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Research Article
Impact of Putrescine and 24-epibrassinolide on Growth, Yield and Chemical Constituents of Cotton (*Gossypium barbadense* L.) Plant Grown under Drought Stress Conditions

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Abstract

Background: Field experiment was carried during the two successive seasons (2014 and 2015) to study the effects of putrescine (Put) and 24-epibrassinolide (EBL) foliar applications on the growth, yield and chemical composition of Egyptian cotton (*Gossypium barbadense* L., cv., Giza 90) plants grown under drought stress conditions. **Materials and Methods:** The soil of the experimental site was clay loam and plants were grown under three irrigation regimes, i.e., 100% as control, 75 and 50% from well watering. Three different doses of Put (0, 1 and 2 ppm) and EBL (0, 10^{-9} and 10^{-7} M) were sprayed on plants 5 times started at the day 40 after planting (DAP) and repeated every 15 days. **Results:** Drought caused a significantly and gradually decreases in the growth characters, yield and its components and chemical compositions e.g., N, P and K concentrations and CAT activity by increasing drought level, while increased lint percentage, Na percentage, total sugars, total soluble phenols, total free amino acid and proline concentrations, antioxidant enzymes POX, PPO and SOD activities. Moreover, the mean values for Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) tended to be lower under drought stress especially 50% drought level compared to control non-stressed plant but its increased at 75% drought level between 60 and 90 DAP. Applications of Put and EBL positively affected cotton growth and yield under drought stress conditions. These treatments resulted an increase in morphological characters e.g., plant height, number of leaves/plant, leaf area/plant and total plant dry weight. Also, Put and EBL increase chemical constituents related to drought tolerance either inorganic (N, P, K and Na) or organic constituents e.g., total free amino acids, total sugars, total soluble phenols, antioxidant enzymes CAT, POX and SOD activities, while proline concentration, PPO activity as well as lint percentage were decreased. As a result of promoting growth induced by previous foliar applications, yield components e.g., number of open bolls, fiber length, fiber strength and micronaire value increased. **Conclusion:** Generally, Put at 2 ppm and EBL at 10^{-7} M applications recorded the highest values of growth and yield characters.

Key word: Cotton, drought, putrescine, 24-epibrassinolide, growth characters, antioxidant enzymes, plant growth analysis, RGR, NAR, fibers quality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Increasing scarcity of irrigation water is a principle threat to sustainable production of cotton *Gossypium* ssp[1]. Water-deficit stress is a major environmental factor limiting more than a third of the arable land around the world[2]. Drought is a common abiotic stress during the cotton growing season, which causes a series of negative effects on cotton growth, yield and fiber quality[3]. Cotton is dreadfully drought sensitive crop causing incentive reduction in yield, because drought stress is a complex phenomenon that affects the physiology of cotton plant[4]. Also, cotton is a very susceptible plant to the quantity of irrigation water and therefore, irrigation management is very complicated[5]. The flowering and boll-forming stage is the key yield determinant period of cotton plants. Water stress occurring during this stage will undoubtedly seriously affect cotton development and final productivity[6].

During the last decade, the foliar application of plant growth regulators and biomolecules, such as brassinosteroids and polyamine has become an established procedure in crop production to increase yield and quality of the crop under abiotic stresses as drought[7]. Brassinosteroids (BRs) are a class of steroidal plant hormones (class on new plant hormones) that play diverse roles in plant growth and developmental processes[8]. Brassinosteroids that were discovered more than thirty years ago but their physiological function has yet to be fully explained[9] but BRs play important roles in a wide range of developmental phenomena and recently they became an alleviation agent for stress tolerance in plants[10]. On the other hand, it is also evident that BRs interact with other phytohormones such as auxin, cytokinin, ethylene, gibberellin, jasmonic acid, abscisic acid, salicylic acid and polyamine in regulating wide range of physiological and developmental processes in plants[8]. Furthermore, brassinosteroids play important roles in the complex network of plant signal transduction that regulates plant growth and development. Field and greenhouse trials have shown that exogenous BRs can also improve plant tolerance to abiotic and biotic stress[11]. Also, brassinosteroids have been proposed to increase the resistance of plants to drought stress. In this respect, 24-epibrassinolide (EBL) as a type of brassinosteroids has a protective role of on chlorophyll content, the photochemical activity of photosystem 2, membrane lipids and proteins. The ameliorative effects by 24-epibrassinolide were closely associated with EBL-induced changes in anti-oxidative enzyme activities and antioxidant contents and they suggested that EBR could improve plant growth under drought stress[12].

Polyamines including spermidine (Spd), spermine (Spm) and putrescine (Put) are small ubiquitous nitrogenous compounds which are involved in several plant growth and developmental processes[13]. They are the recent additions to the class of plant growth regulators and also considered as a secondary messenger in signaling pathways[14]. Polyamines, putrescine, spermidine and spermine are implicated in plants responses under conditions of abiotic stress[15]. Exogenous application of putrescine improved tolerance against abiotic stress as drought[16]. Positive response of exogenously applied polyamines has been reported in cotton that's grown under drought and salinity stress conditions and found to enhance growth, yield and chemical composition under these stress conditions[7,17]. Accordingly, Loka *et al.*[2] mentioned that polyamines are endogenous plant growth promoters that affect a variety of physiological and metabolic functions and in many crops, there is a relationship between changes in polyamine metabolism and drought tolerance, also in cotton, that polyamines play an important role in protection its under adverse environmental conditions including salinity, high or low temperatures and drought and changes in their concentrations, especially putrescine (Put) and spermine (Spm) could be used as potential markers for selection of drought-tolerant cultivars.

The objective of this study was to investigate the effect of 24-epibrassinolide and putrescine on growth, yield and it constituents and chemicals components of Egyptian cotton (*Gossypium barbadense* L., cv., Giza 90) plants growing under different irrigation levels (drought stress).

MATERIALS AND METHODS

This experiment was carried out in the Agricultural Experiments and Research Station, Faculty of Agriculture, Cairo University, Giza governorate (30°02'N latitude and 31°13'E longitude with an altitude of 22.50 m a.s.l.), Egypt, as well as in the Plant Analysis Laboratory of the Plant Physiology Section, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive seasons (2014 and 2015) to determine the effects of putrescine and 24-epibrassinolide foliar applications on the growth, yield and chemical composition of Egyptian cotton (*Gossypium barbadense* L., cv., Giza 90) plants grown under drought conditions. The planting date for both seasons was 20th April and all experimental units were 4.0×2.4 m each units contained 4 rows (60 cm wide) and 25 cm between plants in the rows. A split plot design in randomized complete blocks (RCBD) arrangement with four replications was used in the two successive seasons. Main plots were devoted to the three irrigation regimes and sub-plots were assigned to treatments of five concentrations with 24-epibrassinolide and putrescine.

The soil of the experimental site was clay loam and plants were grown under three irrigation regimes, i.e., well-watering (100%) (Control treatment) where irrigation was applied every 14 days, 2nd irrigation regime was applied every 21 days (75% from well watering) and 3rd irrigation regime was applied every 28 days (50% from well watering). Each main plot was surrounded with a wide alley (3 m width) to avoid interference between the three irrigation water levels. Three different doses of putrescine (0 'tap water', 1 and 2 ppm) and 24-epibrassinolide (0 'tap water', 10^{-9} and 10^{-7} M) were sprayed 5 times started at the day 40 after planting and repeated every 15 days. In the both successive seasons three plant samples were taken at 60, 90 and 120 DAP and recorded the growth characters (plant height, number of leaves/plant, leaf area/plant and total plant dry weight) and chemical compositions of shoot at 90 DAP plant sample (total sugars, total soluble phenols, total free amino acids, proline concentrations, N, P, K and Na percentages). Antioxidant enzymes activities, catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO) and superoxide dismutase (SOD) were determined only at 90 DAP of 2nd season (2015). Moreover, from the dry weight and leaf area, the plant growth analysis between 60-90 DAP and 90-120 DAP were also recorded e.g., Relative Growth Rate (RGR) mg g^{-1} day^{-1} and Net Assimilation Rate (NAR) mg $(cm^2)^{-1}$ day^{-1}, according to the equation described by Hunt[18].

In the both successive seasons, the first pick of cotton yield was performed by hand, on October 18 (180 days after planting), while the second pick was on November 7 (210 days after planting), yield and its components and fibers quality were recorded e.g., number of open bolls, seed cotton yield/plant, lint percentage, fiber length, fiber strength and micronaire value.

Ethanol extract of fresh shoots was used for the determination of total sugars by using the phenol-sulphuric method according to DuBois *et al.*[19], total soluble phenols were estimated using the folin-ciocalteau colorimetric method[20] and total free amino acids were determined by using ninhydrin reagent according to Moore and Stein[21]. Free proline concentration was determined colorimetrically according to Bates *et al.*[22] in extraction of dry materials by using ninhydrin reagent.

Determinations of N, P, K and Na were carried out on the dry material. The wet digestion of 0.2 g plant material with sulphuric and perchloric acids was carried out on shoots as reported by Piper[23]. Nitrogen concentration was determined using the micro Kejeldahl apparatus of Parns-Wagner as described by Van Schouwenburg and Walinga[24]. Phosphorus was estimated calorimetrically by using the chlorostannous reduced molybdophosphoric blue color method according to Jackson[25]. Potassium and sodium concentrations were determined by using the flame photometer apparatus (GENWAY PFP-7).

Extraction of antioxidant enzymes: Cotton leaf tissue (0.5 g) was homogenized in 5 mL of 100 mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidine and 1 mM EDTA and then centrifuged at 15,000$\times$g for 10 min at 4°C. The supernatant was collected and used for determination of antioxidant enzyme activities.

The SOD (EC: 1.15.1.1) activity was determined by nitro-blue tetrazolium (NBT) photochemical assay following Dhindsa *et al.*[26]. Three milliliters of the reaction mixture contained 13.33 mL methionine, 75 mL nitroblue tetrazolium chloride, 0.1 mL EDTA, 50 mL phosphate buffer (pH 7.8), 50 mL sodium carbonate, 0.05 mL enzyme extract and 0.95 mL of water. The reaction was started by adding 2 mL riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal color, served as control. Switching off the light and placing the tubes in the dark stopped the reaction. A non-irradiated complete reaction mixture served as a blank. The absorbance was recorded at 560 nm (Mapada UV 1200) and 1 U of enzyme activity was taken as that amount of enzyme which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme.

Catalase (CAT, EC: 1.11.1.6) was measured according to the method described by Jaleel *et al.*[27] as follows: The assay mixture contained 2.6 mL of potassium phosphate buffer solution (50 mM, pH 7.0), 0.4 mL of H_2O_2 solution (15 mM) and 0.04 mL of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Peroxidase (POX, EC: 1.11.1.7) was assayed as described by Jaleel *et al.*[27] as follows: The assay mixture of POX contained 2 mL of phosphate buffer solution (0.1 M, pH 6.8), 1 mL of pyrogallol solution (0.01 M), 1 mL of H_2O_2 solution (0.005 M) and 0.5 mL of enzyme extract. The solution was incubated for 5 min at 25°C, after which the reaction was terminated by adding 1 mL of H_2SO_4 solution (1.25 M). The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of H_2SO_4 solution at zero time. The activity was expressed in U mg^{-1} protein. One unit is defined as the change in the absorbance by 0.1 min mg^{-1} protein.

Polyphenol oxidase (PPO, EC: 1.14.18.1) PPO activity was assayed by the method described by Liu *et al.*[28]. The standard reaction mixture contained 1.0 mL of 0.1 M catechol, 1.9 mL 0.1 M phosphate buffer (pH 7.0) and 0.1 mL of extract and the reaction mixture incubated for 10 min at 30°C. The increase in absorbance was measured at 420 nm with a spectrophotometer (UNICO UV-2000). The PPO activity was expressed as units in which one unit is defined as the change in absorbance of 0.001 min^{-1} mL^{-1} enzyme extract at 420 nm[29].

The combined analysis of the two growing seasons was done as the homogeneity test proved that both seasons followed a similar trend according to Steel and Torrie[30]. All statistical analysis was performed by using analysis of variance technique of MSTAT-C[31] computer software.

RESULTS AND DISCUSSION

Plant growth characteristics and plant growth analysis: All discussed data are the mean values of the two successive seasons. Plants subjected to drought stress at 75 and 50% comparing with optimum irrigation (100%) had a significant reduction in plant height, number of leaves/plant, leaf area/plant and total plant dry weight comparing with control non-stressed plants for all samples (at 60, 90 and 120 DAP) as data mentioned in Table 1. The most effective drought application in decreasing the plant growth characteristics were found under level 50% of drought, followed by level 75% drought and the 100% recorded the highest value of all characters of the three samples. These results are in

Table 1: Influence of foliar application with 24-epibrassinolide (EBL), putrescine (Put), drought stress and their interaction on plant height, number of leaves/plant, leaf area/plant and whole plant dry weight of *Gossypium barbadense* L., cv., Giza 90 at (60, 90 and 120) days after planting, combined analysis of the two successive seasons (2014 and 2015)

	60 DAP				90 DAP				120 DAP			
Irrigation spray	14	21 (days)	28	Mean B	14	21 (days)	28	Mean B	14	21 (days)	28	Mean B
Plant height (cm)												
C	55.13	51.75	46.38	51.08	87.13	77.13	61.63	75.29	102.63	85.50	69.25	85.79
Put 1	58.13	53.13	50.13	53.79	90.63	80.75	66.50	79.29	117.25	93.38	74.63	95.08
Put 2	62.00	58.13	51.13	57.08	94.63	84.75	71.75	83.71	125.38	102.88	80.13	102.79
EBL 1	62.75	58.50	52.00	57.75	92.88	84.13	68.75	81.92	122.75	96.25	81.25	100.08
EBL 2	65.25	61.25	54.13	60.21	100.38	88.13	73.50	87.33	133.63	106.75	85.75	108.71
Mean A	60.65	56.55	50.75		93.13	82.98	68.43		120.33	96.95	78.20	
LSD 5%	A = 2.08	B = 2.01	A×B = NS		A = 1.56	B = 1.49	A×B = NS		A = 2.69	B = 2.36	A×B = 4.09	
No. of leaves												
C	13.00	10.50	8.00	10.50	25.00	18.50	14.63	19.38	39.00	32.88	22.50	31.46
Put 1	15.13	11.38	8.25	11.58	29.88	20.88	15.63	22.13	41.50	33.75	25.25	33.50
Put 2	16.50	12.50	9.88	12.96	33.50	21.25	16.63	23.79	45.00	35.63	28.50	36.38
EBL 1	15.63	12.00	10.00	12.54	30.00	21.00	17.13	22.71	43.88	34.25	26.75	34.96
EBL 2	18.38	14.00	11.88	14.75	36.63	24.88	18.38	26.63	48.38	39.13	32.00	39.83
Mean A	15.73	12.08	9.60		31.00	21.30	16.48		43.55	35.13	27.00	
LSD 5%	A = 1.09	B = 1.09	A×B = NS		A = 1.22	B = 1.61	A×B = 2.79		A = 1.46	B = 1.67	A×B = NS	
Leaf area (cm^2)												
C	1251	939	473	888	1594	1153	693	1147	2536	2028	1383	1982
Put 1	1373	1018	644	1011	1770	1324	818	1304	2744	2253	1623	2206
Put 2	1518	1098	792	1136	2050	1383	1040	1491	2882	2354	1716	2317
EBL 1	1406	976	729	1037	1976	1364	997	1445	2892	2293	1699	2294
EBL 2	1940	1254	957	1384	2416	1487	1126	1676	3178	2415	1858	2484
Mean A	1497	1057	719		1961	1342	935		2846	2269	1656	
LSD 5%	A = 114.9	B = 180.3	A×B = NS		A = 80.52	B = 98.18	A×B = 170.10		A = 41.78	B = 77.68	A×B = 134.50	
Whole plant DW (g)												
C	11.76	7.88	5.24	8.29	34.28	24.57	12.66	23.83	78.54	48.65	31.87	53.02
Put 1	14.16	9.33	5.83	9.77	36.91	27.31	15.70	26.64	85.40	50.93	32.97	56.43
Put 2	13.80	9.82	6.51	10.04	39.21	27.61	17.74	28.19	91.60	55.73	36.05	61.13
EBL 1	14.72	9.94	6.36	10.34	37.55	26.63	16.26	26.81	90.25	52.84	36.35	59.81
EBL 2	18.06	11.97	7.86	12.63	43.81	30.41	21.21	31.81	105.47	66.08	39.71	70.42
Mean A	14.50	9.79	6.36		38.35	27.30	16.71		90.25	54.84	35.39	
LSD 5%	A = 0.35	B = 0.53	A×B = 0.92		A = 0.44	B = 1.03	A×B = NS		A = 2.21	B = 1.89	A×B = 3.28	

A: Irrigation, B: Spray, A×B: Interaction, C: Control agreement with the results of many researchers[7,32-35] on cotton plants, who mentioned that the plant height, root length, fresh and dry biomass, number of leaves per plant and total leaf area were found to be decreased under drought stress comparison to optimum irrigation plants. In this respect, Farooq *et al.*[36] stated that the drought severely affects plant growth and development with consequence reductions in the rate of cell division and elongation, leaf area, root and stem growth, interrupted stomatal conductance and water use efficiency, which makes photosynthesis very sensitive to drought. Furthermore, Farooq *et al.*[37] mentioned that the detrimental effects of drought on plant growth and development depend on the severity of stress and the crop growth stage. Also, the researchers stated that nutrients require water for uptake and translocation. As water supply decreases, nutrient uptake does.

For plant growth analysis e.g., RGR and NAR the data presented in Table 2 showed that RGR and NAR were decreased with increasing levels of drought stress from optimum irrigation to 50% drought level but its increased at 75% level of watering between 60-90 DAP and then decreased in 50% drought level and that might referred of the plants tried to ending its life cycle under drought in comparison to control unstressed plants. In this connection, Ahmed[38] reported on spinach plants grown under abiotic stress as nitrogen and phosphorus deficiency that RGR were lower under lowering levels of nitrogen and phosphorus compared to control. However, N and P deficiency plants had relatively high values of NAR at early stage of growth at N deficiency but for P deficiency the increased of NAR were recorded throughout whole course of growth. Also, the reseracher mentioned that the abiotic stress as nitrogen and phosphorus deficiencies were accompanied by a change in the chemical composition (organic and non-organic) of the different parts of spinach plants especially in the shoot and the researcher expected that plants under stress tend to terminate their life cycle as quickly as possible and these results in agreement with finding by Ahmed[39] on sweet pepper grown under potassium deficiency.

The individual foliar treatments of EBL and Put on cotton plants were significant increase of all growth characters were shown in Table 1 under the two levels of drought 50 and 75% as well as in control non-stressed plants, the individual treatment of 10^{-7} M of EBL and 2 ppm of Put recorded the highest values comparing with non-sprayed plants under all levels of irrigation. On the other hand, RGR and NAR were decreased when the plants sprayed with EBL and Put at two doses compared with control unsprayed plants and 2 ppm of Put and 10^{-7} M of EBL recorded the low levels of RGR and NAR in comparison to control unsprayed plants as showed in Table 2, it might be suggested that the increasing of plant dry weight related to increasing in leaf area per plant in comparison to increasing the weight in leaf area unit. Likewise, Shallan *et al.*[7] reported that foliar application of cotton plants under drought stress with putrescine caused enhancement of growth characters and that accepted with founding by Ahmed *et al.*[17] on cotton plants grown under salinity soil conditions. Moreover, Galston and Sawhney[40] added that the polyamines, Put, Spd and Spm have been shown to be involved in a variety of plant growth and developmental

Table 2: Influence of foliar application with 24-epibrassinolide (EBL), putrescine (Put), drought stress and their interaction on Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) of *Gossypium barbadense* L., cv., Giza 90, combined analysis of the two successive seasons (2014 and 2015)

	Relative growth rate ($_{1-2}$ RGR) mg g^{-1} day^{-1}				Relative growth rate ($_{2-3}$ RGR) mg g^{-1} day^{-1}			
Irrigation	14	21	28		14	21	28	
spray	(days)			Mean B	(days)			Mean B
C	35.66	37.91	29.40	34.32	27.63	22.77	30.77	27.06
Put 1	31.94	35.80	33.02	33.59	27.96	20.77	24.73	24.49
Put 2	34.81	34.46	33.42	34.23	28.28	23.41	23.64	25.11
EBL 1	31.22	32.85	31.29	31.78	29.23	22.84	26.82	26.30
EBL 2	29.54	31.08	33.09	31.24	29.29	25.87	20.90	25.35
Mean A	32.63	34.42	32.04		28.48	23.13	25.37	
	Net assimilation rate ($_{1-2}$ NAR) mg (cm^2)$^{-1}$ day^{-1}				Net assimilation rate ($_{2-3}$ NAR) mg (cm^2)$^{-1}$ day^{-1}			
	14	21	28		14	21	28	
	(days)			Mean B	(days)			Mean B
C	0.530	0.534	0.429	0.498	0.727	0.518	0.641	0.629
Put 1	0.485	0.515	0.452	0.484	0.728	0.451	0.490	0.556
Put 2	0.478	0.480	0.411	0.457	0.715	0.513	0.452	0.560
EBL 1	0.454	0.480	0.386	0.440	0.730	0.489	0.508	0.576
EBL 2	0.396	0.450	0.428	0.424	0.739	0.621	0.422	0.594
Mean A	0.469	0.492	0.421		0.728	0.518	0.503	

A: Irrigation, B: Spray, C: Control, ($_{1-2}$): 60-90 DAP, ($_{2-3}$): 90-120 DAP processes, including cell division, vascular differentiat ion, root initiation, shoot formation, flower initiation and development, fruit ripening and senescence and embryo format ion in tissue cultures. Also, Osman and Salim[41] showed spermidine has a positive effect on vegetative growth parameters under stressed and non-stress conditions, which reflects its importance in growth and development of plants under most environmental conditions. Increasing both Relative Water Content (RWC) and Membrane Stability Index (MSI) by Spd application under all stress levels to reach the highest values over all other treatments. On the other hand, El-Bassiouny and Bekheta[42] found that Put modulates ABA biosynthesis and stimulated growth by increasing auxin, gibberellins and cytokinins, which accompanied by ABA inhibitors content and activity reduction in response to abiotic stress.

Water deficiency significantly impaired the growth related traits in terms of maize plant height, leaves number per plant, total leaf area per plant, dry weight of shoot per plant, dry weight of root per plant, number of grains per plant and grain yield per plant. However, EBL and/or Spm foliar applications alleviated the detrimental effects of water stress and considerably increased the plant growth[43]. In this respect, Mousavi *et al.*[44] reported that 24-epibrassinolide alleviated the negative effect of water deficit on colza (*Brassica napus* L.) plant growth and significantly increased fresh and dry weight of plants. On the other hand, as other plant hormones, BRs are involved in a many of important vital processes i.e., cell division and elongation, synthesis of DNA, RNA and proteins, the growth and development of plant organs, senescence and stresses responses[45,46].

Yield characters and fiber technology: The yield and its components as well as fiber technology of cotton plants grown under different levels of drought and spraying with different levels of Put and EBL represented in Table 3. The results pointed out that number of open bolls per plants, seed cotton yield, fibre length, fibre strength and fineness (micronaire value) were decreased with increasing the level of drought from optimum irrigation 100-50% level, while lint percentage was increased. The obtained results are in

Table 3: Influence of foliar application with 24-epibrassinolide (EBL), putrescine (Put), drought stress and their interaction on number of open bolls, seed cotton yield/plant, lint percentage, fiber length, fiber strength and micronaire value of *Gossypium barbadense* L., cv., Giza 90, combined analysis of the two successive seasons (2014 and 2015)

	No. of open bolls per plant				Seed cotton yield per plant (g)			
Irrigation	14	21	28		14	21	28	
spray	(days)			Mean B	(days)			Mean B
C	15.25	13.50	9.88	12.88	36.17	28.56	17.01	27.24
Put 1	17.13	14.25	10.50	13.96	41.51	30.47	17.40	29.79
Put 2	18.50	15.25	12.50	15.42	47.64	36.04	23.04	35.57
EBL 1	17.63	14.63	11.63	14.63	43.86	33.00	20.19	32.35
EBL 2	19.38	16.75	13.63	16.58	51.75	40.39	26.19	39.44
Mean A	17.58	14.88	11.63		44.19	33.69	20.76	
LSD 5%	A = 0.610	B = 0.832	A×B = NS		A = 0.597	B = 0.770	A×B = 1.334	
	Lint percentage				Fiber length (mm)			
	14	21	28		14	21	28	
	(days)			Mean B	(days)			Mean B
C	35.55	37.25	38.55	37.12	29.26	28.11	27.35	28.24
Put 1	35.24	37.01	38.28	36.84	29.40	28.37	27.60	28.45
Put 2	34.96	36.62	37.95	36.51	29.95	29.00	27.81	28.92
EBL 1	35.13	36.83	38.20	36.72	29.56	28.66	27.65	28.62
EBL 2	34.70	35.73	37.61	36.01	30.48	29.37	28.22	29.35
Mean A	35.12	36.69	38.12		29.73	28.70	27.72	
LSD 5%	A = 1.025	B = NS	A×B = NS	A = 0.371	B = 0.673	A×B = NS		
	Fiber strength (g tex^{-1})				Micronaire value			
	14	21	28		14	21	28	
	(days)			Mean B	(days)			Mean B
C	35.58	34.41	30.76	33.58	4.07	3.93	3.82	3.94
Put 1	36.12	35.13	31.16	34.14	4.10	3.96	3.85	3.97
Put 2	36.63	35.68	33.36	35.22	4.15	4.05	3.90	4.03
EBL 1	36.49	35.35	31.77	34.54	4.10	4.00	3.83	3.97
EBL 2	36.96	36.05	33.60	35.53	4.20	4.11	3.96	4.09
Mean A	36.35	35.32	32.13		4.12	4.01	3.87	
LSD 5%	A = 0.486	B = 0.705	A×B = NS	A = 0.086	B = 0.111	A×B = NS		

A: Irrigatio, B: Spra, A×B: Interaction, C: Control harmony with those reported by Shallan *et al.*[7], Yagmur *et al.*[47], Mittal *et al.*[48] and Luo *et al.*[35] on cotton plants were grown under drought stress. In this respect, Lv *et al.*[6] stated, the flowering and boll-forming stage is the key yield determinant period of cotton plant. Water stress occurring during this stage will undoubtedly seriously affect cotton development and final productivity. Furthermore, Shamim *et al.*[49] reported that drought is the major limiting factors for fibre and lint quality after flowering and severe water losses can slowdown plant maturity, affecting bolls and ultimately reduce yield. Also, Mittal *et al.*[48] showed the delays in boll cracking and leaf senescence have the effect of extending the boll-filling period, which impacts fiber and seed quality positively. Extended flowering duration and boll filling can be an important trait for cotton growing under dry-land conditions, whereby plants can exploit late season precipitation for production and maturation of additional bolls but drought stress accelerated flowering and cracking of bolls cotton. On the other hand, stresses tend to induce early flowering through an elaborate network of floral signaling pathways[50]. In this connection, Soeda *et al.*[51] showed that plants under drought stress may alter the direction of metabolism process by accelerating the translocation process of sucrose from leaves to seeds and also accelerate the conversion process from sucrose to starch in seeds. As seeds have more starch content, they have advanced level of maturity. The matured seeds can survive under stress more than immature seeds. Also, Lecoeur and Guilioni[52] pointed out that moderate water stress in pea marks the beginning of the modification of the physiological status of plant tissues. Stomatal conductance falls with an increase in ABA content, reduces the size of all developing vegetative organs on the plant at the time of its occurrence and reduces the final number of reproductive branches.

Data in Table 3 revealed that the best concentrations of EBL and Put for the maximum values of most yield characters of cotton plants under drought conditions were 10^{-7} M of EBL and 2 ppm of Put, respectively under optimum irrigation as compared with the non-sprayed plants. That spraying cotton plants with (1 and 2 ppm) of Put and (10^{-9} and 10^{-7} M) of EBL under drought stress conditions increased yield characters, except lint percentage was decreased in compared to untreated plants under the same drought and normal condition. In this study, Shallan *et al.*[7] reported that cotton plants grown under drought stress with foliar application of putrescine caused amelioration of yield and its constitutes and that in good agree with results that reported on cotton plants grown under salinity soil conditions by Ahmed *et al.*[17]. Also, Nayyar *et al.*[53] reported that exogenous application of Put and Spd significantly enhanced soybean drought tolerance. Furthermore, Zulkarami *et al.*[54] on rice reported that the foliar application of polyamines at 35 and 55 days after sowing at glasshouse and 180 L ha^{-1} at the field conditions produced significantly higher grain filling and yield, even under conditions of water stress (increased by 51% in field trials and 41% in a greenhouse under cyclic water stress at 10 days intervals. However, polyamines are involved in various physiological activities in plants such as growth, senescence and stress responses[55] and polyamines reduce water stress in plants through osmotic adjustment[56]. Likewise, Ashraf *et al.*[57] demonstrated that the better yields of rice with the foliar application of polyamines are presumably due to their ability to maintain the turgor pressure of cells in water-stressed rice plants.

For the effect of BRs, under drought stress Salram[58] demonstrated that increased water uptake, membrane stability and higher CO_2 and nitrogen assimilation rates seemed to be related to homobrassinolide induced drought tolerance. Anjum *et al.*[59] concluded from working on maize plants grown under drought stress, that brassinolide application 0.1 mg L^{-1} partially improved the detrimental effects of drought by modulating the activity of enzymatic antioxidants and gas exchange traits, which helped in sustaining plant growth and yield. Furthermore, Mahesh *et al.*[60] also studied the effect of 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) on the germination and seedling growth of radish subjected to water stress and found that BR application ameliorated the inhibitory effect of water stress. The BRs positive effect on radish growth improvement under desiccation stress was associated with elevated levels of soluble proteins and nucleic acids accompanied by lowered RNase activities. Besides decreased fertility, most of the BR mutants also appear an extended life span and delayed senescence, while a typical wild-type arabidopsis plant senesces after appoximately 60 days, BR mutants can remain green and initiating new flowers well after 100 days[61]. On the other hand, Talaat *et al.*[43] on maize found, that the plant growth and its production were improved by EBL and/or Spm treatments in stressed and non-stressed plants. Under stressful conditions, maximum growth and yield attributes were recorded in plants treated with the dual application (25 mg L^{-1} Spm+0.1 mg L^{-1} EBL), which clearly reflect its positive role in drought tolerance.

Chemical compositions: Cotton plants growing under drought stress conditions, exhibited increase in the concentration of total sugars, total soluble phenol, total free amino acids and proline, also increased of Na percentage while N, P and K percentages were decreased compared with control non-stressed plants as shown data in Table 4. The

Table 4: Influence of foliar application with 24-epibrassinolide (EBL), putrescine (Put), drought stress and their interaction on total sugars, total soluble phenols, total free amino acids, proline concentrations, N, P, K and Na percentages in shoots of *Gossypium barbadense* L., cv., Giza 90 at 90 days after planting, combined analysis of the two successive seasons (2014 and 2015)

	Total sugars concentration in shoot (mg g^{-1} FW)				Total soluble phenols concentration in shoot (mg g^{-1} FW)			
Irrigation	14	21	28		14	21	28	
spray	(days)			Mean B	(days)			Mean B
C	12.25	15.69	20.37	16.10	2.15	2.43	2.58	2.39
Put 1	13.05	16.84	21.00	16.96	2.20	2.45	2.66	2.44
Put 2	15.56	20.07	25.05	20.23	2.35	2.56	2.83	2.58
EBL 1	13.99	16.91	23.54	18.15	2.23	2.49	2.76	2.49
EBL 2	17.93	22.29	27.89	22.70	2.56	2.65	3.10	2.77
Mean A	14.55	18.36	23.57		2.30	2.51	2.78	
LSD 5%	A = 0.582	B = 0.646	A×B = 1.119		A = 0.109	B = 0.142	A×B = NS	
	Total free amino acids concentration in shoot (mg g^{-1} FW)				Proline concentration in shoot (mg g^{-1} DW)			
	14	21	28		14	21	28	
	(days)			Mean B	(days)			Mean B
C	5.92	6.27	6.84	6.34	0.98	1.11	1.26	1.12
Put 1	6.01	6.45	6.95	8.14	0.93	1.04	1.19	1.05
Put 2	6.33	6.75	7.23	6.77	0.92	0.98	1.07	0.99
EBL 1	5.89	6.60	7.09	6.53	0.94	1.08	1.13	1.05
EBL 2	6.63	6.96	7.55	7.05	0.85	0.97	1.03	0.95
Mean A	7.15	6.61	7.13		0.92	1.03	1.13	
LSD 5%	A = 0.289	B = 0.309	A×B = NS					
	Nitrogen percentages in shoot				Phosphorus percentages in shoot			
	14	21	28		14	21	28	
	(days)			Mean B	(days)			Mean B
C	4.42	4.14	3.05	3.87	0.319	0.298	0.239	0.285
Put 1	4.42	4.18	3.09	3.89	0.323	0.307	0.246	0.292
Put 2	4.63	4.25	3.27	4.05	0.347	0.325	0.265	0.312
EBL 1	4.51	4.18	3.19	3.96	0.324	0.307	0.255	0.295
EBL 2	4.80	4.28	3.28	4.12	0.363	0.344	0.273	0.327
Mean A	4.55	4.20	3.17		0.335	0.316	0.255	
	Potassium percentages in shoot				Sodium percentages in shoot			
	14	21	28		14	21	28	
	(days)			Mean B	(days)			Mean B
C	3.40	3.15	2.73	3.09	0.28	0.33	0.39	0.33
Put 1	3.42	3.21	2.79	3.14	0.25	0.35	0.41	0.33
Put 2	3.47	3.33	2.92	3.24	0.24	0.39	0.46	0.36
EBL 1	3.48	3.25	2.79	3.17	0.25	0.34	0.44	0.34
EBL 2	3.61	3.29	3.00	3.30	0.23	0.42	0.50	0.38
Mean A	3.47	3.24	2.84		0.25	0.36	0.44	

A: Irrigation, B: Spray, A×B: Interaction, C: Control

highest values of total sugars, total soluble phenol, total free amino acids, proline and Na percentage were recorded on 50% drought level, while the lowest at control plants grown under optimum irrigation but in contrast for N, P and K percentage. These results were in agreement with that finding by Lv *et al.*[6], Shallan *et al.*[7], Zhang *et al.*[34], McWilliams[62] on cotton plants and Neseim *et al.*[63] on sugar beet plant. They reported that the total sugars, total soluble phenol, total free amino acids, proline and Na concentrations increased significantly in response to drought treatment compared to control unstressed plants, while N, P and K concentrations were decreased. Under drought conditions, plants alter metabolic and physiological function to minimize negative impacts and maximize survival[64]. In this respect, Farooq *et al.*[37] stated that low-molecular-weight osmolytes, including glycinebetaine, proline and other amino acids, organic acids and polyols also play vital roles in sustaining cellular functions under drought. Furthermore, Jungklang *et al.*[65] mentioned that osmotic adjustment is a factor of physiological machinery when plants respond to water-deficit or salinity stresses and the accumulation of proline in leaves of plants could possibly play a protection role apart from osmoregulation during drought stress. Moreover, sodium can also to some extent, replace K in its role as osmoticum[66]. Another way in which cotton plants seemingly attempt to cope with drought stress is through the active accumulation of inorganic such as potassium and calcium to lower the osmotic potential in a process called osmotic adjustment that helps cells retain water and maintain turgor pressure[67]. Also, it might be suggested that, drought reduces nutrient uptake among which N, P and K by roots and their transport from roots to shoots because of restricted transpiration rates, impaired active transport and membrane permeability[68]. From a mentioned above, it can be suggested that plant tried to growth under drought stress by increasing organic and inorganic solutes and these solutes play a vital role to maintain turgor of plant cells and that related to continuous metabolism in plants and that in good agree with Helal *et al.*[68], who stated plants may increase its drought tolerance by decreasing osmotic potential by accumulation of solutes, which allows cell enlargement, plant growth, keeps open stomata and CO_2 assimilation under water stress. Accordingly, Giri[69] stated under environmental stresses such as drought, plants accumulate many of low molecular weight water-soluble compounds, which are known as compatible solutes, osmolytes or osmoprotectants, which decrease the cell water potential without decreasing actual water contents. The most common compatible solutes are betaines (glycine betaine, as the original betaine), soluble sugars (sucrose, trehalose, mannitol and sorbitol), polyamines, proline and amino acids. Also, Farooq *et al.*[37] found that these compatible solutes not only maintain the turgor pressure within cells but also protect the enzymes and macromolecules from oxidation by ROS. Also, Marschner and Marschner[70] mentioned that K plays essential roles in enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance.

Exogenous application BRs or Put to stressed and non-stressed cotton plants caused further increase in the ions and osmoprotectants e.g., total sugars, total soluble phenol, total free amino acids, N, P, K and Na compared to control untreated plants, while proline concentration was decreased. The 10^{-7} M of EBL and 2 ppm of Put recorded the highest values of all above characters with absence or presence of drought stress as data showing in Table 4. These results in harmony of findings by Shallan *et al.*[7] on cotton, Zeid *et al.*[71] on desi ajwain and Talaat *et al.*[43] on maize plants, who reported that foliar application of polyamines increasing the protectants with under all levels of drought stress and that might improve tolerance plant to water deficit stress. In this respect, Liu *et al.*[72] on two wheat cultivars treated with PEG, found that PAs conjugated to tonoplast vesicles correlated with the maintenance of tonoplast H^+-ATPase and H^+-PPase activities in roots as well as with the improved osmotic stress tolerance of plant.

Similarly, Mousavi *et al.*[44] reported that electrolyte leakage and lipid peroxidation significantly decreased when stressed colza (*Brassica napus* L.) plants were preliminary treated with 24-epibrassinolide, which showed that less oxidative damage occurred in pretreated plants under water stress conditions. Further EBL alleviated the negative effect of water deficit on plant growth and significantly increased fresh and dry weight of plants. 24-epibrassinolide application considerably improved ion uptake by plants, as well as accumulation of osmolytes like proline and reduced sugars. The results showed that EBL ameliorated the water stress effects and increased the plant tolerance and BR application could be used to improve crops in harsh conditions[44]. Also, Mahesh *et al.*[60] reported that 24-epibrassinolide and 28-homobrassinolide enhanced the accumulation of the osmolyte free proline in radish seedlings challenged with drought stress. Furthermore, Talaat *et al.*[43] demonstrated in maize plants, besides antioxidants and antioxidant enzymes, certain compatible solutes are actively involved in water stress amelioration. That drought conditions increased proline and glycinebetaine accumulation. This phenomenon may be a part of a mechanism that prevents loss of water in the plant through osmotic adjustment. Moreover, EBL and/or Spm application as individually or dual to water-stressed plants significantly induced a burst of these organic solutes. Also, Farooq *et al.*[73] exposed rice plant grown under water stress to EBL (0.01 µm) and Spm (10 µm) as foliar spray, they reported while drought stress enhanced the H_2O_2, malondialdehyde (MDA) and relative membrane permeability, foliar spray of EBL and Spm improved growth possibly because of the improved carbon assimilation, enhanced synthesis of metabolites and maintenance of tissue water status.

Todorova *et al.*[74] concluded evidence has been accumulated that foliar or root application of PAs and/or BRs, as well as seed priming with these chemicals, benefited growth because of the improved carbon assimilation, enhanced synthesis of metabolites and maintenance of tissue water status under drought stress conditions. Additionally, a reduction in the levels of stress markers was also noted in plants treated with PAs and BRs.

The results in Fig. 1 revealed that drought stressed cotton plants 50 and 75% had higher record in POX, PPO and SOD activities than those optimum irrigation, while CAT lower than this optimum irrigation. These results agree with the results mentioned by Vardhini *et al.*[75] on sorghum seedlings,

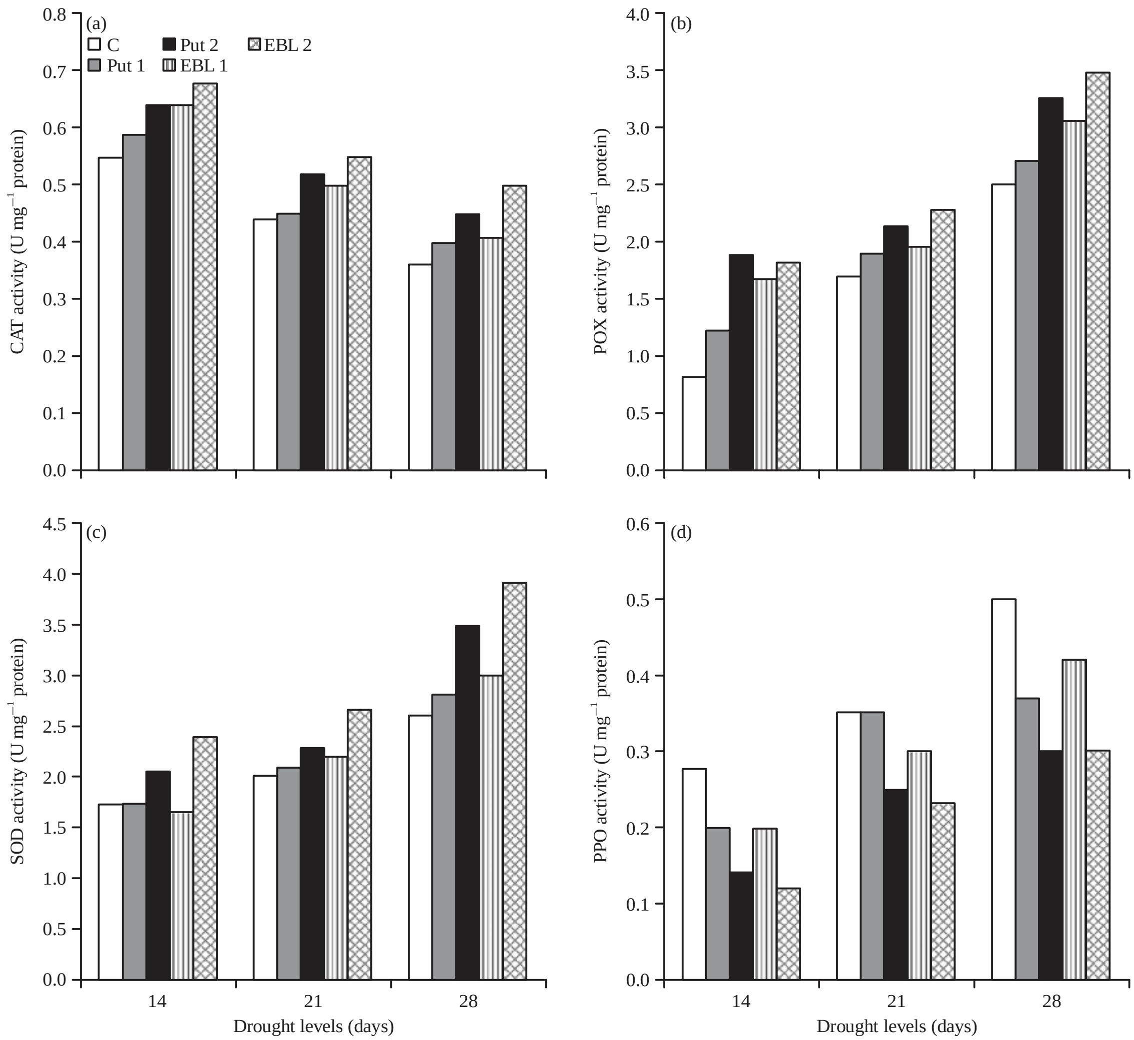

Fig. 1(a-d): Influence of foliar application with 24-epibrassinolide (EBL), putrescine (Put), drought stress and their interaction on antioxidant enzymes activities, (a) Catalase (CAT), (b) Peroxidase (POX), (c) Superoxide dismutase (SOD) and (d) Polyphenol oxidase (PPO) of *Gossypium barbadense* L., cv., Giza 90 leaves at 90 days after planting of the second seasons (2015)

Anjum *et al.*[76] on maize, Shallan *et al.*[7] on cotton plants, Osman[77] on pea plants and Shahana *et al.*[78] on pigeon pea seedlings who found the drought stress reduced catalase activity, while increased peroxidase, superoxide dismutase and polyphenol oxidase activities in comparison with non-stressed plants as control. In this respects, Gill and Tuteja[79] stated various abiotic stresses lead to the overproduction of Reactive Oxygen Species (ROS) ($O_2^{\cdot -}$, superoxide radicals, OH^-, hydroxyl radical, $HO_2^{\cdot}$, perhydroxy radical and $RO^{\cdot}$, alkoxy radicals, H_2O_2, hydrogen peroxide and $^1O_2$, singlet oxygen) in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress. However, Verhagen *et al.*[80] reported that plants are able to protect their tissue from the harmful effects of drought-accumulated ROS using enzymes such as SOD, CAT and POX.

Exogenous application of EBL or Put enhanced the SOD, POX, PPO and CAT under drought as well as well watered control. The highest values of the enzymes activities of all studied enzymes were recorded at 10^{-7} M of EBL and 2 ppm of Put dose compared to other doses of the two materials. In this connection, Shallan *et al.*[7] on cotton plants, stated that catalase and peroxidase are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals ROS and that the foliar application of Put to cotton plants under drought conditions increased the activities of catalase and peroxidase in comparison with control plants non-sprayed plants. Furthermore, Ahanger *et al.*[81] reported that, polyamines serve as messengers of stress signals. As a result of acid neutralizing and antioxidant capability, polyamines show anti-senescence, anti-stress effects and membrane and cell wall stabilizing abilities. Exogenous application of polyamines has been suggested as an effective approach for enhancing stress tolerance of crops and crop productivity as well. Moreover, Talaat *et al.*[43] working on maize stated, that dual application of (25 mg L^{-1} Spm+0.1 mg L^{-1} EBL) alleviated the detrimental effects of drought on the electrolyte leakage. Activities of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase and levels of ascorbate, glutathione, proline and glycinebetaine were increased in response to drought treatments as well as foliar applications. Dual application significantly alleviated drought-induced inhibition in the activities of monodehydroascorbate reductase and dehydroascorbate reductase as well as in the ratios of AsA/DHA and GSH/GSSG. Overall, dual application improved the plant drought tolerance and decreased the accumulation of ROS by enhancing their scavenging through elevation of antioxidant enzymes activity and improving the redox state of ascorbate and glutathione.

For EBL and drought stress, the results were in harmony with those fining by Vardhini *et al.*[75] on sorghum seedlings and Shahana *et al.*[78] on pigeon pea seedlings, they reported that drought stress induced hydrogen peroxide production, lipid peroxidation and electrolyte leakage was significantly counteracted by both EBL and HBL treatments. Supplementation of EBL and HBL enhanced the activities of antioxidative enzymes viz., catalase, peroxidase, superoxide dismutase glutathione reductase and ascorbate peroxidase in both unstressed and drought stressed seedlings, while decreased the PPO activity. Also, Semida and Rady[82] resulted that EBL treatment of seeds further increased these enzyme activities in the presence of abiotic stress as NaCl, compared to the controls non-treated plants. Even in the absence of abiotic stress, EBL increased SOD, CAT and POX enzyme activities compared to the controls non-treated plants. Additionally and in context, Behnamnia *et al.*[83] found that lipid peroxidation and H_2O_2 content were also reduced by 24-epibrassinolide treatment of the water-stressed tomato plants. So, the researchers concluded that EBL application mitigated water stress damages, also, an increase of the content of non-enzymatic antioxidants as ascorbate, carotenoids and proline accompanied with an increase in the activity of antioxidant enzymes catalase, superoxide dismutase and ascorbate peroxidase was observed in EBL-treated tomato plants subjected to drought stress and same results reported on radish by Mahesh *et al.*[60]. Also, Farooq *et al.*[73] exposed rice plant grown under water stress to EBL (0.01 µm) and Spm (10 µm) as foliar spray, they reported that drought tolerance was sturdily associated with the greater tissue water potential, increased synthesis of metabolites and enhanced capacity of antioxidant system and foliar spray with Spm was the most effective followed by BR.

From the above mentioned results it can be suggested that, EBL or Put induced all antioxidant enzyme activities in cotton plants under drought conditions, which may be related to the induction of antioxidant responses enzymatic and non-enzymatic that protect the plant from oxidative damage and that caused protect the membrane and vital organisms from ROS and prevent plant from osmotic and oxidative stress with presence of water deficit. Consequently that well maintained the plant cells turgor and that caused progressive growth and finally yielding of cotton plants under stress.

CONCLUSION

It can be concluded that the exogenous application of EBL or Put to cotton plant resulted in improvements of growth and yield characters and increasing of total soluble sugars, proline, total free amino acids, total soluble phenols, N, P, K, Na and antioxidant enzyme, SOD, POX, CAT and PPO activities during water stress as compared to control untreated plants, especially that EBL at 10^{-7} M and Put at 2 ppm dose.

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