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EFFECT OF FOLIAR APPLICATION OF NON-ENZYMATIC ANTIOXIDANTS ON PEANUT YIELD AND ITS QUALITY UNDER INDUCED WATER STRESS CONDITIONS

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ABSTRACT

This study was conducted during summer seasons of 2011 and 2012 in a sandy soil to study the effect of foliar application of non-enzymatic antioxidants (ascorbic acid; AsA and thiamine, Th) on peanut yield and its quality for sprinkler-irrigated peanut. A field experiment was performed at the Experimental Farm, Faculty of Agriculture, Suez Canal University at Ismailia using split plots design with two irrigation rates *i.e.* 1.00 (5100) and 0.70 (3570 m^3 ha^{-1}) of the estimated evapotranspiration and five levels of non-enzymatic antioxidants (0.0, 50 and 100ppm Th, 50 and 100ppm AsA) as the main and split plots, respectively. Drought caused significant reduction in each of leaf area index, total chlorophyll, relative water content, pod, seed and oil yields. However, water stress increased the activity of antioxidative enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX) as well as seed oil and protein contents (%). Meanwhile, exogenous application of AsA by a suitable level (100 ppm) enhanced growth, yield and its quality compared to thiamine. In the other hand, exogenous application of AsA to stressed and un-stressed plants and Th to stressed plants improved seed protein content (%) and protein molecular weight as well as number of protein bands. Application of AsA alleviated the oxidative stress damage of drought, reflected by improving above mentioned parameters as well as decreasing the activity of these above antioxidant enzymes in the leaves by approximately 14-16% compared to untreated plants under water stress. Seed, pod and oil yields were increased by about 0.65, 1.08 t/ha and 326 kg/ha when plants received 100 ppm AsA under normal irrigation, respectively. Also, under water stress (3570 m^3 ha^{-1}), the relative increases were about 0.84, 1.10 t/ha and 426kg/ha compared to unsprayed plants, respectively. Therefore, using of 100 ppm AsA can save about 1530 m^3/ha of irrigation water without reduction of pod yield and its quality .

Key words: Peanut, water stress, estimated crop evapotranspiration, antioxidants, productivity, quality, protein electrophoresis.

INTRODUCTION

Drought is being the most important environmental stress, severely impairs plant growth and development as well as limits plant production and the performance of crop plants, more than any other environmental factor (Shao *et al.*, 2008; 2009; Anjum *et al.*, 2011). Water availability is limiting factor for peanut production and seed quality. Water stress reduces leaf area by slowing leaf expansion and reducing the supply of carbohydrates. Development of optimal leaf area is important to photosynthesis and dry matter yield. Water deficit stress mostly reduce leaf growth and in turn the leaf area in many plant species (Farooq *et al.*, 2009). Severe drought stress decreased the levels of chlorophyll a, b and total chlorophyll in peanut leaves (Reddy and Rao, 1968) and caused changes in the ratio of chlorophyll a and b and carotenoids in barley and wheat leaves (Anjum *et al.*, 2003; Farooq *et al.*, 2009). The decrease in chlorophyll was attributed to the inhibition of chlorophyll synthesis as well as to acceleration turnover of chlorophyll already present. Under stress conditions, the

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photosynthesis rate of higher plants is known to decrease as the relative water content and leaf water potential decreases (Lawlor and Cornic, 2002; Kotb and Elhamahmy, 2013).

Furthermore, oxidative stress is a common effect of adverse environmental conditions including water stress (Apel and Hirt, 2004). Plants have developed an antioxidant defense system to counteract stress-induced oxidative stress. It is now believed that drought tolerance in most crop plants is associated with a more efficient antioxidant system (Kotb and Elhamahmy, 2013). The antioxidative system includes both enzymatic and non-enzymatic systems. The non-enzymatic system includes carotenoids, vitamins, phenols, flavonoids, dietary glutathione and endogenous metabolites (Krishnaiah *et al.*, 2011), whereas the enzymatic antioxidative system includes superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and polyphenol oxidase (PPO), *etc.* The function of this antioxidant system is to scavenge the injurious radicals produced during oxidative stress and thus help the plants to survive under such conditions (Mandal *et al.*, 2009).

Ascorbic acid (vitamin C) also has been associated with several types of biological activities in plants such as in enzyme co-factors, antioxidant, and as a donor / acceptor in electron transport at the plasma membrane or in the chloroplast (Conklin, 2001). A high level of endogenous ascorbate is essential effectively to maintain the antioxidant system that protects plants from oxidative damage (Cheruth, 2009). It affects plant growth and development, and plays an important role in the electron transport system (El-Kobisy *et al.*, 2005). Furthermore, it has also been implicated in regulation of cell elongation (Zauberman *et al.*, 1991). Further, Farahat *et al.* (2007) reported that foliar application of ascorbic acid caused pronounced increases in vegetative growth and chemical constituents as well as essential oil percent of *Cupressus sempervirn* L. Also, peanut plants sprayed with ascorbic acid, significantly surpassed unsprayed plants in number of seeds / plant, dry weight of pods and seeds / plant, 100-seed weight, seed oil percentage as well as pod, seed, straw and oil yields / fad. (Yakout *et al.*, 2013).

Thiamine (vitamin B1) is a necessary factor for the biosynthesis of co-enzyme, in thiamine pyrophosphatase, which plays an important role in carbohydrate metabolism in plant. It synthesis in leaves and transported to roots where it controls growth. Thiamine is an important factor for the translocation reactions of the pentose phosphate cycle which provides pentose phosphate for nucleotide synthesis and for the reduction of NADP required for various synthetic pathways (Jaleel *et al.*, 2007). Although roots of some plant species can synthesize thiamine, those of other plants cannot synthesize this vitamin (Mittler, 2002). Absorption of thiamine by plant roots has been reported (Mateikene *et al.*, 1988) and leaf applied thiamine can transport in both acropetal and basipetal directions (Mozafar and Oertli, 1992; Mozafar and Oertli, 1993). Youssef and Talaat (2003) recorded pronounced increases in vegetative growth and chemical constituents of rosemary plants due to foliar application of thiamine.

The aim of this work was to examine the effects of non-enzymatic antioxidant compounds such as ascorbic acid and thiamine on some of physiological parameters and antioxidant enzymes activities as well as the productivity and quality of peanut grown under water regimes.

MATERIALS AND METHODS

Experimental Site and Conditions

Field experiments of sprinkler irrigation system were performed during the 2011 and 2012 summer growing seasons in a sandy soil in the Experimental farm, Faculty of Agriculture, Ismailia, Egypt (30° 58′ N latitude, 32°23′ E longitude and 13 m above sea level). The climate in this region is almost arid with a scarce annual rainfall of 20 mm during December to March. The temperature averages approximately 28.5°C in summer; and the relative humidity averages approximately 56.5%. The predicted monthly climatic data at Ismailia region during the growing seasons of peanut are presented in Table 2. Before beginning of the experiment, soil samples were obtained with an auger from soil depths of 0-60 cm to determine the physical and chemical properties of the experimental field (Table 1). The soil texture at this site was

Table 1. Soil physical and chemical properties of the experimental field soil over the two seasons

Soil depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture class	Bulk density (g cm^{-3})
0-60cm	71.51	21.36	4.03	3.10	Sand	1.58
Soil depth (cm)	**Field capacity (%)**	**Wilting point (%)**	**pH**	**Organic matter (%)**	**EC (dS m^{-1})**	
0-60cm	7.38	1.41	7.8	0.21	2.23	

Table 2. The predicted monthly climatic data at Ismailia Governorate during the growing periods of peanut in 2011 and 2012 seasons

Months	Average temperature ^{0}C						Average RH (%)		Average wind speed (Km/hr)	
	Maximum		Minimum		Average					
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
May	31	32	18	20	24.5	26.0	50	49	13	14
June	33	34	21	22	27.0	28.0	57	53	12	14
July	37	38	25	24	31.0	31.0	57	59	12	13
August	38	37	25	24	31.5	30.5	57	62	12	11
September	34	33	21	22	27.5	27.5	61	60	10	12

Data collected from Agriculture Research Center Meteorological Station in Ismailia

RH: Relative Humidity

predominantly sandy throughout its profile (71.51% coarse sand, 21.36% fine sand, 4.03% silt and 3.10% clay). The soil bulk density was determined according to Grossmann and Reinsch (2002). While both field capacity and wilting point were determined following the method of Cassel and Nielsen (1986).

Experimental Design, Treatments and Agronomic Practices

A split-plots design with three replications was used in each season. The sprinkler irrigation system was made inuse, where different treatments of water application rates and levels of exogenous application of ascorbic acid and thiamine were randomly assigned to the main and split plots, respectively. Pressure valve and flow meter were used to control the operating pressure and to measure the irrigation water quantity. The two irrigation treatments were W0: 1.00 and W1: 0.70 of the estimated crop evapotranspiration (ETc), which represented 5100 and 3570 m^3 ha^{-1} of water, respectively. In the two growing seasons, the amount of irrigation water applied, W, was determined from the calculated water requirement for peanut (mm) as determined from the crop coefficient (Kc) and the daily reference potential evapotranspiration (ETo). The latter was determined according to the Penman-Monteith equation (Allen *et al.*, 1998) depending on the predicted climatic factors at each irrigation time and the growth stage of peanut plant.

The Kc is defined as the ratio of the crop evapotranspiration rate to the reference evapotranspiration rate. Because localised Kc values were not available for the study area, the values of Kc suggested by FAO- 56 (Allen *et al.*, 1998) were used. The values of Kc of peanut plant (0.45 for initial stage, 0.75 for crop development stage, 1.05 for mid-season stage and 0.70 for last-season stage) represent the

recommended values for a sub-humid climate (minimum relative humidity, RHmin≈ 45%) with a moderate wind speed (U2≈ 2 m s^{-1}). These recommended values must be adjusted in other areas, where the RHmin differs from 45% and the wind speed is sometimes either greater or less than 2 m s^{-1}. The Kc value (large than 0.45) for the mid season stage was adjusted. After adjustment, the average Kc values for the two growing seasons in the initial, development, mid and late season stages were 0.30, 0.70, 1.15 and 0.60, respectively.

To guarantee full germination and complete establishment of seedlings, 33 mm of water was applied to all test plots at sowing with an additional irrigation of 38 mm applied 10 to 20 days later. To avoid deep percolation losses, irrigation was performed three times during the germination and seedling stage, respectively. Thereafter, the irrigation treatments began at twenty-three days from sowing and performed every 3 days throughout the growing season.

Five exogenous application levels of 50 and 100ppm Th, 50 and 100ppm AsA as well as water (control) were randomly nested within each main plot of irrigation treatments as a split plots. Different levels of aqueous AsA and Th containing 0.1% Tween-20 ($C_{58}H_{114}O_{26}$) were sprayed using a back-mounted pressurized sprayer that was calibrated to deliver 15 ml s^{-1} at a pressure of 1 bar. Also, the control treatment was sprayed with water containing 0.1% Tween-20 to expose all of the plants to the same conditions. Foliar spraying treatments were done at three equal doses, after 34, 54 and 75 days from sowing with volume spray of 475 liters/ ha.

The sub-plot area was 12 m^2 included 6 rows of 4 m long and 50 cm apart. The sowing date was on 1st May in both seasons. All seeds of peanut (*Arachis hypogeae* L. cv. Giza 6) were coated by Arab gum and inoculated with specific Rhizobium strain immediately before sowing, in hills 10 cm apart. After 20 days from sowing, peanut plants were thinned to one plant per hill. The recommended cultural practices for growing peanut crop in this region were followed.

Sampling and Traits Studied

At 85 days from sowing (ten days after the 3rd foliar application of Th and AsA), ten guarded plants from the 2nd row in each sub plot were randomly taken for determining dry weight/ plant, leaf area index (LAI), total chlorophylls and relative water content (RWC). Leaves samples of each season were also collected, washed and stored at −20°C pending biochemical analyses. The central area in each sub plot was kept to determine yield, seed quality and SDS-PAGE Electrophoresis of seed protein. Leaf area index (LAI) was calculated by dividing leaf area/plant in cm^2 by land area in cm^2 according to Bonhomme *et al.* (1974). Relative water content (RWC) was estimated using the following formula: RWC= (FW-DW)/ (TW-DW) x100, where FW is the average weight of freshly twenty leaf disks collected from leaves, TW the weight of disks after hydration for 12 hr at room temperature under low light conditions and DW is the average weight of the same disks after drying at 80°C for 48hr, according to Henson *et al.* (1981). Total chlorophyll (μMm^{-2}), was determined using the Minolta SPAD-502 chlorophyllmeter according to Markwell *et al.* (1995).

Preparation of enzyme leaves extracts

According to Urbanek *et al.* (1991), 0.5 g fresh leaves was homogenized by using a mortar and pestle with 0.1M phosphate buffer (pH 6.5) at 4°C and stirred for 20 min. The suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 18,000×g for 15 min at 4°C. The supernatant was used to determine activity of enzymes as follows:

Superoxide dismutase (SOD,EC1.15.1.1) assay

The reaction mixture contained 100 μl riboflavin (1 μM), 100 μl L-methionine (12 mM), 100 μl EDTA (0.1 mM) (pH 7.8), 100 μl Na_2CO_3 (50mM) (pH 10.2), and 100 μl nitroblue tetrazolium (75 μM) in 2300 μl sodium phosphate buffer (25mM) (pH 6.8), with 200 μl enzyme extract in a final volume of 3 ml. The absorbance was measured at 560 nm. The SOD activity of the extract was expressed as SOD units per milligram of protein according to Giannopolitis and Ries (1997).

Ascorbate peroxidase (EC 1.11.1.11) assay

The activity of ascorbate peroxidase (APX) was measured according to the method of Nakano and Asada (1981) by estimating the rate

of ascorbate oxidation by changes in optical density at 430nm (Beckman DK-2 Spectrophotometer). The 3ml reaction mixture contained 50mM phosphate buffer (pH 7.0), 0.1mM H_2O_2, 0.5mM sodium ascorbate, 0.1mM EDTA and 100 µL enzyme extract. The unit of APX activity was expressed as µmol of ascorbate oxidized mg^{-1} protein min^{-1} at 25 ± 2°C.

Yield measurements

At maturity, after 125 days from sowing, pod, seed and straw yields (t ha^{-1}) were estimated from plants of the two middle rows (the 3rd and 4th rows) in each sub plot.

Seed quality

The following seed quality were determined, seed oil content (%) was determined by using the Soxhelt continuous extraction apparatus with petroleum ether (60–80°C) as an organic solvent for a period of 12 hours according to AOAC (1975), oil yield/ha was calculated by multiplying seed oil content (%) by seed yield/ha, seed crude protein content (%) was determined by using the modified micro kjeldahl apparatus according to the methods described by AOAC (1975), and then the obtained values were multiplied by 6.25.

SDS-PAGE Electrophoresis of seed protein

Seed samples for determining Electrophoretic separation of peanut proteins (SDS-PAGE electrophoresis) were randomly taken at harvest for the second season. Polyacrylamid gel electrophoresis (PAGE) was conducted according to the method of (Laemmli, 1970), in order to find out the effect of water stress, foliar spraying with AsA and Th on the number and molecular weights of protein bands. Seed sample of 0.5g was ground in a mortar and defatted twice with 70% (v/v) ethanol (50ml/g) for 10 min at 40°C. Total soluble proteins were extracted with extraction buffer (0.1g/ml) containing, 20mM Na-borate buffer, 0.5 M NaCl, 1 mM of ethylendiaminetetra acetic acid (EDTA), pH 8.9; after 12 hours of stirring, the extract was centrifugation at 10.000 r.p.m./min. for 20 min. The supernatant (10 µl) was taken for electrophoresis according to Matta *et al.* (1981) and Tucci *et al.* (1991). SDS-PAGE was carried out in 10 % acrylamide slab gels, with a current of 25 mA and 130 V per gel until the bromophenol blue marker reached to the bottom after 3 hours, according to Laemmli (1970). After electrophoresis the gel was stained using silver staining as described by Blum *et al.* (1987). After staining, the slab gel was washed to remove the excess of staining solution in acetic acid (7%) and distilled water. Then the gel was photographed and scaned. Moreover, number of bands and molecular weight were calculated according to Ladizinsky and Waines (1982).

Statistical Analysis

The analysis of variance of split plots design was used according to Snedecor and Cochran (1982). The combined analysis of variance was performed for the data of the two seasons after test the homogeneity of error by Bartellet test (Steel *et al.*, 1997). Means followed by the same alphabetical letters are not statistically different according to Duncan's Multiple Range Test at the 5% level of significance (Duncan, 1955).

RESULTS

The combined analysis of variance for the data over the two seasons reveled that, there were significant interaction effects between water stress treatments and foliar application of thiamine and ascorbic acid on leaf area index, total chlorophyll (Table 3), relative water content, activities of some key enzymes of oxidative defense system (Table 4), seed oil and protein contents, pod, seed and oil yields (Tables 5 and 6). The results and discussion were focus on the interaction effect of water stress and spraying treatments on all measured parameters as shown in Table 7 and Fig. 1 as follow:

Leaf Area Index (LAI)

Peanut plants exposed to water stress without AsA and Th application showed a significant decrease in leaf area index (LAI) (5.83) in comparison to well watered (8.15) as shown in Table 7. Under normal irrigation, the maximum value of LAI (10.58) was significantly recorded in plants foliared with 100 ppm AsA, the followed LAI 9.50 and 9.33 were obtained from both treatments of 50 ppm Th and AsA, respectively without significant differences between them. Plants exposed to water stress and sprayed with 100ppm of AsA did not differ significantly from untreated plants under well

Table 3. Effect of water stress and spraying treatments on some growth attributes of peanut at 85 days from sowing.

Items	Dry weight (g/plant)			Leaf area index			Total chlorophyll (μMm^{-2})		
	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Water treatments (R)									
W0	89.72a	86.98a	88.35a	9.70a	8.56a	9.13a	44.42a	44.79a	44.61a
W1	63.81b	60.79b	62.30b	7.00	6.34b	6.67b	32.29b	34.27b	33.28b
Spraying treatments (F)									
A0	69.12d	66.11d	67.62d	86.97a	86.97a	86.97a	86.97a	86.97a	86.97a
A1	80.31b	78.66b	79.48b	86.97a	86.97a	86.97a	86.97a	86.97a	86.97a
A2	69.49d	66.24d	67.87d	86.97a	86.97a	86.97a	86.97a	86.97a	86.97a
A3	76.11c	73.29c	74.70c	86.97a	86.97a	86.97a	86.97a	86.97a	86.97a
A4	88.81a	85.13a	86.97a	86.97a	86.97a	86.97a	86.97a	86.97a	86.97a
Interaction (R×F)	NS	NS	NS	**	*	*	*	*	*

W0: well-watered (5100 m^3/ha); W1: 0.7 of W0; A0: untreated plants, A1: 50ppm Th, A2: 100ppm Th, A3: 50ppm AsA, A4: 100ppm AsA.

Table 4. Effect of water stress and spraying treatments on relative water content and some enzyme activities (units mg^{-1} protein) of peanut at 85 days from sowing

Items	Relative water content (%)			SOD activity			APX activity		
	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Water treatments (R)									
W0	84.39a	82.27a	83.33a	53.61b	59.64b	56.63b	203.06b	197.35b	200.21b
W1	70.53b	66.87b	68.70b	78.64a	83.45a	81.05a	253.58a	242.85a	248.21a
Spraying treatments (F)									
A0	72.42c	69.50c	70.96c	68.99a	77.51a	73.25a	226.42abc	216.38b	221.40b
A1	77.67b	74.92b	76.29b	67.77ab	71.27c	69.52b	224.29c	217.37b	220.83b
A2	71.29c	69.58c	70.44c	70.22a	75.38b	72.80a	224.82bc	218.85b	221.83b
A3	79.24b	75.67b	77.45b	64.90b	70.13c	67.52c	232.15ab	223.65a	227.90a
A4	86.70a	83.17a	84.94a	58.76c	63.44d	61.10d	233.92a	224.25a	229.08a
Interaction (R×F)	**	**	**	*	**	*	*	*	*

W0: well-watered (5100 m^3/ha); W1: 0.7 of W0; A0: untreated plants, A1: 50ppm Th, A2: 100ppm Th, A3: 50ppm AsA, A4: 100ppm AsA.

Table 5. Effect of water stress and spraying treatments on straw, pod and seed yields of peanut at harvest

Items	Straw yield (t ha^{-1})			Pod yield (t ha^{-1})			Seed yield (t ha^{-1})		
	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Water treatments (R)									
W0	15.03a	14.13a	14.58a	5.97a	5.63a	5.80a	4.56a	4.18a	4.37a
W1	11.71b	10.35b	11.03b	4.73b	4.49b	4.61b	3.42b	3.23b	3.33b
Spraying treatments (F)									
A0	12.55c	10.97d	11.76b	4.94c	4.66d	4.80d	3.65b	3.42c	3.53c
A1	13.01bc	12.04c	12.52b	5.27bc	4.88c	5.08c	4.09a	3.72b	3.90b
A2	12.78bc	10.58d	11.68b	4.91c	4.64d	4.78d	3.49b	3.29c	3.39c
A3	14.01ab	13.32b	13.66a	5.55b	5.43b	5.49b	4.30a	4.01a	4.16ab
A4	14.49a	14.28a	14.38a	6.10a	5.67a	5.89a	4.45a	4.10a	4.27a
Interaction (R×F)	NS	NS	NS	*	*	*	**	*	**

W0: well-watered (5100 m^3/ha); W1: 0.7 of W0; A0: untreated plants, A1: 50ppm Th, A2: 100ppm Th, A3: 50ppm AsA, A4: 100ppm AsA.

Table 6. Effect of water stress and spraying treatments on seed oil and protein contents (%) and oil yield of peanut at harvest

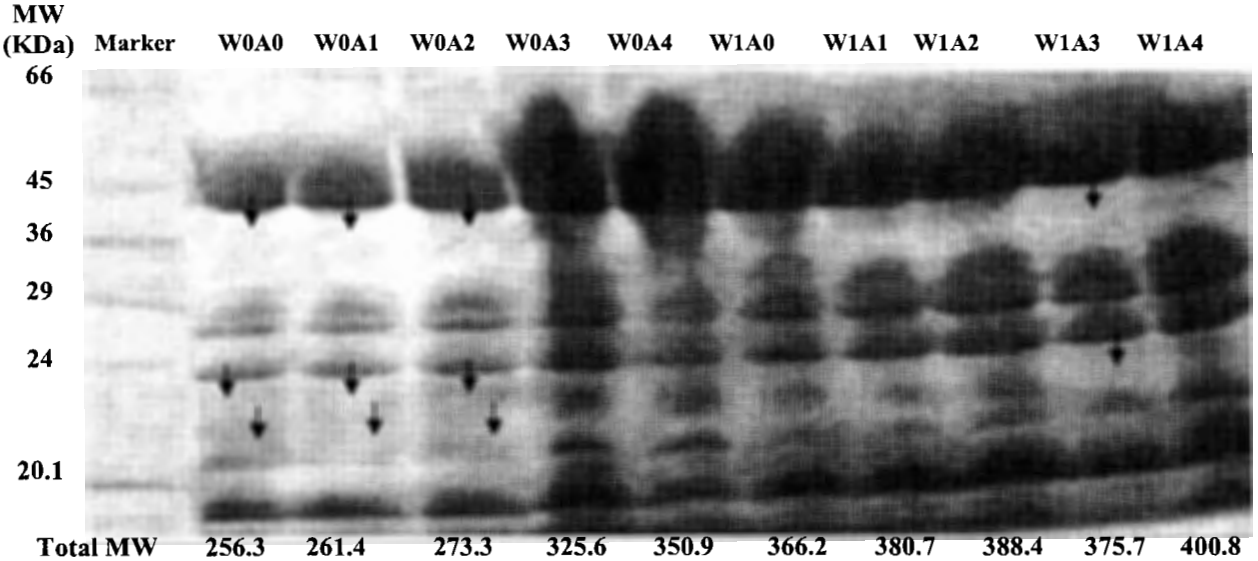
Items	Seed oil content (%)			Oil yield (kg ha^{-1})			Seed protein content (%)		
	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Water treatments (R)									
W0	44.55b	45.41b	44.98b	2034.52a	1902.85a	1968.68a	22.65b	21.80b	22.23b
W1	50.15a	50.93a	50.54a	1720.55b	1646.20b	1683.38b	25.05a	24.60a	24.83a
Spraying treatments (F)									
A0	46.76bc	47.87b	47.32bc	1684.92b	1619.86c	1652.39b	23.00b	22.07c	22.53b
A1	48.86a	49.45a	49.15a	1980.29a	1824.17b	1902.23a	24.08a	23.60b	23.84a
A2	45.64c	46.40c	46.02c	1579.93b	1514.49c	1547.21b	22.90b	22.20c	22.55b
A3	48.12ab	48.85ab	48.48ab	2050.83a	1948.99a	1999.91a	24.67a	23.93ab	24.30a
A4	47.37ab	48.28ab	47.82ab	2091.70a	1965.13a	2028.41a	24.60a	24.22a	24.41a
Interaction (R×F)	**	*	**	**	*	*	*	*	*

W0: well-watered (5100 m^3/ha); W1: 0.7 of W0; A0: untreated plants, A1: 50ppm Th, A2: 100ppm Th, A3: 50ppm AsA, A4: 100ppm AsA.

Table 7. Effect of the interactions between water stress and spraying treatments on some growth attributes and enzyme activities in leaves at 85 days from sowing as well as yield and its quality of peanut at harvest (combined data)

Water treatments	Spraying treatments	Leaf area index	Total chlorophyll (μMm^{-2})	Relative water content (%)	SOD (units mg^{-1} protein)	APX (units mg^{-1} protein)	Pod yield (t ha^{-1})	Seed yield (t ha^{-1})	Seed oil content (%)	Oil yield (kg ha^{-1})	Seed protein content (%)
	A0	8.15c	41.33c	79.75cd	60.60d	181.37d	5.44cd	4.12c	44.20d	1823.23b	21.03f
	A1	9.50b	45.33b	82.75bc	56.80de	181.23d	5.66c	4.48b	46.61c	2087.74a	22.35e
W0	A2	8.08c	40.30cd	80.37cd	60.30d	181.67d	5.26d	3.82d	43.37e	1655.22c	21.33f
	A3	9.33b	45.36b	83.74b	55.17e	223.00c	6.12b	4.67ab	45.60cd	2127.82a	23.37cd
	A4	10.58a	50.73a	90.04a	50.27f	233.77b	6.52a	4.77a	45.11d	2149.39a	23.05d
	A0	5.83f	30.60g	62.17f	85.90a	261.44a	4.15g	2.94f	50.43a	1481.55d	24.03b
	A1	6.50e	32.63f	69.83e	82.23ab	260.42a	4.49f	3.32e	51.70a	1716.72c	25.33a
W1	A2	5.92f	29.13g	60.50f	85.30a	262.00a	4.30fg	2.96f	48.67b	1439.19d	23.77bc
	A3	7.17d	35.27e	71.17e	79. .87b	232.80b	4.86e	3.64d	51.37a	1871.99b	25.23a
	A4	7.92c	38.77d	79.83d	7193c	224.40c	5.25d	3.78d	50.53a	1907.43b	25.77a

W0: well-watered (5100 m^3/ha); W1: 0.7 of W0; A0: untreated plants, A1: 50ppm Th, A2: 100ppm Th, A3: 50ppm AsA, A4: 100ppm AsA.



Total MW	256.3	261.4	273.3	325.6	350.9	366.2	380.7	388.4	375.7	400.8
N. of bands	6	6	6	8	8	8	8	9	7	9
Protein (%)	21.03	22.35	21.33	23.73	23.05	24.03	25.23	25.77	25.33	25.77

Fig. 1. Variation in protein bands, total molecular weight and protein percentage on polyacrylamide gel of peanut seed at harvest. The marker used was SDS-70 from Sigma Chemical Corporation. The arrows represent the absence of bands. W0: well-watered (5100 m^3/ha); W1: 0.7 of W0, A0: untreated plants. A1: 50 ppm Th, A2: 100 ppm Th, A3: 50 ppm AsA, A4: 100 ppm AsA, MW: molecular weight

watered. Also, 100 ppmAsA application improved LAI by approximately 30-36% under both well watered and water stressed plants (W0 and W1) compared to unsprayed plants (W0 A0 and W1 A0), respectively.

Chlorophyll Content

Over the two successive seasons, 0.7 of the estimated crop evapotranspiration (ETc) treatment had a significant negative effect on the content of photosynthetic pigments (Table 7). Peanut leaves exposed to water stress, either treated or not with AsA and Th, showed a significant decrease in total content of chlorophyll (CHL) compared with control plants (well- watered). Under water stress, untreated plants or treated with 100 ppmTh (W1 A0 and W1 A2) showed a less values of CHL content (30.6 and 29.13 μMm^{-2}), respectively compared to well watered plants (41.33 and 40.30 μMm^{-2}). Under well- watered and water stress, the application of 100ppm AsA significantly ameliorated the content of total CHL, with a higher content of total CHL in well- watered (50.73 μMm^{-2}) than in water stress (38.77 μMm^{-2}). Application of 100ppm AsA saved about 8 μMm^{-2} of CHL from degradation under water stress.

Relative Water Content (RWC)

In stressed plants (Table 7), the relative water content of peanut leaves significantly decreased. It reached to 62.17 and 60.50% with A0 and A2 (untreated plants and treated with 100 ppm Th) compared to 79.75% and 80.37% in normal irrigation, respectively. Under stress and normal conditions, increasing the level of AsA from 50 to 100 ppm, significantly ameliorated the values of RWC, but raising Th level decreased RWC significantly in stressed plants and insignificantly under normal irrigation. On the other hand, RWC in stressed plants which was sprayed with 100 ppm AsA (W1 A4) equaled the same content of untreated well-watered plants (W0 A0).

Antioxidant Enzymes

Decreasing of water irrigation from 5100 to 3570 $m^3\ ha^{-1}$, produced significantly increases in the activities of SOD and APX by 0.42 and 0.44 times, respectively in peanut leaves compared to control plants (Table 7). Increasing level of AsA was associated with a significant increase in APX activity (28.89%) in non-stressed plants (W0 A4); however, SOD activity was decreased by 17.05%. Application of 100 ppm AsA to stressed plants (W1 A4) caused a significant decrease on the activity of these above mentioned antioxidant enzymes in the leaves by approximately 14-16% compared to untreated plants under water stres. In general, spraying treatments of Th was not significantly modulated the activities of these enzymes under stressful conditions as well as full irrigation.

Yield Measurements

It was clearly evident from Table 7 that exposed peanut plants to water stress showed a statistically significant reduction in pod yield (4.15 t ha^{-1}) and seed yield (2.94 t ha^{-1}) compared with the control plants, 5.44 and 4.12 t ha^{-1}, respectively, *i.e.* drought reduced seed yield by about third amount. Foliar nutrition with 50 or 100 ppm AsA to stressed and non-stressed plants showed a significant increase in these traits compared to unsprayed plants. Data in Table 7 showed that there were significant increases in pod and seed yields/ ha by spraying stressed peanut plants with 50 ppm Th, but the increase was significant in seed yield/ha alone under normal irrigation as compared to untreated control. The maximum values of seed yield were obtained from well watered with 50 or 100 ppm AsA. Seed and pod yields were increased by about 0.65 and 1.08 t/ha by spraying with 100 ppm AsA under normal irrigation. Also, under water stress, peanut plants treated with 100 ppm AsA showed an increment by about 0.84 and 1.10 ton for seed and pod yields/ha compared to unsprayed plants, respectively. These results also mean that peanut plants responded to high levels of AsA more than Th under stress conditions as well as normal irrigation.

Yield Quality

The changes in seed oil and protein contents (%) during water shortage and normal irrigation are shown in Table 7. They significantly enhanced in response to water stress by about 14.1 and 14.3% relative to control (W0 A0). In general, effect of 50 ppm AsA and Th did not differ significantly from 100 ppm AsA treatment on these traits in stressed plants, but applying

100 ppm AsA overcome significantly 50 ppm Th in protein percentage under normal irrigation. Moreover, spraying AsA gave significant increase in seed protein content (%) of stressed and unstressed peanut plants compared to untreated control.

As shown from the data presented in Table 7, in stressed plants (0.7 Etc water treatment), the oil yield was decreased due to water stress treatment, recorded 1481.55kg/ha compared to 1823.23kg/ha in control plants (well-watered plants). Meanwhile, the maximum values of oil yield were obtained from well watered plants with 100 ppm AsA, 50 ppm AsA and 50 ppm Th, respectively without significant differences among them. Also, the oil yield obtained from well-watered plants (control) didn't show a significant differences from the oil yield obtained from water stressed plants which spraied with 50 and 100 ppm AsA. It is obvious from our results that peanut plants responded to high levels of AsA more than Th under water stress as well as full irrigation.

SDS-PAGE of Seed Protein Profiles

Seed protein of peanut cv. Giza 6 was analyzed to identify protein patterns involved in response to interactions between water stress and AsA or Th treatments. Total proteins extracted from seeds of 10 different interactions treatments were separated by electrophoresis on one dimensional SDS-PAGE gel (Fig. 1). In general, Proteins were resolved as four groups (conarachin: larger than 45kDa, acidic arachin: between 35 to 45 kDa, basic arachin: between 20 to 35 kDa, and smaller than 20 kDa). The protein profiles revealed few difference protein bands among all tested treatments (Fig. 1). These protein profiles obtained from the treatments were distributed along the gel with molecular weights ranging from 256.3 to 400.8 KDa (Fig.1). The number of protein bands obtained from each treatment ranged from 6 to 9 bands depending on the differences between treatments under study. The arrows on protein profiles in Fig 1 refer to the missing protein bands. As shown from protein profiles, all these interactions W0A0, W0W1, W0W2 and W1A3 had about 3, 3, 3 and 2 absent bands inside the range of 35 to 45 kDa and 20 to 24 kDa which corresponds to acidic arachins and basic arachins, respectively. The lowest values of molecular weight, number of protein bands and seed protein content (%) were obtained from W0A0, W0W1, W0W2, respectively. Decreasing amount of irrigation water without spraying treatments increased the values of these traits by about 100kDa, 2 bands and 14% compared to well-watered and unsprayed plants, respectively. Foliar nutrition with 50 ppm and 100 ppm AsA to non-stressed plants showed increase in these traits compared to (control) and sprayed with Th. Meanwhile, under stress conditions, foliar application of 100 ppm AsA and 100 ppm Th gave the highest molecular weight, number of protein bands and seed protein content (%) relative to other interactions treatments.

DISCUSSION

The experiment was conducted under semi-arid conditions in sandy soil with temperature averages approximately 28.5 °C in the summer; and the relative humidity averages approximately 56.5% (Tables 1 and 2). Results indicated that, water stress caused a reduction in each of leaf area index, total chlorophyll, relative water content, pod, seed and oil yields while, activities of antioxidant enzymes and seed contents from oil and protein were increased. Drought stress has adverse influence on water relations (Babu and Rao, 1983), photosynthesis (Bhagsari *et al.*, 1976), mineral nutrition, metabolism, growth and yield of groundnut (Suther and Patel, 1992; Yakout *et al.*, 2013). Water stress causes directly or indirectly, reduction in total dry mass accumulation, limitation on total chlorophyll content, leaf relative water content and leaf area through molecular damage in plant cells by the formation of reactive oxygen species (ROS). (Lissner *et al.* 1999; Reddy *et al.*, 2004; Farooq *et al.*, 2013).

Our data indicated that ascorbic acid was effective in promoting growth and yield of peanuts under drought stress compared to the other biomolecule thimine (Table 7). Also, 50-100 ppm of AsA was an appropriate concentration as an immune-modulator for many plants (Shalata and Neumann, 2001; Amin *et al.*, 2008; Raafat *et al.*, 2011; Kotb and Elhamahmy,

2013). AsA-induced increase in growth under stressful conditions may attributed due to its role in increasing cell division and/or cell enlargement (Athar *et al.*, 2008). For example, AsA has been reported to accelerate cell division and cell enlargement in different plants such as onion (Cabo *et al.*, 1996), pea (Citterio *et al.*, 1994), and wheat (Athar *et al.*, 2008).

Peanut plants under full irrigation as well as water stress responded to AsA application followed by Th due to their nutritive, antioxidant and cellular reductant properties (Noctor and Foyer, 1998). So, AsA-treated well watered plants recorded maximum value in each of LAI, total chlorophylls and RWC. Furthermore, under water stress, addition of 100 ppm AsA and 50 ppm Th improved LAI by 36% and 12% compared to the unsprayed plants, respectively (Table 7). Also, under water stress, effect of 100 ppm of AsA on wheat plants improved LAI by approximately 30% compared to non-sprayed plants (Kotb and Elhamahmy, 2013).

The reduction of chlorophylls (CHL) under drought stress is mainly due to the membrane disintegration and damage to chloroplasts by ROS (Jung, 2004). Also, CHL degradation under drought associated with the formation of proteolytic enzymes such as chlorophyllase (Sabater and Rodriquez, 1978). Non-enzymatic antioxidants (AsA and Th) had a protective effect on CHL from degradation under water stress (Table 7). Under stress condition, the spraying of 100 ppm AsA and 50 ppm Th significantly ameliorated the content of total CHL by about 27 and 7% relative to unsprayed plants, respectively.

Relative water content (RWC) is considered an alternative measure of plant water status, reflecting the metabolic activity in tissues (Flower and Ludlow, 1986). The capacity to maintain high RWC values under drought was observed in drought tolerant bean cultivars (Zlatev, 2005) and in *Astragalus gombiformis* Pom. and *Medicago sativa* L. (Gorai *et al.*, 2010). In the present study, leaf RWC declined significantly due to drought treatment compared to control. This could be due to a reduction of water supply to the leaves (Tuna *et al.*, 2008). Our results confirmed that AsA and Th application increased the RWC in peanut leaves by about 28% and 12% compared to non-treated plants under water stress, respectively, as shown in Table 7. Farooq *et al.* (2013) found that AsA-treated wheat plants, accumulated high amount of proline under drought and it as an osmoregulatory solute improved water status in plants under water and salinity stress.

These responses may be due to AsA protects plant cell as non-enzymatic antioxidant and regulates plant growth owing to its effect on differentiation and cell division (Price, 1966). Also, AsA induced Gibberellin 3-B dioxgenase responsible for synthesis of plant growth promoting hormone, Gibberelin (Coles *et al.*, 1999).

In the current study, thiamine application is able to enhance tolerance to water stress. Thiamine acts as a potential coenzyme in many key metabolic pathways, including plant pigment biosynthesis (Friedrich, 1987). Our results are analogous to what has been earlier observed in sunflower by Sayed and Gadallah (2002) who showed that root or shoot applied thiamine improved growth, which was found to associated with thiamine-induced reduced membrane injury and increased leaf RWC, chlorophyll content, soluble sugars and total free amino acids. Also, Tunc-Ozdemir *et al.* (2009) have shown that thiamine application confers enhanced tolerance to oxidative stress in *Arabidopsis* caused by multiple stresses.

Drought affects not only water relations, but also induces stomatal closure, decreases the photosynthesis rate and hence growth. Closure of stomata decreases CO_2 in rate and hence concentration in leaf mesophyll tissue and results in an accumulation of $NADPH^+H^+$ from light reaction, probably due to an increase in mesophyll resistance as well as stomatal resistance. Under such conditions, where $NADP^+$ is a limiting factor, oxygen acts as an alternate acceptor of electrons from the thylakoid electron transport chain, resulting in formation of superoxide radical ($O2^{\cdot-}$) (Cadenas, 1989). Superoxide radical and its reduction product H_2O_2 are potentially toxic compounds, and can also combine by the Haber-Weiss reaction to form the highly toxic hydroxyl radical ($OH^{\cdot}$) (Sairam *et al.*, 1997). ROS are

highly active molecules that can easily damage membrane and oxidize photosynthetic pigments, proteins and nucleic acids (Gong *et al.*, 2005).

Changes in the activities of various antioxidant enzymes under water stress have been reported (Nayyar and Gupta, 2006). The primary free radical scavenger in plant cells is superoxide dismutase (SOD). This enzyme converts $O_2^{\cdot-}$ to H_2O_2, which is eliminated by APX in association with dehydroascorbate reductase, this process regenerates AsA (Asada, 1994). APX plays an important role in eliminating H_2O_2 by utilizing ascorbate as its specific electron donor to reduce H_2O_2 to water, with the concomitant generation of monodehydroascorbate (MDHA). MDHA is converted to AsA by MDHA reductase, or is disproportionated non-enzymatically to AsA and dehydroascorbate. Hydrogen peroxide is also scavenged by catalase (CAT) (Comba *et al.* 1998).

Antioxidative enzymes such as SOD, CAT and APX are reported to increase under various environmental stresses (Khanna-Chopra and Selote, 2007; Ardc *et al.*, 2009). As a confirmation, in the present work, we also observed enhancing the activities of SOD and APX (42 % and 44%, respectively) under water-stressed condition comparing to plants under well watered conditions (Table 7). The results are in accordance with other authors reporting that, these activities also increased in drought tolerant genotypes of pea (Gillham and Dodge, 1987), tomato (Walker and McKersie, 1993), Sorghum (Jagtap and Bhargava, 1995), bean (Zlatev *et al.*, 2005) and wheat (Kotb and Elhamahmy, 2013).

A role of AsA in the ascorbate–glutathione cycle in mitochondria and peroxisomes has been described (Jime´nez *et al.*, 1997). In the current study, foliar application of non-enzymatic antioxidative compound (AsA) enhanced the activity of APX in non-stressed plants; however, SOD activity was decreased (Table 7). Under stress water condition, exogenous application of AsA partially inhibits these toxic effects of ROS because AsA is a scavenger of ROS (Noctor and Foyer, 1998). Application of 100 pm AsA to stressed plants had a significant decrease on the activity of SOD and APX in the leaves by approximately 16-14% compared to unsprayed plants. It is noteworthy that the activities of these enzymes were less stimulated by thiamine, vitamin B1 as compared to vitamin C under all conditions (Table 7). AsA decreased the activity of these enzymes may be by elimination of free radicals. It has been found to be loaded in the phloem of source leaves and is then transported to other tissues (Tedone *et al.*, 2004).

It has also been reported that water stress is one of the major causes of crop loss worldwide, falling average yields for most major crop plants by more than 50% (Bray, 1997). Our data also suggest that drought can reduce peanut productivity by about 1.3 ton pods/ha, 1.2 ton seeds/ha and 342kg oil/ha compared with control plants (Table 7). Meanwhile, under water stress conditions, plants treated with AsA showed an improvement in these traits by about 1.1, 0.8 t/ha and 426kg/ha in comparison to untreated plants, respectively. Pod and oil yields obtained from 100 ppm AsA-treated peanut irrigated with 3570 m^3/ha was the same obtained from well irrigated (5100m^3/ha) without AsA. Therefore, using of AsA can save about 1530m^3/ha of irrigation water without yield reduction, especially we have serious problem in water sources. In the other hand, the present results confirmed that both of AsA and Th significantly enhanced the content of seed protein under stressful conditions as well as normal irrigation (Table 7). The beneficial effect of ascorbic acid on growth and yield of peanut plants might be due to that ascorbic acid plays essential role in several physiological processes in plant growth, differentiation, plant cell division and metabolism (Foyer, 1993). Also, it plays an important role in cell protection against oxidative stress (Luwe *et al.*, 1993) and photoprotection (Rautenkranz *et al.*, 1994). These results are in harmony with those reported by Abdel-Halim (1995); Shehata *et al.* (2002); Helal *et al.* (2005); El-Banna *et al.* (2006); Dolatabadian *et al.* (2010) and Yakout *et al.* (2013).

Previous results showed also that exogenous application of AsA to stressed and un-stressed plants and Th to stressed plants enhanced seed content from protein (%), protein molecular weight and number of protein bands (Fig. 1).

Increasing number and molecular weight of protein bands may be due to the role of ascorbic acid and thiamine in reducing membrane injury, increasing both leaf RWC, chlorophyll content, soluble sugars and total free amino acids which play an important role in forming the different kinds of protein in seeds (Brady, 1990). Also, they play essential role in several physiological processes in plant growth, differentiation, plant cell division, metabolism and cell protection against oxidative stress (Foyer, 1993; Luwe *et al.,* 1993; Noctor and Foyer, 1998) which are reflected on vegetative growth, yield and its quality.

It could be concluded that subjecting peanut plants to water stress decreased significantly growth, yield and its attributes. Meanwhile, exogenous application of AsA by a proper level (100 ppm) enhanced growth, yield and its quality compared to thiamine. Moreover, the interactions between water regimes and levels of AsA were significant; indicating that foliar application of AsA can increase the survival capacity of peanut plants under stress conditions. Improving resistance to drought is associated with essential role of ascorbic acid in several physiological processes in plant growth, differentiation, plant cell division, metabolism and cell protection against oxidative stress more than thiamine in plants. Consequence of this, AsA can save about 1530m^3/ha of irrigation water without yield reduction

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تأثير الرش الورقى بمضادات الاكسدة غير الانزيمية على محصول الفول السودانى وجودته تحت ظروف استحداث إجهاد مائي

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نفذت هذه الدراسة خلال موسمى ٢٠١١، ٢٠١٢ بأرض رملية لدراسة تأثير الرش الورقى بمضادات الاكسدة غير الانزيمية من مركبى حمض الاسكوربيك والثيامين على محصول الفول السودانى وجودته تحت نظام الرى بالرش، أجريت تجربة حقلية بمزرعة كلية الزراعة جامعة قناة السويس بالإسماعيلية باستخدام تصميم القطع المنشقة بمعاملتين رى بالرش بالقطع الرئيسية (١,٠ و٠,٧ من قيمة البخر نتح لمحصول الفول السودانى والتى تعادل ٥١٠٠ و٣٥٧٠م^3/ هكتار على الترتيب و٥ مستويات من الرش بحمض الاسكوربيك والثيامين بالقطع المنشقة بتركيزات (صفر، ٥٠ ، ١٠٠ و ٥٠ ، ١٠٠ جزء فى المليون من حمض الإسكوربيك والثيامين على الترتيب)، وجد أن الاجهاد المائى ادى الى نقص معنوى فى كل من دليل المساحة الورقية ومحتوى الكلوروفيل الكلى ومحتوى الماء النسبى وكذلك محصول القرون والبذوروالزيت، بينما ادى الى زيادة نشاط بعض الانزيمات المضادة للأكسدة مثل السوبراوكسيد ديسميوتيز والاسكوربات بيروكسيديز وكذلك محتوى البذرة من الزيت والبروتين. ادى الرش الورقى بالتركيز المناسب (١٠٠ جزء فى المليون) من حمض الاسكوربيك الى تشجيع النمو والمحصول وجودته مقارنة بمركب الثيامين، من ناحية اخرى، أدى الرش الورقى بحمض الاسكوربيك للنباتات المجهدة وغير المجهدة وكذلك الثيامين للنباتات المجهدة الى تحسين محتوى البذرة من البروتين وعدد الحزم البروتينية والوزن الجزيئى للبروتين، أدت المعاملة بحمض الاسكوربيك الى التغلب على الأضرار الناجمة عن الإجهاد التأكسدى الناتج عن الجفاف الذى انعكس فى نقص الانزيمات المضادة للاكسدة فى الاوراق بحوالى ١٤-١٦% مقارنة بالنباتات المجهدة وغير المعاملة، حيث زاد محصول البذوروالقرون والزيت بحوالى ٠,٦٥ و ١,٠٨ طن/هكتار و٣٢٦ كجم/هكتار فى النباتات المعاملة بحمض الاسكوربيك (١٠٠ جزء فى المليون) تحت ظروف الرى المعتاد على الترتيب. بينما تحت ظروف الإجهاد (٣٥٧٠ م٣/هكتار) ادت المعاملة بنفس المستوى من حمض الاسكوربيك الى زيادة تلك الصفات بمقدار ٠,٨٤ و ١,١ طن/هكتار و٤٢٦ كجم/هكتار مقارنة بالنباتات غير المعاملة على الترتيب، أدى استخدام ١٠٠ جزء فى المليون من حمض الاسكوربيك إلى توفير حوالى١٥٣٠ م٣/هكتار من ماء الرى دون تقليل كمية محصول القرون وجودته.

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