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Research Article

Effect of Ascorbic Acid antioxidant on Soybean (*Glycine max* L.) plants grown under water stress conditions

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Abstract

Pot experiments were conducted to determine the effect of different water regimes (100%, 80%, 60% and 40% of field capacity, FC) on vegetative growth (plant height, leaf area and plant fresh and dry weights), photosynthetic pigments (chl a, chl b and carotenes), mineral content of leaves (N, P and K), proline content, yield components (pod and seed numbers/plant, seed weight/plant and 100 seed weight), nutritional value of produced seeds (N, P, K and Ca soluble sugars, total carbohydrate, total amino acids and protein contents). The role of ascorbic acid (0, 100, 200 and 300 mg l^{-1}) in alleviating the effect of water stress was also studied. All growth parameters were significantly reduced with low treatment of FC (60% and 40%) while ascorbic acid, particularly at 100 and 200 mg l^{-1}, tended to mitigate the harmful effect of high water deficit on growth and chemical constituents in soybean plants. It is suggested that Ascorbic acid could be a promising material used to reduce the harmful effect of water stress on the growth and yield of *Glycine max* plants grown in Saudi Arabia**.**
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Keywords: water stress, ascorbic acid, soybean, growth, chemical contents.

Introduction

Soybean (Glycine max (L.) Merrill) is an important dicot crop due to the high content of oil and protein in its seeds. Because of its potential for large-scale production, soybean has excelled in the world agricultural economy as a major oilseed crop. At present, soybeans are grown primarily for oil extraction and for use as a high protein meal for animal feed (Singh and Shivakumar, 2010). According to Li-Juan and Ru-Zhen (2010), soybean has a protein content of approximately 40% and an oil content of approximately 20%. Soybean production has been increased from about 26 million tons in 1960 to 223 million tons in 2010 due to increases in harvest area and yield (FAO, 2012). One major factor influencing growth and yield of soybean is water deficit and drought stress.

Drought is a regular and common feature in Saudi Arabia. Severe water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Abdalla, 2011; Azadeh *et al*., 2014). Water stress has been found to activate the production of the reactive oxygen species (ROS) which is toxic for plant cells (Miyake, 2010). The most obvious effect of water stress is growth reduction (Amin, *et al*., 2009 ; Azadeh *et al*., 2014). Thus, a common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Lisar *et al*., 2012). Drought stress causes closing stoma and reducing leaf area (Kumudini, 2010); consequently decreasing photosynthetic pigments and activity.

Antioxidants molecules such as ascorbic acid have been suggested as signal transducers or messengers. These substances have obtained particular attention because of inducing protective effects on plants under abiotic stress. Shalata and Newman (2001) found that ascorbic acid acts directly to neutralize superoxide radicals, and as a secondary anti-oxidant during reductive cycling of the oxidized form of α-tocopherol.

It is now widely accepted that Reactive Oxygen Species (ROS) are responsible for various stress-induced damages to macromolecules and ultimately to cellular structure. Ascorbic acid is an important antioxidant, which reacts not only with H2O2 but also with O2, OH and lipid hydroperoxidases. On the other hand, ascorbic acid has been implicated in several types of biological activities in plants such as, an enzyme co-factor, as an antioxidants, as a donor/acceptor in electron transport at the plasma membrane or in the chloroplast, all of which are related to oxidative stress resistant (Conklin, 2001).

To the best of our knowledge there has been no previous reports regarding the effects of foliar application of ascorbic acid on soybean growth and yield. Therefore, the purpose of this study was to determine the ability of antioxidant "ascorbic acid" to alleviate the deleterious effects of water stress on soybean (*Glycin max* L. Merrill) plants grown in the Kingdom of Saudi Arabia.

Materials and Methods

Experiments

Pot experiments were conducted during the two successive summer seasons of 2013 and 2014 to investigate the effect of the antioxidant 'ascorbic acid" on growth, chemical contents and yield of soybean (Glycin max) plants under drought stress conditions. Seeds of soybean were obtained from authorized agriculture company and were sterilized with 1.5% Clorox, washed three times with distilled water, and coated with N-fixing bacteria (rhizobia). Seeds were then sown in plastic pots (30 cm inner diameter) filled with 10 kg sandy soil. Physical and chemical properties of the soil used in the study were recorded in Table (1). After sowing, irrigation was applied to supply seedlings with 100% available water, at two-day intervals until the seedlings reached the fourth leaf stage. Plants were fertilized with Sangral complete fertilizer (Sinclair Horticulture LTD, England). The fertilizer consists of macro elements, total nitrogen 20% N (4.4% Ammonia - 5.8% Nitrate - 9.8% Urea), Phosphorus (20% P2O5), Potassium (20% K2O) , Mg (0.012%) Sulphur (0.04%) , and microelements (as ppm) Fe (70), Zn (14), Cu (13), Mn (13), B (12) and Mo (12)), in two equal portions; the first during the seedling stage and the second at the start of flowering. After that the pots were divided into four groups for water-stress treatments, with each group divided into four subgroups for ascorbic acid foliar application. The soil moisture for all pots was kept at 80% field capacity until 15 days after sowing. After that, the water stress treatments were initiated.

Pots were subjected to one of the 3 water-stress treatments: 100%(FC1), 80% (FC2), 60% (FC3) and 40% (FC4) of field capacity. All pots were weighed every two days. Losses in pot weight represent transpiration and evaporation. Cumulative water losses were added to each pot to compensate for transpiration and evaporation. Accumulated water loss was calculated as the differences in pot weight between successive weightings. At 40, 50 and 60 days from sowing, the plants were sprayed with either tap water (control) and/or ascorbic acid (ASC) at 100, 200 or 300 mg/l until dripping, using a small pressure pump after adding Tween 20 (0.5%) as a wetting agent.

Vegetative measurements

Three uniform plants were uprooted from each treatment at the full blooming stage (90 days from sowing) to measure morphological and physiological characteristics. The plants were cleaned and plant height, number of branches, number of leaves and leaf area (using a leaf-area meter) were determined. Fresh and dry weights were estimated by drying each plant in electrical oven at 70°C to a constant weight.

Chemical measurements

Photosynthetic pigments were determined spectrophotometrically in 80% acetone extracts as described by Metzner *et al.* (1965). Total carbohydrate and soluble sugars were determined spectrophotometerically using the phenol-sulfuric acid method described by Dubois *et al*. (1956. Standard curves with glucose were prepared and the contribution of soluble sugars was calculated based on the dry weight bases.Total soluble amino acids were determined in 80% ethanol extracts according to Sadasivam and Manickam (1996). Free proline content was determined colorimetrically in aqueous sulfosalicylic acid as described by Bates *et al*. (1973). In a mixture of sulfuric and perchloric acid total nitrogen was determined by the micro-Kjeldahl method (Bremner 1996), Potassium was determined by flame-photometry (Kalra, 1998), Phosphorous was estimated using ammonium molybdate and ascorbic acid (Cooper, 1977) and Calcium was measured according to (Leggett and Westermann, 1973).

Total protein was analyzed by measuring nitrogen concentrations using Kjeldahl procedure then the percentages of total nitrogen were converted to protein values by multiplying nitrogen content by the factor 6.25 according to to Sadasivam and Manickam (1996).

Protein electrophoresis.

SDS-polyacrylamide gel electrophoresis was performed using 10% acrylamide slab gel following Laemmli (1970). Gels were photographed, scanned and analyzed using GelDoc 2000 Bio Rad system.

Yield and its quality

At harvest time (120 days from sowing) yield components (number of pods plant^{-1}, no. of seeds plant^{-1}, total yield plant^{-1} and weight of 100 seeds) were determined. Harvest index was obtained according to Kobraee et al. (2011) by dividing economic yield (grain dry weight) by biological yield (plant dry matter) multiplied by (100 * 0.25). Seed quality was represented by the concentrations of nitrogen, phosphorous, potassium, calcium, carbohydrates, amino acids and protein determined in the dry seeds as described above.

Statistical analysis

The experiment was arranged in a completely randomized design and was analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test using COSTAT software according to Snedecor and Cochran (1980). The values presented are all mean for three samples in each group. Differences among treatments were tested with LSD at 5% level of significance.

Results and discussion

Vegetative growth

Data registered in Tables 2and 3 indicated, in general that, severe reductions in plant growth, manifested by vegetative parameters, were recorded due to water stress. All plant growth characters including plant height, number of branches, number of leaves, leaf area per plant (Table 1) or shoot fresh and dry weights (Table 3), significantly decreased due to water deficit. The highest RWC % records for soybean leaves obtained under 80% FC and 100 FC (Table 3). The largest reduction in growth parameters was observed under severe water stress (40% FC). On the other side, foliar application of ascorbic acid (ASC), especially at 100 and 200 mg l^{-1}, improved all plant growth parameters compared to untreated control plants. Regarding the interaction effects, it is clear that ascorbic acid at 100 and 200 mg l^{-1} significantly increased all growth features of soybean with an increasing effect at 80% and 60% FC. However, while application of ascorbic acid at 100 and 200 mg l^{-1} counteracted the harmful effect of water stress on plant growth, there is no significant effect on growth characters at 300 mg l^{-1} of ascorbate. The obtained data indicated also that all ascorbic acid concentrations showed significant increase in RWC% compared with untreated plants. Where the highest significant increase in RWC% obtained in plants sprayed with 200 ppm ascorbic acid. Regarding the effect of interaction between water stress and ascorbic acid concentrations, the data illustrated that the highest significant means of RWC% obtained under 80% FC combined with 200 ppm ascorbic acid.

The inhibiting effects of water stress on plant growth have previously been reported for soybean (Abdalla, 2011; Heba and Samia, 2014) and white lupins (Hefny, 2011). It is well known that water stress conditions cause a multitude of molecular, biochemical and physiological changes, thereby affecting plant growth and development (Boutraa, 2010). A decline in plant growth in response to water stress might be due either to decreases in cell elongation resulting from the inhibiting effect of water shortage on growth-promoting hormones which, in turn, lead to decreases in cell turgor, volume and eventually growth. Water-stress conditions cause a marked suppression in plant photosynthetic efficiency, mainly due to the closing of stomata and inhibition of (Rubisco) enzyme (Lawlor and Cornic, 2002). The depressive effect of water stress on growth parameters may also be attributed to a drop in leaf water content, as indicated in Table (3), and a reduction in the assimilation of nitrogen compounds (Reddy *et al.*, 2003), affecting the rate of cell division and enlargement. Drought stress also reduced the uptake of essential elements and photosynthetic capacity as well as the excessive accumulation of intermediate compounds such as reactive oxygen species (Yazdanpanah *et al.*, 2011) which cause oxidative damage to DNA, lipid and proteins and consequently a decrease in plant growth. Finally, water stress leads to increases in abscisic acid which cause an inhibition of the growth (Abdalla, 2011). In addition, a secondary aspect of water stress in plants is the stress-induced production of ROS (Manchanda and Garg 2008; Azadeh *et al*., 2014). The enhanced production of ROS during water stress lead to the progressive oxidative damage and ultimately cell death and growth suppression (Ruiz-Lozano *et al*. 2012).

Table (1). Physical and chemical analyses of the soil used in the experiment.

Physical properties	Chemical properties	
Particle size distribution:	CaCO3 (%) 0.41	Soluble anions (meq/l)
Sand (%) 92.3	OM (%) 0.26	CO_3^{2-} 0.22
Silt (%) 6.2	Ec (dsm^{-1}) 0.53	HCO_3^- 0.86
Clay (%) 1.5	Soluble cations (meq/l)	Cl^- 1.83
Soil texture (Sandy)	Ca^{2+} 2.96	Avail. elements (mg/kg)
	Mg^{2+} 1.68	N 19.2
pH 8.01	Na^+ 2.04	P 8.3
	K^+ 0.21	Fe 2.4
Soil suspension (1 soil : 2.5 water)		

Table (2): Effect of different concentrations of ascorbic acid (ASC) on plant height, No. of branches, No. of leaves and leaf area of soybean plants grown under different levels of field capacity (FC%).

Treatments		**Growth characters**			
FC%	**ASC (ppm)**	**Plant ht (cm)**	**Branch/ plant**	**Leaves/ plant**	**Leaf area (cm^2)**
100%	00	55.2	3.5	14.5	457.5
	100	60.4	4.2	17.5	476.3
	200	65.1	4.6	19.3	523.8
	300	50.4	3.8	15.2	469.2
80%	00	45.5	3.2	12.7	422.6
	100	50.6	4.4	14.4	441.5
	200	55.3	4.6	16.6	466.2
	300	48.6	3.5	13.2	438.7
60%	00	40.2	2.8	10.8	364.6
	100	46.3	3.1	12.4	382.8
	200	50.4	3.5	14.3	396.3
	300	42.5	3.1	11.2	372.4
40%	00	30.6	2.2	8.3	276.5
	100	35.3	2.5	9.0	292.1
	200	40.4	2.8	9.7	311.4
	300	32.2	2.4	8.5	285.5
LSD 5%		9.77	0.45	4.12	98.77
Mean of main effects:					
FC%	100%	57.7	4.1	16.6	481.7
	80%	50.1	3.9	14.2	442.3
	60%	44.8	3.1	12.2	379.1
	40%	34.6	2.4	8.8	291.4
LSD 5%		12.45	0.66	3.24	95.64
ASC (ppm)	00	42.9	2.9	11.6	380.3
	100	48.2	3.6	13.3	398.2
	200	52.8	3.9	15.0	424.4
	300	43.4	3.2	12.0	391.5
LSD 5%		2.85	0.26	0.74	6.85

Table (3): Effect of different concentrations of ascorbic acid (ASC) on shoot fresh and dry weights, RWC% and dry mass of soybean plants grown under different levels of field capacity (FC%).

Treatments					
FC%	ASC (ppm)	Shoot Fwt (g)	Shoot Dwt(g)	RWC%	Total dry biomass(g)
100%	00	35.5	6.4	81.9	8.5
	100	42.4	7.2	83.0	9.5
	200	48.2	8.8	81.9	11.3
	300	40.6	6.8	83.3	9.0
80%	00	32.2	5.8	81.9	8.8
	100	38.7	6.5	83.2	9.3
	200	40.4	8.1	80.1	12.4
	300	34.6	6.1	82.3	10.2
60%	00	28.8	5.2	81.9	9.5
	100	32.3	6.1	81.0	10.0
	200	36.4	7.0	80.7	12.8
	300	30.2	5.6	81.5	9.8
40%	00	21.5	5.0	76.7	6.2
	100	24.3	5.8	76.1	6.8
	200	26.2	6.2	76.3	7.2
	300	22.3	5.3	76.2	7.0
LSD 5%		11.55	0.87	3.42	0.64
Mean of main effects:					
FC%	100%	41.7	7.3	82.5	9.5
	80%	36.5	6.3	82.9	9.3
	60%	31.5	5.8	81.7	9.9
	40%	24.6	5.4	77.6	7.5
LSD 5%		9.22	0.86	1.77	0.82
ASC (ppm)	00	29.5	5.6	80.6	8.3
	100	34.4	6.4	80.8	8.9
	200	37.8	7.5	79.8	10.9
	300	31.9	5.9	78.8	9.0
LSD 5%		6.12	0.76	0.48	0.37

Foliar spray of ascorbic acid in most cases resulted in a significant increase in soybean plant growth parameters under normal or stressed conditions. The effect is more pronounced at the intermediate level (200 mg l^{-1}) of ascorbic acid. In addition, data of the interaction between water stress and ascorbic acid concentrations indicated that the highest significant increases in growth criteria observed mostly under 60% soil moisture level interacted with 100 or 200 ppm ascorbic acid where the difference between the two treatments was insignificant. These results are similar to those reported by Ghoname *et al.* (2010) for sweet pepper and and Azooz and Al-Fredan (2009) for *Vicia faba* plants. The stimulating effect of ascorbic acid on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals (ORS) by increasing antioxidants and enzyme activities (Farouk *et al.,* 2011). In addition, the positive effect of ascorbic acid on plant growth may be due to its effect on increasing nutrient uptake and increase elements content such as nitrogen, phosphorous, and potassium, as was found in the present study (discussed latter). Phosphorous and potassium are essential nutrients playing an important role in the biosynthesis and translocation of carbohydrates, and necessary for stimulating cell division, cell turger and forming DNA and RNA (Saeidi-Sar *et al*., 2013). These results are in agreement with those obtained by others (Azooz, 2009; Ekmekçi and Karaman, 2012). They indicated that, vitamins (such as ascorbic acid) could accelerate cell division and cell enlargement and induce improvement of membrane integrity, which may have contributed in reducing ion leakage, and consequently improving growth. Increasing of leaves relative water content (RWC) and reduction in transpiration rate indicated that these vitamins probably reflect the efficiency of water uptake and utilization or reduction of water loss, which consequently causes increase in leaf water potential. Hence, it could be concluded that the beneficial effect of ascorbic acid on growth parameters of soybean plants has been related to the efficiency of their water uptake and utilization. Many studies have reported that vitamins, when used with optimal concentration, exhibited beneficial effect on growth and yield of some crop plants grown under saline conditions (Khan *et al*., 2011; Ekmekçi and Karaman, 2012).

Photosynthetic pigments

Data recorded in Table (4) showed that the concentration of photosynthetic pigments i.e. chl a, chl b and carotenoids were decreased significantly by increasing soil moisture deficit. The most decrease in the pigment concentration was recorded at 40% FC. On the other side, plants sprayed with ascorbic acid showed significant increases in photosynthetic pigments content compared with control ones. However, increasing ascorbic acid concentration above 200 mg l^{-1} caused a significant decrease in photosynthetic pigments compared with the other concentrations of ascorbic acid. Concerning the effect of interaction between water stress and ascorbic acid concentrations, the data showed that plants grown under 60% FC and sprayed with 200 mg l^{-1} ascorbic acid showed mostly the highest significant increases in photosynthetic pigments.

The decrease in chlorophyll content under drought is a commonly observed phenomenon (Kumar *et al.,* 2011; Heba and Samia, 2014), and might be attributed to reduced synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family (Nikolaeva *et al.,* 2010), or to destruction of the pigment protein complexes which protect the photosynthetic apparatus, or to oxidative damage of chloroplast lipids and proteins, therefore formation of chlorophyll a, b and carotenoids decreases. In this regard Akça and Samsunlu (2012) reported that the negative effects of abiotic stress on photosynthetic pigments could be due to the inhibition of chlorophyll biosynthesis or increase of its degradation by chlorophyllase enzyme, which is more active under stresses. An oxidative stress could happen due to water stress leading to deterioration in chloroplast structure, and consequently decrease in chlorophyll content (Kumar *et al.,* 2011).

Among the positive effects of ascorbic acid in the counteraction of the adverse effects of water stress are the stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidization (Khan *et al*. 2011). Ascorbic acid can mitigate the adverse effects of drought through increasing the content of IAA and GA3 and decreasing ABA level, which may be involved in protecting the photosynthetic apparatus and consequently increasing the photosynthetic pigments (Saeidi-Sar *et al*., 2013). Ascorbic acid has a major role in photosynthesis, acting in the Mehler peroxidase reaction with ascorbate peroxidase to regulate the redox state of photosynthetic electron carriers and as a co-factor for violaxanthin de-epoxidase, an enzyme involved in xanthophyll cycle-mediated photoprotection (Khan *et al*., 2011). Consequently, in ascorbic acid treated plants, high level of carotenoids can synergistically function with ascorbic acid to provide an effective barrier against oxidation under salinity stress. These results reinforce the results obtained by other investigators (Azooz and Al-Fredan, 2009). They concluded that, chlorophyll content of plants treated with vitamins (such as ascorbic acid) was increased due to the protection effect of these vitamins. Azzedine *et al*. (2011) reported that, ascorbic acid can detoxify and neutralize the reactive oxygen species by prevention of free radicals activity, leading to increase in chlorophyll content of vitamin- treated plants. They also found that, application of vitamin C (ascorbic acid) was effective to mitigate the adverse effect of abiotic stress on plant growth due to increased leaf area and improved chlorophyll and carotenoids contents.

Nutrient contents

The concentration of nitrogen, phosphorus and potassium in soybean plants were determined under different field moisture capacities and were evaluated on a percentage basis (Table 5). It is a fact that water stress is generally recognized as injurious to plants by disturbing the electrolyte balance, resulting in deficiency of some nutrients. In this respect, water stress decreased significantly the percentages of nitrogen, phosphorus and potassium (Table 5) in plant tissues. The largest reduction occurred under severe water stress (40% FC). However, statistical analysis showed highly significant increases in all nutrient concentrations occurred after exogenous application of ascorbic acid, especially at 200 mg l^{-1}. As with other measurements, application of ascorbic acid at 300 mg l^{-1} reduced this positive effect. It is clear that the impact of ascorbic acid was greater in water-stressed than unstressed plants.

Table (4): Effect of different concentrations of ascorbic acid (ASC) on Chl a, Chl b, total Chl and carotenoids of soybean leaves under different levels of field capacity (FC%).

Treatments		Chlorophyll (mg.g Fwt)			
FC%	ASC (ppm)	Chl a	Chl b	Total Chl	Caroten.
100%	00	0.62	0.42	1.04	0.28
	100	0.68	0.46	1.14	0.32
	200	0.74	0.53	1.27	0.36
	300	0.65	0.44	1.09	0.30
80%	00	0.53	0.40	1.93	0.35
	100	0.57	0.46	1.03	0.33
	200	0.62	0.48	1.10	0.34
	300	0.55	0.42	0.97	0.36
60%	00	0.43	0.32	0.75	0.28
	100	0.46	0.35	0.81	0.26
	200	0.48	0.38	0.86	0.27
	300	0.45	0.34	0.79	0.25
40%	00	0.36	0.25	0.61	0.16
	100	0.38	0.29	0.67	0.18
	200	0.40	0.31	0.71	0.21
	300	0.38	0.26	0.64	0.18
LSD 5%		0.21	0.16	0.26	0.11
Mean of main effects:					
FC%	100%	0.67	0.46	1.13	0.31
	80%	0.56	0.44	1.00	0.35
	60%	0.46	0.34	0.80	0.26
	40%	0.38	0.27	0.65	0.18
LSD 5%		0.25	0.34	0.36	0.14
ASC (ppm)	00	0.49	0.35	0.84	0.26
	100	0.52	0.39	0.91	0.28
	200	0.56	0.43	0.99	0.31
	300	0.51	0.36	0.87	0.27
LSD 5%		0.05	0.05	0.0.8	0.03

It is well known that, water stress affects the availability of nutrients in the soil by its effects on the solubility and precipitation of salt, and alters physiological processes within the plant, including nutrient uptake and translocation (Scheiber *et al*., 2008). The decrease in N content due to water stress has been reported in various crops (Scagel *et al*., 2011). Phosphorus is one of the most important nutrients in the growth and development of plants. It plays a key role in cellular energy transfer, respiration, photosynthesis. Phosphorus uptake decreases with decreasing soil moisture in various crops such as chickpea (Goldani and Rezvani, 2007) pepper (Çimrin *et al*., 2010). The role of ascorbic acid in increasing ionic content may be due to its effects on stabilizing cellular membranes through increasing antioxidants substances, saving cell membranes from oxidative stress and hence improving plant cell permeability (Farouk *et al.*, 2011). As shown in Table (5), external application of ascorbic acid increased significantly N, P and K concentrations in soybean The magnitude of activation was increased with increasing ascorbate concentration. These results are in good harmony with those obtained by Akça and Samsunlu (2012) and they reported that foliar application of some growth regulators enhance the uptake of N, P and K, also Gallie (2012) suggested that one of the main roles of ascorbic acid is to maintain a cation-anion balance in plant tissues by stabilizing cell membranes at high external abiotic stress. In this concern, Saeidi-Sar et al. (2013) found that, exogenous supply of ascorbic acid enhanced potassium concentration in water-stressed *Phaseolus* plants. This increase was attributed to the positive effect of ascorbic acid on the root growth, which consequently increased the absorption of different nutrients and alleviated the harmful effects of water stress.

Table (5): Effect of different concentrations of ascorbic acid (ASC) on the N, P, K and Proline concentrations in soybean leaves under different levels of field capacity (FC%).

Treatments		Chemical contents (%)			
FC%	ASC (ppm)	N	P	K	Total proline
100%	00	3.5	0.65	1.2	15.7
	100	4.6	0.74	1.5	17.3
	200	5.2	0.83	1.9	18.2
	300	3.8	0.67	1.4	16.2
80%	00	3.3	0.58	0.90	20.5
	100	4.2	0.67	1.1	21.6
	200	4.8	0.74	1.5	23.2
	300	3.7	0.61	0.94	20.8
60%	00	2.7	0.43	0.73	35.6
	100	3.5	0.55	0.94	37.8
	200	4.1	0.62	1.1	39.2
	300	2.9	0.45	0.80	36.6
40%	00	2.1	0.35	0.66	22.2
	100	2.4	0.39	0.70	23.2
	200	3.1	0.41	0.86	25.5
	300	2.2	0.37	0.68	22.8
LSD 5%		0.88	0.19	0.25	9.68
Mean of main effects:					
FC%	100%	4.3	0.72	1.5	16.6
	80%	4.0	0.65	1.1	21.5
	60%	3.3	0.51	0.89	37.3
	40%	2.5	0.38	0.73	23.4
LSD 5%		0.66	0.13	0.34	8.77
ASC (ppm)	00	2.9	0.50	0.87	23.5
	100	3.7	0.59	1.1	24.9
	200	4.3	0.65	1.3	26.5
	300	3.2	0.53	0.92	23.9
LSD 5%		0.87	0.08	0.42	1.77

Proline content

Data in Table (5) showed that proline increased significantly when plants subjected to water stress up to 80% and 60% FC, as compared with control plants (100% FC), but at severe stress (40% FC) proline levels tended to go back. Data illustrated also that when plants were kept under drought stress, the amount of proline would increase. Ascorbic acid treatments increased proline concentration whether plants were under water stress or not. However, the most effect of ascorbic acid on proline content was more pronounced in stressed than in non-stressed plants. The most increase in proline concentration was observed at 60% FC when plants were treated with 200 mg l^{-1} ascorbic. Concerning the interaction between water stress and ascorbic acid concentrations, the obtained data revealed that different concentrations of ascorbic acid caused a significant increase in proline accumulation under different water stress levels compared with control plants. Furthermore, the highest significant increase in proline accumulation obtained in 60% FC treatment, while the lowest accumulation obtained under 40% FC.

The present study showed that mild water stress increased proline while severe water stress affected negatively the level of proline in soybean plants. In this regard, Yazdanpanah *et al*. (2011) reported that the amount of proline increased under mild drought stress because proline is a key in osmosis regulation. Moreover, our study showed that ascorbic acid stimulated proline accumulation in water stressed plants. Increasing the amount of proline and sugars in the plants would lead to the resistance against loosing water, protect turger, reduce the membrane damage and accelerate the growth of plants under stress conditions (Amin *et al*., 2009; Gallie, 2012). The higher accumulation of proline under stress conditions was attributed to enhanced activities of proline biosynthesis enzymes, ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes, proline oxidase and proline dehydrogenase(Ramezani *et al*., 2011). Proline have been shown involved in osmotic regulation in plant, playing an important role in tolerance of plant to abiotic stress (Bartels and Sunkar, 2005). The accumulation of proline and amino acids in the cytoplasm plays an important role in the osmotic balance of plants and are good indicators of tolerance (Uiddin *et al*., 2012). These conclusions are confirmed with the results of this study. The increased proline content in soybean suggests an excellent mechanism to mitigate the injurious effect of water stress. This supports the assumption that proline accumulation is a part of physiological response of plant to intense stress (Uiddin *et al*., 2012).

The yield and its components

Data in Table (6) showed that the number of pods and seeds per plant, seed yield per plant and 100 seed weight were affected by water stress and spraying plants with ascorbic acid counteracted this negative effect of drought. All yield components decreased under different water stress levels. It is well known that water deficit affects negatively all growth parameters and development, therefore, yield and its components were significantly depressed under drought stress. Pod yield per plant and seed quality significantly decreased with increasing water deficit, while foliar application of ascorbic acid, especially the treatment of 200 mg l^{-1}, tended to reverse this negative effect and increased the yield of soybean. Decreasing soil water content induced gradual reduction in soybean yield as compared with non-stressed plants. The most negative effect of water stress on yield was recorded at 40% FC, at which, number of pods/plant, number of seeds/plant and seed yield/plant were decreased by about 48.4%, 51.5% and 32.6%, respectively as compared to 100% FC. The weight of 100 seeds showed also a decrease of about 16.3% at 40% FC as compared with control plants (100% FC).

Previous studies reported that water stress reduced the yield of many crops (Hefny, 2011; Farouk and Amany, 2012; Zhwan and Mohammed, 2014). In legume plants such as soybean, seed yield is determined by several factors, the number of pods is one of these factors. The reduced yield may by due to the negative effect of water stress on the number of branches and leaves per plant as well as leaf area (Table 2), resulting in a reduction in the supply of carbon assimilate and photosynthetic rate by plants and consequently less biomass produced as well as decreased translocation of assimilates towards the developing fruits (Vurayai *et al.*, 2011). In addition, yield may be reduced under drought conditions due to increasing the rate of flower abscission and pod abortion (Liu *et al.*, 2003). The depressive effect of water stress on yield may be attributed to the inhibitory effect of drought on vegetative growth as discussed above (Table2). In this connection, Taffouo *et al*. (2009) reported that, the significant decrease of yield components observed under abiotic stress in cowpea would be partly related to a significant reduction of foliar chlorophyll contents and nutrients concentration. Exogenous application of ascorbic acid under drought stress caused increase in all parameters of yield components as compared to the corresponding water stress level. This positive effect of ascorbic acid on yield components may be attributed to its role as a cofactor for enzymes involved in photosynthesis, hormone biosynthesis, and the regeneration of antioxidants (Gallie, 2012).

The result of harvest index (HI) analysis (Fig. 1) show this trait was affected by irrigation regime significantly at level of 5 %. Among factors of irrigation omitting, 40% FC had the lowest harvest index, also among various treatments, it seems that ASC treatments caused a slight enhancing effect on HI of soybean under different water deficit treatments. In present study, withholding irrigation reduced harvest index, but ASC treatments had little effect. In this regard Kobraee et al. (2011) stated that water stress decreased harvest index, while Spaeth et al. (1984) pointed out water stress had no effect on harvest index. Ascorbic acid treatments, on the other side, caused an improvement in the HI of wheat under newly reclaimed sandy soil (Bakry et al., 2013).

Chemical analysis of seeds

It is clear from the results in Table (7) that water stress decreased soluble sugars, total carbohydrates, total amino acid contents and protein percentage of soybean seeds as compared to plants grown under 100% FC (control). The magnitude of reduction was increased with increasing water stress level. The reduction in soluble sugars and total carbohydrates in soybean seeds could be attributed to the nutritional imbalance and reduced photosynthesis as recorded by Ramezani *et al*. (2011). Moreover, the reduction of sugar content under water deficit may be attributed to the negative effect of drought stress on photosynthetic pigments and photosynthesis (Yazdanpanah *et al*., 2011) who found that net photosynthesis, transpiration rate and stomatal conductance were significantly affected by stress due to changes in chlorophyll content and chloroplast structure. In a previous study by Jalal *et al*. (2012) they found that 60% and 40% FC decreased chl a, chl b, carotenoids and caused stomatal closure in *P. tenuiflorus* plants. Stomatal closure, in turne, restricts CO_2 entry into leaves thereby decreasing CO_2 assimilation and rate of transpiration (Chaves, 2002).

The present study showed that ascorbic acid treatments improved plant tolerance against water stress and sugars approached near its normal condition. Increasing amount of sugars and thus the osmosis gradient in plant tissues treated with ascorbic acid would lead to the resistance against loosing water, protect chloroplasts and accelerate plant growth under stress conditions (Amin *et al*., 2009). The exogenous application of ascorbic acid could counteracte the drought deleterious effects on sugars in okra (Amin *et al*., 2009).

Total amino acid content in soybean seeds was also found to be adversely affected by water stress conditions. Same results were obtained by Sarwat and Sherif (2007) who stated that, amino acid appeared to be decreased with water stress treatments. The reduction in protein percentage in seeds of soybean plants grown under water stress may be attributed to the reduction in leaf nitrogen content (Table 5). This result was in line with that found by El-Hindi and El-Ghamry (2005) on cherry gold plants.

Data in Table (7) showed also the role of ascorbic acid in ameliorating the adverse effect of water stress on all chemical compositions studied in soybean seeds. Ascorbic acid treatments increased significantly total soluble sugars, total carbohydrates, total amino acid and protein contents of soybean seeds under water-stress or non-stress conditions. The magnitude of increment was much pronounced by using 200 mg l^{-1} ascorbic acid under all levels of field capacity (FC). These results could be supported by those of Sadak *et al*., 2010 ; Ramezani *et al*., 2011; Zhwan and Mohammed , 2014)

The adverse effect of water stress on N, P, K and Ca contents in soybean seeds increased as drought became more severe (Table 8). The lowest values of these elements were recorded at 40% FC treatment. On the other side, ascorbic acid enhanced the accumulation of these nutrients in both water-stressed and non-stressed seeds. The highest values of N, P, K and Ca in seeds were recorded at 200 mg l^{-1} of ascorbic acid. This study cleared that drought stress was associated with reduction in nutrient concentrations in soybean seeds and when levels of dryness increased, nutrient content decreased also.

The positive effect of water stress on amino acids and proline content in soybean seeds in response to ascorbic acid treatments has significantly magnified. These results confirm the earlier findings of Fercha *et al*. (2011). The effect of ascorbic acid on proline content suggests that this vitamin probably improves growth of stressed plants. Moreover, Azzedine *et al*. (2011) reported that application of vitamin C (ascorbic acid) was effective to mitigate the adverse effect of salt stress on plant growth of durum wheat due to increased leaf area and enhanced proline accumulation.

In summary, soybean yield and its components were significantly depressed with water deficit. while foliar application of ascorbic acid, especially at 200 mg l^{-1}, tended to reverse this negative effect and increased the yield and improved seed quality as assigned by carbohydrates, amino acids and protein contents (Table 7) or nutrient element contents of soybean seeds (Table 8). The interaction treatment indicated that application of ascorbic acid (200 mg/l^{-1}) under moderate water deficit (80% - 60% FC) significantly increased the yield and its quality. The role of ascorbic acid in alleviating the harmful effect of water stress on yield may be attributed to an increase in stomatal conductance and net photosynthetic CO2-fixation activity under water stress and also to its role as an antioxidant, a cofactor for enzymes involved in photosynthesis and hormone biosynthesis (Gallie, 2012).

Protein electrophoretic pattern

Changes of protein patterns have been analyzed in leaves of soybean plants (Fig. 2), in order to follow any possible alterations in gene expression in plants subjected to drought stress at different field capacity levels (100%, 80%, 60% and 40% FC) in the absence or presence of ascorbic acid treatments (100, 200 and 300 mg l^{-1}) comparing with non-stressed control at 100% FC. It is clear that water stress and ascorbic acid treated plants induced variations in the appearance of new protein bands and in disappearance of others with different high molecular weights, whereas no changes in protein patterns with low molecular weights were

Table (6): Effect of different concentrations of ascorbic acid (ASC) on the yield and its components of soybean plants grown under different levels of field capacity (FC%).

Treatments		Yield			
FC%	**ASC (ppm)**	**No. of pods/plants**	**No. of seeds/plant**	**Seeds (g/plant)**	**100 seed wt. (g)**
100%	00	22.5	46.3	9.8	14.6
	100	26.4	52.2	11.4	14.8
	200	30.5	62.7	13.5	14.6
	300	24.5	50.5	10.7	14.2
80%	00	21.6	44.4	10.2	14.2
	100	23.5	46.7	10.8	15.4
	200	27.7	51.3	12.5	14.6
	300	21.8	47.4	10.1	14.4
60%	00	18.2	33.8	8.3	13.2
	100	19.8	41.2	9.7	13.9
	200	22.1	45.3	10.1	14.2
	300	18.8	38.8	9.2	13.8
40%	00	11.6	22.5	6.6	12.2
	100	12.8	26.6	7.8	12.8
	200	14.5	30.3	8.6	13.4
	300	12.4	24.6	7.2	12.6
LSD 5%		3.55	9.14	1.72	1.33
Mean of main effects:					
FC%	100%	25.9	52.9	11.3	14.5
	80%	23.6	47.4	10.9	14.6
	60%	19.7	39.7	9.3	13.7
	40%	12.8	26.0	7.5	12.7
LSD 5%		3.11	8.25	1.65	1.28
ASC (ppm)	00	18.4	36.7	8.7	13.5
	100	20.6	41.6	9.9	14.2
	200	23.7	47.4	11.1	14.4
	300	19.3	40.3	9.3	13.7
LSD 5%		2.12	3.43	1.23	0.56

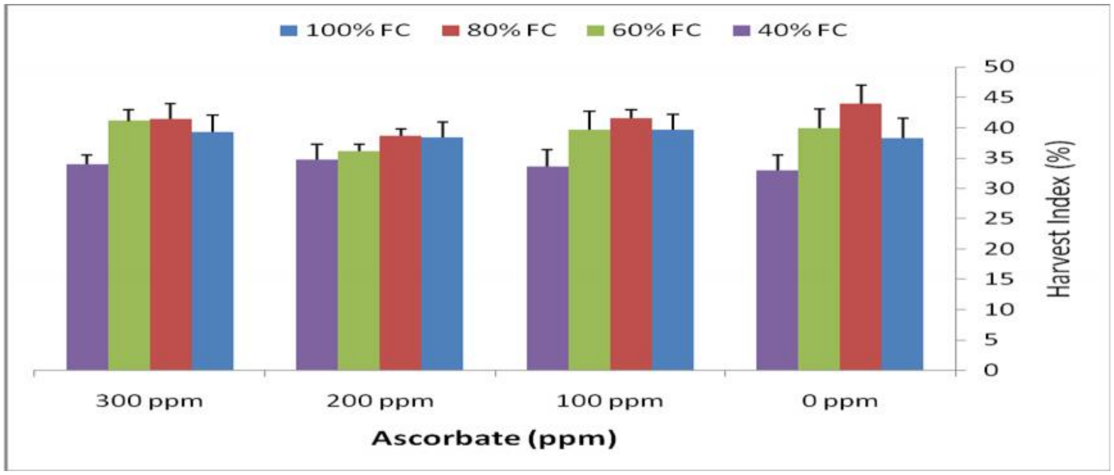

Fig (1). Effect of different concentrations of ascorbic acid (ASC) on the Harvest Index of soybean plants grown under different levels of field capacity (FC%).

Table (7): Effect of different concentrations of ascorbic acid (ASC) on the chemical contents of soybean seeds under different levels of field capacity (FC%).

Treatments		Chemical contents (mg/g dry wt)			
FC%	ASC (ppm)	soluble sugars	Total carboh	Total amino acid	Protein (%)
100%	00	41.2	67.2	60.4	43.6
	100	44.6	72.3	62.2	44.5
	200	48.1	76.2	65.5	45.2
	300	42.3	68.4	61.3	44.0
80%	00	52.3	72.8	66.3	38.2
	100	55.2	74.6	68.2	40.2
	200	58.3	78.5	70.1	43.3
	300	53.1	73.5	66.8	38.9
60%	00	54.6	78.4	80.5	35.5
	100	57.7	81.2	82.3	37.6
	200	60.2	86.6	85.5	40.2
	300	56.4	79.8	81.2	36.5
40%	00	28.8	42.6	52.3	30.5
	100	32.2	47.3	53.3	33.4
	200	35.5	52.1	55.1	36.5
	300	30.4	44.2	52.9	31.3
LSD 5%		13.23	11.55	16.24	6.55
Mean of main effects:					
FC%	100%	44.1	71.1	62.3	44.3
	80%	54.7	74.8	67.8	40.2
	60%	57.2	81.5	82.3	37.5
	40%	31.7	46.6	53.4	32.9
LSD 5%		9.23	6.02	7.14	3.68
ASC (ppm)	00	44.2	65.2	64.8	36.9
	100	47.4	68.8	66.5	38.9
	200	50.5	73.3	69.1	41.3
	300	45.5	66.4	65.6	37.6
LSD 5%		3.55	5.21	3.36	2.56

observed. The ascorbic acid treatment indicated the following results: (1) in non-stressed plants, the application of ascorbic acid did not change the pattern of protein bands, while (2) In drought-stressed plants; ascorbic acid treatment induced the appearance of two new polypeptides (73 and 76 kDa). It appears clearly that ascorbic acid treatment at 80%, 60% and 40% FC enhanced the formation of a 73 and 76 kDa proteins. In contrast, synthesis of these proteins was negatively affected by sufficient watering regime (100% FC) and also by water stress without ascorbic acid treatments as shown in panel (a).

The new bands of high molecular weight proteins in drought stressed plants treated with ascorbic acid might be due to *de novo* synthesis of these proteins (Khalil *et al.*, 2009). These new proteins may have a specific function to protect soybean plants from further dehydration damage and considered as a defense mechanism to drought stress. Drought induced polypeptides have been observed in many studies and are assumed to play a role in water stress tolerance (Sadiq *et al.*, 2013). Disappearance of certain polypeptides in drought-stressed plants with the absence of ascorbic acid may be related to increase the hydrolyzing enzyme RNAase activity (Kong-ngern *et al*, 2005). The effect of ascorbic acid was clear, suggesting an interaction between the protein synthesis and ascorbate function.

Table (8): Effect of different concentrations of ascorbic acid (ASC) on N, P, K and Ca in soybean seeds under different levels of field capacity (FC%).

Treatments		Chemical content (%)			
FC%	ASC (ppm)	N	P	K	Ca
100%	00	14.2	0.62	3.4	1.3
	100	14.8	0.65	3.7	1.8
	200	15.2	0.71	4.2	2.2
	300	14.6	0.64	3.5	1.5
80%	00	13.8	0.60	3.1	1.1
	100	14.2	0.63	3.5	1.4
	200	14.4	0.68	3.8	1.8
	300	14.0	0.62	3.3	1.2
60%	00	13.2	0.54	2.7	0.91
	100	13.4	0.57	3.1	1.3
	200	13.6	0.61	3.5	1.6
	300	13.3	0.55	2.9	1.1
40%	00	12.5	0.37	2.0	0.75
	100	12.8	0.39	2.4	0.84
	200	13.2	0.42	2.6	1.1
	300	12.6	0.38	2.2	0.80
LSD 5%		1.33	0.23	0.76	0.12
Mean of main effects:					
FC%	100%	14.7	0.66	3.7	1.7
	80%	14.1	0.63	3.4	1.4
	60%	13.4	0.56	3.1	1.2
	40%	12.8	0.39	2.3	0.87
LSD 5%		1.44	0.28	0.36	0.42
ASC (ppm)	00	13.4	0.53	2.8	1.0
	100	13.8	0.56	3.2	1.3
	200	14.1	0.61	3.5	1.7
	300	13.6	0.54	2.9	1.1
LSD 5%		0.24	0.05	0.45	0.25

It is also clear that the severe drought stress treatment (40% FC) did not affect the accumulation of the protein subunits between 25 and 38 KDa (panel a), however, these subunits, particularly that found at 38 KDa, were so condensed when water stressed plants were treated with ascorbic acid (Panel b). These data suggest that accumulation of the low MW subunits proteins (between 37- 36 KDa) was insensitive to drought stress, therefore they appear either under water unstressed or water stressed samples. Nevertheless, drought stress might have accounted for the delayed onset of high MW protein subunits, relative to the onset of the low MW subunits, under water stressed in the absence of SA treatments, therefore the 73 and 76 KDa subunits were formed in ascorbic acid-treated samples even though they were under severe water stress condition. These results were consistent with Samarah *et al*. (2006), who reported that, soybean seeds produced under drought stress had a variation in β-subunit of the β-conglycinin, probably because of degradation of proteins in the shriveled seeds produced under drought stress. In this regard, Azooz and Al-Fredan (2009) reported that, vitamin treatments induced alterations in the enzymes related to protein metabolism. These enzymes might act as activators of protein synthesis that appeared in seedlings treated with salinity or/and vitamins. This may play an inductive role in triggering a special defense system helping these seedlings to improve their stress tolerance and consequently their growth and productivity (Sadiq *et al*., 2013).

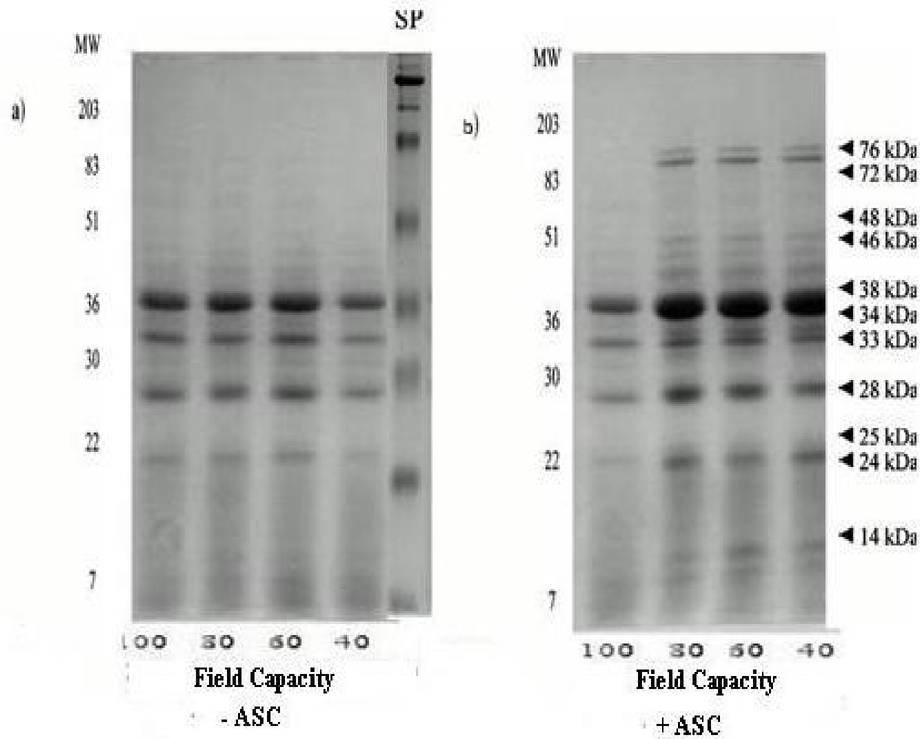

Fig (2) Analysis of protein patterns by one-dimensional SDS-PAGE extracted from leaves of soybean (*Glycine max* L.) plants grown under different watering regimes (100%, 80%, 60% and 40% FC) and untreated (-ASC)or treated (+ASC) with ascorbic acid (ASC). The effect of ascorbic acid on the approximately 72 and 76 MW protein in the leaves of soybean plants (lanes 2b to 4b) is shown. Lanes (SP) on the left contained marker proteins whose molecular masses (kilodaltons) are shown on the right side of the panels. Ascorbic acid was sprayed at 200 mg l^{-1} (b) on soybean plants grown under different watering regimes (100%, 80%, 60% and 40% FC). The lane on the left side indicates the molecular weights (MW) of standard proteins (SP). The lane on the right is used to specify MW of proteins indicated by arrows

It can be concluded that ascorbic acid can play an important role in the growth and productivity of *Glycine max* plants grown under water stress conditions, perhaps through maintaining nutritional elements and other chemical compositions within plant tissues, and because it has the potential to stimulate the production of various metabolites which cause a reduction in transpiration and thus more water become available to plants for better growth and productivity

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