American-Eurasian J. Agric. & Environ. Sci., 12 (9): 1252-1265, 2012
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2012.12.09.1902

Effect of Sodium Niroprusside, Putrescine and Glycine Betaine on Alleviation of Drought Stress in Cotton Plant

[1]Magdy A. Shallan, [1]Hazem M.M. Hassan, [2]Alia A.M. Namich and [2]Alshaimaa A. Ibrahim

[1]Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Cotton Research Institute, Agricultural Research Center, Giza, Egypt

Abstract: Drought stress is one of the major abiotic stresses in agriculture worldwide. This study was carried out to investigate the effect of sodium niroprusside (SNP), putrescine (Put) and glycine betaine (GB) on alleviation of drought stress in cotton plant. The cotton plants pre-treated with three concentrations of SNP (0.05, 0.1 and 1 m*M*), Put (200, 400 and 600 ppm) or GB (400, 600 and 800 ppm) then exposed to drought stress. In general, the drought stress reduced the growth characters, yield characters, pigments, total soluble sugars, total free amino acids, total phenols, total soluble proteins and catalase activity, while increased proline content, total antioxidant capacity and peroxidase activity in comparison with control. The results showed that pretreatment of cotton plants under drought stress with SNP, Put or GB caused enhancement of growth and yield characters and increasing of pigments content, total soluble sugars, proline content, total free amino acids, total phenols, total soluble proteins, total antioxidant capacity and antioxidant enzyme activities. The optimum concentration of SNP, Put and GB to alleviate the drought stress in cotton plant was 0.05 m*M*, 600 ppm and 800 ppm, respectively. Finally, it can be concluded that foliar application of SNP, Put or GB could improve the drought tolerance of cotton plants.

Key words: Drought stress · Cotton · Sodium niroprusside · Putrescine · Glycine betaine

INTRODUCTION

Cotton crop has been associated with ancient civilizations, which has contributed greatly to the industrial and economic development of many countries. Cotton is the most valuable major cash crop. The need for cotton products have ensured its survival as one of the world's most widely cultivated crop, despite the stiff competition it faces from man-made fibers. Cotton is the most important fiber crop of the world [1, 2]. Egyptian cotton (*Gossypium barbadense* L.) is the most important commercial fiber crop in Egypt. Cotton plays a key role in the economic activity. It is the oldest among the commercial crops and is regarded as white gold. Egyptian cotton is preferred around the world because it is long fiber cotton that makes it softer and stronger at the same time [3, 4].

Drought is the most severe abiotic stress factor limiting plant growth and crop production. When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) are produced. However, under various abiotic stresses the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages [5]. One of the main reasons why environmental stress inhibits growth and photosynthetic abilities of plants is the breakdown of the balance between the production of reactive oxygen species (ROS) and the antioxidant defense [6]. These activated oxygens injure the cellular components of proteins, membrane lipids and nucleic acids [7]. To mitigate and repair damage initiated by ROS, plants have developed a complex antioxidant system [8]. Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation [9].

During the last decade, the foliar application of plant growth regulators and biomolecules has become an established procedure in crop production to increase yield and quality of the crop under abiotic stresses. Nitric oxide (NO) is as mall and lipophilic gas and a bioactive molecule

Corresponding Author: Hazem M.M. Hassan, Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

that play an important role in different physiological processes. NO can also mediate plant growth regulators and ROS metabolism and increasingly evident have shown, which is involved in signal transduction and responses to biotic and abiotic stress such as drought, low and high temperatures, UV and ozone exposure, heavy metal, herbicides, cold and salt stress [10-12]. Applications of NO donors (sodium nitroprusside, SNP) enhance plant tolerance to specific stresses [13, 14]. For example, SNP (0.1 m*M*) delays the senescence of wheat leaves by inhibition of the degradation of chlorophyll and soluble proteins [15]; promotes the germination of wheat seeds under osmotic stress by improving antioxidant capacity [16]; decreases heavy metal induced oxidative damage in many plants [17,18], NO is associated with induced plant tolerance to salinity [19], moreover, it has been proved that NO can ameliorate UV-induced damage by lowering H_2O_2 content and ion leakage and enhancing the activities of scavenging enzymes [20,21].

Polyamines including spermidine, spermine and putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and developmental processes [22]. They are the recent additions to the class of plant growth regulators and also considered as a secondary messenger in signaling pathways [23]. Polyamines are involved in abiotic stress tolerance in plants [24]. Increased polyamines level in stressed plants are of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss and stimulation of protein, nucleic acid and protective alkaloids [25]. Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions [26]. Polyamines also protect the membranes from oxidative damages as they act as free radical scavengers [27, 28]. Transgenic plants with over production of polyamines are reported to display better stress tolerance than their wild counterparts [23]. Exogenous application of polyamines improved tolerance against several abiotic stresses [29, 30]. Positive response of exogenously applied polyamines has been reported in olive, rice, soybean, alfalfa and pomegranate [24, 25, 31-33]. Polyamines were found to enhance productivity in wheat under water stress conditions [34].

Glycine betaine is a quaternary ammonium. It is nontoxic, highly water soluble and readily absorbed in various tissues [35]. GB is naturally biosynthesized under stressful conditions [36]. Efforts are underway to metabolically engineer plants with enhanced capacity to accumulate GB [37]. Glycine betaine, a member of the effective compatible solutes, is involved in higher plants as a defensive response to extreme conditions of salt, drought, temperature or light stress [38, 39]. Possible mechanisms for the GB-enhanced tolerance of plants to various types of abiotic stresses include the protection of the photosynthetic machinery [40], enhanced photosynthetic rate [40-42], induction of specific genes involved in stress tolerance, reductions in levels of reactive oxygen species (ROS) under stress and regulation of the activities of ion-channel proteins either directly or via protection of the plasma membrane [43]. It has also destabilizing effects on photorespiration [44]. Significant advances have been made in mitigating the inhibitory effects of environmental stresses by exogenously applied glycine betaine in different crops such as rice [45], cotton [46], sunflower [47], wheat [42], maize [48] and soybean [49].

The present study aims to investigate the influence of sodium niroprusside, putrescine and glycine betaine on growth characteristics, Yield and chemical constituents of cotton plant under drought stress.

MATERIALS AND METHODS

Materials

Plant Materials: Cotton (*Gossypium barbadense* L. cv. Giza 90) seeds were obtained from the Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Giza, Egypt.

Chemicals: Sodium nitroprusside was purchased from Sigma Chemical Co., USA. Putrescine was purchased from Acmatic Co., Egypt. All other chemicals were of analytical reagent grade.

Methods

Experimental Design and Treatments: A pot experiment was conducted in summer season 2011 at wire green house of Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Giza, Egypt. This experiment was carried out to study the effect of spraying cotton plants with different concentrations of sodium nitroprusside (0.05, 0.1 and 1 m*M*), putrescine (200, 400 and 600 ppm) and glycine betaine (400, 600 and 800 ppm) on growth, yield and chemical composition of cotton plants under drought conditions. Seeds of cultivar Giza 90 were sown in clay loam soils on 12th April 2011 and plants were thinned to leave two plants per pot, 40-cm in diameter containing 16 kg of soil taken from

the upper 10-cm of the Agricultural Experimental Station Farm of the Agriculture Research Center, Giza. All pots received an adequate amount of fertilizer in order to produce healthy plants. Fertilization was carried out according to recommendation of Cotton Research Institute, each of pot before sowing received 2.2 g calcium superphosphate (15.5% P_2O_5) and 1.1 g potassium sulphate (48% K_2O). After 30 days planting each pot received 2.0 g urea (46% N). Irrigation was carried out regularly at the plant needs using tap water until the start of flowering stage, then the pots preventing water supply for 10 days till the appearance of sing of permanent wilting (drought stress) to take samples and back to irrigation plants. Plants were sprayed with sodium nitroprusside, putrescine and glycine betaine at start of flowering stage and the untreated pots (control) were irrigated with tap water continuously.

Plant Samples: Plant samples (whole plant and leaves) were taken at flowering stage (60 days from sowing) during the experimental period. In this stage, 4 plants were taken from each treatment (2 pots). The soil particles were washed off the roots by a stream of tap water. At harvest stage (180 days after sowing), samples from four pots were taken.

Growth Characteristics: Four plants were randomly taken after flowering stage to determine the growth characteristics, including plant height (cm), number of main stem nodes/plant, inter-node length (cm), number of fruiting branches/plant, root/shoot ratio, dry weight of stem and branches, roots, leaves and square parts (g) and leaf area (cm^2). Leaf area was determined by leaf area meter Model L1-3100.

Yield and its Components: Yield and its components, including number of flowers/plant, number of open bolls/plant, boll setting (%), boll weight (g), lint percentage, seed index (g) and seed cotton yield/plant (g) were recorded.

Chemical Analysis: Cotton leaves were taken randomly after flowering stage to determine the chemical analysis as follows:

Determination of Pigment Content: The chlorophyll a, b and total chlorophyll were determined according to the method of Arnon [50] and carotenoids using the method of Robbelen [51].

Determination of Total Soluble Sugars: Total soluble sugars were determined in ethanol extract of cotton leaves by the phenol-sulfuric acid method according to Cerning [52].

Determination of Reducing Sugars: Reducing sugars were determined colormetrically according to Folin and Wu method as reported in AOAC [53].

Determination of Non-Reducing Sugars: Non-reducing sugars were calculated by the difference between total soluble sugars and total reducing sugars.

Determination of Total Free Amino Acids: Total free amino acids were determined in ethanol extract of cotton leaves by ninhydrin method according to Rosen [54]

Total Phenols: Total phenols were determined in ethanol extract of cotton leaves using Folin-Ciocalteau method according to Simons and Ross [55]

Determination of Proline Content: Proline content of cotton leaves were determined according to method of Bates *et al*. [56] as described by Shyam and Aery [57] as follows: The fresh leaves (0.5 g) 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 2 ml of acid ninhydrin and 2 ml 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 4 ml of toluene and mixed vigorously using a test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and thawed to room temperature and the absorbance of the solution was measured at 520 nm 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 ìmoles of proline/g of fresh weight.

Determination of Antioxidant Enzymes

Extraction of Antioxidant Enzymes: Crude enzyme extract was prepared for assay of catalase (CAT) and peroxidase (POX) activities according to Chance and Maehly [58] as described by Choudhury and Panda [59] as follows:

Extraction of enzymes was done by homogenizing the cotton leaves in 0.1 *M* phosphate buffer (pH 6.8) in pre-chilled mortar and pestle under cold condition. The extract was centrifuged at 4°C for 15 min at 14,000 rpm in a cooling centrifuge. The supernatant was used for the assay of catalase (CAT), peroxidase (POX) and protein content.

Determination of Catalase Activity: Catalase (EC 1.11.1.6) was measured according to the method described by Jaleel *et al*. [60] as follows: The assay mixture contained 2.6 ml of potassium phosphate buffer solution (50 m*M*, pH 7.0), 0.4 ml of H_2O_2 solution (15 m*M*) 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 protein (U = 1 m*M* of H_2O_2 reduction/min/mg protein).

Determination of Peroxidase Activity: Peroxidase (EC 1.11.1.7) was assayed as described by Jaleel *et al*. [60] 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 protein. One U is defined as the change in the absorbance by 0.1 min/mg protein.

Determination of Total Soluble Protein: Total soluble proteins were estimated according to the method of Lowry-Folin as described by Dawson *et al*. [61].

Determination of Total Antioxidant Capacity: Total antioxidant capacity was assayed in ethanol extract of cotton leaves by the phosphomolybdenum method as described by Kumaran and Karunakaran [62] as follows: A known volume (0.01 ml) of extract was added to test tube then completed to a constant volume (0.3 ml) with DW. 3.0 ml of reagent solution (0.6 *M* sulfuric acid, 28.0 m*M* sodium phosphate and 4.0 m*M* ammonium molybdate) were added to each tube and mixed well then incubated at 95°C for 90 min. Blank was prepared by the same procedure without extract. After cooling to room, the absorbance of the solution was measured at 695 nm using spectrophotometer against blank. Increased absorbance of the reaction mixture indicated increased total antioxidant capacity.

Statistical Analysis: The results were analysed by an analysis of variance ($P<0.05$) and the means separated by Duncan's multiple range test. The results were processed by CoStat computer program (1986).

RESULTS AND DISCUSSION

Application of SNP, Put and GB on Cotton Plant under Drought Stress

Growth Characters: The effect of spraying the SNP, Put and GB on growth characters of cotton plants under drought stress is shown in Table 1. The obtained results showed that the all growth characters (plant height, number of nodes, inter-node length, etc.) of cotton plants were decreased under drought stress conditions in comparison with control plants. Spraying of cotton plants with SNP (0.05, 0.1 and 1 m*M*), Put (200, 400 and 600 ppm) and GB (400, 600 and 800 ppm) under drought stress conditions increased the growth characters of cotton plants to be near of untreated plants (control). There were many differences in growth characters of cotton plants under drought conditions in response to different concentrations of SNP, Put and GB. No significant differences between treatments in the plant height, number of nodes and inter-node length were also observed. In general, it could be concluded that 800 ppm of GB improved most growth characters (number of nodes, number of fruiting branches and dry weight of stem and branches, roots, leaves and square part). On the other hand, the concentration 0.05 m*M* of SNP improved the plant height and internode length, while the concentration 600 ppm of Put improved the ratio of root to shoot and leaf area of cotton plants under drought stress to be near of untreated plants under normal conditions (control).

Yield Characters: Data in Table 2 show the effect of foliar application of SNP, Put and GB on yield characters of cotton plants under drought conditions. As shown previously, many differences in yield characters of cotton plants in response to different concentrations of SNP, Put and GB were reported. Data revealed that the best concentrations of SNP, Put and GB for the maximum

Table 1: Effect of foliar application of sodium nitropruside (SNP), putrescine (Put) and glycine betaine (GB) on growth characters of cotton cv. Giza 90 under drought stress

Treatments	Conc.	Plant height (cm)	Number of nodes/plant	Inter-node length (cm)	Number of Fruiting branches	Root/shoot	Dry weight (g)				Leaf area (cm^2)
							Stem and branches	Roots	Leaves	Square part	
Control		49.5^{a}±5.16	16.0^{a}±2.65	3.09^{a}±0.62	7.2^{ab}±1.17	17.8^{cd}±1.19	5.88^{a}±0.31	5.30^{a}±0.23	4.12^{a}±0.14	1.58^{cde}±0.11	351^{cd}±17.71
Drought stress		40.2^{a}±2.6	13.0^{a}±2.52	3.09^{a}±0.68	4.0^{b}±0.58	18.4^{cd}±0.95	2.21^{f}±0.26	2.00^{f}±0.33	1.86^{d}±0.17	1.30^{ef}±0.14	270^{f}±7.21
Drought stress											
SNP	0.05 m*M*	48.1^{a}±1.21	13.9^{a}±2.08	3.46^{a}±0.34	6.0^{ab}±1.53	18.4^{cd}±0.67	3.86^{bcd}±0.52	3.10^{cd}±0.21	2.95^{c}±0.19	1.80^{cd}±0.12	370^{bc}±12.51
	0.1 m*M*	45.5^{a}±3.75	13.1^{a}±3.18	3.47^{a}±0.48	4.5^{ab}±0.74	13.0^{e}±1.53	2.27^{f}±0.27	2.23^{bef}±0.17	2.08^{d}±0.15	0.93^{f}±0.06	313de±18.50
	1 m*M*	43.2^{a}±1..56	13.09^{a}±2.33	3.30^{a}±0.30	4.6^{ab}±0.88	15.9^{de}±1.43	2.34^{ef}±0.18	2.25^{ef}±0.23	1.89^{d}±0.21	1.10^{ef}±0.16	260^{f}±9.61
Put	200 ppm	45.5^{a}±5.49	13.9^{a}±2.33	3.27^{a}±0.27	6.2^{ab}±0.60	12.7^{e}±1.39	2.99^{cdef}±0.45	2.71^{cde}±0.18	2.65^{c}±0.10	1.36^{def}±0.15	270^{f}±8.19
	400 ppm	45.9^{a}±1.73	13.92^{a}±1.45	3.29^{a}±0.42	6.9^{ab}±0.97	27.5^{a}±1.42	3.24^{cdef}±0.49	2.82^{cde}±0.21	2.80^{c}±0.15	1.28^{ef}±0.24	320^{d}±11.55
	600 ppm	46.3^{a}±3.84	14.0^{a}±1.73	3.31^{a}±0.09	7.2^{ab}±1.17	26.2^{a}±0.81	3.62^{bcde}±0.26	2.90^{cde}±0.15	2.94^{c}±0.17	0.97^{f}±0.12	436^{a}±3.22
GB	400 ppm	43.0^{a}±2.89	14.0^{a}±0.99	3.07^{a}±0.20	6.0^{ab}±1.15	21.0^{bc}±1.08	2.62^{def}±0.56	2.60^{def}±0.10	2.96^{c}±0.14	2.20^{bc}±0.09	276^{cd}±9.16
	600 ppm	45.5^{a}±2.03	14.5^{a}±1.76	3.14^{a}±0.05	7.0^{ab}±0.95	23.9^{ab}±0.81	4.00^{bc}±0.41	3.39^{c}±0.09	3.54^{b}±0.17	2.30^{b}±0.21	323^{d}±9.54
	800 ppm	46.6^{a}±3.01	14.9^{a}±1.15	3.12^{a}±0.07	7.5^{a}±0.87	27.2^{a}±2.19	4.87^{ab}±0.55	4.70^{b}±0.26	4.48^{a}±0.12	3.38^{a}±0.26	403^{ab}±7.81
L.S.D 0.05		9.322	3.967	0.575	2.932	3.788	1.198	0.612	0.466	0.437	38.278

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

Table 2: Effect of foliar application of sodium nitropruside (SNP), putrescine (Put) and glycine betaine (GB) on yield characters of cotton cv. Giza 90 under drought stress

Treatment	Conc.	Number of flowers	Number of bolls	Boll setting %	Boll weight (g)	Lint %	Seed index(g)	Seed cotton yield (g/plant)
Control		15.0^{ab}±1.73	11.3^{ab}±0.9	76.5^{c}±2.14	1.5^{a}±0.15	39.0^{ab}±0.43	8.5^{a}±0.4	16.59^{abcd}±0.70
Drought stress		11.3^{b}±1.45	7.7^{bc}±1.42	68.1^{e}±1.44	1.3^{a}±0.08	37.4^{b}±1.17	7.9^{a}±0.81	10.49^{e}±0.74
Drought stress								
SNP	0.05 m*M*	11.5^{b}±0.88	8.0^{bc}±1.16	70.4^{de}±1.64	1.6^{a}±0.12	37.9^{ab}±0.21	8.0^{a}±0.58	12.83^{bcde}±1.16
	0.1 m*M*	11.4^{b}±1.45	7.8^{bc}±1.02	69.3^{e}±2.61	1.5^{a}±0.23	37.4^{ab}±0.43	8.2^{a}±0.08	11.80^{de}±2.17
	1 m*M*	13.8^{ab}±1.45	6.6^{c}±0.91	69.4^{e}±2.02	1.3^{a}±0.12	37.9^{ab}±0.64	8.2^{a}±0.12	8.60^{e}±0.58
Put	200 ppm	12.5^{ab}±1.86	8.5^{abc}±0.99	75.6^{cd}±1.68	1.5^{a}±0.3	38.6^{ab}±0.59	8.3^{a}±0.09	12.19^{cde}±0.83
	400 ppm	13.3^{ab}±0.88	8.3^{abc}±1.02	78.1^{bc}±0.58	1.6^{a}±0.17	38.7^{ab}±0.91	8.3^{a}±0.15	13.50^{bcde}±0.96
	600 ppm	15.5^{ab}±1.45	11.7^{a}±1.28	82.5^{ab}±1.68	1.6^{a}±0.12	38.7^{ab}±0.63	8.4^{a}±0.25	18.80^{a}±1.56
GB	400 ppm	14.3^{ab}±1.20	8.8^{abc}±0.49	78.3^{bc}±2.37	1.4^{a}±0.06	39.4^{a}±0.15	8.4^{a}±0.32	12.98^{bcde}±0.51
	600 ppm	15.6^{ab}±0.88	10.5^{ab}±1.26	80.6^{abc}±1.55	1.6^{a}±0.23	38.4^{ab}±0.8	8.6^{a}±0.15	16.93^{abc}±2.78
	800 ppm	16.6^{a}±0.88	11.1^{ab}±1.16	85.0^{a}±2.31	1.6^{a}±0.12	38.3^{ab}±0.73	8.7^{a}±0.15	17.60^{ab}±2.34
L.S.D 0.05		3.899	3.167	5.566	0.493	1.909	1.049	4.392

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

values of most yield characters of cotton plants under drought conditions were 0.05 m*M*, 600 ppm and 800 ppm, respectively as compared with the plants grown under normal irrigation. Some yield characters of cotton plants (number of flowers, boll setting, boll weight and seed index) were affected by concentration 800 ppm of GB higher than 0.05 m*M* of SNP and 600 ppm of Put, whilst reversed results were observed with number of bolls, lint percentage and seed cotton yield. In general, it could be concluded that spraying cotton plants with 800 ppm of GB and 600 ppm of Put under drought stress conditions increased yield characters compared to untreated plants under the same drought condition.

In conclusion, results obtained indicated that foliar application of SNP, Put and GB on cotton plants under drought conditions decreased the adverse effects and enhanced growth and yield characteristics. In the present study, growth and yield characteristics of cotton plants were reduced due to water stress. The reduction in growth and yield characteristics of stressed cotton plants can be attributed to the plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, decrease in photosynthetic pigments, carbohydrates accumulation and nitrogenous compounds [63-65]. The decrease in yield and yield components in cotton crop under drought

conditions has also been reported by Namich [66], Gorham *et al*. [67], Meek *et al*. [68] and Gebaly [69]. Our finding showed that pre-treatment of cotton plants under drought stress with SNP, Put and GB decreased adverse effects of drought stress; supporting that NO, Put and GB are actively involved in the regulation of plant growth. Previous studies have demonstrated that the exogenous NO, Put and GB mitigated decrease in plant growth caused by drought is through increasing antioxidant system, alleviating oxidative damage and accelerate proline accumulation, augmented the synthesis of compatible solutes, enhance photosynthesis [39,70-73]. Tian and Lei [74] investigated the physiological and biochemical responses of wheat seedlings to drought, UV-B radiation and combined stress. The results showed that the addition of 0.2 m*M* sodium nitroprusside (SNP) enhanced wheat seedling growth under drought, UV-B and combined stress. Aftab *et al.* [75] reported that treatment of NO donor favoured growth and improved the photosynthetic efficiency in stressed as well as non-stressed plants and exogenous application of SNP promoted root elongation in both stressed and non-stressed plants. AbdEI-Wahed [76] showed that cotton seeds soaked in spermidine solutions caused significant increases of root fresh and dry biomass especially at vegetative and flowering stages. The increases of root fresh and dry biomass were related to increase spermidine concentration. This effect might be due to spermidine regulation of growth or the consequence of spermidine biosynthesis at low concentration. In addition, the consequence of spermidine degradation can be the products of a precursor for other growth substances in plant. Putrescine induced increase in chlorophyll content, water status, photosynthesis, membrane properties have been reported in rice [77]. Liu *et al.* [78] suggested that polyamines target KAT1 like inward K^+ channel in guard cell and modulate stomatal movement, providing a link between stress conditions, polyamine levels and stomatal regulation. This property may be linked to the increased transpiration rate and stomatal conductance caused by polyamine application in wheat. In soybean, foliar spray of 10^{-3} *M* polyamines at 50% flowering stage increased number of pod/plant, 100-seed weight, seed and oil yield [25]. Gupta and Gupta [34] investigated the efficacy of putrescine (0.01, 0.1 and 1.0 m*M* concentrations) was applied as seed treatment, one or two foliar sprays on wheat under water stress condition. The results showed that putrescine application enhanced plant height, leaf area, grain number, grain weight, grain yield and biological yield under non-stress as well as under water stress conditions. Naidu *et al*. [46] reported a highly significant role of supplied GB in increasing the yield of cotton. However, the positive effects of foliar spray of GB 100-achene weight of sunflower lines grown under water limited environment as observed in the present study has also been however also reported in different crops such as soybean [79], cotton [67, 80], wheat [35] and sunflower [47]. It is clear from the results of the experiment that foliar application of SNP, Put and GB ameliorated the negative effects of water stress on growth and yield characteristics of cotton plants.

Chemical Constituents of Cotton Leaves: Cotton leaves obtained from this experiment were employed to determine their contents of chlorophyll a, b, total chlorophyll, carotenoids, total soluble sugars, reducing sugars, non-reducing sugars, proline, total free amino acids, total phenols and total soluble proteins in addition to determine the antioxidant enzyme activities (catalase and peroxidase) and total antioxidant capacity. The obtained results are presented graphically in Figs. 1, 2, 3 and 4. Data presented in Fig. 1 showed that the contents of chlorophyll a, b, total chlorophyll, carotenoids of stressed cotton plants were decreased in comparison with control plants. Spraying of cotton plants with different concentrations of SNP, Put and GB under drought conditions increased chlorophyll a, b, total chlorophyll and carotenoid contents of cotton plants to be near control plants under normal conditions. This increasing in pigments content of cotton plants is varied between treatments. The results generally indicated that chlorophyll a, b, total chlorophyll and carotenoid contents were significantly increased as a result of foliar application of Put (600 ppm) in comparison with GB and SNP. Spraying of cotton plants by GB (800 ppm) was more effective on pigment content than SNP (0.05 m*M*). Similar results were obtained by Zeid and Shedeed [32] and Youssef *et al*. [81]. The obtained results also support the suggestion of Galston and Kaur-Sawhney [82] and Flores [83] who reported that senescence is extremely affected by polyamines, which are commonly known as antisenescene agents and observed that polyamines retained chlorophyll and inhibted RNase and protease activity. Besford *et al*. [27] attributed the positive effects of polyamines on chlorophyll and carotenoids levels to preservation of the thylakoid membranes at site of chlorophyll-protein complex.

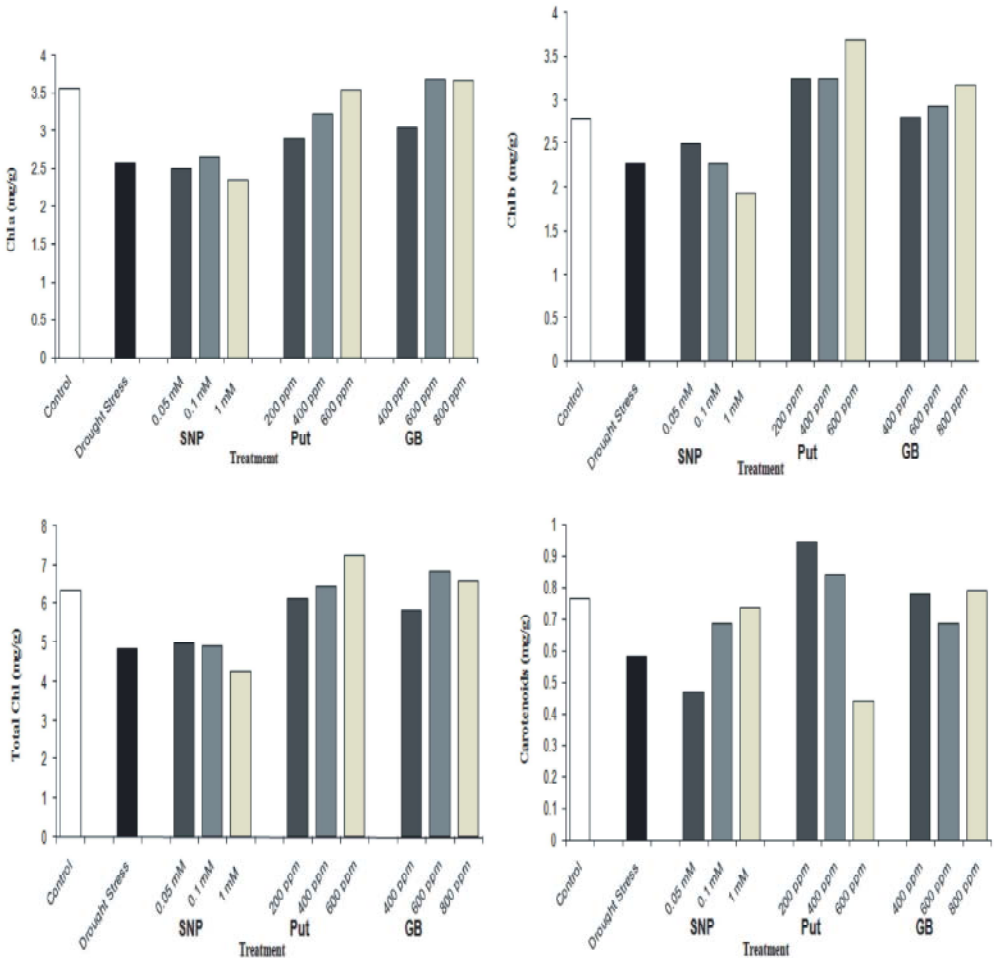

Fig. 1: Effect of foliar application of sodium nitroprusside (SNP), Putrescine (Put) and Glycine betaine (GB) on Chlorophyll (Chl) a, b, total chlorophyll and carotenoids contents in leaves of cotton plant under drought stress.

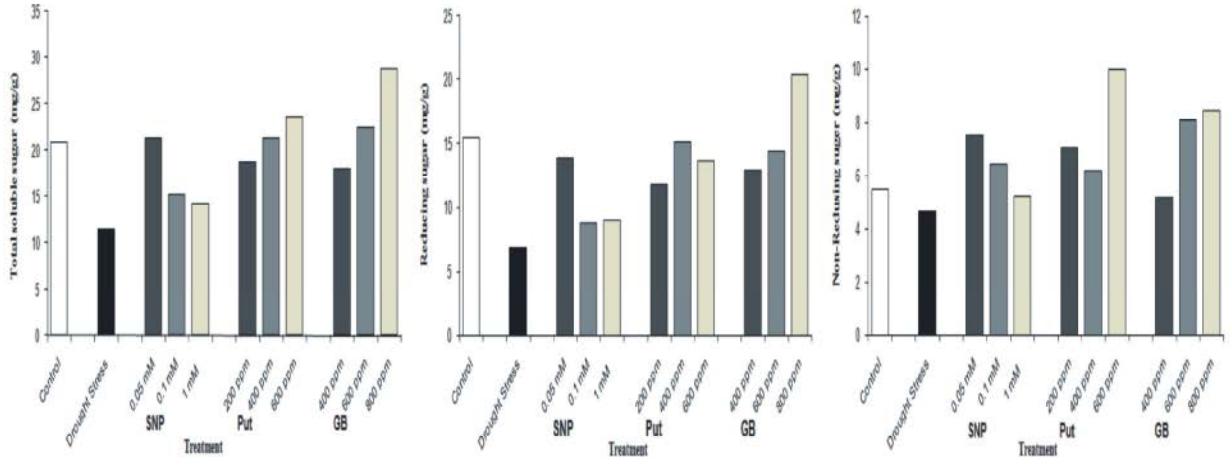

Fig. 2: Effect of sodium nitroprusside (SNP), Putrescine (Put) and Glycine betaine (GB) on total soluble sugars, reducing and non-reducing sugars contents in leaves of cotton plant under drought stress.

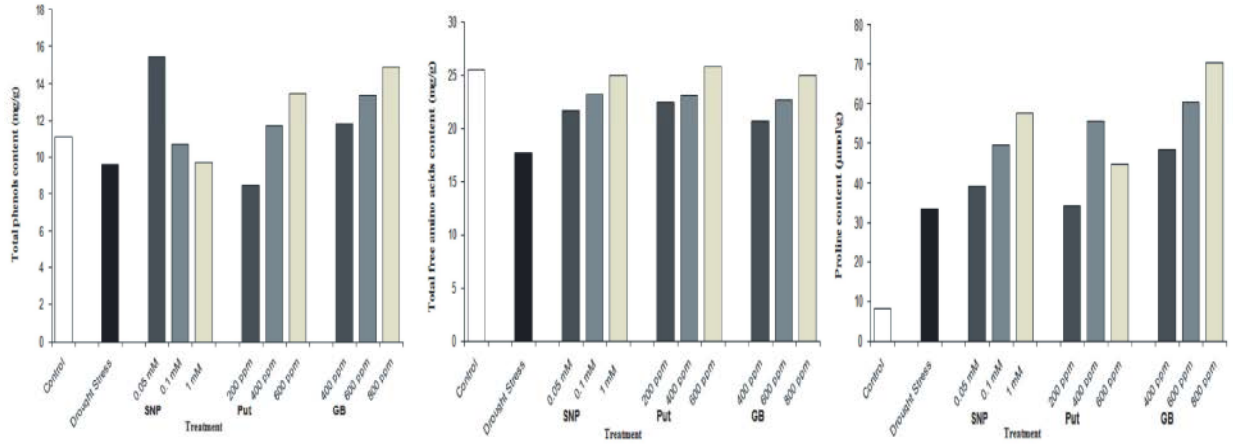

Fig. 3: Effect of sodium nitroprusside (SNP), Putrescine (Put) and Glycine betaine (GB) on total phenol, total free amino acids and proline contents in leaves of cotton plant under drought stress.

Carbohydrates that represent one of the main organic constituents of the dry matter were found to be affected by water stress. As shown in Fig. 2, the foliar application of SNP, Put and GB to cotton plants under drought conditions increased the contents of total soluble sugars, reducing sugars and non-reducing sugars as compared with untreated stressed cotton plants. The results generally showed that the spraying of cotton plants with GB (800 ppm) was more effective in increasing the contents of total soluble sugars, reducing and non-reducing sugars in comparison with Put and SNP. These results are in line with the findings of Namich [80] and Meek *et al*. [68]. This was in agreement with the finding of Ibrahim and Aldesuquy [84] and Aldesuquy *et al*. [85] who found that droughted sorghum and wheat plants treated with GB accumulated more soluble sugars than the droughted plants only.

Data presented in Fig. 3 indicated that the foliar application of SNP, Put and GB to cotton plants under drought conditions increased the contents of proline, total free amino acids and total phenols in comparison with untreated stressed plants. The results revealed that spraying of cotton plants with GB (800 ppm) was more effective in increasing the proline content than SNP and Put whilst the spraying with SNP (0.05 m*M*) was more effective in increasing the total phenols than GB and Put. There are no significant differences between treatment with SNP, Put and GB on total free amino acids content of cotton plants under drought conditions. In this study, higher level of proline accumulation in cotton plants enabled the water-stressed plants to maintain low water potentials. By decreasing water potentials, proline accumulation involved in osmoregulation appeared to allow additional water to be taken up from the environment, thus counteracting the influence of drought stress on the plant tissues [86]. Moreover, the accumulation of proline under drought stress by GB treatment is consistent with earlier findings of Hussain *et al*. [87]. Foliar application of GB caused marked increases in the soluble nitrogen and enhancing the total nitrogen as well as soluble protein in the droughted wheat plants of the two cultivars. GB application may lead to increase free amino acids especially proline in the water stressed wheat plants and consequently increased the soluble nitrogen as well as total-N. In this respect, Ibrahim [88] found that, in correcting the N concentration to total shoot dry weight found that, salinity had a negative effect on N content and GB improved total-N of salinity stressed sorghum plants. Results in Fig. 3 indicated that total phenols content was decreased in droughted cotton leaves. These results are in accordance with those of Yi *et al*. [89] who reported that water stress reduced total phenols content as compared to these found in control plants. Phenolic compounds as well known can be synthesized through three pathways, i.e., acetate, mevalonate and shikimic acid. The main precursors for phenol synthesis in plant tissue are carbohydrates, especially soluble carbohydrate in which lead to the formation of the essential substances required for simple and poly phenols synthesis. Thus, the reduction in phenolic compounds in which was observed may be due to the reduction in soluble carbohydrate under drought conditions (Fig. 2). These results are in harmony with those obtained by Ahmed *et al*. [90] and Gebaly [69].

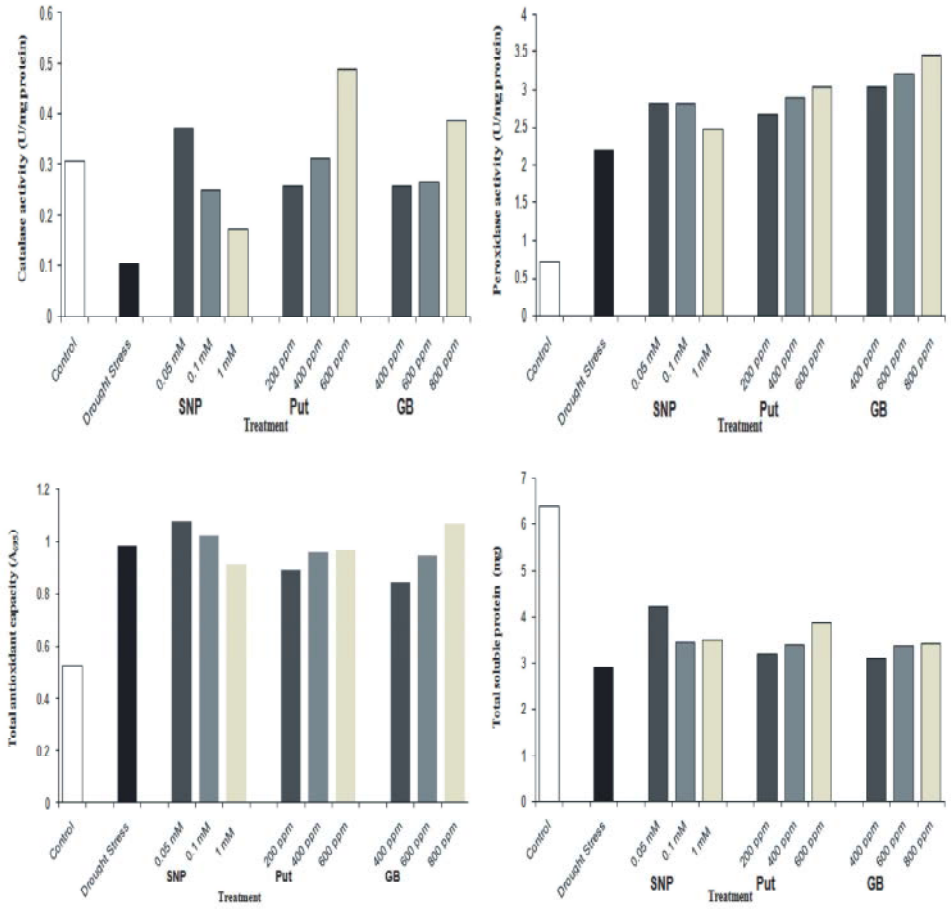

Fig. 4: Effect of sodium nitroprusside (SNP), Putrescine (Put) and glycine betaine (GB) on the activities of catalase and peroxidase, total antioxidant capacity and total soluble protein content in leaves of cotton plant under drought stress.

They concluded that phenolic content of cotton leaves decreased sharply when the cotton plants were subjected to water stress.

Catalase and peroxidase are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals. The results obtained in Fig. 4 showed that the foliar application of SNP, Put and GB to cotton plants under drought conditions increased the activities of catalase and peroxidase in comparison with control plants. The results revealed that spraying of cotton plants with Put (600 ppm) was more effective in increasing the activity of catalase than GB (800 ppm) and SNP (0.05 m*M*) whilst the spraying with GB (800 ppm) was more effective in increasing the activity of peroxidase than Put (600 ppm) and SNP (0.05 mM). Regarding total soluble protein content, data revealed that the total soluble protein content was increased when spraying the cotton plants by SNP (0.05 m*M*) in comparison with Put (600 ppm) and GB (800 ppm) under drought conditions. Concerning total antioxidant capacity, data showed that the total antioxidant capacity of cotton plants was increased when spraying with SNP, Put and GB in the following order: SNP (0.05 m*M*) > GB (800 ppm) > Put (600 ppm). The total antioxidant capacity of untreated stressed cotton plants was more than untreated control plants.

Plants are able to protect their tissue from the harmful effects of drought-accumulated reactive oxygen species (ROS) using enzymes such as SOD, CAT and POX [91]. The results showed that the SNP, Put and GB have induced all antioxidant enzyme activities and increased total antioxidant capacity 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.

There is data supporting that application of SNP, Put and GB increases the activity of antioxidant enzymes such as CAT, POX and SOD and total antioxidant capacity [75, 92, 93] which in turn protect plants against ROS generation and lipid peroxidation. On the other hand, it was reported an increase in the activity of POX but an inhibition of CAT activity in untreated cotton plants under drought conditions. This finding was in agreement with those reported by Khalil *et al*. [93] who found that the activities of the antioxidant enzymes (peroxidase (POX) in wheat shoots exposed to the high temperature stress for 4 or 8 hrs were significantly increased, while superoxide dismutase (SOD) and catalase (CAT) were decreased as compared with those of the control plants. Water stress led to massive decreases total nitrogen and soluble protein (Fig. 4). These results were in good conformity with that reported by Khalil and Mandurah [94] who studied the effect of water stress on nitrogen metabolism of plants. They observed that water stress decreased shoot total-N and protein-N but increased the soluble-N content. This change in nitrogen content may be related to the inhibition of translocation from root to shoot, inhibition of protein synthesis or the increase of protease activity. Mohammadkhani and Heidari [95] found that the decrease in total soluble proteins during drought stress was due to a severe decrease in photosynthesis. Photosynthesis decreased in drought stress and materials for protein synthesis weren't provided; therefore, protein synthesis dramatically reduced or even stopped. Foliar application of SNP, Put and GB caused marked increases the total soluble proteins in the droughted cotton plants. These results are supported by many authors [12, 32, 80,85].

Finally, it can be concluded that the exogenous application of SNP, Put or GB to cotton plant resulted in enhancement of growth and yield characters and increasing of pigments content, total soluble sugars, proline content, total free amino acids, total phenols, total soluble proteins, total antioxidant capacity and antioxidant enzyme activities during water stress as compared to untreated plants.

REFERENCES

1. Texier, P.H., 1993. Le-cotton, cinquieme producteur mondial d huile alimentaire. Cotton Development, 8: 2-3.
2. Saleem, M.F., M.F. Bilal, M. Awais, M.Q. Shahid and S.A. Anjum, 2010. Effect of nitrogen on seed cotton yield and fiber qualities of cotton (*Gossypium hirsutum* L.) cultivars. The Journal of Animal and Plant Sciences, 20(1): 23-27.
3. Smith, C.W., 1995. Cotton (*Gossypium hirsutum*, L.). In: Crop Production, Evolution, History and Technology, C.W. Smith, (Ed.), John Wiley and Sons, Inc., New York, USA, pp: 287-349.
4. Mehasen, S.A.S., S.G. Gebaly and O.A. Seoudi, 2012. Effectiveness of organic and inorganic fertilization in presence of some growth regulators on productivity and quality of Egyptian cotton. Asian Journal of Biological Sciences, 5: 171-182.
5. Almeselmani, M., P.S. Deshmukh, R.K. Sairam, S.R. Kushwaha and T.P. Singh, 2006. Protective role of antioxidant enzymes under high temperature stress. Plant Science, 171: 382-388.
6. Iturbe-Ormaetxe, I., P.R. Escuredo, C. Arrese-Igor and M. Becana, 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiology, 116: 173-181.
7. Foyer, C.H., L. Maud and K.J. Kunert, 1994. Photooxidative stress in plants. Physiologia Plantarum, 92: 696-717.
8. del Rio, L.A., F.J. Corpas, L.M. Sandalio, J.M. Palma, M. Gomez and J.B. Barroso, 2002. Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. Journal of Experimental Botany, 53: 1255-1272.
9. Mafakheri, A., A. Siosemardeh, B. Bahramnejad, P.C. Struik and Y. Sohrabi, 2011. Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. Australian Journal of Crop Science, 5(10): 1255-1260.
10. Neill, S.J., R. Desikan and J. Hancock, 2003. Nitric oxide signaling in plants. The New Physiology, 159: 11-35.
11. Fan, H.F., S.R. Guo, J. Li, C.X. Du and B.J. Huang, 2007. Effects of exogenous nitric oxide on *Cucumis sativus* seedlings growth and osmotic adjustment substances contents under NaCl stress. Journal of Chemical Ecology, 26: 2045-2050.
12. Nasrin, F., F. Nasibi and R. Rezazadeh, 2012. Comparison the effects of nitric oxide and spermidin pretreatment on alleviation of salt stress in chamomile plant (*Matricaria recutita* L.). Journal of Stress Physiology and Biochemistry, 8(3): 214-223.

13. Delledonne, M., Y. Xia, R.A. Dixon and C. Lamb, 1998. Nitric oxide functions as a signal in plant disease resistance. Nature, 394: 585-588.
14. Zhao, M.G., Q.Y. Tian and W.H. Zhang, 2007. Nitric oxide synthase dependent nitric oxide production is associated with salt tolerance in Arabidopsis. Plant Physiology, 144: 206-217.
15. Tu, J., W.B. Shen and L.L. Xu, 2003. Regulation of nitric acid on the aging process of wheat leaves. Acta Botanica Sinica, 45: 1055-1062.
16. Zhang, H., W.B. Shen and L.L. Xu, 2003. Effects of nitric oxide on the germination of wheat seeds and its reactive oxygen species metabolisms under osmotic stress. Acta Botanica Sinica, 45: 901-905.
17. Yu, C.C., K.T. Hung and C.H. Kao, 2005. Nitric oxide reduces Cu toxicity and Cu-induced NH_4^+ accumulation in rice leaves. Journal of Plant Physiology, 162: 1319-1330.
18. Rodríguez-Serrano, M., M.C. Romero-Puertas, D.M. Pazmiño, P.S. Testillano, M.C. Risueño, L.A. Del Río and L.M. Sandalio, 2009. Cellular response of pea plants to cadmium toxicity: cross talk between reactive oxygen species, nitric oxide and calcium. Plant Physiology, 150: 229-243.
19. Molassiotis, A., G. Tanou and G. Diamantidis, 2010. NO says more than 'YES' to salt tolerance: Salt priming and systemic nitric oxide signaling in plants. Plant Signaling and Behavior, 5: 209-212.
20. Shi, S.Y., G. Wang, Y.D. Wang, L.G. Zhang and L.X. Zhang, 2005. Protective effect of nitric oxide against oxidative stress under ultraviolet-B radiation. Nitric Oxide: Biology and Chemistry, 13: 1-9.
21. Wang, B., H. Liu, C. Li, Y. Zhu, X. Tian, G. Ma and H. Zou, 2011. Effects of nitric oxide on some physiological characteristics of maize seedlings under water logging. African Journal of Agricultural Research, 6(19): 4501-4504.
22. Farooq, M., A. Wahid and D.J. Lee, 2009. Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiologia Plantarum, 31: 937-45.
23. Kusano, T., T. Berberich, C. Tateda and Y. Takahashi, 2008. Polyamines: essential factors for growth and survival. Planta, 228: 367-81.
24. Nayyar, H., S. Kaur, S.S. Kumar, K.J. Singh and K.K. Dhir, 2005. Involvement of polyamines in the contrasting sensitivity of chickpea (*Cicer arietinum* L.) and soybean (*Glycine max*) to water deficit stress. Botanical Bulletin of Academica Sinica, 46: 333-338.
25. Sharma, M.L., 1999. Polyamine metabolism under abiotic stress in higher plants: salinity, drought and high temperature. Physiology and Molecular Biology of Plants, 5: 103-13.
26. Roberts, D.R., F.B. Dumbroff and J.E. Thompson, 1986. Exogenous polyamines alter membrane fluidity in bean leaves-a basis for their potential misinterpretation of their true physiological role. Planta, 167: 395-401.
27. Besford, R.T., C.M. Richardson, J.L. Campos and A.F. Tiburcio, 1993. Effect of polyamines on stabilization of molecular complexes in thylakoid membranes of osmotically stressed oat leaves. Planta, 189: 201-206.
28. Verma, S. and S.N. Mishra, 2005. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. Journal of Plant Physiology, 162: 669-677.
29. Basra, R.K., A.K. Basra, C.P. Malik and I.S. Grover, 1997. Are polyamines involved in the heat shock protection of mungbean seedlings? Botanical Bulletin of Academica Sinica, 38: 165-169.
30. Çakmak, T. and O. Atici, 2009. Effects of putrescine and low temperature on the apoplastic antioxidant enzymes in the leaves of two wheat cultivars. Plant, Soil and Environment, 55(8): 320-326.
31. Yang, J., J. Zhang, K. Liu, Z. Wang and L. Liu, 1997. Involvement of polyamines in the drought resistance of rice. Journal of Experimental Botany, 58: 1545-1555.
32. Zeid, L.M. and Z.A. Shedeed, 2006. Response of alfalfa to putrescine treatment under drought stress. Biologia Plantarum, 50: 635-640.
33. Amri, E., M. Mirzaei, M. Moradi and K. Zare, 2011. The effects of spermidine and putrescine polyamines on growth of pomegranate (*Punica granatum* L. cv 'Rabbab') in salinity circumstance. International Journal of Plant Physiology and Biochemistry, 3(3): 43-49.
34. Gupta, S. and K.N. Gupta, 2011. Field efficacy of exogenously applied putrescine in wheat (*Triticum aestivum*) under water-stress conditions. Indian Journal of Agricultural Sciences, 81(6): 516-519.
35. Diaz-Zorita, M., M.V. Fernandez-Canigia and G.A. Grosso, 2001. Application of foliar fertilizers containing glycinebetaine improved wheat yields. Journal of Agronomy and Crop Science, 186: 209-215.
36. Jagendorf, A.T. and T. Takabe, 2001. Inducers of glycinebetaine synthesis in barley. Plant Physiology, 127: 1827-1835.

37. Quan, R., M. Shang, H. Zhang, Y. Zhao and J. Zhang, 2004. Improved chilling tolerance by transformation with beta A gene for the enhancement of glycinebetaine synthesis in maize. Plant Science, 166: 141-149.
38. Abdul-Wahed, S. and S. Asma, 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with Glycinebetaine. Plant Growth Regulators, 46: 133-141.
39. Farooq, M., T. Aziz, M. Hussain, H. Rehman, K. Jabran and M.B. Khan, 2008. Glycinebetaine improves chilling tolerance in hybrid maize. Journal of Agronomy and Crop Science, 194(2): 152-160.
40. Zhao, X.X., Q.Q. Ma, C. Liang, Y. Fang, Y.Q. Wang and W. Wang, 2007. Effect of glycinebetaine on function of thylakoid membranes in wheat flag leaves under drought stress. Biologia Plantarum, 51(3): 584-588.
41. Ma, Q.Q., W. Wang, Y.H. Li, D.Q. Li and Q. Zou, 2006. Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum* L.) by foliar-applied glycinebetaine. Journal of Plant Physiology, 163: 165-175.
42. Mahmood, T., M. Ashraf and M. Shahbaz, 2009. Does exogenous application of glycinebetaine as a pre-sowing seed treatment improve growth and regulate some key physiological attributes in wheat plants grown under water deficit conditions? Pakistan Journal of Botany, 41: 1291-1302.
43. Chen, T.H. and N. Murata, 2008. Glycinebetaine: an effective protectant against abiotic stress in plants. Trends of Plant Science, 13(9): 499-505.
44. Sulpice, R., Y. Gibon, G. Cornic and F.R. Larher, 2002. Interaction between exogenous glycine betaine and the photorespiratory pathway in canola leaf discs. Physiologia Plantarum, 116: 460-467.
45. Rahaman, S.M., H. Miyake and Y. Takeoka, 2002. Effects of exogenous glycinebetaine on growth and ultrastructure of salt stressed rice seedlings (*Oryza sativa* L.). Plant Production Science, 5: 33-44.
46. Naidu, B.P., D.F. Cameron and S.V. Konduri, 1998. Improving drought tolerance of cotton by glycine betaine application and selection. In: Proceedings of 9th Australian Agronomy Conference, Wagga Wagga, pp: 1-5.
47. Iqbal, N., M.Y. Ashraf and M. Ashraf, 2005. Influence of water stress and exogenous glycine betaine on sunflower achene weight and oil percentage. International Journal of Environmental Science and Technology, 2: 155-160.
48. Ali, Q. and M. Ashraf, 2011. Exogenously applied glycinebetaine enhances seed and seed oil quality of maize (*Zea mays* L.) under water deficit conditions. Environmental and Experimental Botany, 71: 249-259.
49. Rezaei, M.A., B. Kaviani and H. Jahanshahi, 2012. Application of exogenous glycine betaine on some growth traits of soybean (*Glycine max* L.) cv. DPX in drought stress conditions. Scientific Research and Essays, 7(3): 432-436, 23.
50. Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. Plant Physiology, 24: 1-15.
51. Robbelen, G., 1957. Ztscnr Unter Suchungen an strahlenin duzierten Blaltt arbumtaten Von Arahidopsis thaliana (L.) Heynt. Z. Indutet. Absbanm u Verbungaleherett, 88: 189-202.
52. Cerning, B.J., 1975. A note on sugar determination by the anthrone method. Cereal Chemistry, 52: 857-860.
53. A.O.A.C., 1975. Official Methods of Analysis of the Association of Official Analytical Chemists, 12th ed. Washington D.C., USA.
54. Rosen, H., 1957. A modified ninhydrin colormetric analysis for amino acid nitrogen. Archives of Biochemistry and Biophysics, 67: 10-15.
55. Simons, T.J. and A.F. Ross, 1971. Change in phenol metabolism associated with enclosed systemic resistance to tobacco mosaic Virus Samson N. tobacco. Phytopathology, 61: 1261-1265.
56. Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress. Plant and Soil, 39: 205-207.
57. Shyam, R. and N.C. Aery, 2012. Effect of cerium on growth, dry matter production, biochemical constituents and enzymatic activities of cowpea plants [*Vigna unguiculata* (L.) Walp.]. Journal of Soil Science and Plant Nutrition, 12(1): 1-14.
58. Chance, B. and A.C. Maehly, 1955. Assay of catalase and peroxidase. Methods of Enzymology, 2: 764-775.
59. Choudhury, S. and S.K. Panda, 2004. Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. Bulgarian Journal of Plant Physiology, 30(3-4): 95-110.
60. Jaleel, C.A., R. Gopi, P. Manivannan and R. Panneerselvam, 2007. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. Turkish Journal of Botany, 31: 245-251.
61. Dawson, R.M.C., D.C. Elliott, W.H. Elliott and K.M. Jones, 1986. Biochemical procedures. In: Data for Biochemical Research, Clarendon press, Oxford, pp: 543.

62. Kumaran, A. and R.J. Karunakaran, 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Science and Technology, 40(2): 344-352.
63. Radin, J.W., 1981. Water relations of cotton plants under nitrogen deficiency. IV. Leaf senescence during drought and its relation to stomatal closure. Physiologia Plantarum, 51: 145-149.
64. Ball, R.A., D.M. Oosterhuis and A. Mauromoustakos, 1994. Growth dynamics of the cotton plant during water-deficit stress. Agronomy Journal, 86: 788-795.
65. Mafakheri, A., A. Siosemardeh, B. Bahramnejad, P.C. Struik and Y. Sohrabi, 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science, 4(8): 580-585.
66. Namich, A.A.M., 1997. Biochemical studies on cotton plant. Ph.D. Thesis, Faculty of Agriculture, Cairo University.
67. Gorham, J., J. Bridges, M.N. Malik and I.A. Khan, 1998. Physiological response of cotton to water deficit in Pakistan. 2nd World Cotton Conference, Athens, pp: 316.
68. Meek, C., D. Oosterhis and J. Gorham, 2003. Does foliar applied glycine betaine effect endogenous betaine levels and yield in cotton on line? Crop Management, 10-904/CM-0804-02-RS.
69. Gebaly, S.G., 2007. Effect of foliar application of methanol under two levels of irrigation regime on cotton productivity. Egypt. Journal of Agriculture Research, 85(2): 615-628.
70. Yang, J., J. Zhang, K. Liu, Z. Wang and L. Liu, 2007. Involvement of polyamines in the drought resistance of rice Journal of Experimental Botany, 58(6): 1545-1555.
71. Tan, J., H. Zhao, J. Hong, Y. Han, H. Li and W. Zhao, 2008. Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. World Journal of Agricultural Sciences, 4(3): 307-313.
72. Hao, G.P., Y. Xing and J.H. Zhang, 2008. Role of nitric oxide dependence on nitric oxide synthase-like activity in the water stress signaling of maize seedling. Journal of Integrative Plant Biology, 50: 435-442.
73. Anjum, S.A., X. Xie, L. Wang, M.F. Saleem, C. Man and W. Lei, 2011. Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research, 6: 2026-2032.
74. Tian, X. and Y. Lei, 2006. Nitric oxide treatment alleviates drought stress in wheat seedlings. Biologia Plantarum, 50(4): 775-778.
75. Aftab, T., M. Masroor, A. Khan, M. Naeem, I. Mohd, Moinuddin, T. da Silva, A. Jaime and M. Ram, 2012. Exogenous nitric oxide donor protects *Artemisia annua* from oxidative stress generated by boron and aluminium toxicity. Ecotoxicology and Environmental Safety, 8: 80-60.
76. Abd EI-Wahed, M.S.A., 2006. Exogenous and Endogenous Polyamines Relation to Growth, (X-cellulose Precipitation in Fibres and Productivity of Cotton Plant. World Journal of Agricultural Sciences, 2(2): 139-148.
77. Farooq, M., A. Wahid and D.J. Lee, 2009. Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiologia Plantarum, 31: 937-945.
78. Liu, K., H. Fu, Q. Bei and S. Luan, 2000. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiology, 124: 1315-1326.
79. Agboma, P.C., T.R. Sinclair, K. Jokinen, P. Peltonen-Sainio and E. Pehu, 1997. An evaluation of the effect of exogenous glycine betaine on the growth and yield of soybean: timing of application, watering regimes and cultivars. Field Crops Res., 54: 51-64.
80. Namich, A.A.M., 2003. Effect of glycine betaine on growth, yield, yield components and some chemical constituents of cotton plant of Giza 80. Egyptian Journal of Applied Science, 18(1): 91-101.
81. Youssef, A.A., M.H. Mahgoub and I.M. Talaat, 2004. Physiological and Biochemical aspects of *Matthiola incana* L. plants under the effect of putrescine and kinetin treatments. Egyptian Journal of Applied Science, 19: 492-510.
82. Galston, A.W. and R. Kaur-Sawhney, 1988. Polyamines as Endogenous Growth Regulators. In: Plant Hormones and their Role in Plant Growth and Development, P.J. Davies, (Ed.). Kluwer Academic Publisher, Dordrecht, Netherlands, pp: 280-295.
83. Flores, H., 1991. Changes in Polyamine Metabolism in Response to Abiotic Stress. In: Biochemistry and Physiology of Polyamines in Plants, R.D. Slocum and H.E. Flores (Eds.). CRC Press, Boca Raton, pp: 213-228.

84. Ibrahim, A.H. and H.S. Aldesuquy, 2003. Glycine betaine and shikimic acid-induced modifications in growth criteria, water relation and productivity of droughted Sorghum bicolor plants. Phyton (Austria), 43: 351-363.
85. Aldesuquy, H.S., S.A. Abo-Hamed, M.A. Abbas and A.H. Elhakem, 2012. Role of glycine betaine and salicylic acid in improving growth vigour and physiological aspects of droughted wheat cultivars. Journal of Stress Physiology and Biochemistry, 8(1): 149-171.
86. Kumar, S.G., A. Mattareddy and C. Sudhakar, 2003. NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Science, 165: 1245-1251.
87. Hussain, J., A.L. Khan, N. Rehman, M. Hamayun, T. Shah, T. Nisar, T. Bano, Z.K. Shinwari and I.J. Lee, 2009. Proximate and nutrient analysis of selected vegetable species: A case study of Karak Region Pakistan. African Journal of Biotechnology, 8(12): 2725-2729.
88. Ibrahim, A.H., 2004. Efficacy of exogenous glycine betaine application on sorghum plants grown under salinity stress. Acta Botanica Hungarica, 46: 307-318.
89. Yi, L.H., Y.Q. Zhou and T.M. Hua, 2000. The effect of soil moisture on the chlorophyll content and photosynthetic rate of different cotton cultivars. China Cottons, 27(2): 21-22.
90. Ahmed, F.M., M.S. Ismail and M.H. Abdel-Al, 1989. Effect of drought conditions at bolling stage on some chemical constituents of cotton plant. Journal of Agronomy and Crop Science, 163: 167-173.
91. Verhagen, J., J. Put and M. Yool, 2004. Climate change and drought risks for agriculture, Environment and Policy, 39: 49-60.
92. Islam, M.M., M.A. Hoque, E. Okuma, M.N.A. Banu, Y. Shimoishi, Y. Nakamura and Y. Murata, 2009. Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. Journal of Plant Physiology, 166: 1587-1597.
93. Khalil, S.I., H.M.S. El-Bassiouny, R.A. Hassanein, H.A. Mostafa, S.A. El-Khawas, A.A. Abd El-Monem, 2009. Antioxidant Defense System in Heat Shocked Wheat Plants Previously Treated with Arginine or Putrescine. Australian Journal of Basic and Applied Sciences, 3(3): 1517-1526.
94. Khalil, S. and H.M. Mandurah, 1990. Effect of water deficiency and kinetin on growth and nitrogen metabolism of cowpea plants. Journal of Agronomy and Crop Science, 164: 93-99.
95. Mohammadkhani, N. and R. Heidari, 2008. Drought-induced Accumulation of Soluble Sugars and Proline in Two Maize Varieties. World Applied Sciences Journal, 3(3): 448-453.