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Effect of Drought Tolerance Inducers on Growth, Productivity and some Chemical Properties of Cotton under Prolonging Irrigation Intervals

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COTTON is known as the king of fibers and an important source of oil. The aim of this study was to evaluate the mitigating effects of three osmoprotectants, including glycine betaine (GB), proline, and chitosan each at two concentrations of 400 and 200 parts per million (ppm), 100 and 50 ppm, and 300 and 100 ppm, respectively, on water deficit stress after irrigation intervals of 10-, 15-, and 20-days, relative to the untreated (control) plants. Data indicated that prolonged irrigation interval significantly decreased growth, yield, yield components, fiber parameters, total chlorophyll, Chl. *a*, and Chl. *b*, while, proline and total soluble sugars significant increased. All drought tolerance inducers showed significant increases in cotton growth and productivity traits. Generally, GB treated plants at 400 ppm showed superior traits of all studied parameters. For example, the interaction between irrigation intervals and GB application caused significant effects on growth and productivity as well as fiber quality and chemical properties. The application of drought tolerance inducers mitigated the effects of prolonged irrigation intervals in cotton, and all drought tolerance inducers achieved higher yield and yield component values at 15 days irrigation interval compared with the control at 10 days irrigation interval. Application of 400 ppm GB could improve cotton plants endurance against the negative effect of prolonged water intervals.

Keywords: Chitosan, Cotton, Glycine betaine, Irrigation intervals, Proline, Water stress.

Introduction

Cotton is a key fiber producer in the world, and it represent one of most economically important crops in Egypt for both local industry and export trade, which earned it the moniker of "White Gold." The crop grows in a variety of environmental conditions, soils, and cultural practices, and it is a key source of fiber and oil. Expanding cotton production by increasing the productivity unit and cultivation areas can improve its economic output. Consequently, increasing cotton yield and reducing production costs are major objectives of growers (Drwish et al., 2018).

Water is an integral part of the plant biochemical responses, cell development, phytonutrient translocation, and transpiration. Thus, deficit irrigation water (DIW) can cause unfavorable modifications in plant life systems and morphology, as well as in their physicochemical properties and overall efficiency (Hsiao, 1979; Abd El-Mageed et al., 2017; Badran, 2022). In addition, DIW can cause osmotic stretch and membrane enlargement due to rapid accumulation of toxic reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and hydroxyl radicals (OH^-), in cell organelles, such as peroxisome, mitochondria, and chloroplasts, leading to impeded plant development and efficiency (Rady et al., 2021). ROS generation caused by DIW may be the reason for plant cell passing, thus, hindering the alleviation of ROS by antioxidants (Abd El-Mageed et al., 2017; Rady et al., 2021). The cotton plant physiology, metabolism, and yield are significantly influenced by deficit water conditions (Pettigrew, 2004; Basal

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et al., 2009). During the delicate development stages, such as blooming, flowering, and fruit setting, DIW can inhibit plant growth , and fiber quality attributes, including length, consistency, quality, and fiber micronaire values, leading to lowered degree of daintiness and development (Pettigrew, 2004; DeTar, 2008; Eid et al., 2022).

GB and proline are partially charged, low atomic weight compounds generated in plants, and are thought to be osmoprotectants that stabilize and protect cell layers and proteins during stresses (Khan et al., 2015; Hasanuzzaman et al., 2019), with the amino acid proline as the most important osmoprotectants with vital roles in ion homeostasis and redox balance in plants (Kaur & Asthir, 2015; Roychoudhury et al., 2015; Zandalinas et al., 2018). Other proline functions, include osmotic balance during stress, scavenging free radicals, stabilizing macromolecules, and signaling pathways (Verslues & Sharma, 2010; Hayat et al., 2012; Loutfy et al., 2022). In addition, proline has been shown to regulate embryo/ seed development, increase stem length, and are important in transitioning plants from vegetative to maturity stage (Emamverdian et al., 2015). Under DIW, proline can increase plant osmotic pressure and regulate water potential (Zandalinas et al., 2017; Ferreira Júnior et al., 2018; Singh et al., 2018).

GB is produced as a reaction to water deficit, and it is primarily accumulated in the chloroplast to protect the thylakoid film by altering osmotic control and maintaining photosynthetic efficiency. It is also induced alongside other osmoprotectants in plants under stress conditions. As a low-molecular weight metabolite, GB show significant activity against DIW (Rasool et al., 2013; Roychoudhury & Banerjee, 2016). It is synthesized through two diverse pathways that utilize glycine and choline as substrates (Rasool et al., 2013; Gupta & Third, 2015). As a highly compatible solute, GB effectively dissolve in water, and show no harmful effects at any concentration (Giri, 2011). Its fundamental role in plants is plant cell protection by ensuring, protein stability, osmotic balance, and ROS detoxification (Roychoudhury & Banerjee, 2016). Studies have shown that GB at low condensation can secure macromolecules counting nucleic acids, proteins, and lipids, which are sufficient in nitrogen and carbon to be used as energy sources (Umezawa et al., 2006). Increased GB might also be associated with response to stress resistance by increasing superoxide dismutase (SOD) and catalase (CAT), as well as decreasing cell membrane damage after arranging of lipid peroxidation and ion homeostasis (Alasvandyari et al., 2017). Exogenous GB application as foliar spray can decrease membrane permeability and enhance plant growth, yield components, phenolic content, ascorbic acid, pigments, osmolyte concentration, and the activities of ROS scavenging enzymes, including SOD, CAT, and peroxidase (Hamani et al., 2021; Shafiq et al., 2021) (Szabados & Savoure, 2010). A previous study revealed that proline is a proteinogenic α-amino acid harboring an auxiliary amine group, and with basic functions in primary metabolism.

Chitin is found in a few organisms, such as shrimp and crab. Commercially, chitosan is prepared by demineralization of chitin with acids after the deproteinization process (Kaya et al., 2015). The probability of getting values of these naval squanders has persuaded inquire about around the world to discover utilize of chitin and it is attached, chitosan. Chitosan and its oligosaccharides have received wide prospects in agrarian application, biomedicine, and biotechnology because of their biodegradability and biocompatibility (Katiyar et al., 2014). This study aimed to determine the effect of three drought tolerance inducers, including glycine betaine(GB), proline, and chitosan, on the physicochemical properties, growth, and productivity of the "Giza 94" cotton cultivar, during extended irrigation intervals 10-, 15-, and 20 days.

Materials and Methods

Two field experiments were carried out during the two successive summer seasons of 2019 and 2020 at the Nubaria Station, Agricultural Research Center, El-Behira, Egypt (30°55′27.6″N, 29°56′57.8″E). The effects of three drought tolerance inducers on the growth, productivity and chemical properties of the "Giza 94" cotton cultivar were each evaluated at two concentrations, including GB (400 and 200ppm), proline (100 and 50ppm), and chitosan (300 and 100ppm), then compared with the control (untreated plants) under prolonged water stress intervals of 10-, 15-, and 20-days throughout the growing season starting after the first irrigation. A split experimental plot with three replications was used, with the irrigation treatments being conducted in the main plots, while the anti-stress treatments and the control being carried out in the sub plots. All osmoprotectants were exogenously sprayed three times at 55-, 75-, and 95-days after sowing. The experimental unit included seven ridges (5m long and 65cm apart) occupying an area of 22.75-m². Representative soil samples were taken before planting, then prepared for analysis according to Chapman & Pratt (1978), and soil analysis results are shown in Table 1. Cotton seeds were planted on April 29, and harvested after 170 days (October 15), 2019 and 2020. Hills were spaced at 25cm within rows and seedlings were thinned at 2 plants/hill after 35 days of planting. The climatic measurements (Table 2), for the two growing seasons were obtained from a local weather station adjacent to the experimental site. The characteristics of the "Giza 94" cultivar are shown in Table 3.

During the seed bed preparation, superphosphate (15.5%, P_2O_5) was supplied at a rate of 22.5-kg P_2O_5/fed (fed = 4200 what is the measuring unit). Nitrogen (N) fertilizer at a rate of 65kg N/fed in the form of ammonium nitrate (NH_4NO_3) (33.5%, N), was added immediately before the first and second irrigation in two equal doses, while potassium (K) fertilizer in the form of potassium sulfate (K_2SO_4) (48%, K_2O) was side-dressed at a rate of 24kg K_2O/fed before the second irrigation. Throughout the growing seasons, other recommended agricultural practices were followed.

Measurement of plant parameters

To measure growth and yield characteristics, all samples were randomly selected from each sub plot. At harvest, six guarded plants were randomly taken from the central ridge to determine growth traits, including plant height (cm), number of fruiting branches per plant, earliness traits (Position of first node, number of day to first flower, and number of day to first open boll), as well as yield and yield components, such as boll weight (gm), number of open bolls per plant, lint percentage, seed index (gm), and seed cotton yield (ken/fed) was estimated as the weight of seed cotton yield (kg) picked from the three central ridges, then converted to yield per fed in kentar (1 Kentar= 157.5kg).

TABLE 1. Physical and chemical properties of the experimental site in the 2019 and 2020 seasons

Season	Soil depth (cm)	Mechanical analysis			Soil texture	Soil moisture (%)		
		Sand (%)	Silt (%)	Clay (%)		Field capacity	Wilting point	Available water
2019	0-40	58.67	23.11	18.22	Sandy loam	35.13	14.61	21.31
2020	0-40	56.13	24.93	18.94	Sandy loam	36.91	14.71	21.30

Season	Soil depth (cm)	Soil E.C (ds/m)	Soil pH (1: 2.5)	Total Caco3	Organic matter (%)	Available macronutri-ents (%)			Total N (%)
						N	P	K	
2019	0-40	1.74	8.10	20.90	0.23	36.7	4.6	89	0.12
2020	0-40	1.69	8.00	21.14	0.25	38.7	5.0	96	0.15

TABLE 2 . Climatic data for the study area for both 2019 and 2020 seasons

MO	TMax		Tmin		RH		Rain		Wind	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
APR	27.83	26.87	12.21	11.82	46.75	57.24	0.043	2.86	4.46	3.99
MAY	36.43	32.56	17.97	15.76	32.13	50.76	0.001	0.00	4.75	4.46
JUN	37.77	36.53	21.49	19.10	40.97	41.49	0	0.00	4.67	4.32
JUL	38.68	38.81	22.16	21.57	42.30	42.90	0	0	4.15	4.14
AUG	38.37	38.88	22.55	21.99	43.16	45.32	0	0	3.75	3.79
SEP	35.42	38.17	20.37	21.84	51.59	50.59	0	0	4.10	4.32
OCT	31.92	33.30	18.31	19.13	57.10	57.27	0.53	0.02	3.98	4.08

TMax= Maximum temperature at 2 meters (°C), TMin= Minimum temperature at 2 meters (°C), RH= Relative humidity at 2 meters (%), Rain= Precipitation (mm day^{-1}) and Wind= Wind speed at 2 meters (m s^{-1}),

TABLE 3. Characterized the Egyptian cotton variety Giza 94

Variety name	Giza 94
Species	Barbadense
Category	Long staple variety
Pedigree	Crossing between G86 x 10229
Characteristics	Long staple characterized by high yielding, early maturity, resistance to Fuzariam and high lint (%)
Vegetative characters	The stem has a medium length with polygon shape also has green color mixed by dim red with medium length internodes. The leaves have palmate shape with large size with no deep lobes and leather fell. The node of the first fruiting branch ranged from 8 - 9. A flower petal has tubular shape. The boll size is large and pyramid shape with drawn summit. Seed is big-sized and the fuzz covers about fuzz less to ¼ from the whole size and fuzz color is gray-greenish
Varity breed by	Breeding Res. Section, Cotton Res. Inst., Agric. Res. Center, Giza, Egypt

Analyzed fiber properties, included fiber length in (mm) at 2.5% span length. Uniformity index was measured using a digital Fibrograph according to the standard method for testing the fiber length (American Society for Testing and Materials, ASTM (1) 1447-63). Fiber fineness was expressed in Micronaire (ASTM D-1448-59), while fiber strength (as flat-bundles of fibers) was measured using the Pressley tester at zero gage length, and recorded as (Pressley index) values (ASTM D-1445-67). All fiber property tests were measured in the laboratories of the Cotton Technology Research Division, Cotton Research Institute, Cairo, Egypt.

Evaluation of chemical properties

Fifteen plants were randomly taken at the blossoming stage (110 days after sowing) from each duplicate plots of each drought tolerance inducer treatment under DIW to determine the chemical properties, including proline content (mg g^{-1} fresh weight, FW), which was determined as reported by Peters et al. (1997). Carbohydrate, Chl. *a*, Chl. *b*, and total chlorophyll contents (mg g^{-1} leaf FW) were determined as reported by Hiscox & Israelstam (1979). In addition, total soluble sugars (mg g^{-1} FW) was estimated.

Statistical analysis

The obtained data from each split plot were statistically analyzed in three replications. For comparison of means was performed with least significance difference, with the overall significance level set at 0.05 according to Gomez & Gomez (1984). All the results were analyzed using MSTAT statistical software (ref)

Results and Discussion

The results of growth attributes, earliness, yield traits, fiber parameters, and chemical properties as affected by irrigation intervals, the application of drought tolerance inducers materials, and their interaction on the cotton “Giza 94” cultivar during the 2018 and 2019 seasons are shown in Tables 4 to 7.

Effects of irrigation intervals, drought tolerance inducers, and their interaction on cotton growth and earliness traits

Growth traits, including plant height and the number of fruiting branches per plant were significantly affected by irrigation intervals and drought tolerance inducers (Table 4). Plant height and the number of fruiting branches per plant decreased with prolonged irrigation intervals from 10 to 20 days by 5.0% and 14.4% in the 2019 season, and by 7.2% and 10.4% in the 2020 season, respectively. The reduction in plant height due to water deficit could be due to the irregularity in physiological processes induced by water deficit.

Earliness indicators, such as number of days to first flower or first open boll showed significant variations with irrigation intervals and drought tolerance inducers (Table 4). Prolonged irrigation intervals from 10 to 20 days exhibited fewer number of days to the first flower by 3.2% and 2.2% and number of days to first open boll by 4.8% and 2% in both the 2019 and 2020 seasons, respectively. Similarly, the position of the first node was significantly affected by irrigation intervals. In contrast, foliar application with drought tolerance inducers delayed the number of days to the first flower, number of days to first open boll, and the position of the first node compared to those of control treatment in the 2019 growing season. Foliar application of 400ppm GB showed the strongest effects on delayed number of days to first flower, number of days to first open boll, and the position of the first node by 6.1%, 3.3%, and 13.6%, respectively compared to the untreated (control) in the 2019 season. Foliar application of 100ppm chitosan exhibited the shortest number of days to first flower and the number of days to first open boll by 0.35% and 4.5%, respectively, while the control and foliar application with 100 and 300 ppm chitosan during the 2020 season generated the shortest position of the first node.

The interaction between irrigation intervals and drought tolerance inducers (Table 4) significantly affected the plant height in the 2019 and 2020 seasons, with GB foliar application at 400ppm under 10 days irrigation interval recording the highest plant height. In addition, the number of fruiting branches per plant and the position of the first node were significantly affected by the interaction between irrigation intervals and drought tolerance inducers in the two planting seasons. The combination of untreated (control) with a 20 day irrigation interval showed the best interaction that achieved the shortest number of days to the first flower, while the combination of 100ppm proline with a 20 day irrigation interval revealed the best interaction that generated the earliest number of days to the first open boll in the 2020 season. However, the interaction of this combination generated insignificant effects in the 2019 season.

Effects of irrigation intervals, drought tolerance inducers, and their interaction on seed cotton yield and its components

Irrigation intervals caused significant variations in the number of open bolls per plant, weight of bolls, weight of 100 seeds, lint percentage, and cotton yield per fed in the 2019 and 2020 seasons (Table 5). Extending the irrigation intervals from 15 to 20 days in both growing seasons, significantly reduced the number of open bolls per plant by 4.8% – 23.4%, and 2.2% –13.5%, boll weight by 7.2% –13.1%, and 7.1% –8.3%, seed index by 9.4% –13.6%, and 7.1% –19.5%, seed cotton yield per fed. by 6.1% –40.8%, and 5.9% – 36.1%, respectively, while lint percentage was significantly increased by 0.6% – 1.4% in the 2019, and 0.2% – 1.1% in 2020 compared with the irrigation interval of 10 days.

Drought tolerance inducers also significantly affected the number of open bolls per plant, weight of boll, weight of 100 seeds, lint percentage, and cotton yield per fed in the 2019 and 2020 seasons (Table 5). Seed cotton yield attributes were enhanced by drought tolerance inducers, while 400 ppm GB application enhanced the number of open bolls per plant by 24.2% and 19.5%, weight of boll by 23.6% and 13.8%, seed index by 16.8% and 12.5%, seed cotton yield per fed. by 20.8% and 21.7% compared with untreated (control) in both the 2019 and 2020 seasons, respectively. In contrast, the untreated plants showed the highest values of lint percentage in the 2019 and 2020 seasons.

Significant interaction between drought tolerance inducers and irrigation intervals on earliness treats was observed (Table 5). Data also showed that GB foliar application with an irrigation interval of 10 days gave the highest significant values for the number of open bolls per plant, boll weight, and weight of 100 seeds, in the 2019 and 2020 seasons. The highest value of lint percentage was obtained with cotton plants under 20 days irrigation interval in the 2019 and 2020 seasons. Evidently, the application of drought tolerance inducers mitigated the effect of prolonged irrigation intervals in cotton plants (Table 5). Foliar application with all drought tolerance inducers achieved higher values of the number of open bolls per plant, boll weight, weight of 100 seeds, and cotton yield per fed. under 15 days irrigation interval compared with untreated plants under 10 days irrigation interval in the 2019 and 2020 seasons.

The prolonged irrigation intervals negatively affected cotton yield components, with the highest reductions being observed with 20 days prolong irrigation intervals (Table 5), which was consistent with previously reported trends (Abdel-Kader et al., 2015; Eid et al., 2022). Yield is the result of integrated plant metabolic responses, thus, factors that affect metabolic action at any plant organ during development can antagonistically influence yield. Water deficit conditions can diminish yield characteristics, such as seed yield, the weight of 100 seeds, and lint percentage. Cotton yield was greatly affected by DIW conditions. Soil water deficit during the sensitive growth, blooming, flowering, and fruiting stages, can affect plant growth and yield (Abdel-Kader et al., 2015; Eid et al., 2022). This study showed that exogenous foliar supplementation with GB, proline, and chitosan could maintain the level of cotton yield components under DIW conditions, with GB application generating the strongest enhancement effects on cotton yield components, which was consistent with previously reported trends (Bhuiyan et al., 2019; Hamani et al., 2021; Shafiq et al., 2021). In addition, exogenous GB application enhanced plant growth and yield qualities, Chls, osmolyte concentration, total phenolics, ascorbic acid, and the activities of ROS scavenging enzymes, including SOD, peroxidase, and CAT, but decreased the leaf relative membrane permeability and malondialdehyde (MDA) concentration (Shafiq et al., 2021; Hamani et al., 2021).

TABLE 4. Effect of irrigation intervals, drought tolerance inducers and its interaction on cotton growth and earliness traits of Giza 94 cultivar at 2019 and 2020 seasons

Treat-ments irrigation intervals (A)	Drought tolerance inducers (B)	Growth traits				Earliness traits					
		Plant height at harvest (cm)		Number of fruiting branches / plant		Position of first node		No. of day to first flower		No. of day to first open boll	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
After 10 day	Control	133.33	144.33	12.66	12.33	6.00	6.33	55.00	58.66	130.00	133.00
	400ppm Glycine betaine	154.66	154.66	15.66	14.66	7.66	7.66	58.66	58.33	134.66	130.66
	200ppm Glycine betaine	148.33	151.66	13.33	14.00	7.33	7.33	58.00	57.33	134.33	130.33
	Proline 100ppm	143.33	148.66	14.66	14.00	7.00	7.00	57.33	56.66	133.66	130.00
	50ppm Proline	141.66	147.66	14.33	12.33	6.66	6.66	57.00	56.66	133.00	129.66
	300ppm Chitosan	136.66	145.33	13.66	13.00	6.33	6.33	56.66	56.33	132.66	128.00
	100ppm Chitosan	136.66	145.00	13.00	12.33	6.33	6.33	56.66	55.33	132.33	124.00
	Mean	142.09	148.19	13.90	13.23	6.76	6.81	57.04	57.04	132.95	129.38
After 15 day	Control	131.67	139.00	12.00	12.00	6.00	6.00	54.66	55.33	125.66	131.00
	400ppm Glycine betaine	148.33	151.66	15.33	14.00	7.33	7.33	57.33	58.00	130.00	130.33
	200ppm Glycine betaine	146.66	150.66	14.66	13.66	7.00	7.00	57.00	57.66	129.33	130.00
	Proline 100ppm	141.66	147.66	13.66	13.33	6.66	6.66	56.66	57.33	129.33	129.66
	50ppm Proline	140.00	146.33	13.33	13.00	6.33	6.33	56.00	56.66	127.66	129.33
	300ppm Chitosan	133.33	145.66	12.66	12.66	6.33	6.00	56.33	56.33	127.33	129.00
	100ppm Chitosan	133.33	144.66	12.33	12.33	6.00	6.00	55.00	56.66	125.66	128.66
	Mean	139.28	146.52	13.42	13.00	6.52	6.47	56.14	56.85	127.85	129.71
After 20 day	Control	129.66	131.33	10.00	10.33	6.00	6.00	53.00	54.33	124.66	129.00
	400ppm Glycine betaine	143.33	143.00	13.33	13.00	6.66	6.66	56.66	57.66	128.33	128.33
	200ppm Glycine betaine	143.33	141.66	13.00	12.66	6.66	6.66	56.00	56.66	127.66	128.00
	Proline 100ppm	138.33	140.00	12.66	12.33	6.33	6.33	56.66	56.00	127.33	124.00
	50ppm Proline	133.33	138.33	12.00	12.00	6.33	6.33	56.33	56.00	127.00	127.00
	300ppm Chitosan	128.33	135.00	11.33	11.66	6.00	6.00	54.33	55.00	125.66	126.33
	100ppm Chitosan	128.33	133.66	11.00	11.00	6.00	6.00	54.00	54.66	125.66	124.66
	Mean	134.95	137.51	11.90	11.85	6.28	6.28	55.28	55.76	126.61	126.76
General mean of drought tolerance inducers (B)	Control	131.56	138.22	11.55	11.55	6.00	6.11	54.22	56.11	126.78	131.00
	400ppm Glycine betaine	148.78	149.78	14.78	13.89	7.22	7.22	57.55	58.00	131.00	129.77
	200ppm Glycine betaine	146.11	148.00	13.67	13.44	7.00	7.00	57.00	57.22	130.44	129.44
	Proline 100ppm	141.11	145.44	13.66	13.22	6.66	6.66	56.89	56.66	130.11	127.88
	50ppm Proline	138.33	144.11	13.22	12.44	6.44	6.44	56.44	56.44	129.22	128.66
	300ppm Chitosan	132.77	142.00	12.56	12.44	6.22	6.11	55.77	55.89	128.55	127.78
	100ppm Chitosan	132.77	141.11	12.11	11.00	6.11	6.11	55.22	55.56	127.89	125.11
LSD at 0.5 for	A	0.21	0.77	0.60	0.45	N.S	N.S	0.45	0.88	0.77	0.73
	B	0.61	0.69	1.00	1.15	0.76	0.80	0.88	0.99	0.92	1.4
	AxB	1.06	1.19	N.S	N.S	N.S	N.S	N.S	1.7	N.S	2.5

TABLE 5. Effect of irrigation intervals, drought tolerance inducers and its interaction on yield and its components of Giza 94 cotton cultivar stages at 2019 and 2020 seasons

Treatments irrigation intervals (A)	Drought tolerance inducers (B)	No. of open bolls / plant		Boll weight (g)		Seed index (g)		Lint %		Seed cotton yield /fed	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
After 10 day	Control	15.00	14.00	2.29	2.27	9.43	9.89	39.05	39.05	7.75	8.54
	400ppm Glycine betaine	17.66	16.33	2.80	2.62	10.45	10.44	37.92	37.65	9.35	10.35
	200ppm Glycine betaine	16.33	16.00	2.65	2.55	10.53	10.38	38.00	38.00	9.04	10.04
	Proline 100ppm	16.00	15.66	2.59	2.41	10.33	10.36	37.75	38.32	8.41	9.40
	50ppm Proline	15.66	15.33	2.51	2.35	10.38	10.32	38.53	38.57	8.31	9.29
	300ppm Chitosan	15.33	14.67	2.44	2.32	9.72	10.31	38.13	38.70	7.92	8.89
	100ppm Chitosan	15.00	14.33	2.32	2.30	9.63	10.05	38.41	38.85	7.69	8.61
	Mean	15.85	15.`19	2.51	2.40	10.06	10.25	38.25	38.45	8.35	9.30
After 15 day	Control	13.00	13.66	2.09	2.11	8.25	8.46	39.20	39.11	6.96	7.65
	400ppm Glycine betaine	16.66	16.00	2.60	2.41	10.17	10.20	37.52	37.85	8.41	9.43
	200ppm Glycine betaine	16.33	15.66	2.55	2.37	10.20	10.12	37.75	37.81	8.32	9.30
	Proline 100ppm	15.66	15.33	2.41	2.22	9.93	9.90	38.40	38.51	8.17	9.12
	50ppm Proline	15.00	15.00	2.34	2.20	9.70	9.86	38.46	38.65	7.86	8.67
	300ppm Chitosan	14.66	14.33	2.19	2.19	9.33	9.11	38.90	38.75	7.39	8.35
	100ppm Chitosan	14.33	14.00	2.14	2.16	9.00	9.02	39.00	38.93	7.81	8.73
	Mean	15.09	14.85	2.33	2.23	9.11	9.52	38.46	38.51	7.84	8.75
After 20 day	Control	10.66	11.66	2.00	2.00	7.85	7.82	39.73	39.18	4.61	5.52
	400ppm Glycine betaine	13.66	14.66	2.39	2.21	9.20	8.81	38.23	38.33	5.59	6.62
	200ppm Glycine betaine	13.33	14.33	2.33	2.19	9.00	8.72	38.28	38.60	5.52	6.40
	Proline 100ppm	12.33	13.33	2.22	2.14	9.13	8.28	38.50	38.90	4.77	5.70
	50ppm Proline	12.00	13.00	2.20	2.10	8.95	8.15	38.76	38.95	4.65	5.59
	300ppm Chitosan	11.66	12.66	2.08	2.50	8.45	8.03	39.00	39.00	4.80	6.10
	100ppm Chitosan	11.33	12.33	2.06	2.30	8.26	8.00	39.13	39.10	4.66	5.70
	Mean	12.14	13.14	2.18	2.20	8.69	8.25	38.80	38.86	4.94	5.94
General mean of drought tolerance inducers (B)	Control	12.88	13.11	2.12	2.12	8.51	8.72	39.32	39.11	6.44	7.23
	400ppm Glycine betaine	16.00	15.66	2.60	2.41	9.94	9.81	37.89	37.94	7.78	8.80
	200ppm Glycine betaine	15.33	15.33	2.51	2.37	9.91	9.74	38.01	38.13	7.62	8.58
	Proline 100ppm	14.66	14.77	2.41	2.25	9.79	9.51	38.21	38.57	7.11	8.07
	50ppm Proline	14.22	14.44	2.35	2.21	9.67	9.44	38.58	38.72	6.94	7.85
	300ppm Chitosan	13.89	13.89	2.23	2.33	9.16	9.15	38.67	38.81	6.70	7.77
	100ppm Chitosan	13.55	13.55	2.17	2.25	8.96	9.02	38.84	38.96	6.72	7.68
LSD at 0.5 for	A	1.2	2.3	0.05	0.02	0.08	0.05	0.01	0.11	0.04	0.1
	B	1.4	1.5	0.05	0.02	0.13	0.06	0.02	0.12	0.05	0.12
	AxB	2.3	2.7	0.08	0.03	0.24	0.11	0.04	0.2	0.09	0.2

TABLE 6. Effect of irrigation intervals, drought tolerance inducers and its interaction on cotton fiber properties of Giza 94 at 2019 and 2020 season

Treatments Irrigation intervals (A)	Drought tolerance inducers (B)	Fiber length (mm)		Uniformity index		Fiber strength (g/tex)		Micronare Value	
		2019	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
After 10 day	Control	32.63	32.20	85.56	85.63	40.40	38.66	3.55	3.50
	400ppm Glycine betaine	34.20	32.93	86.83	85.43	42.16	42.33	3.38	3.84
	200ppm Glycine betaine	33.53	32.93	86.10	85.40	41.73	42.13	3.72	3.63
	Proline 100ppm	33.36	32.90	86.06	85.30	41.33	41.46	3.69	3.63
	50ppm Proline	33.60	32.36	85.93	85.20	41.10	40.10	3.67	3.62
	300ppm Chitosan	33.20	32.36	85.86	85.10	41.00	39.66	3.55	3.52
	100ppm Chitosan	32.63	32.30	85.80	85.03	40.86	39.13	3.55	3.51
	Mean	**33.31**	**32.57**	**86.02**	**85.30**	**41.22**	**40.50**	**3.59**	**3.61**
After 15 day	Control	32.10	31.83	84.93	84.23	37.96	37.93	3.42	3.36
	400ppm Glycine betaine	32.63	32.13	85.53	84.90	40.33	38.63	3.51	3.42
	200ppm Glycine betaine	32.43	33.06	85.53	84.80	40.20	38.46	3.50	3.41
	Proline 100ppm	32.43	32.03	85.30	84.63	39.86	38.36	3.47	3.40
	50ppm Proline	32.26	32.00	85.23	84.43	39.16	38.36	3.45	3.38
	300ppm Chitosan	32.70	32.66	85.16	84.43	38.20	38.00	3.43	3.37
	100ppm Chitosan	32.06	31.90	85.06	84.40	38.20	37.96	3.43	3.37
	Mean	**32.37**	**32.23**	**85.25**	**84.54**	**39.13**	**38.24**	**3.46**	**3.39**
After 20 day	Control	30.90	30.86	80.83	83.43	36.60	34.10	2.63	3.17
	400ppm Glycine betaine	31.46	31.80	84.66	84.23	36.20	36.73	3.41	3.35
	200ppm Glycine betaine	31.36	31.76	84.40	84.13	37.46	36.63	3.40	3.35
	Proline 100ppm	31.13	31.33	83.96	84.00	36.73	36.40	3.27	3.35
	50ppm Proline	31.90	31.30	83.93	83.93	36.73	36.20	3.25	3.27
	300ppm Chitosan	30.46	31.06	83.60	83.80	36.66	36.06	2.98	3.27
	100ppm Chitosan	30.83	31.06	83.16	83.63	36.60	35.90	2.78	3.19
	Mean	**31.15**	**31.31**	**83.51**	**83.88**	**36.71**	**36.00**	**3.11**	**3.28**
General mean of drought tolerance inducers (B)	Control	**31.87**	**31.63**	**83.77**	**84.43**	**38.32**	**36.90**	**3.20**	**3.34**
	400ppm Glycine betaine	**32.76**	**32.28**	**85.67**	**84.85**	**39.56**	**39.23**	**3.43**	**3.54**
	200ppm Glycine betaine	**32.44**	**32.58**	**85.34**	**84.77**	**39.80**	**39.07**	**3.54**	**3.46**
	Proline 100ppm	**32.31**	**32.08**	**85.11**	**84.64**	**39.31**	**38.74**	**3.48**	**3.46**
	50ppm Proline	**32.58**	**31.88**	**85.03**	**84.52**	**39.00**	**38.22**	**3.45**	**3.42**
	300ppm Chitosan	**32.12**	**32.03**	**84.87**	**84.44**	**38.62**	**37.91**	**3.32**	**3.39**
	100ppm Chitosan	**31.84**	**31.75**	**84.67**	**84.35**	**38.55**	**37.66**	**3.28**	**3.36**
LSD at 0.5 for	A	**0.54**	**0.25**	**0.55**	**0.40**	**1.1**	**2.4**	**0.14**	**0.11**
	B	**0.49**	**0.62**	**N.S**	**N.S**	**N.S**	**N.S**	**0.19**	**N.S**
	AxB	**N.S**	**N.S**	**N.S**	**N.S**	**N.S**	**N.S**	**0.33**	**N.S**

TABLE 7. Effect of irrigation intervals, drought tolerance inducers and its interaction on chemical properties on cotton at 2020 season

Treatments irrigation intervals (A)	Drought tolerance inducers (B)	Chlorophyll (mg g^{-1}fw)			Proline	Total soluble sugars (mg g^{-1}fw)
		a	b	Total		
After 10 day	Control	3.18	2.64	5.81	4.40	26.34
	400ppm Glycine betaine	4.16	2.18	7.34	11.71	29.03
	200ppm Glycine betaine	4.01	2.94	7.10	9.98	28.71
	Proline 100ppm	4.04	2.81	6.85	8.70	28.21
	50ppm Proline	3.76	2.73	6.69	7.93	27.92
	300ppm Chitosan	3.43	2.88	6.31	7.24	27.20
	100ppm Chitosan	3.14	2.78	5.92	6.55	26.86
	Mean	3.67	2.85	6.57	8.07	27.75
After 15 day	Control	2.38	2.43	5.26	15.98	29.48
	400ppm Glycine betaine	4.00	2.80	6.82	23.45	31.91
	200ppm Glycine betaine	3.58	2.76	6.34	21.67	31.25
	Proline 100ppm	3.24	2.81	6.20	20.93	30.74
	50ppm Proline	3.18	2.69	5.87	19.21	30.38
	300ppm Chitosan	3.12	2.53	5.65	19.03	30.17
	100ppm Chitosan	2.91	2.51	5.42	18.62	29.92
	Mean	3.26	2.64	5.93	19.84	30.55
After 20 day	Control	2.14	1.89	4.03	38.76	32.65
	400ppm Glycine betaine	3.03	2.57	5.60	44.32	34.18
	200ppm Glycine betaine	2.78	2.16	5.01	43.18	33.92
	Proline 100ppm	2.66	2.23	4.89	42.87	33.64
	50ppm Proline	2.57	2.04	4.61	41.54	33.25
	300ppm Chitosan	2.48	1.99	4.47	40.98	32.88
	100ppm Chitosan	2.29	1.95	4.24	19.61	32.79
	Mean	2.56	2.11	4.69	38.75	33.33
General mean of drought tolerance inducers (B)	Control	2.71	2.32	5.03	19.71	29.49
	400ppm Glycine betaine	3.73	2.85	6.58	26.49	31.70
	200ppm Glycine betaine	3.45	2.62	5.15	24.94	31.29
	Proline 100ppm	3.31	2.61	5.98	24.16	30.86
	50ppm Proline	3.17	2.48	5.72	22.89	30.51
	300ppm Chitosan	3.01	2.46	5.47	22.41	30.08
	100ppm Chitosan	2.78	2.41	5.19	14.92	29.85
LSD at 0.5 for	A	0.01	0.01	0.07	3.2	0.08
	B	0.01	0.01	0.2	3.6	0.12
	AxB	0.03	0.03	0.3	6.2	0.2

Effects of irrigation intervals, drought tolerance inducers, and their interaction on cotton fiber properties

DIW conditions showed significant gradual decrease in fiber length, uniformity index, fiber strength, and micronare values when the irrigation intervals were extended from 10 to 20 days (Table 6). The reductions in fiber length, uniformity index, fiber strength, and micronare values under 15 and 20 days irrigation intervals in the 2019 and 2020 seasons were 2.8%; 6.5%, 1.0%; 3.9%, 0.9%; 3.1%, 0.9%; 1.7%, 5.1%; 10.9%, 5.6%; 11.1%, 3.6%; 13.4%, and 6.1%; 9.1%, respectively. For osmoprotectants, 400ppm GB improved fiber length by 2.8% in the 2019 season and by 4.1% with 200ppm GB in the 2020 season relative to the untreated group. Similarly, 200ppm GB showed the highest significant values for micronare in the 2019 season. In contrast, the effects of drought tolerance inducers were insignificant for uniformity index and fiber strength in the 2019 and 2020 seasons, and for micronare in the 2020 season. For the interactions between irrigation intervals and osmoprotectants, insignificant effects were detected for all fiber properties except for micronare, which recorded the highest value after 200ppm GB application under 10 days irrigation interval in the 2019 season. The prolong of irrigation intervals caused significantly in the fiber properties under drought tolerance inducers tested in current study (Table 6) similar with (Abdel-Kader et al., 2015; Eid et al., 2022).

Effects of irrigation intervals, drought tolerance inducers, and their interaction on physio-chemical properties

Irrigation intervals caused significant gradual decreases in Chl. *a*, Chl. *b*, and total Chls., while proline application showed a significantly increasing trend in total soluble sugars with prolonged irrigation intervals between 10-, 15-, and 20 days in the 2020 season (Table 7). The reductions in Chl. *a*, Chl. *b*, and total Chls. were 11.2% and 30.3%, 7.4% and 26.0%, and 9.7% and 28.6% under both 15- and 20-days irrigation intervals, compared with 10 days irrigation interval. In contrast, proline and total soluble sugars increased by 145.9% and 380.2%, and 10.1% and 20.2% under both irrigation intervals of 15 and 20 days, respectively, compared with normal irrigation days (10 days).

For drought tolerance inducers, 400 ppm GB application caused a significant and progressive increase in Chl. *a*, Chl. *b*, total Chls., proline, and total soluble sugars (Table 7). Notably, foliar application of 400ppm GB significantly outperformed all other treatments on physicochemical traits, with Chl. *a*, Chl. *b*, total Chls., proline, and total soluble sugars by 41.6%, 36.0%, 39.0%, 14.3%, and 4.7%, respectively, relative to the contro treatment

Significant interaction between the effects of osmoprotectants with irrigation intervals was observed (Table 7). Results showed that 400ppm GB foliar application under 10 days irrigation interval had the highest values for chlorophyll and total Chls. In contrast, the highest Chl. *b* content was recorded with 200ppm GB application under 10 days irrigation interval. Interestingly, increased irrigation intervales caused an increase in proline and total soluble sugars accumulation in fresh leaves, and the highest contents were recorded with 400ppm GB application under 20 days irrigation interval. Foliar application with 200 and 400ppm GB or 50 and 100ppm proline caused more pronounced plant tolerance under prolonged irrigation intervals, as estimated by chlorophyll content, with higher values being observed under 15 days irrigation interval compared with the control under 10 days (Table 3).

The chlorophyll content showed a reduction in the two seasons under different water intervals. A previous study demonstrated that this could be due to the breakdown of chlorophyll pigments and related compounds (Bhuiyan et al., 2019). Chl *a*, Chl *b*, and total Chls. are key regulators of photosynthesis, which can be used as a positive predictor of cotton productivity (Eid et al., 2022). The foliar application of GB, proline, and chitosan improved chlorophyll pigments under DIW conditions. Similarly, foliar-applied GB in rapeseed was shown to improve the chlorophyll concentration under DIW conditions (Bhuiyan et al., 2019). The enhanced pigment concentrations is likely due to the role of GB and proline in protecting the photosynthetic apparatus, stabilizing the Rubisco structures, as well as plant cell membranes under DIW (Hamani et al., 2021; Shafiq et al., 2021). Moreover, GB has been demonstrated to improve the efficiency of photosynthetic machinery (Hamani et al., 2021; Shafiq et al., 2021)

Evaluation of the interrelationship among the assessed treatments and traits

Principal component analysis (PCA) was used to study the relationship among the assessed treatments and traits (Figs. 1, 2). The irrigation intervals and osmoprotectants were separated into three groups in the 2019 growing season (Fig. 1). The first two PCAs exhibited a total of 90.17% of the observed variability, with PCA1 accounting for 81.33% of the variation, and it was associated with the levels of assessed osmoprotectants under 10-, 15-, and 20-days irrigation intervals. The first group included 400 and 200ppm GB under 10- or 15-days irrigation intervals. The prominent traits in this group were the number of days to first flower, position of the first node, plant height at harvest, boll weight, seed index, and the number of fruiting branches per plant. The second group contained 50 and 100ppm proline under 10- or 15-days irrigation intervals, as well as 100 and 300ppm chitosan under 10- or 15-days irrigation intervals. The dominant traits in this group, included the number of days to the first open boll, the number of open bolls per plant, fiber length, uniformity index, fiber strength, micronare, and seed cotton yield per plant. The third group contained 100 and 300ppm chitosan under 15 days irrigation interval, and the group was dominated with lint percentage. PCA also classified the interaction between irrigation intervals and osmoprotectants into four groups in the 2020 growing season (Fig. 2). The first group contained glycine betaine 400ppm & 200ppm under irrigation interval 10 or 15 days and proline 100ppm under irrigation interval 15days. The obvious traits were No. of day to first flower, Position of first node, plant height at harvest, boll weight, open bolls No. plant^{-1}, fruiting branches No. plant^{-1}, total Chls. and Chl. *a*. On the other hand, the second included proline 100ppm under irrigation interval 10 days, proline 50ppm under irrigation interval 10 or 15 days, control and chitosan 100ppm or 300ppm under irrigation interval 10 days. The prominent traits were No. of day to first open boll, seed index, fiber length, uniformity index, fiber strength, micronare, Chl. *b* and seed cotton yield plant^{-1}. The third group included control under irrigation interval 15 or 20 days, chitosan 100ppm & 300ppm under irrigation interval 15days, chitosan 100ppm under irrigation interval 20 days, it was influential with lint%. The fourth group contained glycine betaine 400ppm & 200ppm, proline 50ppm & 100ppm and chitosan 300 under irrigation interval 20 days, the prominent traits were proline and total soluble sugars (Eid et al., 2022) estimated the PCA in cotton and found that there are response of cotton treats to Deficit irrigation water.

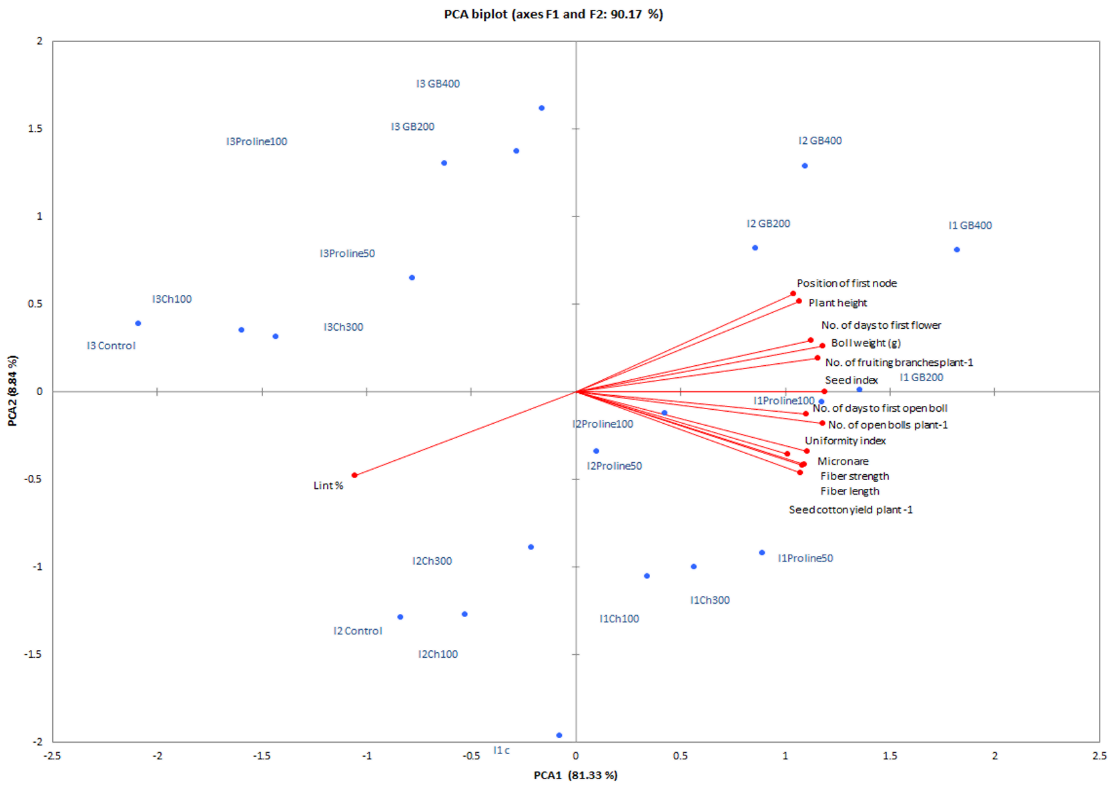

Fig. 1. PCA biplot for the assessed treatments of DW and drought tolerance inducers tested and the evaluated traits of cotton in 2019 season [Whereas: I1= Irrigation every 10 days, I2= Irrigation every 15 days , I3= Irrigation every 20 days, C= Control, GB 400= Glycine betaine 400ppm, GB 200= Glycine betaine 200ppm, P 100= Proline 100 ppm, P 50=Proline 50ppm, Ch100 =Chitosan 100ppm, Ch300 =Chitosan 300ppm]

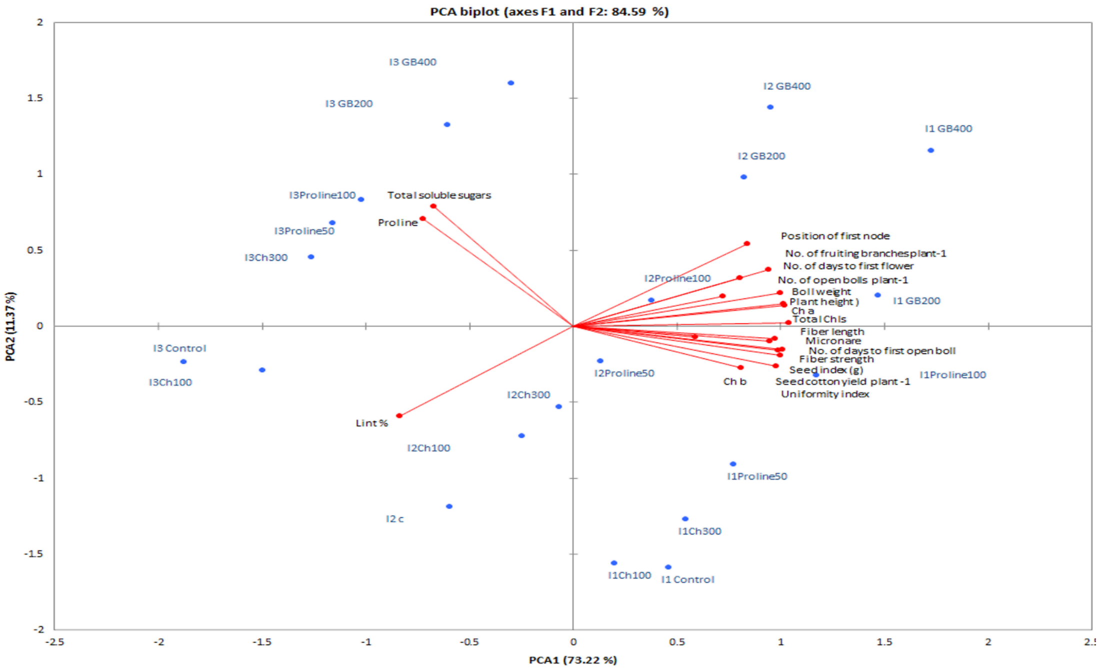

Fig. 2. PCA biplot for the assessed treatments of DW and drought tolerance inducers tested and the evaluated traits of cotton in 2020 season [Whereas: I1= Irrigation every 10 days, I2= Irrigation every 15 days , I3= Irrigation every 20 days, C= Control, GB 400= Glycine betaine 400ppm, GB 200= Glycine betaine 200ppm, P 100=Proline 100ppm, P 50=Proline 50ppm, Ch100 =Chitosan 100ppm, Ch300 =Chitosan 300ppm]

Conclusion

In summary, this study showed that the application of osmoprotectants, including GB, proline, and chitosan under prolonged drought intervals could mitigate the negative effects of drought, improve tolerance of cotton plants, and enhance the growth, earliness indicators, yield, fiber, and chemical properties. Overall, a 15-day irrigation interval coupled with the application of 400ppm GB was determined as the best treatment combination for good growth and high cotton productivity.

Competing interests: The authors report no conflicts of interest regarding this work.

Authors' contributions: ASE-D, MA-F, SAF-H, SA, and ME-t conceived and designed the experiments. ASE-D, MA-F, SAF-H, SA, and ME-t analyzed the data and drafted the manuscript. ASE-D, MA-F, SAF -H, SA, and ME-t wrote and edited the final manuscript. All authors read and approved the final version of the manuscript.

Ethics approval: Not applicable.

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تأثير مستحثات تحمل الجفاف على نمو وإنتاجية وبعض الصفات الكيميائية للقطن تحت اطالة فترات الري

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القطن هو ملك الالياف ومصدر الزيت والعلف .أجريت التجارب بمحطة بحوث النوبارية التابعه لمركز البحوث الزراعية بمحافظة البحيرة خلال موسمي 2019 و 2020 لدراسة تأثير استخدام مستحثات تحمل الجفاف (جليسين بيتاين، برولين وشيتوزان) تحت فترات الري (10، 15 و20 يوم) على النمو والإنتاجية والصفات الكيميائية لصنف قطن جيزة 94. أشارت البيانات إلى أن إطالة فترة الري أدت إلى انخفاض معنوي في النمو والمحصول ومكوناته، وطول التيله والمتانه وكلوروفيل أ، ب والكلوروفيل الكلي بينما زادت قيم البرولين والسكريات الكلية الذائبةً. أظهرت جميع مستحثات تحمل الجفاف زيادة معنوية في صفات النمو والمحصول ومكوناته والصفات الكيميائية حيث أعطت النباتات التي تم رشها بالجليسين بيتاين افضل القيم لهذة الصفات. كان لتفاعل فترات الري مع مستحثات تحمل الجفاف تأثير معنوي على صفات النمو والمحصول ومكوناته وصفات جودة التيله والصفات الكيميائية. استخدام مستحثات تحمل الجفاف يخفف من تأثير إطالة فترات الري على نباتات القطن. اعطت جميع مستحثات تحمل الجفاف مع فترة الري 15 يوم قيم أعلى للمحصول ومكوناته مقارنة مع فترة الري 10 أيام . الجليسين بيتاين بتركيز 400 جزء في المليون ادى لتحسين قدرة نباتات القطن على تحمل التأثير السلبي لإطالة فترات الرى.