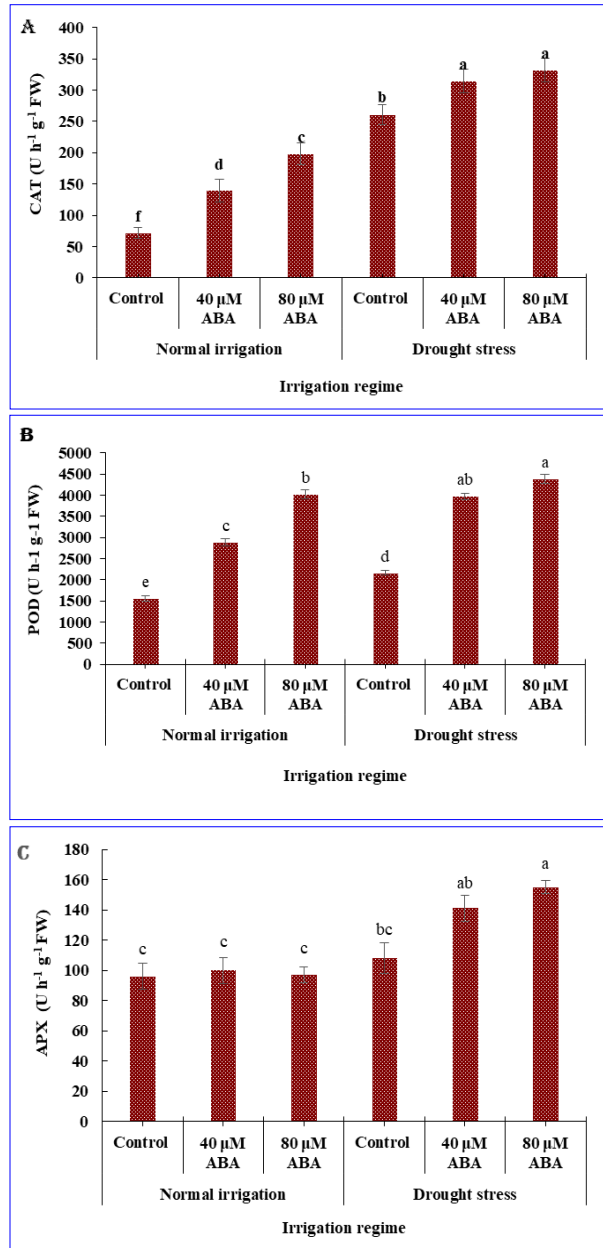


**Figure (4):** Effect of irrigation regimes and abscisic acid (ABA) applications on total soluble sugar contents. The bars represent the mean ± standard error. Bars with different superscript letters are significantly at different  $p \leq 0.05$ .

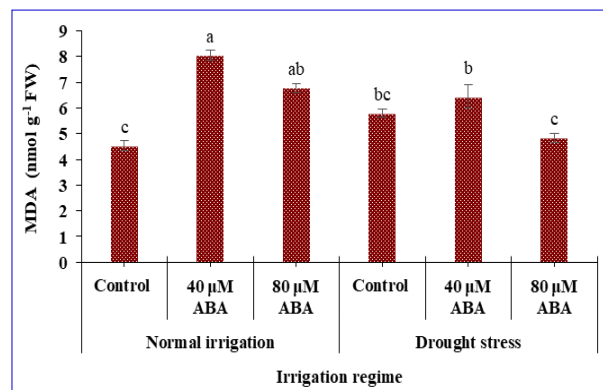
Furthermore, the interaction between drought stress and ABA at both concentrations synthesized the polymorphic polypeptide 235, and 68 KDa in water deficit-stressed cotton plants with respect to other treatments.

### DISCUSSION

Drought is an environmental stress, that limits crop growth and productivity, threatening agriculture due to a disturbance between reactive oxygen species and antioxidants (DaCosta and Huang, 2007). To keep the plant alive in these conditions, it was necessary to find approaches that enhance and promote the plant's hard-



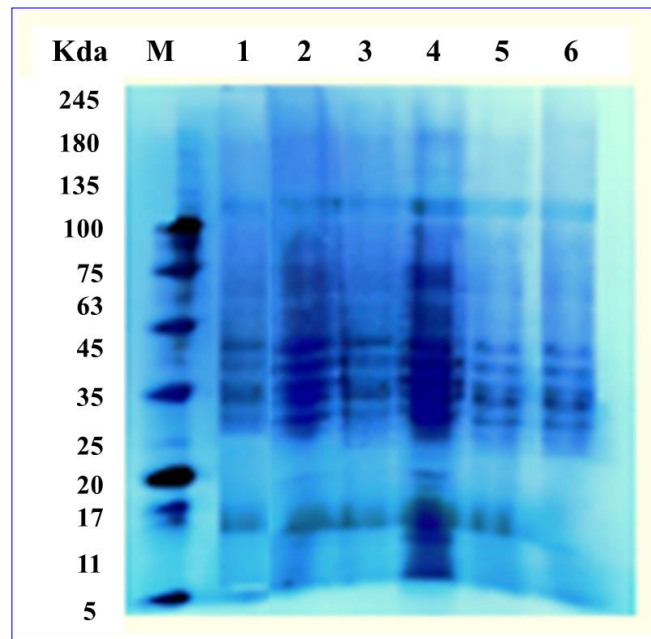
**Figure (5):** Effect of irrigation regimes and abscisic acid (ABA) applications on antioxidant enzymes. A, catalase; B, peroxidase and C, ascorbate peroxidase. The bars represent the mean ± standard error. Means with different letters are significantly at different  $p \leq 0.05$ .



**Figure (6):** Effect of irrigation regimes and abscisic acid (ABA) applications on lipid peroxidation expressed as Malondialdehyde (nmol g<sup>-1</sup> FW).

**Table (2):** Protein profile analysis of cotton plants under drought stress and abscisic acid (ABA) treatments showing band classification. L1, control; L2, 40  $\mu$ M ABA; L3, 80  $\mu$ M ABA; L4, drought stress, L5, drought combined with 40  $\mu$ M ABA, L6, drought condition combined with 80  $\mu$ M ABA.

Band No	M.W (KDa)	Treatment conditions					
		1	2	3	4	5	6
1	235	+	+	+	+	-	-
2	124	+	+	+	+	+	+
3	100	-	-	-	+	-	-
4	87	-	+	-	-	-	-
5	73	+	+	+	+	+	+
6	68	+	+	+	+	-	-
7	56	+	+	+	+	+	+
8	52	+	+	+	+	+	+
9	49	+	+	+	+	+	+
10	38	+	+	+	+	-	-
11	34	+	+	+	+	+	+
12	28	+	+	+	+	+	+
13	20	-	-	-	+	-	-
14	18	+	+	+	+	+	-
15	11	-	-	-	+	-	-
16	9	+	+	+	+	+	+
<b>Total bands</b>		13	15	14	15	11	10
<b>Band classification</b>		<b>Monomorphic bands</b>		<b>Unique bands</b>		<b>Polymorphic bands</b>	
		8		4		4	



**Figure (7):** Protein profile analysis of cotton plants under drought stress and abscisic acid (ABA) treatments showing different protein bands with different molecular weight (KDa).

iness to face these stresses. One of these approaches is the pretreatment with growth regulators. One of the growth regulators is ABA which has a protective, and effective function in plants against adverse effects of oxidative stressors, especially water shortage (Doneva *et al.*, 2021).

In the present experiment, water-deficit conditions inhibited the growth attributes of cotton crop. Our results are agreement with the study of Mekki *et al.* who revealed that drought stress conditions on cotton plants reduced the fresh and dry weights of both shoots and roots (Mekki *et al.*, 2015). A recent study

(Kusvuran, 2021) showed that leaf area and length of shoot and root were suppressed in response to drought. Similarly, it has been documented that leaf areas were significantly inhibited in drought-stressed maize crop compared to un-stressed ones (Li *et al.*, 2018). Moreover, height of plant, leaf area, and leaves number of cotton were reduced due to exposure to drought related to un-stressed plants (Ahmed *et al.*, 2017). Suppression of morphological growth indices due to drought conditions may be associated with the weakness in plants to absorb water and different nutrients.

Foliar pretreatments of ABA had minimal impact on plant growth; except for shoot-fresh weight in unstressed plants, however, drought-stressed plants treated with ABA increased plant growth and plant drought tolerance. The promotive role of ABA at a low level may be due to the regulation of growth mediated by ABA; involving crosstalk with rest hormones and nutritional signaling, regulating cell division, enlargement, differentiation, and physiological processes (Brookbank *et al.*, 2021). In this respect, various organs and tissues need ABA as a factor for growth and development (Humplík *et al.*, 2017). Interestingly, exogenous ABA regulates root elongation in a biphasic manner in *Arabidopsis*. It has been documented that ABA at low concentrations enhances root growth, but at high levels suppresses it (Li *et al.*, 2017). Depending on the concentration, ABA also regulates photosynthesis, carbon metabolism, and transport, affecting growth. In this concern, ABA plays a role in photosynthesis, with tomatoes showing increased net assimilation rates. Moreover, deposition and synthesis of waxes and cutin were enhanced by ABA, binding to and positively regulating wax biosynthetic genes which subsequently increased the plant drought tolerance (Zhao *et al.*, 2016).

Chlorophylls and carotenoids are essential for the photosynthesis process in plants. Photosynthesis is a physicochemical pathway that uses photon energy to produce several organic molecules thus plants grow.

The current study submitted that water-deficit stress decreases photosynthetic pigment contents in cotton plants. The decrease in leaf pigments may be due to a decrease in leaf area, which is responsible for capturing light and enabling photosynthesis. In another study, Cotton plants exposed to drought stress had inhibitions in their chlorophyll (a, b, and total) concentrations (Shallan *et al.*, 2012). Recently, the contents of chlorophylls were reduced when plants exposed to water-deficit stress (Hussein *et al.*, 2023). The change in photosynthetic pigments under drought conditions might be linked to the disturbance in the metabolic pathways caused by denaturation of protein and oxidation of lipid, which are essential for the structure of pigment and chloroplast (Hussien *et al.*, 2015). Exogenous application of ABA markedly increased the pigments content (chlorophyll a, b, and carotenoids) in cotton. Under drought stress, similar findings were documented that exogenous ABA promoted the contents of chlorophyll in the cultivars (An *et al.*, 2014). The simulative role of low concentration of ABA on chlorophyll content may be related to the role of ABA in carotenoid synthesis. Carotenoids, ABA precursor, sustain high photosynthetic pigments, and these might increase the key enzymes  $\beta$ -carotene hydrolase and phytoene synthase (Meier *et al.*, 2011). It was demonstrated that chlorophylls and carotenoid contents of drought-stressed cotton plants were boosted due to the ABA application at different levels; the authors attributed the enhancements of pigments to the role of ABA in maintaining the structure of thylakoid and chloroplast (Shallan *et al.* 2012). Water deficit

stress accumulated soluble sugars, free amino acids, and proline. Our findings are consistent with those of (Hammad and Ali, 2014), who found that free amino acid levels in plants decreased due to drought exposure. Proline has a low molecular weight that effectively organizes redox potential, causes scavenging of hydroxyl radicals, attenuates oxidative harm, and maintain cell membranes under stressful conditions (Skowron and Trojak, 2021). Osmolytes accumulation is a reaction and mechanism by which plants tolerate stress-induced oxidative damage. Proline and total sugars in cotton plants increased when the plants were grown under arid conditions (Ahmed *et al.*, 2017). Quinoa plants exposed to drought stress resulted in significant accumulations in the content of total soluble sugars and free proline (Elewa *et al.*, 2017). It has been suggested that the contents of soluble sugars, amino acids, and proline in cotton plants are increased due to certain levels of ABA treatment and drought stress. For drought-stressed plants, higher levels of sugars were found in drought-stressed + ABA plants, which are important compatible solutes that may contribute to the superior regulatory abilities of ABA-strained plants and are involved in the study in cells. Molecular structure of ROS detoxification and stability (Brito *et al.*, 2020). Recently, proline content in alfalfa plants was increased after ABA application (An *et al.*, 2014). Exogenous abscisic acid enhances proline metabolism in wheat crop, which can be explained by the role of ABA in regulating proline synthesis (Pál *et al.*, 2018). Water stress induces ABA-dependent proline accumulation during post-germination growth in *Medicago truncatula* (Planchet *et al.*, 2011, 2014). Exogenous ABA treatment mimics the accumulation of amino acids induced by PEG (mainly asparagine and proline). This suggests that free amino acids accumulation under water deprivation conditions might be due to the role of ABA contributes to osmoregulation.

Under various environmental stressors, plants develop a variety of physiological and biochemical pathways to counter or cope with these stresses. Under stressful conditions, plant cells utilize phenolic compounds and flavonoids to scavenge stress-induced reactive oxygen species and enhance plant tolerance (Mittler, 2002). Drought stress resulted in the accumulation of phenolics, and flavonoids compared to non-stressed plants. The results are consistent with those of (Shallan *et al.*, 2012) for cotton. Previously, the authors demonstrated the accumulation of phenolic and flavonoid content under water-deficit conditions (Farooq *et al.*, 2009). Several previous studies have documented an increase in flavonoid levels under water stress conditions (Hammad and Ali 2014; Hussein *et al.* 2022). Ibrahim and Jaafar (2013) found that the enhancement in the production of total phenol and flavonoid contents in *Orthosiphon stamineus* is due to the increase in the carbohydrates level. Interestingly, pretreatment with ABA enhanced the production of phenolics while decreasing flavonoids content in cotton plants grown under drought stressor compared with



untreated stressed plants. The results showed that ABA application has a certain effect on mitigating plant stress. The response might be related to the effect of ABA on photosynthesis, which is the source of phenolics (Farooq *et al.*, 2009). The adverse effects of resulted from water stress can be alleviated through the promotion of phenolics because of the function of phenolics as an antioxidant defense system which have could scavenge free radicals thus preventing cell destruction. On the other hand, the decrement in flavonoids may be due to their consumption to overcome the adverse effects of drought.

Reactive oxygen species generation and accumulation rates significantly augmented under stress conditions of drought leading to an increase in lipid peroxidation. Activities of catalase and peroxidase were significantly influenced in drought-stressed cotton plants. In cotton, drought-induced the production of ROS, associated with increasing APX activity to maintain the ROS scavenging process until the cotton plants are relieved from stress conditions (Hussien *et al.*, 2015). The drought-tolerant cultivar has constitutively active antioxidative enzymes, including APX, SOD, CAT, and POD, which inhibit the oxidative stress induced by lipid peroxidation (Sekmen *et al.*, 2014). On the other hand, pretreatment with ABA at 80  $\mu\text{M}$  significantly increased catalase, peroxidase, and ascorbate peroxidase enzymes in stressed plants while decreasing lipid peroxidation. These findings are similar to (Dash *et al.*, 2020) which showed catalase activities were higher levels of observed stress and ABA foliar applied plants compared to the control. Increased antioxidant enzyme activity has been shown to protect plants from oxidative stress.

Protein profile is used to compare the appearance and disappearance of ABA and drought-responsive proteins in drought-stressed cotton plants by comparing protein profiles. Drought stress induced the synthesis of the unique polypeptides (11, 20, and 100 KDa). The results may be because of the presence of three stress-responsive genes attributed to water stress tolerance in cotton cultivar-90. Meanwhile, the interaction between water stress and ABA induces a polymorphic polypeptide (235 and 68 KDa). These results mean that the interaction effect between drought and ABA may have a significant impact on the expression of two distinct genes. The results suggest that ABA pretreatment enhances plant tolerance to stress conditions by modulating the polypeptides responsible. Similarly, a previous study supports the vital role of ABA that is done in the tea plants during water stress, by inducing carbon metabolism, transport and expression of tolerance proteins (Zhou *et al.*, 2014). ABA-pre-treated tea plants may increase photosynthesis by increasing the activity of Rubisco. This could increase photosynthesis and increase sugar levels, which promotes proline accumulation. Moreover, the results refer to a relationship between the accumulation of drought-induced proteins and physiological adaptation

to water-limitation. While, decreasing protein bands under two ABA concentrations might be due to their sensitivity to ABA treatments.

## CONCLUSION

Drought is a major limiting environmental stress, affecting crop growth and productivity. Drought-stressed cotton plants have evolved adaptive strategies to cope with environmental cues. ABA-pretreated cotton plant responses have revealed a key role of ABA in growth and development during drought stress conditions. ABA pretreatment has a potential role in vital physiological processes in cotton plants, such as osmotic balance, membrane stability, and antioxidant mechanisms responsible for oxidative stress. ABA regulates plant growth parameters, photosynthetic pigments, antioxidant activities, soluble sugars, free amino acids, proline, total phenols, flavonoids, and lipid peroxidation, and accelerates the expression of ABA-drought-responsive genes.

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