Foliar application of ascorbic acid improved drought tolerance and productivity of wheat (*Triticum aestivum* L.)

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Abstract: Little is known about ascorbic acid (AsA) role in counteracting the adverse effects of water stress on wheat growth and productivity under field conditions. Two field experiments were conducted in a sandy soil in the Experimental Farm, Faculty of Agriculture, Ismailia, Egypt during 2011/12 and 2012/13 seasons. The work aimed to study the effect of three levels of AsA (0.0, 100 and 200 ppm) on the response of wheat (*Triticum asetivum*, L. cv. Sakha 94) to three surface irrigation rates (1.00. 0.80 and 0.60 of the estimated crop evapotranspiration, which represented 4260, 3408 and 2556 m^3/ha, respectively. Results indicated that, drought caused a reduction in each of leaf area index, total chlorophyll, relative water content, grain and straw yields. However, water deficit increased lipid peroxidation (as malondialdehyde, MDA) and protein oxidation as well as induced the activities of some antioxidative enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). The activity of SOD, APX and CAT was increased by about 74-108%, 44-57% and 107-156%, respectlvely in flag leaf compared to the well watered wheats. Application of 100 -200 ppm of AsA significantly alleviated the oxidative stress damage of drought, reflected by improving above parameters as well as decreasing MDA and protein oxidation, but decreased activity of antioxidative enzymes by about 10-20 %. Consequently, foliar spray of AsA improved productivity and irrigation water use efficiency in wheats. Grain yield was increased by about 0.5 t/ha in AsA-treated plants under normal irrigation. Also, AsA treatments protected about 0.8-0.9 t/ha grains from collapse under water stress. Application of AsA saved approximately 852m^3/h of irrigation water without yield reduction. Modification of some principle tissues in flag leaf and main stem to drought and AsA were also investigated.

Keywords: Water Stress, Antioxidative Enzymes, MDA, Anatomy, Productivity, IWUE.
Abbreviations: AsA, Ascorbic acid, APX, Ascorbate peroxidase, CAT, Catalase, SOD, Superoxide dismutase, MDA, Malondialdehyde, LAI, Leaf area index , RWC, Relative water content, IWUE, Irrigation water use efficiency, Kc, Crop coefficient.

INTRODUCTION

The most important environmental factor inhibiting photosynthesis, decreasing growth and productivity of plants is drought. It is one of the major causes of crop loss worldwide, with an average yields loss for most strategical crops by more than 50% (Bray, 1997). So wheat anti-drought physiology study is of importance to wheat production and biological breeding for the sake of coping with abiotic conditions as it is the staple food for more than 35% of world population (Shao *et al.*, 2005). A consequence of soil water deficits and other types of environmental stress is the limitation of photosynthesis which usually accompanied with the formation of reactive oxygen species (ROS) in various subcellular organelles of plant cell such as the superoxide, hydrogen peroxide and the hydroxyl radical (Zhu, 2000). Increased levels of ROS cause damage to various cellular mechanisms, such as enzyme inhibition, protein degradation, DNA and RNA damage, and membrane lipid peroxidation, which ultimately culminate in cell death (Ishikawa *et al.*, 2010).

A complex antioxidative defense system, composed of both non-enzymatic and enzymatic constituents, is present in all plant cells (Foyer *et al.*, 1994). Low-molecular weight, non-enzymatic, nutrient-derived antioxidants are presented by carotenoids, tocopherols, glutathione and ascorbic acid (AsA). Apart their obvious role as enzyme substrates, they can react chemically with almost all forms of ROS. AsA is a small, water-soluble antioxidant molecule that acts as a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen (Noctor and Foyer, 1998). Also, ascorbate has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion and other developmental processes (Pignocchi and Foyer, 2003), gene regulation, modulation of some enzymes and redox regulation of membrane-bound antioxidant compounds (Horemans *et al.*, 2000). It has also been reported that a progressive increase in each of number of spikes, leaf area, photosynthetic pigments and dry weight (Amin *et al.*, 2008), grain yield and its quality (Raafat *et al.*, 2011) in wheat treated with AsA. As well as, foliar application of exogenous ascorbate can increase plant resistance to salt stress and reduce oxidative stress (Shalata and Neumann, 2001).

Antioxidative enzymes (e.g., superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidases (APX) have been related with water deficit and are considered the main components of anti-oxidative machinery for drought resistance in higher plants (Gogorcena *et al.*, 1995 and Bergmann et al., 1999). Changes in the activities of various antioxidant enzymes under water stress have been reported (Nayyar and Gupta, 2006). Hydrogen peroxide is commonly taken as an indicator of oxidative stress, because it is induced by ROS and also influencing the level of lipid peroxidation (Mittler, 2002). Malondialdehyde (MDA), a product of the decomposition of polyunsaturated fatty acids in biomembranes, shows increased accumulation under water stress (Guo *et al.* 2012). Osmotic adjustment strategy in wheat and cereals under water stress has

been more comprehensively studied (Richter and Wagner, 1982). But rapid quenching of ROS and protective role of AsA on field-cultivated wheats under drought still need more investigations.

Therefore, the present investigation was conducted to examine whether exogenously applied of AsA will improve the drought tolerance of field-growing wheats by modulating the activity of antioxidant enzymes to enhance the growth and productivity or not.

MATERIAL AND METHODS

Experimental site and conditions

Two field experiments of surface irrigation system were conducted during two growing seasons (2011/12 and 2012/13) 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 scarce annual rainfall of about 20 mm concentrated over the months of December to March, the temperature average is about 16.8°C during winter, and the relative humidity average is about 56%. The predicted monthly climatic data at Ismailia region during the growing seasons of wheat are presented in Tables 1. Soil physical and chemical properties were analyzed according to Grossmann and Reinsch (2002) and presented in Table 2. The soil texture was predominantly sandy throughout its profile (89.11% sand, 7.48 % silt and 3.41% clay). Both field capacity and wilting point were determined following the method of Cassel and Nielsen (1986).

Experimental design, treatments and agronomic practices:

Grains of wheat (*Triticum asetivum*, L. cv. Sakha 94) were provided by the Egyptian crops Research Center, Ministry of Agriculture, Egypt. A split plot design with three replicates was used in each season. The irrigation treatments were randomly assigned to the main plots and donated, W_0: 1.00 (4260 m^3/ha), W_1: 0.80 (3408 m^3/ha) and W_2: 0.60 (2556 m^3/ha) of the estimated crop evapotranspiration (ETc). Water application was monitored *via* water meter. Wheat plants were given 11 irrigations at 10 days interval starting from 30 days from sowing. In the two growing seasons, the amount of water needed for each irrigation was calculated according to 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 wheat plant. As recommended by Allen *et al.*, 1996 and Neale et al., 1996, the FAO Kc of wheat plant was 0.35 for initial stage, 0.75 for crop development stage, 1.15 for mid-season stage and 0.45 for last-season stage. At the end of the last irrigation, the amount of water applied for each of the three irrigation treatments was calculated according to the total amount of water added in the 11 irrigations for the two seasons.

Ascorbic acid levels were 0.0, 100 and 200 ppm randomly assigned to the sub plot. Three foliar sprays of AsA were made at the interval of 1 month, i.e. 35, 65 and 95 days from planting. All sprays were applied in the morning (9–10 a.m.). The aqueous of AsA containing 0.1% Tween-20 as a surfactant (1 drop per litre) was sprayed on the leaves of to run-off point, using a back-mounted pressurized sprayer. The control treatment was sprayed with distilled water containing 0.1% Tween-20.

The experimental plot area was 6 m^2 (2m x 3m). Each plot included 10 rows, 20cm apart with 3m long. Grains were sown by drill at the rate of 120 kg/ha. Sowing was done on December 5th in the two growing seasons.

Table (1): The predicted monthly climatic data at Ismailia Governorate during the growing periods of wheat in 2011/12 and 2012/13 seasons.

Months	Average temperature ^{0}C						Average RH (%)		Average Wind speed (Km/h)	
	Maximum		Minimum		Average					
	11/12	12/13	11/12	12/13	11/12	12/13	11/12	12/13	11/12	12/13
December	24.0	23.5	9.3	8.7	16.7	16.1	57.6	55.4	14	15
January	18.9	19.2	7.1	7.4	13.0	13.3	56.9	57.5	13	14
Feb.	20.6	21.3	9.6	9.8	15.1	15.6	54.3	53.8	15	13
Mars	24.9	23.4	10.7	10.3	17.0	16.9	55.4	56.1	13	14
April	28.5	29.5	15.5	15.9	22.0	22.7	53.2	54.7	12	13

Data collected from Agriculture Research Center Meteorological Station in Ismailia

Table (2): Soil physical and chemical properties of the experimental field soil over the two seasons.

Soil depth (cm)	Sand (%)	Silt (%)	Clay (%)	Hydraulic conductivity (cm h^{-1})	Texture class	Bulk density (g cm^{-3})
0-60cm	89.11	7.48	3.41	7.50	Sand	1.65
Soil depth (cm)	**Field capacity (%)**	**Wilting point (%)**	**pH**	**Organic matter (%)**	**EC (dS m^{-1})**	
0-60cm	7.50	1.49	7.62	0.29	2.05	

To ensure full germination, 30 mm of irrigation water was applied to the all experimental field area at sowing. In addition, 33 and 37 mm were applied at 10 and 20 days from sowing for complete establishment of seedlings. To avoid deep percolation losses, irrigation was performed two times during the germination and seedling stages. All cultural practices for wheat in this region were applied as recommended.

Sampling

Ten days after the 3rd foliar application of AsA, wheat plants from an area of 0.5 m^2 from each plot were randomly taken to determine leaf area index (LAI). Meanwhile, relative water content (RWC) and total chlorophylls were determined in flag leaves. Flag Leaf samples of each season were also collected, washed and stored at −20°C pending biochemical analysis. Flag leaf and main stem specimens from second year were removed for anatomical analysis. The central area in each plot was kept to determine yield and irrigation water use efficiency (IWUE).

Leaf area index

Leaf area index (LAI) was calculated according to Bonhomme *et al.*, 1974.

Relative Water Content

Relative water content 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 flag leaves, TW the weight of disks after hydration for 12 h at room temperature under low light conditions and DW is the average weight of the same disks after drying at 80°C for 48h, according to Henson *et al.*, (1981).

Chlorophyll assay

According to Arnon (1949), 0.5 g fresh flag leaves was ground with 10ml aceton 85% and filtered. Optical density was measured at 644 and 662 nm using a Beckman DK-2 Spectrophotometer. Concentration of total chlorophylls as mg/100 g FW was calculated.

Preparation of enzyme leaves extracts

According to Urbanek *et al.*, (1991), 0.5 g fresh flag leaf 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, 4 °C. The supernatant was used to determine activity of enzymes as follows:

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.

Catalase (CAT, EC 1.11.1.6) assay: The reaction mixture consisted of 0.01mL enzyme extract and 2.99mL hydrogen peroxide-phosphate buffer (pH 6.8) prepared after dilution of 0.16mL of H_2O_2 (10%, w/v) to 100mL phosphate buffer (Urbanek et al., 1991). The oxidation of H_2O_2 was measured by changes in optical density at 240nm in 30 s intervals for 5min (Beckman DK-2 Spectrophotometer). The unit of CAT activity was defined as the amount of enzyme, which decomposes 1mmol H_2O_2 per minute at 25°C.

Superoxide dismutase (SOD, EC 1.15.1.1) assay: The reaction mixture contained 100 µl riboflavin(1 µM), 100 µl L-methionine (12mM), 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 (Beckman DK-2 Spectrophotometer). The SOD activity of the extract was expressed as SOD units per milligram of protein according to Giannopolitis and Ries (1997).

Protein assay

Total protein content (mg/g FW) was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford (1976), using 1 ml Bradford solution and 100 µl leaf extract.

Lipid peroxidation assay

The level of membrane damage was determined by measuring malondialdehyde (MDA) as the end product of peroxidation of membrane lipids (De Vos *et al.*, 1991). Leaves were homogenized in an aqueous solution of trichloroacetic acid (10 % w/v), and aliquots of the filtrates were heated in 0.25 % thiobarbituric acid. The amount of MDA was determined from the absorbance at 532 nm, followed by correction for the non-specific absorbance at 600 nm.

Anatomical studies

Flag leaf and main stem specimens from second season plants were killed and fixed in F.A.A., dehydrated in ethyl alcohol series, embedded in Paraffin wax, sectioned to thickness of 15 µm, double stained with Safranin and Light green, cleared in Xylene and mounted in Canada balsam according to Willey (1971). All measurements were calculated by eyepiece micrometer.

Yield measurements and irrigation water use efficiency (IWUE)

At maturity, data collected from 1m^2 area for each plot was used to determine number of spikes/m^2, number of grains $spike^{-1}$, grain yield and straw yield. In addition, irrigation water use efficiency, defined as the ratio of grain yield to the seasonal amount of irrigation water applied, was calculated as IWUE = (Y/I), where Y is the economical yield (kg ha^{-1}) and I is the amount of applied water (m^3/ha) for each irrigation treatment according to Burt *et al.*, (1997).

Statistical analysis

All data were analyzed using the CoStat software, version 6.311 (CoHort software, Berkeley, CA 94701). 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 over 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). Graphical presentation of data was carried out using Microsoft Excel program (Microsoft Corporation, Los Angeles, CA, USA).

RESULTS

The combined analysis of variance for the data over the two seasons reveled that, there were significant interaction effects between water stress and foliar application of ascorbic acid on leaf area index, relative water content, total chlorophyll, protein content (Table, 3), activities of some key enzymes of oxidative defense system and malondialdehyde content (Table, 4), yield measurements and irrigation water use efficiency (Table, 5). Interaction effect of water stress and AsA application on measured parameters can discuss as follow:

Leaf area index (LAI)

Water stressed wheats without AsA application showed a significant decrease in leaf area index (LAI) (2.4-3.0) compared to control ones (3.5) as shown in Fig.1. The maximum values of LAI (4.0-4.2) were recorded in 100 or 200 ppm AsA-treated plants under normal irrigation. Although effect of 100ppm of AsA did not differ significantly from 200ppm treatment, but they improved LAI by approximately 21-30% under both water stress levels (W_1 and W_2), respectively compared to non-sprayed plants.

Relative water content (RWC)

Relative water content (RWC) of wheat flag leaves were significantly decreased under drought treatments (Fig. 1). Over the two successive seasons, 0.6 ETc treatment resulted in lower RWC (34.6%) than those in the 0.8 (41.0%) and 1.00 (70.2%) of ETc, but AsA application alleviated the adverse effect of water deficit. AsA application increased the RWC in flag leaves by approximately 32-33 % compared to non-treated plants under the both two water stress levels (W_1 and W_2), respectively. However, there are no significant changes in RWC of both levels of AsA.

Chlorophyll content

Drought treatments had a significant negative effect on the content of photosynthetic pigments (Fig. 1.). Wheats exposed to water stress without AsA application showed a significant decrease in total content of total chlorophyll (CHL) compared with control plants. Plants exposed to moderate and severe water stress (W_1 and W_2) showed minimum significant values of CHL content (24.1 and 16.6 mg/100g FW), respectively relative to well watered plants (30.7 mg/100g FW). Under moderate and severe water stress, the application of AsA significantly ameliorated the content of total CHL, with a higher level of total CHL in moderate stress (29.4 mg/100g FW) than in sever stress (21.2 mg/100g FW). AsA application saved about 4-5 mg/100gFW of CHL from degradation under water stress.

Total protein content

The changes in leaf protein contents during water shortages are shown in Fig.1. It significantly reduced in response to moderate stress (by about 1.5 mg/g FW) and severe stress (by about 2.5 mg/g FW) relative to controls (W_0A_0). Effect of 100ppm of AsA did not differ significantly from 200ppm treatment, but they significantly prevented the reduction of total protein in wheat flag leaves by about 28-32% under both water regimes (W_1 and W_2), respectively compared to non-sprayed plants.

Antioxidant enzymes

The decrease of the amount of irrigation water produced gradually and significantly increases activities of SOD, APX and CAT by 0.74-1.1, 0.44-0.57 and 1.1-1.6 times, respectively in wheat flag leaves compared to control plants (Fig. 2). Foliar application of AsA was associated with a significant increase in CAT and APX activity in non-stressed plants; however, SOD activity was decreased. Application of AsA to stressed plants resulted in a significant decrease regarding the activity of all above mentioned antioxidant enzymes in the flag leaves by approximately 10-20% compared to water stressed ones. Foliar application of AsA in 100ppm did not differ significantly from 200ppm on the activity of all antioxidant enzymes under non-stressed and stressed plants.

Lipid peroxidation

The occurrence of oxidative stress induced by drought was monitored by analyzing membrane damage through measurement of malondialdehyde (MDA) levels. The level of lipid peroxidation of flag leaves, measured as MDA content, is shown in Fig. (2). In stressed plants, the MDA content was increased by water stress treatments, reach to 8.2 -11.4 nmol/g FW compared to 4.6 nmol/g FW in control plants. AsA protected lipids of cell membranes in wheats from degradation under drought. Application of AsA decreased the MDA content in the leaves of plants exposed to normal irrigation by 24% as well as moderate and sever stress by 51 and 38%, respectively.

Anatomical characters

Flag leaf blade

Measured anatomical characters of flag leaf blade were significantly differed under water stress than control (Table 6 and Fig.5). Reduction of mesophyll thickness by approximately 27-34 µ in water stressed wheats was observed compared to control plants. Application of AsA alleviated the adverse effect of drought on mesophyll thickness. Plants sprayed with 100 and 200ppm of AsA preserved approximately 34-14 and 56 – 47 % of mesophyll tissue under W_1 and W_2 levels compared to water stressed plants. Drought increased thickness of cuticle on epidermis cells 5-times (reached to 6.1 µ), but AsA application increased it only in well watered plants. Maximum significant values of metaxylem radius was recorded in 100ppm AsA-sprayed wheats under well irrigation treatment, but minimum ones was observed in non-sprayed wheats under second level of water stress. 200ppm AsA application was increased area of both sub-stomatal

Table 3. Effect of water stress and spraying treatments on leaf area index; relative water content, total chlorophyll and protein content in flag leaves of wheat plants at 105 days from planting.

	Leaf area index (LAI)			Relative water content (%)			Total chlorophyll (mg 100g^{-1} FW)			Protien content (mg g^{-1} FW)		
Factors	**11/12**	**12/13**	**Comb.**	**11/12**	**12/13**	**Comb.**	**11/12**	**12/13**	**Comb.**	**11/12**	**12/13**	**Comb.**
Water stress (W)												
W0	4.31a	3.51a	3.91a	76.28a	70.00a	73.14a	37.28a	32.02a	34.65a	7.85a	6.55a	7.20a
W1	3.67b	3.11b	3.39b	56.87b	43.29b	50.08b	31.89b	23.33b	27.61b	6.42b	5.72b	6.07b
W2	3.02c	2.66c	2.84c	38.93c	44.83c	41.88c	18.12c	20.64c	19.38c	5.32c	4.56c	4.94c
F. test	*	**	**	**	**	**	**	*	**	*	*	*
Spraying treatments (A)												
A0	3.42b	2.50b	2.96b	53.64b	43.58b	48.61b	27.58b	20.02b	23.80b	5.94b	4.58b	5.26b
A1	3.75a	3.31a	3.53a	60.35a	56.73a	58.54a	30.47a	27.75a	29.11a	6.75a	6.11a	6.43a
A2	3.84a	3.46a	3.65a	58.09a	57.81a	57.95a	29.25a	28.21a	28.73a	6.90a	6.14a	6.52a
F. test	**	*	*	**	*	**	*	**	**	*	*	*
Interaction (A*B)	*	**	**	*	**	**	*	*	*	**	*	**

W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

Table 4. Effect of water stress and spraying treatments on activities of some key enzymes of oxidative defense system (units mg^{-1} protein) and malondialdehyde content in flag leaves of wheat plants at 105 days from planting.

Factors	**SOD activity**			**CAT activity**			**APX activity**			**Malondialdehyde content (nmol g^{-1} FW)**		
	11/12	**12/13**	**Comb.**	**11/12**	**12/13**	**Comb.**	**11/12**	**12/13**	**Comb.**	**11/12**	**12/13**	**Comb.**
Water stress (W)												
W0	34.17c	30.87c	32.52c	22.51c	15.61c	19.06c	176.82c	189.36c	183.09c	3.42c	4.46c	3.94c
W1	50.46b	59.28b	54.87b	32.65b	23.55b	28.10b	216.25b	213.53b	214.89b	4.95b	6.19b	5.57b
W2	65.93a	70.69a	68.31a	36.39a	33.45a	34.92a	224.84a	246.36a	235.60a	9.21a	8.49a	8.85a
F. test	**	**	**	*	**	**	*	*	**	**	*	*
Spraying treatments (A)												
A0	55.14a	57.54a	56.34a	31.33a	26.51a	28.92a	208.56a	225.82a	217.19a	9.05a	7.17a	8.11a
A1	48.11b	50.69c	49.40b	28.62c	24.58b	26.60b	200.26b	212.96b	206.61b	4.00c	6.10b	5.05b
A2	47.32b	52.60b	49.96b	31.61b	21.50c	26.56b	209.08a	210.48b	209.78b	4.54b	5.87b	5.21b
F. test	*	**	*	**	**	**	*	*	*	*	*	*
Interaction (A*B)	**	*	**	**	*	**	*	*	*	*	**	**

W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

Table 5. Effect of water stress and spraying treatments on yield measurements and irrigation water use efficiency (IWUE) of wheat plants at harvest.

Factors	Number of spikes m^{-2}			Number of grains $spike^{-1}$			Grain yield (t ha^{-1})			Straw yield (t ha^{-1})			Irrigation water use efficiency (kg m^{-3})		
	11/12	12/13	Comb.	11/12	12/13	Comb.	11/12	12/13	Comb.	11/12	12/13	Comb.	11/12	12/13	Comb.
Water stress (W)															
W0	235.32a	215.94a	225.63a	45.65a	41.97a	43.81a	4.658a	4.049a	4.354a	7.987a	7.725a	7.856a	1.093b	0.950b	1.022b
W1	182.75b	192.39b	187.57b	38.69b	35.71b	37.20b	4.038b	3.468b	3.752b	6.559b	6.703b	6.631b	1.185a	1.018a	1.101a
W2	165.36c	162.00c	163.68c	29.68c	28.24c	28.96c	2.659c	2.436c	2.548c	4.652c	4.416c	4.534c	1.040b	0.953b	0.997b
F. test	**	**	**	**	**	**	*	**	**	**	**	**	**	*	**
Spraying treatments (A)															
A0	170.25b	177.27c	173.76b	32.38b	31.70c	32.04b	2.958b	3.206b	3.082b	5.569b	5.392c	5.481b	0.868b	0.941b	0.904b
A1	207.60a	193.36b	200.48a	40.48a	38.26a	39.37a	4.165a	3.490a	3.828a	6.728a	6.974a	6.851a	1.222a	1.024a	1.123a
A2	205.59a	199.69a	202.64a	41.16a	35.96b	38.56a	4.230a	3.258b	3.744a	6.900a	6.477b	6.688a	1.241a	0.956b	1.099a
F. test	**	*	*	*	**	**	**	*	**	**	**	**	*	**	*
Interaction (A*B)	*	**	**	**	**	**	**	**	**	*	**	**	*	*	*

W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

Table 6. Average of some anatomical characters of flag leaf as affected by AsA application under drought treatments at 105 days from planting.

Water stress	Spraying treatments	Thickness (µ)		Metaxylem radius (µ)	Area (μ^2) of		
		Mesophyll	Upper epidermal cuticle		Sub-stomatal chamber	Phloem	Bulliform cells
W0	**A0**	78.3 a	1.2 d	9.3 b	6.36 c	12.2 a	3.6 c
	A1	65.8 c	1.6 c	12.2 a	6.5 c	10.3 b	3.6 c
	A2	59.4 d	3.1 b	9.5 b	6.5 c	9.1 c	3.6 c
W1	**A0**	51.7 e	6.1 a	6.1 c	7.5 b	7.2 d	4.6 b
	A1	69.2 b	3.1 b	6.1 c	5.2 d	6.1 e	3.6 c
	A2	59.1 d	3.1 b	9.1 b	5.3 d	5.9 e	4.6 b
W2	**A0**	43.9 f	6.1 a	6.1 c	7.4 b	4.9 f	4.6 b
	A1	68.6 b	6.1 a	9.1 b	7.4 b	6.1 e	9.3 a
	A2	64.5 c	6.1 a	9.1 b	9.5 a	7.2 d	9.2 a
L.S.D 5%		2.7	0.33	0.29	0.6	0.4	0.78

W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

Table 7. Average of some anatomical characters of main stem as affected by AsA application under drought treatments at 105 days from planting.

Water stress	Spraying treatments	Ground tissue Thickness (µ)	Metaxylem radius (µ)	Area (μ^2) of	
				Chlorenchema	Phloem
W0	**A0**	142.5 b	9.8 a	35.9 a	7.2 a
	A1	162.2 a	9.8 a	23.9 b	6.1 b
	A2	122.9 c	9.8 a	26.8 b	5.9 b
W1	**A0**	102.6 de	7.8 b	17.9 c	2.8 e
	A1	98.6 e	7.9 b	24.8 b	1.9 f
	A2	102.1 de	9.7 a	23.9 b	4.9 c
W2	**A0**	67.9 f	10.1 a	16.5 c	3.9 d
	A1	106.4 d	9.8 a	19.2 c	3.9 d
	A2	120.1 c	9.9 a	19.2 c	6.1 b
L.S.D 5%		3.8	1.1	3.5	0.36

W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

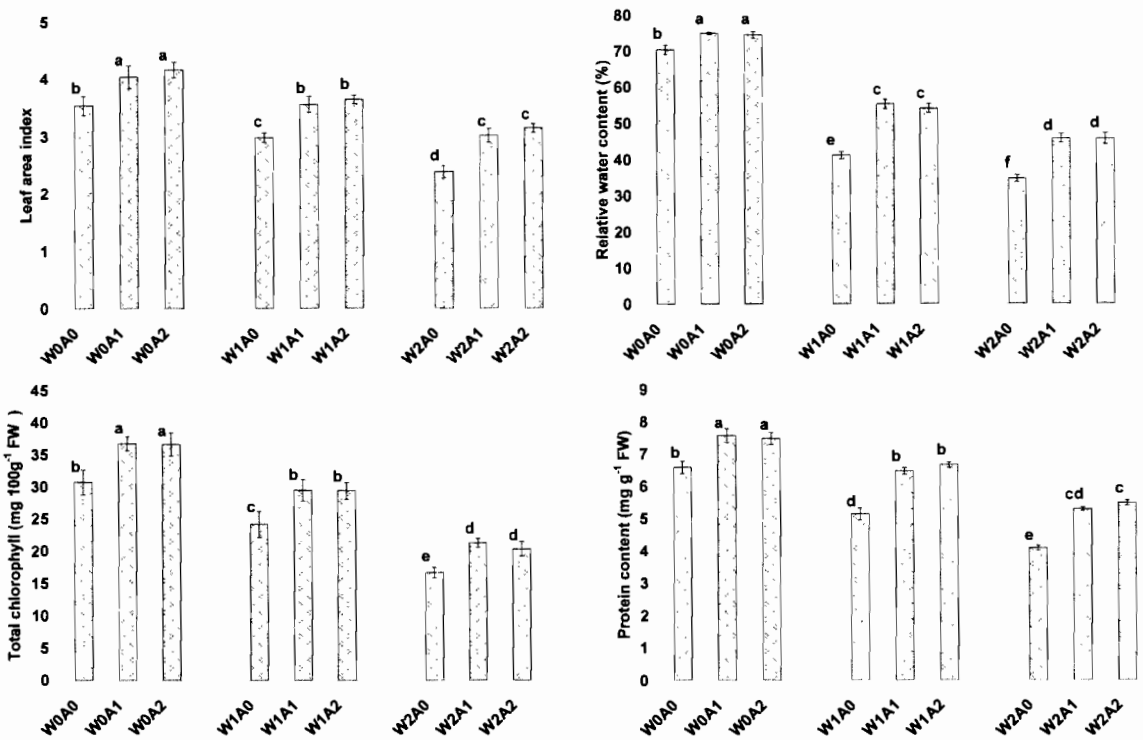

Fig. (1): Interaction effect between water stress and foliar application of ascorbic acid on leaf area index, relative water content, total chlorophyll and protein content in flag leaves of wheats at 105 days from planting (Combined data). Mean values ± SD followed by the same letter in each bar show non-significantly different at the $P < 0.05$ probability level. W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

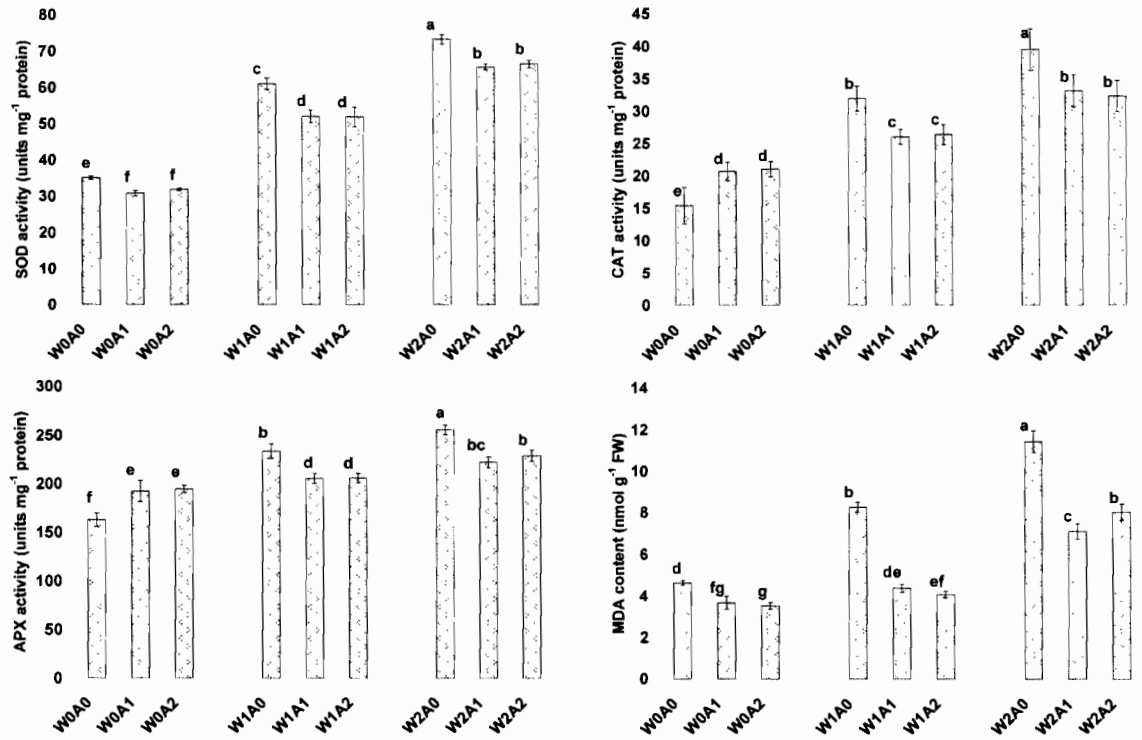

Fig. (2): Interaction effect between water stress and foliar application of ascorbic acid on activities of some key enzymes of oxidative defense system and malondialdehyde content (MDA) in flag leaves of wheats (Combined data) at 105 days from planting. Mean values ± SD followed by the same letter in each bar show non-significantly different at the $P < 0.05$ probability level. W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

chamber and bulliform cells to maximum values (9.5 and 9.3 μ^2, respectively) grown under second level of drought. Although, 2.5-folds reduction of phloem thickness was occurred in stressed-water wheats compared to control plants, but 100 and 200ppm AsA-treated plants showed 20-30% increment in phloem thickness.

Main stem

Table 7 and Fig.6 showed that, the thickness of ground tissue in the main stem of wheat was decreased by 28 to 52 % in water stressed plants compared to well watered plants where its maximum thickness (162.2 μ) was recorded in well watered plants sprayed with 100 ppm of AsA. Also, both of 100 and 200 ppm AsA-treatments protected approximately 36-43 % of ground tissue from collapse under high level of drought. Maximum significant values of metaxylem (10.1 μ) were recorded in stressed water wheats. Application of AsA showed vigorous effect on radius of xylem vessels. Approximately, half of chlorenchyma and phloem area were reduced under water stress compared to control plants. 200ppm-AsA application only showed an increment of both previous parameters (26-14%) for chlorenchyma and 42-56 % for phloem under W_1 and W_2 treatments, respectively compared to water stressed plants.

Yield measurements

As illustrated in Fig. 3, wheat plants exposed to moderate and severe water stress showed a statistically significant reduction on number of grains/spike (30-25), number of spikes/ m^2 (169-141), grain (3.2-2.0 t/ha) and straw (5.3-3.5 t/ha) yields compared with the control plants which had 40, 210, 4 and 7.6 averages, respectively. Therefore drought reduced grain yield by about 50% due to severe drought treatment. Application of AsA to stressed and non-stressed plants showed a significant increase in each of these traits. AsA application alleviated the adverse effect of water stress. The maximum values of yield measurements were obtained from well watered with 100 or 200ppm AsA at harvest. Grain yield was increased by about 0.5 t/ha in AsA-treated plants under normal irrigation. Meanwhile, wheat plants exposed to severe stress without AsA gave the lowest averages of yield measurements compared to the other treatments. In general, increasing the level of AsA from 100 to 200 ppm did not significantly ameliorate the averages of these traits under stressful conditions and full irrigation. Wheat plants treated with AsA showed an increment in all yield measured parameters by about 10-5 grain/spikes, 28-34 for spike number/m^2, 0.9-0.8 ton for grains /ha and 2.0-1.6 ton for straw/ha under moderate or sever water stress, respectively. These results also mean that wheats responded to AsA application under stress conditions as well as normal irrigation.

Irrigation water use efficiency (IWUE)

The changes in values of IWUE during water stress are shown in Fig. 4. IWUE significantly decreased in response to severe stress (0.78 kg/m^3) relative to controls or moderate stress (0.95 kg/m^3). However, in moderate stress treatment with 100 ppm AsA, IWUE showed a highly significant value (1.2 kg/m^3) compared with the other treatments.

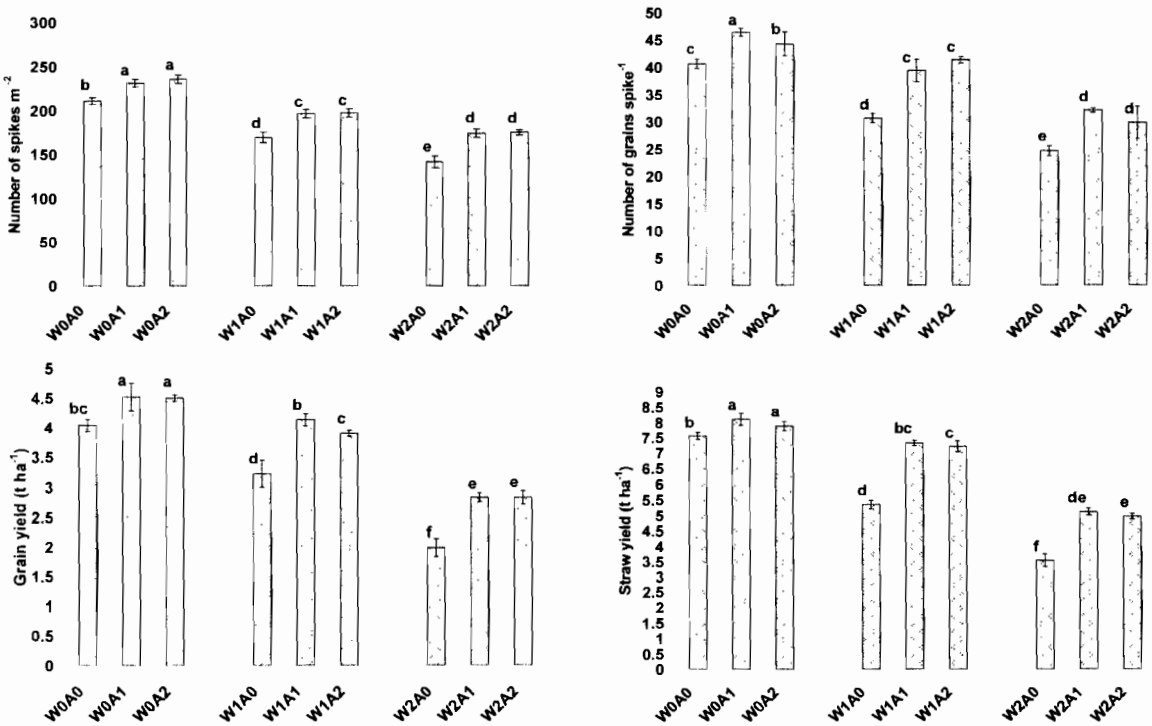

Fig. (3): Interaction effect between water stress and foliar application of ascorbic acid on Yield measurements (Combined data). Mean values ± SD followed by the same letter in each bar show non-significantly different at the $P < 0.05$ probability level. W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

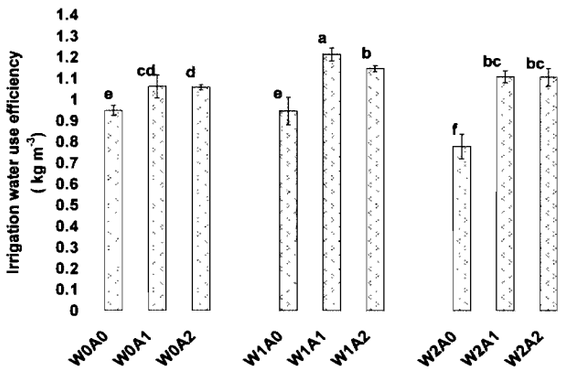

Fig. (4): Interaction effect between water stress and foliar application of ascorbic acid on irrigation water use efficiency (IWUE) (Combined data). Mean values ± SD followed by the same letter in each bar show non-significantly different at the $P < 0.05$ probability level. . W0: well-watered (4260 m^3/ha); W1: 0.8 of W0, W2: 0.6 of W0. A0: untreated plants, A1: 100ppm AsA, A2: 200ppm AsA.

DISCUSSION

All biochemical determinations were conducted on flag leaf as it contributes about 30 to 50% of the assimilates partitioned for grain filling in wheat (Sylvester-Bradley *et al.*, 1990) as well as their onset and rate of senescence are important factors for determining yield potential (Evans, 1993).Table 1 and 2 revealed that, the experiment was conducted under semi-arid conditions in sandy soil with 20mm as annual rain rate during the experimental period. Also, 50-200 ppm of AsA was an appropriate concentration as an immune-modulator for many plants (Amin *et al.*, 2008; Raafat *et al.*, 2011 and Shalata and Neumann, 2001). Furthermore, AsA reaches a concentration over 20mM (≈3500ppm) in chloroplast and occurs in all cell compartments including the cell wall (Smirnoff and Wheeler, 2000).

Results indicated that, water stress caused a reduction in each of leaf area index, total chlorophyll, relative water content, grain and straw yields, but increased lipid peroxidation and protein oxidation in wheat. Water stress causes directly or indirectly reduction in total dry mass accumulation, limitation on total chlorophyll content, leaf relative water content, leaf areas in wheat seedlings through molecular damage in plant cells by the formation of reactive oxygen species (ROS) (Lissner et al. 1999 and Farooq et al., 2013).

Leaf area index (LAI) is the principal mean for light interception and is an important photosynthesis parameter for the plant as well as it is considered the best measure for photosynthesis capacity of plant regarding to the occupied area. Wheats under normal irrigation as well as water stress responsed to AsA application due to its nutritive, antioxidant and cellular reductant properties (Noctor and Foyer, 1998). So, AsA-treated well watered plants recorded maximum value of LAI. Furthermore, under both water stress levels, addition of AsA improved LAI by third amount compared to the control (Fig.1). These response may be due to AsA protect plant cell as non-enzymatic antioxidant and regulate plant growth owing to its effect on cell division and differentiation (Price, 1966). Also, AsA induced Gibberellin 3-B dioxgenase responsible for synthesis of plant growth promoting hormone, Gibberelin (Coles *et al.*, 1999)

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). AsA application increased the RWC in wheat leaves by about 33% compared to non-treated plants under the both two water stress levels as shown in Fig 1. Farooq, *et al.*, 2013, found that, AsA-treated wheat accumulated high amount of proline under drought and it as an osmoregulatory solute improved water status in plants under water and salinity stress.

The reduction of chlorophylls (CHL) under drought stress is mainly due to the membrane disintegration and damage to chloroplasts by ROS (Jung, 2004). As.A had a protective effect on CHL from degradation under water stress (Fig.1). The application of AsA significantly ameliorated the content of total CHL with a higher level in moderate stress than in sever stress. CHL degradation under drought associated with the formation of proteolytic enzymes such as chlorophyllase (Sabater and Rodriquez, 1978).

Oxidative stress due to the existence of the water deficits can be demonstrated by the decreases of protein content. Protein content was decreased by 22-38 % in leaf wheat under moderate and severe water stress. Water deficit enhances proteolytic degradation of Rubisco protein (Lin and Wang, 2002). Hence, decreases of soluble protein contents under water stress could be largely due to a decline in Rubisco protein. Both levels of AsA (100 or 200 ppm) prevented the reduction of total protein in wheat leaves by 28-32 % under both water regimes (Fig.1).

Drought affects not only water relations, but also induces stomatal closure, decreases the photosynthetic 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 the formation 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). AOS are highly active molecules that can easily damage membrane and oxidize photosynthetic pigments, proteins and nucleic acids (Gong *et al*, 2005).

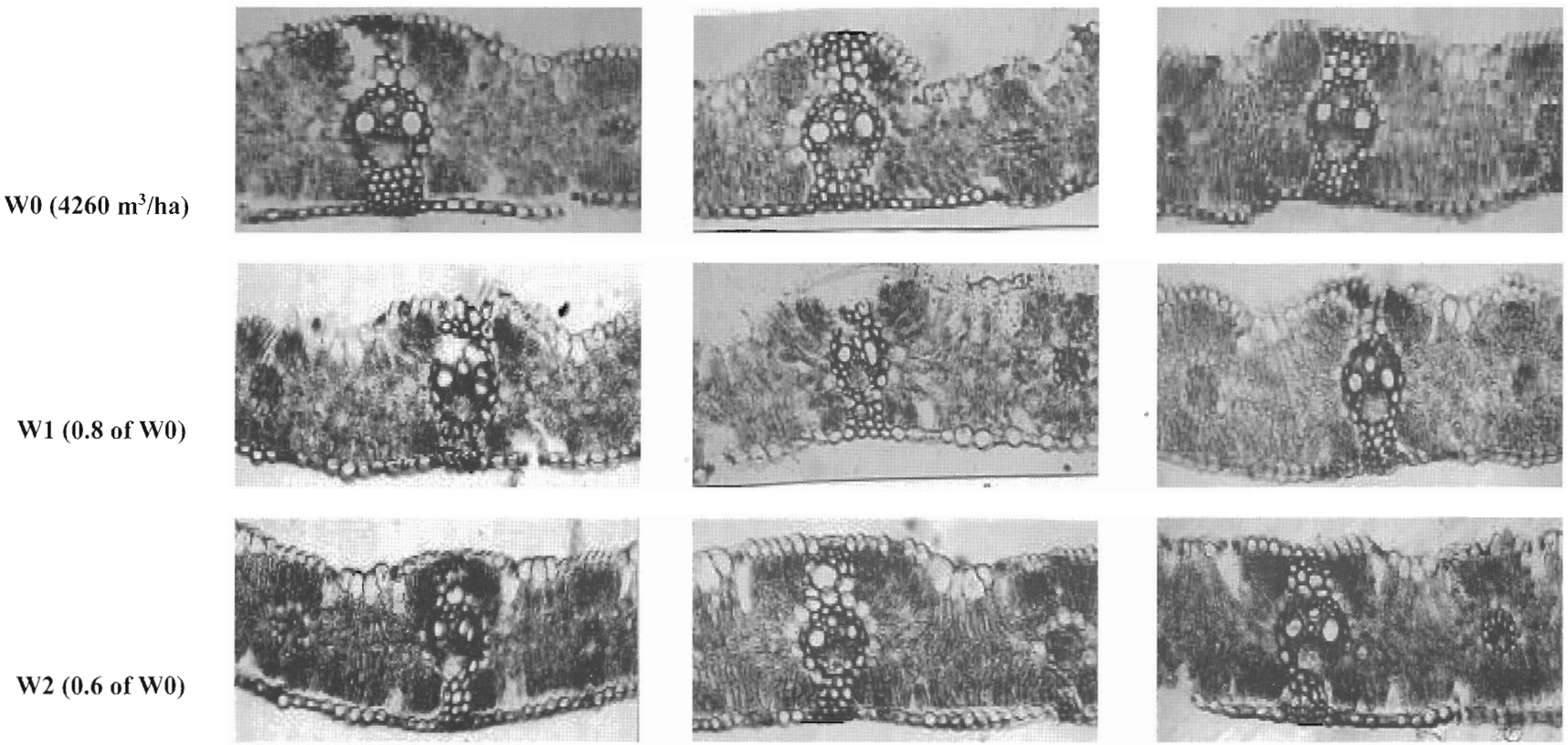

Fig. 5. Wheat flag leaf in cross section as affected by water levels and AsA treatments at 105 days from planting (100X).

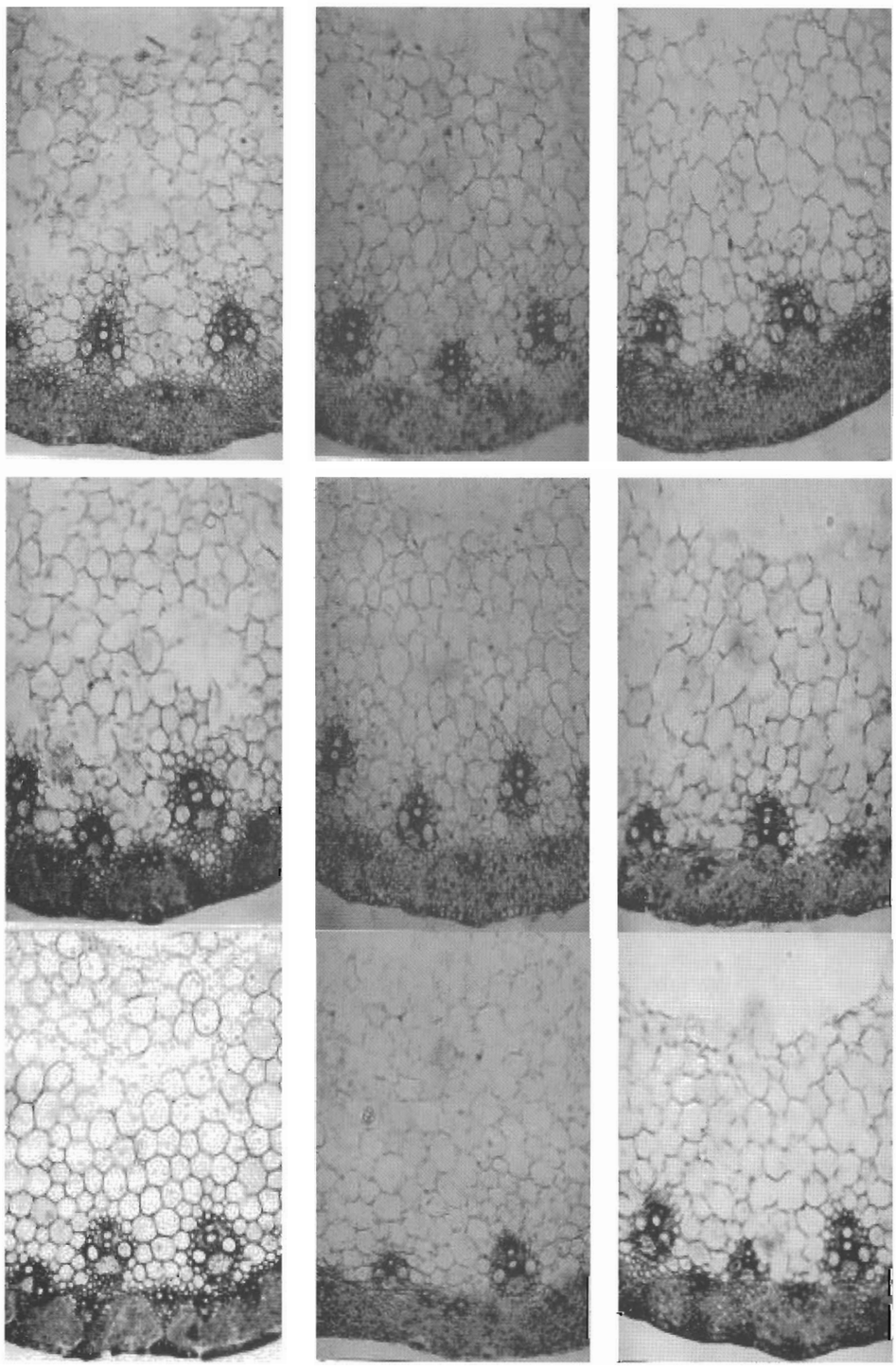

Fig. 6. Wheat main stem in cross section as affected by water levels and AsA treatments at 105 days from planting (100X).

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^- 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, APX and CAT are reported to increase under various environmental stresses (Misra and Gupta, 2006, Khanna-chopra and Selote, 2007, Ardc *et al*, 2009). As a confirmation, in the present work, we also observed enhanced activities of SOD, APX and CAT (74-108 %, 44- 57 % and 107-156 %, respectively) under water-stressed conditions in compared to plants under well watered conditions (Fig. 2). It means that, wheat induced CAT and SOD followed by APX activity under drought. 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, under stress water condition, exogenous application of AsA partially inhibits these toxic effects of AOS because AsA is a scavenger of AOS (Noctor and Foyer 1998). Foliar application of AsA was associated with a significant increase in CAT and APX activity in non-stressed plants, however, SOD activity was decreased. Application of AsA to stressed plants had a significant decrease on the activity of all antioxidant enzymes in the leaves by approximately 10-20% compared to water stressed ones. 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). The results are in accordance with other authors reporting that, APX and CAT activity increased in drought tolerant genotypes of pea (Gillham and Dodge, 1987), tomato (Walker and McKersie, 1993), Sorghum (Jagtap and Bhargava, 1995) and bean (Zlatev *et al.*, 2005).

Acclimation of plants to drought is considered to promote antioxidants defense systems to face the increased levels of AOS, which in turn, cause membrane damage by lipid peroxidation and indicated by malondialdehyde (MDA) content, which is one main parameter for evaluating membrane oxidation extent and are toxic for the cells (Chaves *et al.*, 2003, Shao *et al.*, 2005). MDA, a product of the decomposition of polyunsaturated fatty acids in biomembranes, shows increased accumulation under water stress (Guo *et al.* 2012). In stressed wheats (Fig.2), the MDA content was increased by water stress treatment, reach to about 8.2 - 11.4 nmol/g FW compared to 4.6 nmol/g FW in control plants. But application of AsA decreased the MDA content in the plant leaves exposed to normal irrigation (by 24%) as well as drought stress (by 51-38%). During drought conditions high activities of antioxidant enzymes are associated with lower levels of lipid peroxidation, being connected to drought tolerance (Bowler *et al.*, 1992).

Responses of some tissues in flag leaf and stem in wheat to drought were investigated (Table 6 and Fig.5). The most active photosynthetic tissue in higher plants is the mesophyll of leaves (Ray, 2006). The mesophyll thickness was decreased in water stressed wheats compared to control ones. But, AsA treatments preserved mesophyll from collapse in moderate and severe levels of water irrigation compared to water stressed plants. Moreover, plants from moist habitats often develop thick cuticles when grown under dry conditions (Ray, 2006). In water stressed wheats thickness of cuticle on epidermis cells was increased 5-times, but AsA application increased it only in well watered plants. Synthesis of plant metabolites as cuticle lipids affected by the internal concentration of reducing agents, such as AsA (Taiz and Zeiger, 2002). The two long-distance transport pathways, the phloem and the xylem affect plant growth and productivity (Ray, 2006). Maximum significant values of metaxylem radius was recorded in 100 ppm-sprayed wheats under well irrigation treatment, but minimum ones was observed in non-sprayed wheats under sever water stress. Phloem redistributes water and various compounds throughout the plant body (Taiz and Zeiger, 2002). Although, 2.5-folds reduction of phloem thickness was occurred in stressed water wheats compared to control plants, but 100 or 200 ppm AsA treated plants showed 20-30% increment in phloem thickness. AsA regulates cell division and differentiation (Pignocchi and Foyer, 2003). Grasses are characterized by the presence of large thin-walled cells, bulliform or roll cells. In mature leaves during periods of drought, it seems to participate in involution (the rolling up of leaves) by losing water, becoming flaccid, and thereby facilitating the process. 200ppm of AsA application was increased area of both sub-stomatal chamber and bulliform cells to maximum values grown under second level of drought. Stomatal apparatus has a major regulatory role in gas exchange in leaves and they can often affect yields of agricultural crops (Ray, 2006). Relative cell volume of very rigid cell walls (e.g., mesophyll cells in the leaves of many grasses) decreased by more than 10% as turgor pressure reached to zero (Taiz and Zeiger, 2002). These findings were agreed with, Zagdańska and Kozdój (1994) who found an irreversible reduction in leaf area and thickness, increase frequencies of stomata, higher number of bulliform cells with simultaneous decrease in number of intermediate veins and an increase in the share of the cell walls in total cell volume in flag leaf of wheat under drought. The smaller leaf thickness was due to a diminished number of mesophyll layers and a decreased size of mesophyll cells. Such altered leaf anatomy indicated development of leaf xerophily. The irreversible changes in anatomy of wheat flag leaves play a decisive role in acquiring drought tolerance during wheat acclimation to drought.

Stem ground tissue (a storage region) in the center of the stem may lack chloroplasts but will contain

unpigmented plastids (amyloplasts) in which starch is synthesized (Ray, 2006). Its thickness (Table 7 and Fig. 6) was decreased to half in water stressed wheats compared to well watered plants. 100 or 200ppm of AsA application protected approximately quarter of ground tissue from damage under high level of drought. AsA treatment delays the formation of lysigenous intercellular spaces in wheat stem, may be through inhibition of proteolytic enzyme like, pectinase or AsA may be regulate the programmed cell death in plant under water stress, but this need more studies. Maximum significant values of metaxylem was recorded in stressed water wheats. Application of AsA not showed vigorous effect on radius of xylem vessels. Approximately, half of chlorenchyma and phloem area were decreased under water stress compared to control plants. But, 200ppm-AsA application only showed an increment of both previous parameters under W1 and W2 treatments, respectively compared to water stressed plants.

As stomata closed during early stages of water stress, water-use efficiency may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations. As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases (Gallego *et al.*, 1996 and Milone *et al.*, 2003). With increasing shortage of water resources and worsening ecoenvironment, wheat production is influenced greatly (Vasil, 2003). Our results also suggest that water shortage stress can reduce wheat productivity and Irrigation water use efficiency (Fig.3 and 4). As show in Fig. 3, wheats exposed to water stress showed a statistically significant reduced number of grains/spike, number of spikes/ m^2, grain and straw yields by about 25-40%, 20-32%, 20-51% and 29-53% compared with control plants, respectively. 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). Also, oxidative stress and signs of senescence include loss of CHL and protien and decline in membrane permeability, all of which lead to a progressive reduction in photosynthetic capacity (Thompson et al. 1987). Foliar application of AsA to stressed and non-stressed plants showed a significant increase on these all traits. Our data also confirmed that, AsA application alleviated the adverse effect of water stress on wheat productivity. Under normal conditions, the plants exposed to AsA gave the maximum values of yield measurements compared with other treatments. Also, under water stress conditions, wheats treated with AsA showed an improved in grain and straw yields by about 0.8-0.9 t/ha and 1.6-2.0 t/ha in comparison to untreated plants, respectively. Grain yield produced from AsA-treated wheat irrigated with 3408 m^3/ha (W2) was the same obtained from well irrigated wheat (4260 m^3/ha). Therefore, using of AsA can save about 852 m^3/h of irrigation water without yield reduction; especially we have a serious problem in water resources.

A significant decrease in IWUE was found in response to sever stress relative to water stress. In this context, Gallego *et al.*, 1996 and Milone *et al.*, 2003 reported that, water stress leads to an increase in free radicals in chloroplasts and destruction of CHL molecules by ROS, which results in reduction of photosynthesis, growth, crop productivity and IWUE in plants. Meanwhile our results indicated that wheats treated with 100 ppm showed a highly significant increase in IWUE (1.2 kg/m^3) compared with the other treatments (Fig.4).

These results also indicate that wheats responded to AsA application under stressful conditions as well as normal irrigation. The beneficial effect of AsA on growth analysis and productivity of wheat (Amin *et al.*, 2008, Raafat *et al.*, 2011) under drought might be due to that AsA plays an essential role in several physiological processes in plant, differentiation, plant cell division and metabolism. Also, it plays an important role in cell protection against oxidative stress (Pignocchi and Foyer, 2003).

CONCLUSIONS

The present results demonstrated that foliar application of AsA can increase the survival capacity of wheat plants under conditions of drought stress. The increment of drought tolerance is associated with the antioxidative activity of AsA. This effect was mainly attributed to improvement of irrigation water use efficiency, prevention of growth inhibition, lipid peroxidation, chlorophyll and protein degradation, and modulation of SOD, CAT, and APX activities, as components of the antioxidative machinery that allowed the plant to cope better with drought stress. AsA also protected the principle tissues in flag leaf and stem. According to these results it can be concluded that foliar application of AsA can reduce the harmful effects of ROS, improves plant resistance and maximizing their productivity in marginal lands.

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تحسين مقاومة القمح للجفاف وانتاجيته عن طريق الرش بحمض الاسكوربيك

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هناك نقص فى الدراسات التطبيقية على دور حمض الاسكوربيك فى مجابهة التاثيرات الضارة للجفاف على نمو وانتاجية القمح تحت الظروف الحقلية. لذلك أجريت تجربتان حقليتان بارض رملية بمزرعة كلية الزراعة – جامعة قناة السويس – بالاسماعيلية خلال موسمى ١٢/٢٠١١ -١٣/٢٠١٢. بهدف دراسة تاثير٣ مستويات من الرش بحمض الاسكوربيك بتركيز (٠,٠-١٠٠-٢٠٠ جزء فى المليون) على استجابة القمح (صنف سخا٩٤) الى ٣ معاملات رى سطحى (١,٠و ٠,٨و٠,٦ من قيمة البخر- نتح لمحصول القمح) والتى تعادل٤٢٦٠ – ٣٤٠٨ – ٢٥٥٦ م٣/ هكتارعلى الترتيب. اوضحت النتائج ان الاجهاد المائى للقمح ادى الى نقص دليل المساحة الورقية ومحتوى الكلوروفيل الكلى ومحتوى الماء النسبى لورقة العلم وكذلك محصول الحبوب والقش. بينما ادى لزيادة اكسدة الدهون (على صورة مالون داى الدهيد) واكسدة البروتين بالاضافة الى زيادة نشاط بعض الانزيمات المضادة للاكسدة مثل السوبراوكسيد ديسميوتيز والاسكوربات بيروكسيديز والكتاليزبنسبة تتراواح بين ٧٤-١٠٨% و ٤٤-٥٧% و ١٠٧-١٥٦% على التوالى فى ورقة العلم مقارنة بالرى المعتاد. ادت المعاملة بكلا تركيزى حمض الاسكوربيك الى التغلب على الاضرار الناجمة عن الاجهاد التأكسدى الذى انعكس فى تحسين جميع الصفات السابقة مع نقص نسبة الدهون والبروتين المؤكسدة ولكن استمرت الانزيمات المضادة للاكسدة فى ارتفاع بحوالى ١٠-٢٠% مقارنة بالكنترول. ادى ذلك لتحسين انتاجية القمح وكفاءة استخدام ماء الرى. حيث زاد محصول الحبوب بحوالى نصف طن للهكتارفى النباتات المعاملة بحمض الاسكوربيك تحت ظروف الرى المعتاد . بينما ادت المعاملة للحفاظ على حوالى ٠,٨-٠,٩ طن حبوب للهكتار من الفقد نتيجة الاجهاد المائى . ادى استخدام حمض الاسكوربيك الى توفير حوالى ٨٥٢ م٣/ هكتار من ماء الرى دون اضرار بمحصول الحبوب. تم فحص التحورات التشريحية لانسجة ورقة العلم والساق المستجيبة للجفاف وحمض الاسكوربيك.