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Role of Glutathione, Ascorbic Acid and α-Tocopherol in Alleviation of Drought Stress in Cotton Plants

Bahaa El-Din Mekki[1], Hebat-Allah Hussien[2] and Hanaa Salem[2]

[1]Field Crops Research Department, National Research Centre, 33 El-Bohouth St., Dokki, Giza, Egypt.
[2]Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt.

Abstract: Drought is one of the major abiotic stresses in agriculture worldwide. A pot experiment was carried out in the greenhouse of National Research Centre, Dokki, Giza, Egypt during the summer season of 2014 to investigate the effect of ascorbic acid (ASC), glutathione (GSH) and/or α-tocopherol (α-TOC) and their interactions on cotton plants grown under normal and drought conditions during vegetative growth stage. In general, the drought stress reduced the growth characters, yield characters, pigments, total soluble sugars and total free amino acids were increased in comparison with control. The results showed that treatment of cotton plants under drought stress with ASC, GSH, α-TOC and their interaction caused enhancement of growth and yield characters and increased pigments content, total soluble sugars, total carbohydrates and total free amino acids. Finally, it can be concluded that foliar application of ASC, GSH, α-TOC and their interaction improved the drought tolerance of cotton plants. The GSH+ ASC are the best effective treatment to alleviate the adverse effects of the drought stress on cotton plants.

Key words: Cotton (*Gossypium barbadense* L.), Drought stress, Glutathione, Ascorbic acid, α-Tocopherol, Growth, Seed cotton yield, Photosynthetic pigments, Free amino acids.

Introduction

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[1]. 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[2]. These activated oxygens injure the cellular components of proteins, membrane lipids and nucleic acids[3]. To mitigate and repair damage initiated by ROS, plants have developed a complex antioxidant system[4]. Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation[5].

Exogenous applications of osmoprotectants, plant growth regulators, fertilizers, and antioxidants have been reported to successfully mitigate the adverse effects of drought on plants. Of these, exogenous application of antioxidants has recently gained a ground as a very promising means of mitigating the adverse effects of drought on plant growth and metabolism[6]. Number of enzymatic and non-enzymatic antioxidants is produced in plants in response to abiotic stresses which save plant from oxidative damage caused by ROS[7]. Major enzymatic antioxidants reported are superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) whereas, ascorbic acid (vitamin C), tocopherols and glutathione are the main non-enzymatic antioxidants exploited by plants under stressful conditions to ameliorate the adverse effects imposed by ROS[8]. A few studies reported that exogenously applied ascorbic acid, glutathione and α-tocopherols ameliorate adverse effects of drought[9, 10, 11]. From the earlier mentioned reports it is evident that each of ascorbic acid, glutathione and α-tocopherol plays a key role in the regulation of a number of metabolic processes in plants exposed to drought stress. However, information on how non-enzymatic antioxidants regulate physiological /biochemical processes in cotton plants subjected to drought stress is not much available in the literature. Accordingly, this investigation aimed to study the effect of exogenous application of ascorbic acid, glutathione and α-tocopherol (separately and interactions) on growth, yield and some chemical analysis of cotton plants grown under normal and drought conditions.

Materials and Methods

A pot experiment was carried out during summer season 2014 in the greenhouse of National Research Centre, Dokki, Giza, Egypt in order to investigate the effect of ascorbic acid (ASC), glutathione (GSH) and/or α-tocopherol (α-TOC) and their interactions on alleviation of drought stress in cotton (*Gossypium barbadense* L.) plants. Seeds of cotton cv. Giza 86 were sown in plastic pots (40 cm diameter and 40 cm depth) filled by clay soil and arranged in factorial experiment in complete randomize design with 5 replicates for each treatment. The soil texture is clay, field capacity (FC %), 38.0, pH 7.95, EC dSm^{-1} 0.96, $CaCO_3$% 0.72, OM% 2.89, available N 155.0 ppm, P 5.50 and K 265.0 were carried out according the methods described by Jackson[12]. Phosphorus and potassium fertilizers were added before sowing at a rate of 6.0 and 3.0 g/pot of calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48-50% K_2O), respectively. Thinning was done twice at 21 and 35 days after planting (DAP) to leave one plant per pot till picking time. Nitrogen fertilizer was applied as two equal portions at a rate of 0.60 g/pot for each in the form of ammonium nitrate (33.5% N) at 30 and 60 days after planting. At 45 days after planting (DAP), the plants were subjected under two irrigation regimes e.g. plants irrigated with 100 % full field capacity (as normal irrigation) and the second one plants irrigated after depletion of 50 % field capacity. At 60 days after planting the exogenous application of antioxidant compounds was applied as follows:

1. Without application (Control)
2. Ascorbic acid (ASC) (1mM).
3. Glutathione (GSH) (1mM).
4. Alpha-tocopherol (α-TOC) (1mM).
5. ASC + GSH
6. ASC + α-TOC
7. GSH + α-TOC
8. ASC + GSH + α-TOC

At 90 days after planting a representative sample was taken from each treatment for determining some growth characters: shoot height (cm), root length, number of leaves per plant as well as dry weights of root, stem and leaves/plant. At the same time, the third leaf from the terminal bud was taken from each treatment for determining some chemical analyses as the following:

Pigment determination

Chlorophyll *a*, Chlorophyll *b* and total carotenoids were extracted from one gram of fresh leaves in 85% acetone and measured spectrophotometrically according to Metzner *et al.*[13] and their values were calculated according to the formula of Lichtenthaler[14].

Determination of total soluble sugars

Total soluble sugars were determined in ethanol extract of cotton leaves by anthrone method according to Cerning[15].

Estimation of Polysaccharides

The method used for estimation of polysaccharides in the present study was determined according to Thayurmanavan and Sadasivam[16].

At picking time, the following criteria were recorded:

1. Number of sympodia/plant
2. Number of open bolls/plant

3. Seed cotton yield/ plant (g)
4. Lint yield/ plant (g)
5. Lint percentage %
6. 1000-seed weight (g)
7. Oil percentage in seeds (%)

Statistical analysis

Data obtained was statistically analyzed by using MSTATC programme according to Snedecor and Cochran[17]. The least significant differences (LSD) at 5% level of probability were calculated to compare the means of different treatments.

Results and Discussion

Growth characters

The effect of spraying ASC, GSH, α-TOC and their interactions on growth characters of cotton plants under normal irrigation and drought stress is shown in Table 1. The obtained results showed that all growth characters e.g. shoot height, root length (cm), leaves and stem dry weight (g/plant) of cotton plants were significantly decreased under drought stress conditions in comparison with control plants. Spraying of cotton plants with ASC, ASC + GSH and GSH + α-TOC under normal irrigation conditions increased the growth characters of cotton plants compared to untreated plants (control). Moreover, foliar application of ASC+GSH seemed to be the most effective treatment. This may be due to the antioxidants have synergistic effects on growth of many plant species. These compounds have beneficial effects on catching the free radicals or the active oxygen that produced during photosynthesis and respiration processes[18]. Exogenous application of antioxidants has gained considerable attention as a possible approach to ameliorate the adverse effects of stressed plants for improving plant growth and development[19]. Results obtained indicated that the highest values of growth characteristics in ASC+GSH treated cotton were listed in normal and stressed-cotton (Table 1). Ascorbic acid (ASC) treatment under normal irrigation plants resulted in significantly high values for root length (13.33 cm), dry weights of stem (6.60 g) and root (3.37g), while glutathion (GSH) recorded highest shoot height (89.67 cm) under the same condition of normal irrigation (Table 1). On the other hand, these morphological characters were decreased in ASC+GSH drought-stressed cotton for root length (6.67cm), dry weights of stem (3.22g), root (1.85 g) and shoot height (50.33 cm). Ascorbic acid is involved in the regulation of many critical biological processes such as photo-inhibition and cell elongation[20], many other important enzymatic and non enzymatic reactions[21], as well as in regulating plant growth and development, since it plays an important role as plant growth regulators[22]. Moreover, ascorbic acid is very important for the regulation of photosynthesis, flowering and senescence[23]. In the present study, growth characteristics of cotton plants were reduced due to drought stress. The reductions in growth 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[24]. Low-molecular-weight antioxidants such as ascorbate, glutathione (GSH) and tocopherols can mitigate the harmful effects of elevated reactive oxygen species (ROS) production. They can affect gene expression associated with abiotic stresses, altering acclimation responses. On the other hand, these antioxidants function as redox buffers that interact with ROS and act as a metabolic interface that modulates the appropriate induction of acclimation responses or programmed cell death[25]. In plants, ascorbate is the most abundant antioxidant and also serves as an electron donor to many important reactions[26, 27]. Generally it reaches a concentration of over 20 mM in chloroplasts and occurs in all cell compartments including the cell wall. It is the best known molecule for detoxifying H_2O_2, especially as a substrate of ascorbate peroxidase (APX), an essential enzyme of the ascorbate-glutathione cycle, present in most compartments of the plant cell[28]. Control of ascorbate steady-state levels in plants potentially involves regulation of biosynthesis, catabolism, recycling and transport of this compound.

Table 1: Effect of some antioxidants application on some growth characters in cotton plants grown under normal and water stress conditions.

Irrigation	Treatments	Plant height (cm)	Root length (cm)	No. of leaves/plant	Leaves dry weight (g)	Stem dry weight (g)	Root dry weight (g)
Normal	Control	81.33	13.00	15.00	17.04	8.97	3.35
	ASC (1mM)	79.67	13.33	12.33	11.89	10.60	3.37
	GSH (1mM)	89.67	10.33	12.33	9.91	7.47	2.71
	α-TOC (1mM)	70.00	10.67	10.33	8.25	5.78	2.14
	ASC + GSH	68.67	8.33	14.33	9.39	5.93	3.02
	ASC + α-TOC	76.67	7.67	11.67	9.90	5.58	2.14
	GSH + α-TOC	72.67	13.00	12.00	10.50	6.81	2.88
	ASC + GSH + α-TOC	68.67	10.67	11.00	6.54	4.30	2.21
Mean		**75.92**	**10.88**	**12.38**	**10.43**	**6.93**	**2.73**
Drought	Control	70.33	9.33	11.33	15.34	7.18	2.47
	ASC (1mM)	63.33	8.33	10.67	8.39	5.40	2.75
	GSH (1mM)	63.00	11.00	11.00	12.26	7.41	2.87
	α-TOC (1mM)	75.00	8.33	11.67	8.64	4.83	2.14
	ASC + GSH	50.33	6.67	8.67	6.65	3.22	1.85
	ASC + α-TOC	71.33	9.67	9.67	8.77	7.02	2.36
	GSH + α-TOC	68.33	12.00	11.33	9.33	5.37	2.76
	ASC + GSH + α-TOC	65.00	12.33	10.00	5.13	4.36	2.38
Mean		**65.83**	**9.71**	**10.54**	**9.32**	**5.60**	**2.49**
LSD 0.05	Irrigation (I)	2.95	0.79	0.98	1.27	0.45	NS
	Antioxidants (A)	5.90	1.58	NS	2.53	0.89	NS
	I x A	8.38	2.24	2.78	3.58	1.26	NS

Effect of ASC, GSH, α-TOC on seed cotton yield and its attributes of cotton plants under normal irrigation and drought stress conditions

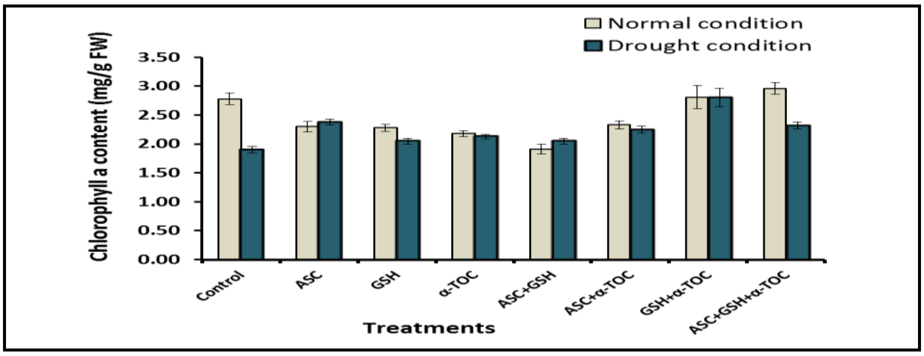
The exogenous application of osmoprotectants/antioxidants has been considered as a shotgun approach to with stand the effects of drought. Data presented in Table 2 indicated that seed cotton yield, number of sympodia/plant and 1000-seed weight was not significantly affected when plants exposed to drought stress (50 % FC), except lint yield, and lint percentage were increased significantly under drought stress in comparison with the plants irrigated with full field capacity (normal irrigation). Ahmad *et al.*[29] reported that seed cotton yield was decreased with increasing moisture stress and was the minimum under severe water stress treatment (40% FC). Only the number of open bolls/plant was decreased significantly when cotton plants subjected under drought stress conditions. Concerning the effect of irrigation treatments combined with the antioxidants compounds, data in Table 2 indicated that seed cotton yield, lint yield, lint % and number of bolls/ plant were significantly affected by the interaction between irrigation regimes and antioxidants compounds. It is clear that from Table 2 application of ASC, α-TOC or ASC+GSH+α-TOC increased seed cotton yield under drought stress, while applied of ASC + GSH increased the lint yield compared to the other treatments under the same condition of drought stress. Also, the lint percentage was increased by using ASC + GSH or by ASC + GSH when cotton plants irrigated with 50% FC compared to the other treatment under the irrigation level or with the same treatments under normal irrigation. Such increases in these traits under drought stress may be indicate enhanced activities of the enzymes of ascorbate-glutathione cycle, signifying a potential role of these enzymes in providing antioxidative defense under drought stress conditions. At higher levels of drought stress, generation of superoxide anion, increased lipid peroxidation, declines in soluble proteins, thiols as well as non-enzymatic antioxidants ascorbate and glutathione were observed compared to mildly stressed rice seedlings[30]. On the other hand, number of bolls/plant was increased under normal irrigation only with untreated plants and also under the same normal irrigation and spraying by ASC + GSH + α-TOC. In contrast, the plants received 50% FC in combination with different antioxidant compounds produced lower number of bolls than that received well water, except the treatment of α-TOC under drought stress had the same number of bolls under normal irrigation. In general, the plants subjected under stress produced the highest seed cotton yield (18.76 g) with the treatment of ASC + GSH + α-TOC, lint yield (7.14g) with ASC + GSH and lint percentage (55.42%) with GSH + α-TOC. However, the highest number of open bolls (10.33) was produced when plants received normal irrigation (100% FC) in combination with ASC + GSH + α-TOC and/ or with untreated plants (Table 2). In cotton, however, there is still debate about the most sensitive period to water-stress during development in relation to yield, even though water sensitivity during flowering and boll development has been well established[31]. According to Reddell *et al.*[32] the early flowering period is the most sensitive to water stress, whereas Orgaz *et al.*[33] concluded that water stress during peak flowering had the most detrimental effects on cotton yield. On the other hand, de Kock *et al.*[34] stated that boll development, particularly well after the end of effective flowering, is the most water-deficit-sensitive period for cotton. The majority of studies have focused on the consequences of water stress on dry matter, boll number and weight, as well as lint yield and their correlations to leaf photosynthesis and plant water relations, without any emphasis on the biochemical and metabolic processes of the reproductive units themselves. Lint yield is generally reduced under water stress because of reduced boll production primarily due to the production of fewer flowers and bolls[35], but also because of increased rates of boll abortion when the stress is extreme during the reproductive growth stage[31].

Table 2: Yield and yield components of cotton plants in response to foliar application with some antioxidants compounds under normal and water stress conditions.

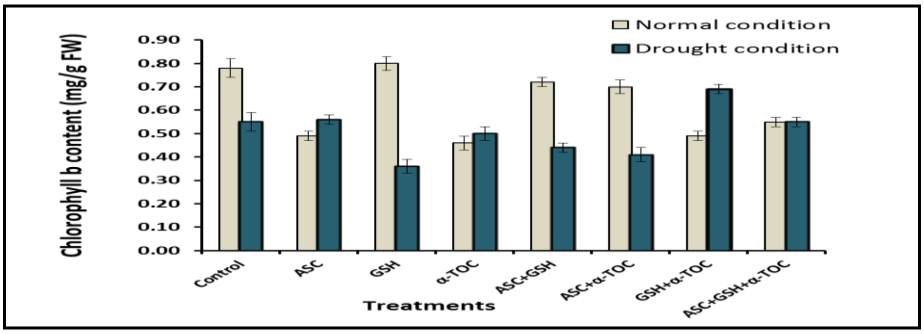
Irrigation	Treatments	Seed cotton yield (g/plant)	Lint yield (g/plant)	Lint %	No. of sympodia /plant	No. of open bolls/plant	1000-seed weight (g)	Oil %
Normal	Control	17.47	5.11	29.16	5.33	10.33	105.15	18.65
	ASC (1mM)	15.35	6.30	41.04	8.67	9.33	120.80	21.29
	GSH (1mM)	12.82	4.15	32.51	9.67	8.33	105.92	19.13
	α-TOC (1mM)	14.02	5.11	36.70	6.67	8.67	95.23	19.62
	ASC + GSH	15.04	6.05	40.28	6.00	8.00	98.74	20.38
	ASC + α-TOC	12.07	4.18	36.24	8.00	7.67	104.77	17.34
	GSH + α-TOC	16.67	5.00	31.29	3.67	9.00	101.09	20.08
	ASC + GSH + α-TOC	16.11	5.93	37.33	7.00	10.33	92.89	20.56
Mean		**14.94**	**5.23**	**35.57**	**6.88**	**8.96**	**103.07**	**19.63**
Drought	Control	14.74	5.55	37.76	6.33	8.00	111.94	18.78
	ASC (1mM)	16.97	6.74	40.08	9.33	8.67	112.32	19.23
	GSH (1mM)	10.36	4.31	42.87	7.33	6.00	117.71	18.95
	α-TOC (1mM)	17.04	6.46	39.54	7.67	8.67	104.91	17.57
	ASC + GSH	13.29	7.14	54.29	8.00	5.67	107.97	21.52
	ASC + α-TOC	10.79	4.54	42.517	9.00	6.67	87.50	19.62
	GSH + α-TOC	8.64	4.47	55.42	3.50	7.00	107.92	18.99
	ASC + GSH + α-TOC	18.76	6.40	34.66	7.00	8.67	112.79	20.06
Mean		**13.83**	**5.70**	**43.39**	**11.21**	**7.42**	**107.88**	**19.34**
LSD 0.05	Irrigation (I)	NS	0.38	4.67	NS	0.32	NS	NS
	Antioxidants (A)	2.84	0.76	9.34	NS	1.44	10.81	NS
	I x A	4.02	1.07	13.20	NS	2.04	NS	NS

Photosynthetic pigments

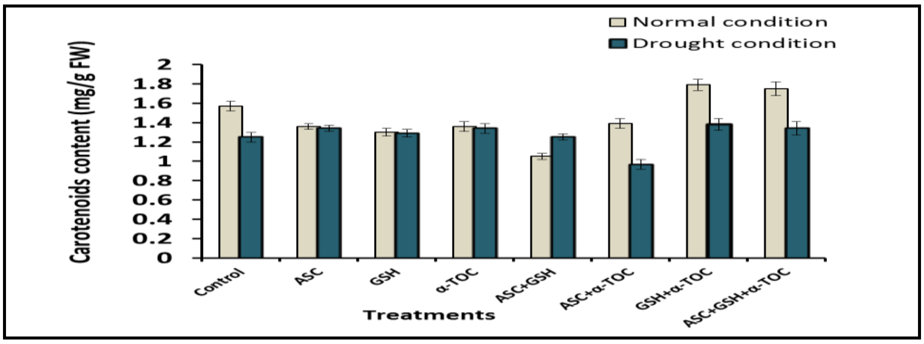
Data presented in Fig. 1 showed that chlorophyll *a*, *b*, total chlorophyll, carotenoids of drought stressed cotton plants were decreased in comparison to control plants. In contrast to other treatments, exogenous application of GSH+ α-TOC or triad interaction on cotton plants grown under normal irrigation increased chlorophyll *a*, *b* and carotenoids contents compared to control. Moreover, spraying of cotton plants with ASC, GSH, α-TOC and their interaction increased chlorophyll *a*, *b* and carotenoids contents compared to untreated drought stressed plants. The GSH+ α-TOC interaction treatment seemed to be the best effective treatment in increasing the chlorophyll *a*, *b* and carotenoids content compared to other treatments under drought stress conditions. The decrease of chlorophyll content under water limited conditions is reported to take place because of its photo-oxidation and degradation under drought[36]. Under drought stress, degradation of chlrorophyll takes place due to the increased activity of chlorophyllase enzyme[37]. Non-stomatal decrease in photosynthesis due to drought stress in the present study may have been as a result of chlorophyll degradation[36, 38]. Reactive oxygen species produced under stress conditions have been reported to cause pigment degradation[36, 39]. The ameliorating effects of ASC, GSH, α-TOC and their interaction on pigments levels of cotton leaves may be due to their effects on either enhancing the photosynthetic activities and chlorophyll biosynthesis or retardation of chlorophyll degradation resulted from the drought stress. However, ascorbic acid and glutathione being an antioxidant actively scavenges these ROS, thereby reducing the chlorophyll degradation under stress[7, 40].



LSD 0.05 for drought= 0.04, for treatments = 0.09, for interaction = 0.16, Vertical bars indicate ± SD.



LSD 0.05 for drought=0.02 , for treatments = 0.03, for interaction =0.05, Vertical bars indicate ± SD.

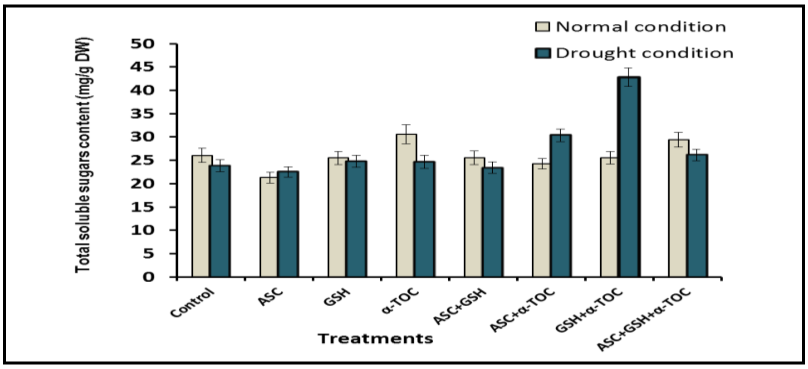


LSD 0.05 for drought= 0.02 , for treatments = 0.05, for interaction =0.07,Vertical bars indicate ± SD.

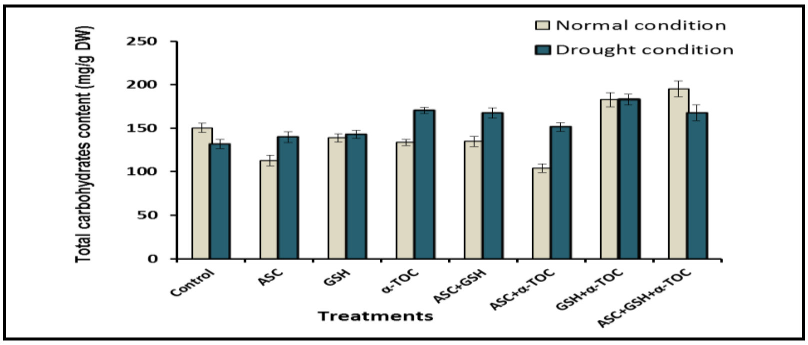
Figure 1. Effect of foliar application of ascorbic acid, glutathion, α-tocopherol and their interactions on chlorophyll *a, b* and carotenoids contents in leaves of cotton plants under normal irrigation and drought stress conditions.

Carbohydrate content

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 ASC, GSH, α-TOC and their interactions on cotton plants under drought conditions increased the contents of total soluble sugars, total carbohydrates compared with untreated stressed cotton plants. In contrast to other treatments, exogenous application of GSH+ α-TOC or triad interaction on cotton plants grown under normal irrigation increased total carbohydrates content compared to control. Moreover, the results showed that total soluble sugars and total carbohydrate contents were highly accumulated in GSH+ α-TOC treated stressed cotton plants (42.8 and 182.97 mg/g dw, respectively). The interactive effects of GSH+α-TOC on accumulation of soluble sugars probably, attributed to the protective effects of glutathione on the photosythetic systems. Also, GSH plays a protective role in drought tolerance by maintenance of the redox status[41]. Noctor and Foyer [42] and Mullineaux and Rausch[43] stated that α-tocopherol and glutathione plays an essential role in plant metabolism and drought stress tolerance and they can influence the functioning of signal transduction pathway by modulating cellular redox state.



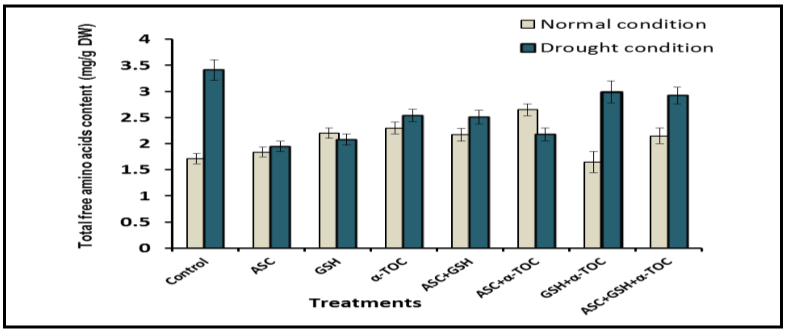
LSD 0.05 for drought=1.1 , for treatments = 1.5, for interaction =2.0, Vertical bars indicate ± SD.



LSD 0.05 for drought= 3.0 , for treatments = 5.0, for interaction =9.0, Vertical bars indicate ± SD.

Figure 2. Effect of foliar application of ascorbic acid, glutathion, α-tocopherol and their interactions on total soluble sugars and total carbohydrates contents in leaves of cotton plants under normal irrigation and drought stress conditions.

Total free amino acids content



LSD 0.05 for drought=0.09 , for treatments = 0.12, for interaction = 0.20, Vertical bars indicate ± SD.

Figure 3. Effect of foliar application of ascorbic acid, glutathion, α- tocopherol and their interactions on total free amino acids contents in leaves of cotton plants under normal irrigation and drought stress conditions.

Data presented in Fig.3 indicated that foliar application of ASC, GSH, α-TOC and their interactions on cotton plants grown under normal condition caused pronounced decreased total free amino acids contents in leaves of cotton plants grown under drought condition compared to control ones. The accumulation in amino acids in cotton plants exposed to drought stress probably attributed to the disturbance in amino acid metabolism. Under water stress, a decrease in proteins could reflect either diminished synthesis or increased breakdown, leading to higher levels of free amino acids[44]. Since the effects of drought depend on species, tissue and age, as well as the nature, duration and degree of the stress, it is not surprising that marked differences have been found in the amino acid pattern for stress conditions[45]. However, Parida *et al.*[45] pointed out the amino acids which contributed most to this increase were aspartate, glutamate, proline, alanine and valine. The free amino acid pool did not change very much in control samples during the entire period of investigation, while in drought induced plants, total free amino acids was increased. Upon recovery from drought, total free amino acid contents decreased significantly and were comparable to the control plants.

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