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The Physiological Response of Some Cotton Cultivars to Water Stress and Growth Inducers

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WATER deficiency is the most common abiotic stress in cotton production in Egypt. Although, cotton plants react strongly to soil moisture, water-deficit conditions during squaring and flowering stages diminish plant growth and productivity. The experiment was conducted to evaluate the physiological response of three Egyptian cotton cultivars (Giza 94, 96 and 97), under irrigation intervals (normal and severe water-deficit conditions) and with spraying with growth inducers [calcium(Ca)-boron(B) and mixed amino acids] and their interactions on the leaves chemical constituents and yield components during the 2020 and 2021 seasons. The experiment design was a split-split plot with three replicates. The main plots included three cotton cultivars, the subplots included two irrigation intervals and the sub-subplots included spraying with growth inducers at squaring and flowering stages (400ppm). The results revealed that the performance of the three cotton cultivars was significantly different in chemical constituents and yield components via their different genetic potentials, with the best results registered by Giza 97 compared to Giza 94 and 96. Water-deficit conditions significantly reduced leaves pigment content and all yield components, but significantly improved all osmolyte compounds (total soluble sugars, total phenol, total free amino acids, free proline and total antioxidant capacity) compared to normal conditions. Spraying with growth inducers significantly enhanced the chemical constituents and yield components compared to untreated plants in relation to their positive effects in improving photosynthesis, sugar biosynthesis and all cotton cultivar yields. Giza 97 sprayed with Ca-B under normal conditions recorded the best results compared to other treatments.

Keywords: Egyptian cotton cultivars, Growth inducers, Leaves chemical constituents, Water-deficit condition, Yield components.

Introduction

Egyptian cotton (*Gossypium barbadense* L.) plays an especially important role in the economy. Egyptian cotton has extra-long staple fibers compared to other kinds of cotton worldwide. Cotton is a source of animal cake, oil and fiber for the textile industry (Iqbal et al., 2011; Mahdy et al., 2017).

Water deficiency is the main challenge for cotton growth and productivity, especially in arid and semiarid areas worldwide. Drought stress is expected to be more repeated and acute due to global climate change, with harmful impacts on the world's crop production (Abbas et al., 2021). Cotton water requirement is 3000 to 3500m^3/f during the fall season to obtain a normal yield. Water is required for all organisms in a specified quantity. The reduction of water availability is an indicator effect on plant growth and productivity leading to adverse effects on ongoing biochemical and physiological processes. Recently, the extent of drought danger has increased in many countries. Also, environmental changes and increasing temperatures are the main causes of drought stress and have contributed to an appreciable reduction in crop productivity (Obidiegwu et al., 2015). In turn, significant agricultural losses lead to the failure of drought-sensitive crops to plant and grow under stress conditions (Athar & Ashraf, 2009). The difference in plant responses to water-

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deficit conditions is commensurate with anatomical, morphological and physiological properties. In response to water-deficit conditions, some plants adjust their leaves structure to decrease transpiration (Hameed et al., 2012). Egyptian cotton cultivars are differentiated in drought stress responses (Yehia & El-Menshawie, 2008; Dahad et al., 2012; Gamez et al., 2019; Pawar et al., 2020; Yehia, 2020; Ibrahim et al., 2021; Zaki & Radwan, 2022).

Drought stress causes molecular, physiological and biochemical changes in plants, such as modification of plant water status, photosynthesis reduction, antioxidant enzyme activity and metabolism of carbohydrates, amino acids and proteins (El-Far et al., 2019). Water-deficit conditions due to stomatal closure and destruction of photosynthetic reaction centers cause serious decreases in photosynthetic rates and biomass accumulation (Ibrahim et al., 2016). In addition, drought stress induces nutrient absorption, redistribution, and transport, resulting in a decline in growth, dry matter content and yield (Anjum et al., 2017; Kim et al., 2019; Gaafar et al., 2022). Drought conditions cause oxidative stress in cells due to a higher leakage of electrons toward O_2^- during photosynthetic processes and an increase in reactive oxygen species (ROS) production (Ali & Ullah, 2020). Hamoda (2012) pointed out that irrigation interval every 3 weeks cause a significant reduction in plant height, number of fruiting branches, number of open bolls/plant and seed cotton yield. Emara et al. (2015) and Gomaa et al. (2019) demonstrated that drought conditions during plant growth and fruit retention are due to a significant reduction in cotton yield and fiber quality properties.

Calcium (Ca) and boron (B) are essential nutrient elements for cotton plant growth and development and partake in different physiological processes. Ca is a multifunctional element in plants, such as cell wall structure, maintenance of membrane integrity, interaction with phytohormones, and countercations for organic and inorganic anions in vacuoles. Also, Ca acts as an intracellular cytosol messenger and increases the activity of many key enzymes. Under stress conditions, Ca increases plant resistance by signaling anti-drought responses; increasing cellular transient Ca participates in the processes of abscisic acid (ABA)-induced drought signal transduction (Batistic & Kudla, 2012). ABA biosynthesis improves water use efficiency and increases plant drought stress tolerance (Ali et al., 2020). Hamoda (2012) and Naeem et al. (2018) stated that Ca foliar application enhances the drought tolerance of plants. Fan (2019) and Abbas et al. (2021) suggested that Ca foliar application improves growth and yield under normal and drought conditions.

B is a static element in plant organs, the most important microelement in all necessary nutrients for cotton plant growth and productivity. Cotton plants need B in comparatively large amounts compared to other plants (Niaz et al., 2002). B assists in cell wall biosynthesis, cell division, and elongation. Besides, B accelerates tissue growth and development, enhances tissue conduction and storage, and helps in the translocation of sugars and nutrients (Eleyan et al., 2014; Rahman et al., 2018). Cotton plants need B during flowering and boll development stages; B stimulates pollen germination and pollen tube growth and causes successful fruit setting (Emara et al., 2015). More et al. (2018) revealed the positive effects of spraying with B on growth, fruit retention, and yield components of cotton. Kassem et al. (2009), Hamoda (2012), and Karademir & Karadmir (2020) illustrated that foliar application of B gives the highest values in leaves content of N, P, K, Mg, B, Fe, Mn, and Zn and significantly increases the seed cotton yield compared to control. Mixed Ca-B is essential for pollen grain germination and pollen tube elongation by helping in successful fertilization or pollination and banning the abortion of flowers. The benefits of Ca and B application depend on a balance between Ca and B levels in the plant; the foliar application of mixed Ca and B is transported in the phloem, preferably for new tissue, with the movement linked to the metabolic activity of plants (Zoz et al., 2016).

Amino acids can directly or indirectly affect the physiological activities of plants. They act as a carbon and energy source and protect plants against stress. Amino acids have functions in the synthesis of amines, pyrimidines, purines, alkaloids, terpenoids, vitamins, enzymes, and others (Rai, 2002). Also, amino acids are well-known biostimulants that have favorable effects on plant growth and yield under normal and stress conditions. They act as coenzymes of certain plant hormones and enhance plant growth by maintaining a favorable pH value within plant cells and improving photosynthesis (Amin et al., 2011). They help mRNA transcription and protein and sugar biosynthesis and form complexes with metal cations, mainly amine ($-NH_2$) and carboxylate (-COO) groups, for the bioavailability of metals in plants (Khattab et al., 2016). El-Desouky et al. (2011) demonstrated that amino acid application significantly increases many growth traits, such as the number of formed branches and leaves/plant, fresh and dry weight of stems and leaves, total leaves area/plant, and specific leaves weight. El-Badawy & Abd El-Aal (2013) reported that spraying with mixed amino acids increased the leaves content of N, P, K, total chlorophyll, total carbohydrate, total indole, and total phenol of plants. El-Gmal et al. (2016) concluded that foliar application of amino acids improves the yield components and quality of plants. Likewise, Ammar et al. (2013) assumed that amino acid foliar application increases the growth and yield components of plants under stress conditions. Sadak et al. (2015) and Mesbah (2016) noticed that spraying with mixed amino acids significantly mitigates the harmful effects of water stress and enhances the growth and yield components of plants under water stress conditions compared to control.

These responses are mostly associated with altering many biochemical and physiological adaptive responses, eventually improving the leaves chemical constituents and productivity of cotton plants. For all these reasons, this study was conducted to evaluate the effects of spraying with growth inducers (Ca-B and mixed amino acids) on the leaves chemical constituents and yield components on three different Egyptian cotton cultivars (Giza 94, 96, and 97) under different two irrigation intervals (normal and severe water-deficit conditions).

Materials and Methods

Experimental design and treatments

The experiment was conducted at the Sakha Research Station of the Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Kafr El-Sheikh, Egypt, situated at 31°6′N latitude, 30°50′E longitude with an elevation of ~6 m above mean sea level, during the 2020 and 2021 seasons to evaluate the physiological response of three Egyptian cotton (*G. barbadense* L.) cultivars (Giza 94, 96, and 97) under two irrigation intervals (normal and severe water-deficit conditions), spraying with growth inducers (Ca-B and mixed amino acids), and their interactions on the leaves chemical constituents and yield components. The experiment design was a split-split plot with three replicates. The main plots included three cotton cultivars (Giza 94, 96, and 97). The subplots included two irrigation intervals (normal and severe water-deficit conditions). The sub-subplots included spraying with growth inducers (Ca-B and mixed amino acids) at 400ppm concentration, twice at squaring and flowering stages. The growth inducers active ingredient and rate of application are shown in Table 1. Seeds of the three cotton cultivars (Giza 94, 96, and 97) were sown in clay loam soils on April 26 in the 2020 season and April 24 in the 2021 season. The characterization of three Egyptian cotton (*Gossypium barbadenes* L.) cultivars are shown in Table 2.

The experimental plot consisted of 7 rows, 3.5m long and 0.6m wide (plot area= 14.70m^2). All plots were fertilized at a rate of 60kg N/fed in the form of urea (46.5% N) in two equal doses. The first dose was added after thinning (before the first irrigation), whereas the second dose was applied before the second irrigation. All plots received an adequate amount of fertilizer to produce healthy plants. Fertilization was carried out according to the recommendations of the Cotton Research Institute. P fertilizer was applied during soil preparation in the form of Ca superphosphate (15.5% P_2O_5) at a rate of 15.5kg P_2O_5/fed. K fertilizer was applied after thinning at a rate of 24kg K_2O/fed in the form of K_2SO_4 (48% kg K_2O). All experimental plots received irrigation, pesticide, and fertilizer as recommended by the Egyptian Ministry of Agriculture for cotton cultivation. Soil analysis was conducted according to Chapman & Pratt (1978). The physical and chemical properties of the experimental field soil are shown in Table 3.

Source of irrigation water

The source of irrigation water of the field experiments is a sub-canal of the Nile River. The chemical analysis of irrigation water showed in Table 4.

TABLE 1. The growth inducers active ingredient and rate of application

Trade name	Active ingredient	Rate of application
Calcium-Boron	1.5% Acetic acid + 12% Calcium Oxide (CaO) + 6% Boron	0.5cm^2/L
Amino acids	85% mixed of 10 different L- free Amino acids (glycine, methionine, lysine, arginine, glutamic acid, proline, tryptophan,cysteine, threonin and histidine) + 8% Potassium + 7% Total nitrogen	0.75cm^2/L

TABLE 2. The characterization of three Egyptian cotton (*Gossypium barbadenes* L.) cultivars

Cultivar name	Genotype	Category
Super-Giza 94	Crossing between Giza 86 x 10229	Long staple
Extra-Giza 96	Crossing between Giza 94 x [Giza 86 x (R101 x Giza 89)]	Extra-long staple
Super-Giza 97	Crossing between [Giza 84 x (Giza 70 x Giza B51) x S62]	Long staple

TABLE 3. Physical and chemical properties of experimental soil during 2020 and 2021 seasons

Properties		2020	2021
pH		8.06	8.04
E.C. (dsm^{-1})		3.53	3.49
Soil mechanical analysis	Clay%	52.24	53.18
	Silt%	27.39	25.92
	Sand%	20.57	19.18
Soil texture		Clayey	Clayey
Available minerals (mg/kg soil)	N	17.12	17.09
	P	23.92	23.88
	K	216.42	215.85
	Cu	7.94	7.86
	Fe	42.43	42.17
	Mn	7.75	7.62
	Zn	14.17	14.02
Soluble anions (meq/L)	CO_3^{2-}	--	--
	HCO_3^-	4.78	4.73
	Cl^-	4.49	4.27
	SO_4^{2-}	7.84	7.79
Soluble cations (meq/L)	Ca^{2+}	6.60	6.57
	Mg^{2+}	3.75	3.72
	Na^+	7.55	7.54
	K^+	0.54	0.51

TABLE 4. Irrigation water chemical properties

Properties		2020	2021
pH		7.62	7.38
E.C. (dsm^{-1})		1.16	1.09
Soluble anions (meq/L)	CO_3^{2-}	--	--
	HCO_3^-	0.67	0.58
	Cl^-	1.85	1.64
	SO_4^{2-}	0.24	0.21
Soluble cations (meq/L)	Ca^{2+}	3.54	3.42
	Mg^{2+}	1.82	1.65
	Na^+	2.71	2.49
	K^+	0.64	0.60

Estimation of water requirements of irrigation intervals and growth inducer applications

Water requirements were computed for irrigation cotton crops based on the CROPWAT8 program (Allen et al., 1998; Tables 5 and 6) for furrow irrigation under normal and severe water-deficit conditions. Normal condition plots were irrigated every 2 weeks (12-15 days), and severe water-deficit condition plots were irrigated every 4 weeks (28 days) from the squaring stage of cotton plants. Plants were sprayed with growth inducers (Ca-B and mixed amino acids) twice at squaring and flowering stages at 400 ppm concentration beside the untreated plots in both irrigation interval conditions.

Mean temperature and heat unit accumulations

Mean temperature and heat unit accumulations were monitored using in Department of Meteorology, Agricultural Research Center. Maximum and minimum and mean air temperature (°C) in the three locations Sakha station during 2020 and 2021 seasons are shown in Table 3. The data covered the period from the start of planting to harvesting stage. Average of air temperatures (°C) through the growing seasons recorded in order to calculate heat units (HU). Heat units (HU) were calculated according to Sutherland (2012) equation as follows:

Heat unit (HU)= Mean daily temperature – Base Temp. (Base Temp. = zero growth =15.6°C).

Monthly heat units (HU) during a six-month cotton growth period in the location of Sakha station during 2020 and 2021 seasons are showed in Table 7.

Chemical analysis

Cotton samples of the fourth upper leaf/plant were taken randomly after 10 days from the last sprayed time (at the flowering stage) with growth inducers (Ca-B and mixed amino acids) to determine the chemical analysis as follows:

Total chlorophyll and carotenoid content

The total chlorophyll (mg/g; FW) and carotenoid content was estimated by the spectrophotometric method recommended by Arnon (1949) and Robbelen (1957), respectively. Leaf samples (0.3g from each replicate were homogenized in 50ml 80% (v/v) acetone and centrifuged at 10,000 × g for 10min. The absorbance of each acetone extract was measured at 665, 649, and 440nm using a UV-visible spectrophotometer.

TABLE 5. Irrigation water requirement for normal furrow irrigation/season

Days from planting	Plant stage	Actual irrigation requirement (m^3/f)	Notes
1	Initial	400	Applied water before planting
21	Seedling	300	Applied water first irrigation
36	Seedling	300	
51	Squaring	300	
66	Squaring	300	
81	Flowering	300	Irrigation intervals every two weeks (12-15 days)
96	Flowering	300	
111	Bolling	300	
126	Bolling	300	
141	Ripening	280	
Total		**3080**	

TABLE 6. Irrigation water requirement for sever water deficit furrow irrigation/season

Days from planting	Plant stage	Actual irrigation requirement (m^3/f)	Notes
1	Initial	400	Applied water before planting
21	Seedling	300	Applied water first irrigation
36	Seedling	300	
66	Squaring	400	Irrigation intervals every four weeks (28 days)
96	Flowering	400	
126	Bolling	400	
Total		**2200**	

TABLE 7. Monthly maximum, minimum, mean temperature and Monthly heat units (HU) in location of Sakha station during 2020 and 2021 seasons

Months	2020			Monthly heat units	2021			Monthly heat units
	Temperature °C				Temperature °C			
	Min	Max	Mean		Min	Max	Mean	
May	17.88	32.95	25.41	294.34	16.74	32.05	24.39	**263.85**
June	18.46	34.71	26.58	329.46	17.82	33.94	25.88	308.40
July	20.58	36.42	28.50	387.08	19.63	36.02	27.82	366.75
August	22.23	38.19	30.21	438.35	21.88	37.79	29.83	427.05
September	20.76	35.68	28.22	378.63	20.14	35.23	27.68	362.55
October	18.44	32.16	25.30	291.02	17.95	31.78	24.86	277.95

Total soluble sugars content

The total soluble sugar content was determined in leaves ethanol extracts by the phenol-sulfuric acid method according to Cerning (1975). A stranded curve was prepared using different concentrations (10–100mg/mL) of pure glucose.

Total phenols content

The total phenols were determined in ethanol of leaves using Folin-Ciocalteau method according to Simons & Ross (1971). One milliliter of sample was mixed with 1mL of Folin and Ciocalten's phenol reagent, after 3min, 1ml of saturated Na_2CO_3 (14%) was added to the mixture and completed to 10mL by adding distilled water. The reaction was kept in the dark for 90min, after which the absorbance was read at 725nm. A calibration curve was constructed with different concentrations of gallic acid (0.01–1mM) as the standard.

Total free amino acids content

The total free amino acid content was determined in the ethanol extract of cotton leaves by the ninhydrin method according to Rosen (1957).

Free proline content

Proline content of cotton leaves was determined according to method of Bates et al. (1973) as described by Shyam & Aery (2012) as follows: The fresh leaves (0.5g) were homogenized in 10 ml of sulfosalicylic acid (3%, w/v). The homogenate was filtered through filter paper. Two milliliter of filtrate was mixed with 2mL of acid ninhydrin and 2mL of glacial acetic acid in a test tube. The resulting mixture was incubated in a boiling water bath for 1 h. The reaction was stopped using an ice bath and the contents were extracted with 4mL of toluene and mixed vigorously using a test tube stirrer for 15-20sec. The chromosphere containing toluene was aspirated from the aqueous phase and thawed to room temperature and the absorbance of the solution was measured at 520nm using a UV-visible spectrophotometer. Blank was prepared by the same procedure without sample. The proline concentration was determined from a standard curve prepared with L-proline. The results are expressed (free proline content) as µmoles of proline/g of fresh weight.

Total antioxidant capacity

Total antioxidant capacity was determined in ethanol extract of cotton leaves using the phosphomolybdenum method of Prieto et al. (1999) as follows: A known volume (0.01mL) of extract was added to test tube then completed to a constant volume (0.3mL) with DW. 3.0mL of reagent solution (0.6M sulfuric acid, 28.0mM sodium phosphate and 4.0mM ammonium molybdate) were added to each tube and mixed well then incubated at 95°C for 90min. Blank was prepared by the same procedure without extract. After cooling to room, the absorbance of the solution was measured at 695nm using spectrophotometer against blank. Increased absorbance of the reaction mixture indicated increased TAC.

Yield and its components

At first pick, a random sample of 10 guarded plants was taken and labeled from each plot to determine the following characters: number of opened bolls/plant, boll weight, seed index (100-seed weight), lint % (weight of lint per plant/weight of seed cotton plant*100), and seed cotton yield/fed (kentar; i.e., 157.5kg).

Statistical analysis

The experimental design was a split-split plot design with three replications in each treatment.

The results were pooled, and the means were taken. The measured variables were analyzed by analysis of variance performed using the M Stat-C statistical package (Gomez & Gomez, 1984) for data analysis. The means were considered significantly different at $P \leq 0.05$.

Results

Cotton cultivar effects on the chemical constituents and yield components of cotton

As for the effects of the three Egyptian cotton cultivars (Giza 94, 96, and 97), the three cotton cultivars significantly affected the leaves chemical constituents and yield components; Giza 97 gave the highest content of all chemical constituents and yield components compared to Giza 94 and 96 (Tables 8 and 9). The best results were registered by Giza 97 in the chemical constituents of total chlorophyll, carotenoids, total soluble sugars, total phenol, total free amino acids, free proline, and total antioxidant capacity (TAC) (8.63, 1.535, 30.14, 12.91, and 19.51mg/g, 19.63µmol/g, and 1.117 O.D.), respectively, compared to other cotton cultivars (Giza 94 and 96). Likewise, Giza 97 gave the heights means in yield components of the number of opened bolls/plant (16.27 and 16.64), boll weight (2.46 and 2.48g), seed index (10.92 and 11.17g), and seed cotton yield (8.75 and 8.95k/f) compared to the other cotton cultivars in the 2020 and 2021 seasons, respectively.

However, Giza 96 registered the lowest results in leaves chemical constituents of total chlorophyll, carotenoids, total soluble sugars, total phenol, total free amino acids, free proline, and TAC (6.38, 0.914, 24.72, 9.92, and 14.45mg/g, 15.14µmol/g, and 0.689 O.D.), respectively, compared to other cotton cultivars. Giza 96 also gave the lowest means in yield components of the number of opened bolls/plant (13.11 and 13.34), boll weight (2.27 and 2.30g), seed index (9.97 and 10.35 g), and seed cotton yield (6.43 and 6.56k/f), respectively, compared to other cotton cultivars in the 2020 and 2021 seasons.

Irrigation interval effects on the chemical constituents and yield components of cotton

As for the effects of irrigation intervals (normal and severe water-deficit conditions) treatments, irrigation intervals significantly affected the leaves chemical constituents and yield components (Tables 8 and 9); water-deficit conditions (every 4 weeks) significantly decreased the leaves content of total chlorophyll by 16.24% and carotenoids by 19.91% but significantly increased the leaves content of total soluble sugars by 29.73%, total phenol by 19.64%, total free amino acids by 19.03%, free proline by 363.43%, and TAC by 88.49% compared to normal conditions. Water-deficit conditions significantly reduced the yield components of the number of opened bolls/plant (12.73% and 13.51%), boll weight (2.51% and 2.89%), seed index (7.81% and 7.13%), and seed cotton yield (14.71% and 16.43%) compared to normal conditions in both seasons, respectively.

Growth inducer effects on the chemical constituents and yield components of cotton

As for the effects of spraying with growth inducers (Ca-B and mixed amino acids), growth inducer foliar applications significantly affected the leaves chemical constituents and yield components compared to untreated plants (Tables 8 and 9); spraying with Ca-B recorded the maximum values, followed by spraying with mixed amino acids, compared to untreated cotton plants in the three cotton cultivars under both irrigation intervals. Ca-B foliar application registered the best results in all leaves chemical constituents of total chlorophyll by 27.21%, carotenoids by 19.25%, total soluble sugars by 24.59%, total phenol by 26.28%, total free amino acids by 22.37%, free proline by 80.42%, and TAC by 47.97% compared to untreated plants. Spraying with Ca-B gave the maximum means of yield components of the number of opened bolls/plant (17.09% and 15.47%), boll weight (3.89% and 3.84%), seed index (5.01% and 4.54%), and seed cotton yield (20.74% and 20.65%) compared to untreated cotton plants in both seasons, respectively.

In contrast, spraying cotton cultivars with mixed amino acids recorded enhancement in all leaves chemical constituents of total chlorophyll by 17.29%, carotenoids by 11.39%, total soluble sugars by 15.85%, total phenol by 19.71%, total free amino acids by 14.86%, free proline by 64.08%, and TAC by 33.24% compared to untreated plants. Spraying plants with mixed amino acids registered the maximum means of yield components of the number of opened bolls/plant (9.75% and 8.54%), boll weight (2.19% and 1.29%), seed index (3.61% and 1.56%), and seed cotton yield (8.86% and 13.04%) compared to untreated cotton plants in both seasons, respectively.

TABLE 8. Effect of main factors (cotton cultivars, irrigation intervals and spraying growth inducers) and their interactions on leaves chemical constituents on cotton

Factors			Total Chl. (mg/g FW)	Carotenoids (mg/g FW)	Total soluble sugars (mg/g FW)	Total Phenols (mg/g FW)	Total amino acids (mg/g FW)	Proline (µmol/g FW)	Total antioxidant capacity ($O.D_{695\ nm}$)
Cultivars (A)	**Irrigation intervals (B)**	**Growth inducers (C)**							
Giza 94	Normal condition	Control	7.59	1.307	19.52	8.20	12.51	4.62	0.583
		Calcium-Boron	9.46	1.565	26.47	10.20	17.02	7.87	0.727
		Amino acids	8.56	1.483	23.32	10.00	15.32	6.65	0.675
	Water deficit condition	Control	5.94	1.073	28.54	10.10	15.28	18.89	0.915
		Calcium-Boron	8.28	1.199	33.12	13.84	19.90	35.46	1.764
		Amino acids	7.59	1.134	30.76	12.90	18.36	31.21	1.427
		Mean	**7.90**	**1.293**	**26.95**	**10.87**	**16.39**	**17.45**	**1.015**
Giza 96	Normal condition	Control	6.31	0.999	18.95	7.70	11.37	3.54	0.569
		Calcium-Boron	7.55	1.183	23.48	9.98	15.42	5.73	0.627
		Amino acids	7.30	1.020	22.42	9.65	14.05	4.72	0.598
	Water deficit condition	Control	4.56	0.602	24.71	9.84	13.60	17.59	0.713
		Calcium-Boron	6.99	0.887	30.73	11.87	16.34	30.34	0.818
		Amino acids	5.59	0.793	28.06	10.48	15.97	28.96	0.809
		Mean	**6.38**	**0.914**	**24.72**	**9.92**	**14.45**	**15.14**	**0.689**
Giza 97	Normal condition	Control	8.66	1.511	21.29	10.42	15.93	5.33	0.640
		Calcium-Boron	9.99	1.721	29.34	13.15	18.89	9.03	0.737
		Amino acids	9.40	1.682	28.88	12.77	17.32	8.13	0.713
	Water deficit condition	Control	6.86	1.303	31.17	12.19	20.94	20.55	1.020
		Calcium-Boron	8.50	1.543	36.54	14.79	22.09	38.78	1.898
		Amino acids	8.38	1.452	33.64	14.16	21.89	36.01	1.694
		Mean	**8.63**	**1.535**	**30.14**	**12.91**	**19.51**	**19.63**	**1.117**
Generally mean of irrigation intervals (B)	Normal condition	Control	7.52	1.272	19.92	8.77	13.27	4.49	0.597
		Calcium-Boron	9.00	1.490	26.43	11.11	17.11	7.54	0.697
		Amino acids	8.42	1.395	24.87	10.80	15.56	6.50	0.662
		Mean	**8.31**	**1.386**	**23.74**	**10.23**	**15.34**	**6.18**	**0.652**
	Water deficit condition	Control	5.78	0.993	28.14	10.71	16.60	19.01	0.883
		Calcium-Boron	7.92	1.210	33.46	13.50	19.44	34.86	1.493
		Amino acids	7.18	1.126	30.82	12.51	18.74	32.06	1.310
		Mean	**6.96**	**1.110**	**30.80**	**12.24**	**18.26**	**28.64**	**1.229**
Generally mean of growth inducers (C)		Control	6.65	1.132	24.03	9.74	14.93	11.75	0.740
		Calcium-Boron	8.46	1.350	29.94	12.30	18.27	21.20	1.095
		Amino acids	7.80	1.261	27.84	11.66	17.15	19.28	0.986
LSD at 0.05 of		A	0.079	0.058	0.155	0.114	0.127	0.455	0.018
		B (T_{test})	**	**	**	**	**	**	**
		C	0.142	0.042	0.141	0.138	0.117	0.328	0.034
		A * B	N.S	0.047	0.239	0.231	0.117	0.264	0.052
		A * C	0.246	N.S	0.245	0.240	0.204	0.568	0.060
		B * C	0.201	0.059	0.200	0.196	0.166	0.464	0.049
		A * B * C	0.349	N.S	0.347	0.339	0.288	0.804	0.085

TABLE 9. Effect of main factors (cotton cultivars, irrigation intervals and spraying growth inducers) and their interactions on yield and its components on cotton

Factors			No. of bolls/p		Boll weight (g)		Seed index (g)		Lint %		Seed cotton yield (k/f)	
Cultivars (A)	**Irrigation intervals (B)**	**Growth inducers (C)**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Giza 94	Normal condition	Control	15.00	15.48	2.37	2.41	10.43	10.77	40.90	40.82	7.81	7.95
		Calcium-Boron	18.00	18.32	2.42	2.44	11.05	11.56	40.62	40.53	9.16	9.68
		Amino acids	16.20	16.64	2.40	2.41	10.78	11.26	40.75	40.64	8.68	8.62
	Water deficit condition	Control	12.80	13.25	2.27	2.30	9.90	10.58	41.53	41.42	6.75	6.43
		Calcium-Boron	15.00	15.42	2.34	2.36	10.55	10.76	41.00	40.57	7.86	7.89
		Amino acids	14.00	14.38	2.31	3.34	10.16	10.59	41.12	40.73	6.92	7.24
	Mean		**15.16**	**15.58**	**2.35**	**2.37**	**10.48**	**10.92**	**40.98**	**40.78**	**7.86**	**7.96**
Giza 96	Normal condition	Control	12.60	13.04	2.25	2.27	10.29	10.48	40.43	40.32	6.20	6.35
		Calcium-Boron	14.20	14.58	2.34	2.38	10.66	10.95	40.02	39.94	7.22	7.46
		Amino acids	13.60	14.13	2.29	2.33	10.53	10.67	40.26	40.15	6.81	7.02
	Water deficit condition	Control	11.80	12.08	2.20	2.24	9.10	9.84	41.73	41.63	5.39	5.76
		Calcium-Boron	13.80	13.26	2.29	2.32	9.83	10.17	41.08	40.95	6.88	6.32
		Amino acids	12.70	12.95	2.28	2.29	9.45	10.00	41.25	41.12	6.13	6.45
	Mean		**13.11**	**13.34**	**2.27**	**2.30**	**9.97**	**10.35**	**40.79**	**40.68**	**6.43**	**6.56**
Giza 97	Normal condition	Control	16.00	16.32	2.41	2.45	11.24	11.46	39.54	38.85	8.32	8.53
		Calcium-Boron	19.00	19.46	2.54	2.59	11.28	12.04	39.10	39.42	10.6	10.77
		Amino acids	18.20	18.61	2.50	2.53	11.67	11.75	39.18	39.00	9.87	10.36
	Water deficit condition	Control	13.60	13.98	2.39	2.42	10.07	10.34	40.83	40.74	7.17	7.15
		Calcium-Boron	15.80	16.13	2.51	2.50	10.74	10.82	39.12	39.03	8.60	8.74
		Amino acids	15.06	15.34	2.41	2.43	10.51	10.65	40.26	40.12	7.97	8.16
	Mean		**16.27**	**16.64**	**2.46**	**2.48**	**10.92**	**11.17**	**39.67**	**39.52**	**8.75**	**8.95**
Generally mean of irrigation intervals (B)	Normal condition	Control	14.53	14.94	2.34	2.37	10.65	10.90	40.29	39.99	7.44	7.61
		Calcium-Boron	17.06	17.45	2.43	2.47	11.00	11.51	39.91	39.96	8.99	9.30
		Amino acids	16.00	16.46	2.39	2.42	10.99	11.22	40.06	39.93	8.45	8.66
	Mean		**15.86**	**16.28**	**2.39**	**2.42**	**10.88**	**11.21**	**40.08**	**39.96**	**8.29**	**8.52**
	Water deficit condition	Control	12.73	13.10	2.28	2.32	9.69	10.25	41.36	41.26	6.43	6.44
		Calcium-Boron	14.86	14.93	2.38	2.39	10.37	10.58	40.40	40.18	7.78	7.65
		Amino acids	13.92	14.22	2.33	2.35	10.04	10.41	40.87	40.65	7.00	7.28
	Mean		**13.84**	**14.08**	**2.33**	**2.35**	**10.03**	**10.41**	**40.88**	**40.70**	**7.07**	**7.12**
Generally mean of growth inducers (C)		Control	13.63	14.02	2.31	2.34	10.17	10.57	40.82	40.63	6.94	7.02
		Calcium-Boron	15.96	16.19	2.40	2.43	10.68	11.05	40.15	40.07	8.38	8.47
		Amino acids	14.96	15.34	2.36	2.38	10.51	10.82	40.47	40.29	7.73	7.97

TABLE. 9. Cont.

Factors			No. of bolls/p		Boll weight (g)		Seed index (g)		Lint %		Seed cotton yield (k/f)	
Cultivars (A)	**Irrigation intervals (B)**	**Growth inducers (C)**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
LSD at 0.05 of		A	0.103	0.019	0.012	0.035	0.219	0.154	0.069	0.201	0.018	0.104
		B (T_{test})	**	**	**	**	**	**	**	**	**	**
		C	0.074	0.052	0.013	0.005	0.171	0.093	0.080	0.163	0.041	0.077
		A * B	0.116	0.024	0.019	0.007	0.291	0.155	0.131	0.138	0.059	0.082
		A * C	0.128	0.090	0.029	0.010	N.S	0.162	0.139	0.282	0.071	0.134
		B * C	0.104	0.073	N.S	0.008	N.S	0.132	0.114	0.230	0.058	0.109
		A * B * C	0.181	0.128	0.032	0.014	N.S	N.S	0.197	0.399	0.100	0.189

Effects of the interactions among cotton cultivars, irrigation intervals, and growth inducers on the leaves chemical constitution, growth, and yield components of cotton

The interaction among the three cotton cultivars (Giza 94, 96, and 97) and irrigation intervals (normal and severe water-deficit conditions) significantly affected the leaves chemical constituents (carotenoids, total soluble sugars, total phenol, total free amino acids, free proline, and TAC) and yield components (number of opened bolls/plant, boll weight, seed index, lint %, and seed cotton yield) in both seasons (Tables 8 and 9). Giza 97 recorded the best results, whereas Giza 96 gave the lowest values under normal and severe water-deficit conditions.

The interaction among the three cotton cultivars (Giza 94, 96, and 97) and spraying with growth inducers (Ca-B and mixed amino acids) significantly affected the leaves chemical constituents (total chlorophyll, total soluble sugars, total phenol, total free amino acids, free proline, and TAC) and yield components (number of opened bolls/plant, boll weight, lint %, and seed cotton yield) in both seasons (Tables 8 and 9). Exogenous Giza 97 with Ca-B recorded the maximum results, whereas Giza 96 without treatment (control plants) gave the minimum values.

The interaction between irrigation intervals (normal and severe water-deficit conditions) and spraying with growth inducers (Ca-B and mixed amino acids) significantly affected the leaves chemical constituents and yield components (number of opened bolls/plant, lint %, and seed cotton yield) in both seasons (Tables 8 and 9). Spraying with Ca-B under drought stress conditions significantly improved almost all leaves chemical constituents of total soluble sugars, total phenol, total free amino acids, free proline, and TAC (33.46, 13.5, and 19.44 mg/g, 34.86 14 µmol/g and 1.493 O.D, respectively; Table 8). However, exogenous cotton plants sprayed with Ca-B under normal conditions enhanced the yield components of the number of opened bolls/plant (17.06 and 17.45) and seed cotton yield (8.99 and 9.3k/f), respectively, in both seasons, whereas boll weight (2.47g) and seed index (11.51g) significantly increased only in the 2021 season compared to other treatments (Table 9).

As for the effects of the interaction among three cotton cultivars, irrigation intervals, and spraying with growth inducers, the interaction among the three factors significantly affected the leaves chemical constituents (total chlorophyll, total soluble sugars, total phenol, total free amino acids, free proline, and TAC) and yield components (number of opened bolls/plant, boll weight, lint %, and seed cotton yield) in both seasons (Tables 8 and 9). Exogenous Giza 97 sprayed with Ca-B under water-deficit conditions registered the maximum values of almost all leaves chemical constituents of total soluble sugars, total phenol, total free amino acids, free proline, and TAC (36.54, 14.79, and 22.09mg/g, 38.78 µmol/g, and 1.898 O.D, respectively; Table 8). Spraying Giza 97 with Ca-B under normal conditions improved the yield components of the number of opened bolls/plant (19 and 19.46), boll weight (2.54 and 2.59g), and seed cotton yield (10.6 and 10.77k/f), respectively, whereas seed index was insignificantly affected in both seasons compared to all cotton cultivars sprayed with Ca-B and mixed amino acids under normal and severe water-deficit conditions (Table 9).

Discussion

Effects of the main factors and their interactions on the leaves chemical constituents of cotton

The different responses of the three Egyptian cotton cultivars (Giza 94, 96, and 97) on the leaves chemical constituents might be attributed to the response of the three genotypes to water stress conditions by increasing the accumulation of soluble sugars, phenol, and free amino acids, especially proline (Table 8). These accumulated compounds acted as cellular osmoregulators agents, protected enzymes and proteins against damage, supported energy supply, and improved the antioxidant activity of plants under stress conditions. A similar trend was obtained by Yehia & El-Menshawie (2008), Yehia (2020), Pawar et al. (2020), and Ibrahim et al. (2021), who stated that Egyptian cotton cultivars differed in drought stress tolerance via the cotton leaves content of proline and their ability for accumulating it in leaves.

Water-deficit conditions adversely affected all cotton cultivar pigment content (Table 8), which might be related to the increased ROS production and oxidative stress, in parallel with water-deficit, close stomatal conductance, and chloroplast damage (Ali & Ullah, 2020; Pawar et al., 2020). The accumulation of osmoprotectant compounds (soluble sugars, phenol, amino acids, proline, and antioxidants) might be attributed to their serving as ROS scavengers, maintaining turgor, and stabilizing cellular membranes under water stress conditions to improve cotton plant tolerance. These findings were supported by Yehia & El-Menshawie (2008), Ibrahim et al. (2016), and Ibrahim et al. (2021), who documented that plants exposed to water stress led to a significantly increased leaves content of sugars, phenols, and proline to alleviate the harmful effects of stress conditions. In this regard, Anjum et al. (2017) suggested that decreasing pigment content might be an important regulatory step to avoid high light absorbance, overreduced photosynthetic electron transport chain, and ROS generation. Ibrahim (2014) stated that proline and soluble sugars accumulate in plants under water stress conditions; these substances might help plants organize the osmotic potential of cells and enhance water absorbance and translocation.

In this respect, the favorable effects of Ca on pigment and soluble sugar content in cotton leaves might be attributed to its role in improving photosynthesis and soluble sugar biosynthesis (Xu et al., 2013; Ibrahim, 2014). Also, Ca is responsible for the biosynthesis of various phenol compounds. Phenolic compounds play a vital role in defense and protection against the harmful effects of plant stresses by scavenging ROS that causes oxidative damage (Weidner et al., 2009). Finally, Ca alleviates the adverse effects of water stress in cotton plants by affecting osmolytes and antioxidants or cell wall structure, enhancing the water status of plants and maintaining the membrane integrity (Abbas et al., 2021). B is necessary for transporting carbohydrates directly and indirectly to plants and contributes to B-cell differentiation and maturation. B is an important element in plant cell division, elongation, and growth. It participates in carbohydrate metabolism and movement by favoring sugar-borate complexes transport to other organs and cells of plants (Eleyan et al., 2014). B engages in nucleic acids (RNA) biosynthesis, so its application increases protein synthesis (Rahman et al., 2018). B improves P and Ca uptake, respiration, indole acetic acid, and phenol metabolism in plants. Zoz et al. (2016) deduced that early bloom by application of Ca and B are key components of pollen tube elongation that help ensure more successful fertilization of flowers and higher yield.

In this context, the favorable role of spraying with amino acids might be due to consideration as sources for C and N and as structural components for enzymes and chlorophyll formation. Exogenous amino acid application regulates watering and improves the photosynthesis rate under normal and stress conditions. Under drought conditions, glutamine and asparagine are inhibited; the roles of ABA in stomatal closure include regulation and decreasing the harmful effects on photosynthesis. Further, amino acids play a role in stress alleviation as an osmolyte, more probable in the case of cotton. Application of amino acids improved proline and protein accumulation as a nontoxic osmolyte connected with amino acid concentration (Table 8). This demonstrated that the effects of spraying with amino acids in cotton as an osmolyte are due to enhancing organic osmoregulators, such as soluble sugars, phenol, amino acids, and proline. Improving drought stress alleviation by increasing proline accumulation that scavenged ROS plays an indirect role in improving plant drought tolerance, thus buffering cellular redox potential under stress conditions. In turn, the rapid increase in proline accumulation by spraying with amino acids results in proline-protein interaction, reducing protein degradation. Also, exogenous amino acid application increased the TAC of cotton plants, especially under drought stress, to enhance plant tolerance against stress conditions. These results were consistent with Rai (2002), who concluded that using amino acids increases the efficient photosynthesis process by increasing leaf pigments and increasing carbohydrate biosynthesis and their storage (Amin et al., 2011). Amino acids are considered the building blocks of protein, act as coenzymes of certain plant hormones, and enhance plant growth by improving photosynthesis. Similar results were obtained by El-Badawy & El-Aal (2013) and Khattab et al. (2016), who noted that amino acids act as a source of carbon and energy and protect plants against stress by synthesizing other organic compounds, such as protein, amines, pyrimidines, purines, alkaloids, vitamins, terpenoids, enzymes, and others. Haghighi et al. (2020) stated that exogenous amino acid application increases pigment, proline, protein, and phenol content and TAC of plants under drought stress.

Effects of the main factors and their interactions on the yield components of cotton

The different performances of the three Egyptian cotton cultivars (Giza 94, 96, and 97) on the yield components might be related to their genetic potential for adaptation under water-deficit conditions (Table 9). The ranking of cultivars according to yield components differed from the genotype responses to normal and severe water-deficit conditions. These results were consistent with Iqbal et al. (2011), Dahad et al. (2012), Pawar et al. (2020), Yehia (2020), Ibrahim et al. (2021), and Zaki & Radwan (2022), who pointed out that the differences in drought stress tolerance according to among the cotton cultivars. That was justified using yield components and stress tolerance indexed to depict the behaviors of genotypes under normal and severe water-deficit conditions.

Prolonged irrigation intervals (severe water-deficit condition) caused a reduction in yield components in all cotton cultivars. This might be due to the lack of nutrient uptake, close stomata, reduced vegetative growth, photosynthesis rate, and biosynthesis of carbohydrates. The reduction in cotton plant growth and metabolism was reflected by the lack of nutrients and cellulose to boll due to increased boll shedding and decreased cotton yield productivity compared to normal irrigation plants in both seasons (Table 9). A similar trend was indicated by Hamoda (2012) and Emara et al. (2015), who showed that water-deficit conditions reduce stomatal conductance to water and CO_2, the main reason for the decreased photosynthesis rate and plant growth and reduced cotton productivity. Also, Gomaa et al. (2019) and Zaki & Radwan (2022) concluded that drought stress affects cotton plant hormonal imbalance (ABA), reducing plant growth and productivity. In this connection, Kim et al. (2019) and Ibrahim et al. (2021) indicated that irrigation deficit conditions during maturation (squaring and flowering stages) are more damaging than deficit during vegetative stages due to the limitations of development.

The increase in the yield components of cotton cultivars sprayed with Ca-B is attributed to their functions as nutrient elements in various physiological and biochemical processes in plants. In this regard, B is essential for cell wall formation; it plays a vital role in the growth and development of new cells and regulates auxin production in plants (Hamoda 2012; Zoz *et al.* 2016). Kassem et al. (2009) and Karadmir & Karademir (2020) stated that spraying with B had positive effects on cotton, which significantly increased plant height, number of fruiting branches, number of opened bolls/plant, boll weight, and seed cotton yield (k/f). In contrast, the favorable effects of Ca in cotton yield components might be attributed to the important role in enhancing photosynthesis and maintaining turgor under drought stress conditions. Ca improves the water status of plants by enhancing the stability of membranes and protecting them from ROS-damaging effects (Emara et al., 2015; Ibrahim et al., 2016; More et al., 2018; Naeem et al., 2018). Ca acts as an anti-drought signaling response by participating in ABA-induced drought signal transduction, which enhances water use efficiency and opposes drought tolerance. Ca foliar application increases intracellular Ca levels, thus organizing the expression of stress-responsive gene levels and their activation (Ali et al., 2020). Fan (2019) and Abbas et al. (2021) suggested that Ca application achieves the highest plant yield under normal and drought stress conditions.

Based on this result, the positive effects of amino acid foliar application on cotton yield components might be related to its vital role in hormone synthesis and increasing the photosynthesis rate. Amino acids could improve leaf pigment content and chelating factor for micronutrient transport and absorption. Exogenous amino acids increase amino acid concentrations in the cell, improving plant growth and development under normal and stress conditions. Also, amino acids are considered precursors and constituents of proteins, which are important for stimulating cell growth. Moreover, they contain basic and acidic groups and act as buffers, maintaining a favorable pH value within the plant cell. Likewise, amino acids are known bio-stimulant with favorable effects on plant growth and yield and alleviate the effects of abiotic stresses. These results were consistent with Sadak et al. (2015), Mebah (2016), Gomaa et al. (2019), and Haghighi et al. (2020), who concluded that spraying crops with amino acids improves growth and yield components, especially under water stress conditions.

Conclusion

It can conclude that cotton water requirement is about 3000-3500m^3/f during the fall season for obtaining normal yield. The results explained as follows:

1- The performance of the cotton cultivars (Giza 94, 96 and 97) were different under normal and water-deficit conditions according to their ability to accumulate the osmolyte compounds in their leaves such as total soluble sugars, total phenol, total free amino acids, especially free proline that serve as antioxidant, removing ROS, alleviating the adverse impacts of drought stress and improving the productivity on plants, which Giza 97 gave the maximum means of chemical constituents and yield components.

2- Under normal irrigation intervals cotton plants used 3080 m^3/ha of water, whereas under water-deficit irrigation intervals used 2200 m^3/ha of water. Water-deficit conditions reduced leaves content of pigments and yield components, while it increased leaves contents of total soluble sugars, total phenol, total free amino acids and free proline of all cotton cultivar to reduce the harmful effect of water deficit conditions. The reduction of water availability indicator effects on plant growth and productivity led to adverse effect on ongoing biochemical and physiological processes such as damaging the chlorophyll and photosynthesis rate reduction causing reduction of carbohydrates, biosynthesis and all metabolism processes in cotton plant. Also, drought stress decrease cotton plant growth and development especially at squaring, flowering and bolling stages that caused to reduction on cotton yield quantity and quality compared to normal irrigation condition.

3- Growth inducers (Ca-B and mixed amino acid) improved all chemical constituents and yield components of cotton that related to the positive effect of Ca-B and amino acids in improving the photosynthesis rate, increasing soluble sugars biosynthesis and storage and eventually increased all cotton cultivars yield comparing with the untreated plants.

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الإستجابة الفسيولوجية لبعض أصناف القطن للإجهاد المائي وبعض منشطات النمو

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ظروف نقص المياه من الاسباب الشائعة الرئيسية للإجهاد الاحيوي المؤثره علي إنتاجية القطن في مصر. بالرغم من أن نباتات القطن تظهر قوية في التربه الرطوبيه إلا أن ظروف الجفاف أثناء مرحلة الوسواس والتزهير تسبب نقص النمو والإنتاجية. التجربة اقيمت خلال موسمين 2020- 2021 لتقييم الإستجابة الفسيولوجية لثلاث أصناف للقطن المصري (جيزه 94 – جيزه 96 – جيزه 97) تحت فترتين من الري (ظروف الري العادي – نقص المياه) والرش ببعض منشطات النمو (مركب الكالسيوم برون – مخلوط الاحماض الامينية) والتفاعلات بينهما علي المكونات الكيميائية للأوراق والمحصول ومكوناته. تصميم التجربه قطع منشقة مرتين مع ثلاث مكررات، حيث تعتبر الثلاث أصناف من القطن القطع المنشقة الرئيسية، وفترات الري القطع المنشقة الفرعية، والرش بمنشطات النمو القطع تحت لمنشقة الفرعية حيت تم الرش مرتين في مرحلتي الوسواس والتزهير بتركيز 400 جزء في المليون. أوضحت النتائج أن أداء الثلاث أصناف القطن المصرية يختلف معنويا في المكونات الكيميائية للأوراق والمحصول ومكوناته بإختلاف قدراتهم الوراثية، حيث أعطي صنف جيزه 97 أعلي النتائج يليه صنف جيزه 94 ثم صنف جيزه 96. ظروف نقص المياه أدت إلي النقص المعنوي في محتوي الأوراق للصبغات والمحصول ومكوناته، بينما أدي إلي زياده محتوي الأوراق من المركبات التي تحافظ علي الاسموزية (كمية السكريات الذائبة والفينولات والأحماض الأمينية و البرولين) مقارنة بالظروف الطبيعية للري. أدي الرش بجميع منشطات النمو إلي زياده معنويه في محتوي الأوراق من المكونات الكيميائية والمحصول ومكوناته، وذلك يرجع الي تأثيرها الإيجابي علي معدل البناء الضوئي وزيادة تخليق الكربوهيدرات وبالتالي المحصول ومكوناته مقارنة بالنباتات الكنترول. أخيرا، أدي الرش بمركب الكالسيوم بورون علي صنف جيزه 97 تحت ظروف الري العادي أفضل النتائج مقارنه بالمعاملات الاخري.