

## Effect of Foliar Application of Ascorbic Acid on Growth, Yield Components and Some Chemical Constituents of Wheat Under Water Stress Conditions\*

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### ABSTRACT

A greenhouse pot experiment was carried out during October 26, 2009 to May 15, 2010. Winter wheat grains (*Triticum aestivum* L.) cv. Rizgary was used to investigate the role of spraying the plant with 200ppm ascorbic acid (AsA) on vegetative, biomass and some chemical and biochemical constituents of leaves under drought condition. The water stress treatments were: irrigation every 3day- well watered control, 7, 14 and 21 days interval during 90 days. Under water deficit conditions, the vegetative growth and yield components of plant were decline, in addition marked changes in the level of mineral ions and biochemical constituents of leaves were observed. Plant height, shoot dry weight, flag leaf area, water content and leaf growth elongation rate decreased significantly by increasing drought stress, while root dry weight and root: shoot increased. Chemical constituents of leaves such nitrogen, phosphorus, potassium and magnesium were decreased, proline and AsA increased significantly with increasing drought stress and the highest values obtained at 21 day water regime. Soluble sugar, total carbohydrates, total protein, chlorophyll a, b and total carotenoid of leaves were also decreased and the highest reduction in parameters recorded at 21days water regime. Spikes length, number of spikes/plant, weight of spikes, 100 grain dry weight, grain numbers and grain yield were also decreased. However, AsA treatments alleviated the inhibitory effect of drought through enhancing above mentioned parameters.

**Keywords:** Ascorbic Acid, Water Stress, Wheat.

### INTRODUCTION

Wheat is cultivated in different environments, in arid and semi arid region of the world is very low due to low precipitation, high temperature and accumulation of salts in soil (Munns, 2002). Due to their sedentary mode of life, plants resort to many adaptive strategies in response to

different abiotic stresses such as high salt, drought, cold and heat, which ultimately affect the plant growth and productivity (Gill *et al.*, 2003). Against these stresses, plants adapt themselves by different mechanisms including change in morphological and developmental pattern as well as physiological and biochemical responses. Adaptation to all these stresses is associated with metabolic adjustments that lead to the modulation of different enzymes (Strain and Fletcher, 2003).

Ascorbate (AsA) has been shown to have an essential role in several physiological processes in plants, including the regulation of growth, differentiation, and metabolism (Horemans *et al.*, 2000). It functions as a reductant for

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many free radicals, thereby minimizing the damage caused by oxidative stress (Smirnoff and Wheeler, 2000). A fundamental role of AsA in the plant defense system is to protect metabolic processes against H<sub>2</sub>O<sub>2</sub> and other toxic derivatives of oxygen (Shao *et al.*, 2008). Induction of drought tolerance in plants by exogenous application of AsA may have a significant practical application in agriculture. Hamada (2000) investigated the effect of AsA application on amelioration of wheat plant from water deficit (100%, 70%, 50% and 30% field capacity). Abdel-Hameed *et al.*, (2004), they found that foliar application of AsA (50- 200ppm) caused significant increases in vegetative growth characters, yield and its components of wheat plants to foliar application of. There was a progressive increase in plant height, number of tiller and spikes, flag leaf area, blades area/plant, spike length, grain and straw yield per plant and per fed with increasing ascorbic acid level up to 400ppm as foliar application (Amin *et al.*, 2008). Most of the protein and free amino acids of shoots were reduced with the reduction of soil moisture content for 15 days. On the other side, the decreasing of soil moisture content induced progressive increase in soluble sugars, starch, proline contents of shoots and roots. Application of AsA 100ppm induced the drought tolerance and improves the performance under normal and stress conditions compared with control plants. Such promoting effects of AsA on growth were also, obtained by (Al-Hakimi and Hamada, 2001). Soaking of wheat grains presowing for 6hrs in 100ppm of AsA synergistically enhanced the stimulatory effects of drought (70%, 50% and 30% field capacity) on photosynthetic pigments. The highly adverse effects of drought stress were clearly demonstrated in wheat plants treated with the lowest level of field capacity (30%). On the other side, soaking presowing of wheat grains in AsA was generally effective in alleviating, partially or completely, these inhibitory effects of drought treatments (Hamad and Hamada, 2004).

Proline accumulated under stressed condition supplies energy for growth and survival and thereby helps the plant to tolerate stress (Mittler, 2002). Proline acts as a compatible solute, and buffers cellular redox potential; stabilizing the macromolecules, the organelles and the structures (proteins, membranes, chloroplasts and liposome against damage caused by stress condition (Wahid and Close, 2007). According to Hanson (1980), proline accumulation in wheat, barley, rice, sorghum and maize, started when the leaf water potential was reduced to -1.0 MPa. Higher proline content in wheat plants after water stress application has been reported by (Vendruscolo *et al.*, 2007). Increased proline accumulation was reported in water stressed wheat (Tatar and Gevrek, 2008).

Water deficit is a primary factor that limits the wheat production in Kurdistan region, since the rainfall during the growing season is usually low and not constant, which leads to decrease ground water and eventually increase the soil and water salinity. The main objectives of the present study therefore were: i) to examine the effects of progressive water deficit and foliar application of AsA on growth criteria and yield attributes of wheat (*Triticum aestivum L.*) cv. Rizgary ii) to examine whether foliar application of AsA could minimize the harmful effects of water stress and could enhance drought tolerance of plant ii) to estimate the changes that occur in mineral ions (N, P, K, Ca, Mg, Na and Cl ) content in the leaves of plant, as well as alteration in the amount of chlorophylls, carotenoids and some other metabolites content such as proline, AsA, soluble sugars, total carbohydrates and proteins under the influence of different water regime and foliar applied AsA.

## METHODOLOGY

The experiment was conducted in a greenhouse of Biology department, College of Education/ Scientific departments-University of Salahaddin-Erbil. The soil

(sandy loam) was air dried and ground to pass through a 2mm sieve then sterilized by formalin (HCHO) 40%. 7 kg of prepared soil was put in plastic pots (24cm diameter, 21cm depth, and 9.4 L volume). Some chemical and physical properties of the soil were analyzed using different methods as described by (Ryan *et al.*, 2001), were shown in table (1). Pots were brought to field capacity (100%) prior to sowing the seeds. Seven seeds of wheat (*Triticum aestivum* L.) cv. Rizgary were sown per pot at the fourth week of October and reduced to three, were used through out the experimental period for vegetative, chemical analysis and the remaining seedling was left to grow for studying yield components. The pots were fertilized with urea fertilizer CO(NH<sub>2</sub>)<sub>2</sub> containing 46.66% N, phosphorus and potassium fertilizer (KH<sub>2</sub>PO<sub>4</sub>) containing 22.70 %P and % 28.60 K were added to the pots as solutions after 10days from germination with the rates of 75, 75, 94.5 mg/Kg soil and for N, P and K, respectively. Soil moisture status was maintained by frequently weighing the pots, and the amount of water equal to the weight loss was added. Average relative humidity in glasshouse ranged from 40% to 70% at day and night, respectively. Monthly average temperatures were recorded at the glasshouse as shown in table (2). Seedlings were sprayed with (200ppm) AsA after 30 and 40days from sowing; stock solution (1000ppm) of pure AsA (1.0g/L) was prepared and then from this stock solution 500ml of diluted 200ppm was prepared. Two drops of Tween 40 were added to each solution as a surfactant agent. The plants were sprayed with solutions at the early morning hours by using manual sprayer and the control plants were sprayed with distilled water and the volume of the spray solution has to cover the plant foliage completely till drip (Dolatabadian *et al.*, 2009). Two days after second spray the plants were subjected to water deficit for 90days. Four period of irrigation was applied as: control (every 3days), after one, two and three weeks of irrigation. After irrigation treatments the plants returned to normal

irrigation that all of the pots were kept at field capacity till harvesting. The experiment was laid out in a randomized complete block design replicated 4 times with 4 irrigation regimes including control (every 3days), after one, two and three weeks of irrigation each with or without spraying with (200ppm) AsA. Analysis of variance (ANOVA) of the data from each attribute was computed using the Statistical Package for the Social Sciences (SPSS) version 16. The Fischer's Least Significant Differences (LSD) test at 5% level of probability for greenhouse measurements and 1% level for the laboratory measurements was used to test the differences among mean values (Snedecor and Cochran, 1989).

Vegetative and biomass growth characteristics were recorded after 85days from sowing day. Plant height was recorded on tagged plant by measuring the height from ground level to the base of youngest fully opened leaf and expressed in centimeters (cm). Flag leaf area was measured by adopting Stickler's linear measurement method (Fang *et al.* 2006a) as given below:

Leaf area (cm<sup>2</sup>) = L x B x 0.78, where L = length of leaf, B = Breadth of leaf

The leaf growth elongation rate (mm day<sup>-1</sup>) was calculated according to Liang *et al.*, (2002) formula as follows:

$$GR = 1/L_0 \times dL/dt$$

L<sub>0</sub>=Stand for the leaf length of selected plants at the beginning of the treatment.

dL/dt= Stand for the length increment of selected leaves per day during the treatment.

The whole plant was uprooted by pouring water into the plant's pot, roots were carefully cleaned with running tap water and finally washed with distilled water then the shoot and root were dried in an oven with drift fan at (70°C) for 48hrs, then shoot and root dry weight per plant were measured and the ratio of shoot dry weight (g plant<sup>-1</sup>)/ root dry weight (g plant<sup>-1</sup>) was attained (Luvaha

*et al.*, 2008). Water content ( $\text{g plant}^{-1}$ ) was calculated by subtracting shoot dry weight from shoot fresh weight (Talebi *et al.*, 2009).

Yield characteristics were recorded at harvesting time which included number of spikes  $\text{plant}^{-1}$ , spikes length (cm), weight of spikes ( $\text{g plant}^{-1}$ ), grain numbers  $\text{plant}^{-1}$ , grain weight ( $\text{g plant}^{-1}$ ) and 100-grains weight (g). Chemical analysis of leaves after 85days from sowing included total nitrogen, phosphorus, potassium, magnesium, calcium, sodium and chloride. Biochemical analysis of leaves after 85days from sowing included proteins, soluble sugars, total carbohydrates, proline, ascorbic acid and photosynthetic pigments.

Total nitrogen ( $\text{mg g}^{-1}$ ) was determined in plant extract by Kjeldahl method as described by (Temminghoff and Houba, 2004). Total phosphorus ( $\text{mg g}^{-1}$ ) was determined in plant extract samples by using spectrophotometer method as described by (Ryan *et al.*, 2001). Total potassium and sodium ( $\text{mg g}^{-1}$ ) were estimated by flame

emission photometer technique as adopted by (Temminghoff and Houba, 2004). Total magnesium and calcium ( $\text{mg g}^{-1}$ ) were determined simultaneously in the plant extract by using atomic absorbance spectrophotometer as described by (Hanlon, 1992). Chloride content was estimated according to Mohr method which was described by Pandey (Pandey *et al.*, 2000). Total protein ( $\text{mg g}^{-1}$ ) was calculated by multiplying the total nitrogen by 5.75 (A.O.A.C., 1995). Soluble sugars ( $\text{mg g}^{-1}$ ) and total carbohydrate ( $\text{mg g}^{-1}$ ) were determined by anthrone method (Sadasivam and Manickam, 1992). Chlorophyll a, b and total carotenoids were extracted from 0.5g of fresh leaves in 85% ethanol and measured spectrophotometrically according to (Lichtenthaler, 1987). Ascorbic acid content in wheat leaves was estimated using 2, 6-dichlorophenol indophenols titration method (Sadasivam and Manickam, 1992). Proline was determined according to the method described by (Bates *et al.*, 1973).

**Table 1. Some physical and chemical properties of the soil used in the experiments.**

| Properties   | Values     |
|--|------------|
| Particle Size  | Sand 701.1 |
| Distribution $\text{g kg}^{-1}$                        | Silt 234.6 |
|  | Clay 64.3  |
| Soil Texture   | Sandy loam |
| Field Capacity%  | 15.87      |
| Organic matter%  | 0.76       |
| pH   | 7.51       |
| Electrical Conductivity ( $\text{dS m}^{-1}$ at 25 0C) | 0.38       |
| Total nitrogen%  | 0.6        |
| Total Phosphorus ( $\text{mg kg}^{-1}$ soil)           | 1.32       |
| Soluble potassium ( $\text{mg kg}^{-1}$ soil)          | 24.20      |
| Soluble Sodium( $\text{mg kg}^{-1}$ soil)              | 19.56      |
| Soluble Chloride( $\text{mg kg}^{-1}$ soil)            | 35.42      |

**Table 2. Minimum and maximum air temperatures throughout the experiments period.**

| Year | Months   | Minimum °C | Maximum °C |
|------|----------|------------|------------|
| 2009 | October  | 18.8       | 30.9       |
|      | November | 12.4       | 21.6       |
|      | December | 7.9        | 16.4       |
| 2010 | January  | 5.8        | 15.2       |
|      | February | 10.4       | 17.6       |
|      | March    | 12.4       | 20.6       |
|      | April    | 16.0       | 25.7       |
|      | May      | 23.7       | 34.8       |

## RESULTS

### *Vegetative and Biomass Characteristics*

Data represented in table (3) illustrated that as the soil moisture decreased the plant height, flag leaf area and shoot dry weight were significantly decreased compared to the well watered plants. The maximum reduction in growth criteria was observed in plants with 21days water regime. The lower values were 46.33cm, 55.20 cm<sup>2</sup> and 1.200g for plant height; flag leaf area and shoot dry weight, respectively. AsA at the level of 200ppm applied as foliar spray could alleviate the inhibitory effect of drought by enhancement plant height, flag leaf area and shoot dry weight. The values of mentioned parameters were 61.33cm, 70.84cm<sup>2</sup>, 2.403g observed in plants irrigated after 3, 7days interval were increased significantly to 63.66cm, 75.13cm<sup>2</sup> and 2.693g, respectively by 7days water regime plus 200ppm AsA. Root dry weight was increased significantly by decreasing soil moisture, the higher value 1.500g obtained by 21days water regime. While AsA application caused a significantly decline in root dry weight compared to control, the higher reduction was 0.355 obtained after 21days irrigation plus 200ppm AsA. The root: shoot ratio increased significantly by increasing drought stress. The higher value was 1.250 obtained after 21days water irrigation. While application of 200ppm AsA caused a

significant increase in root: shoot ratio, the lower value was 0.151 obtained from 3days interval plus 200ppm AsA treatment and the higher value was 0.264 obtained with 21days irrigation plus 200ppm AsA. Water regime for 14, 21 days interval were significantly decrease water content, the lower value of water content per plant was 6.65g obtained from 21days water regime and no difference between 14, 21 days interval was observed. The AsA application could alleviate the harm effect of drought through enhancement plant water content. All treatments of the irrigation day intervals plus 200ppm AsA had significant large water content than their controls.

Moreover, decreasing soil moisture caused a significant gradually decreases in growth elongation rate of leaves; the lower value was 0.285mm day<sup>-1</sup> obtained from sever drought 21days irrigation. Whereas in other hand, spraying the drought- stressed plants with 200ppm AsA caused a significantly increase in leaf growth elongation rate as compared with untreated control except 3 days irrigation. The lower value was 0.285mm day<sup>-1</sup> for plant leaves obtained from 21days irrigation; increased significantly to 0.773mm day<sup>-1</sup> with 21days irrigation plus 200ppm AsA.

**Table 3. Combination effect of irrigation intervals and foliar application of ascorbic acid (AsA) on some vegetative and biomass characteristics of wheat cv. Rizgary after 85days from sowing.**

| Treatments   | Irrigation interval (Days) | Plant height (cm) | Flag leaf area (cm <sup>2</sup> plant <sup>-1</sup> ) | Shoot dry weight (g plant <sup>-1</sup> ) | Root dry weight (g plant <sup>-1</sup> ) | Root: Shoot plant <sup>-1</sup> | Water content (g plant <sup>-1</sup> ) | Growth Rate of leaf (mm day <sup>-1</sup> ) |
|--------------|----------------------------|-------------------|---|---|--|---------------------------------|--|---|
| Controls     | 3                          | 66.66             | 74.64   | 2.480                                     | 0.945                                    | 0.381                           | 12.18                                  | 1.314                                       |
|              | 7                          | 61.33             | 70.84   | 2.403                                     | 1.372                                    | 0.570                           | 11.68                                  | 0.844                                       |
|              | 14                         | 50.33             | 64.63   | 2.355                                     | 1.465                                    | 0.622                           | 6.98                                   | 0.735                                       |
|              | 21                         | 46.33             | 55.20   | 1.200                                     | 1.500                                    | 1.250                           | 6.65                                   | 0.285                                       |
| 200ppm AsA + | 3                          | 68.33             | 79.67   | 3.915                                     | 0.595                                    | 0.151                           | 14.39                                  | 1.737                                       |
|              | 7                          | 63.66             | 75.13   | 2.693                                     | 0.520                                    | 0.193                           | 12.79                                  | 1.507                                       |
|              | 14                         | 51.00             | 65.26   | 2.525                                     | 0.413                                    | 0.163                           | 10.28                                  | 1.346                                       |
|              | 21                         | 47.66             | 57.59   | 1.342                                     | 0.355                                    | 0.264                           | 8.11                                   | 0.773                                       |
| L.S.D 0.05   |                            | 1.58              | 4.26  | 0.246                                     | 0.196                                    | 0.121                           | 0.87                                   | 0.445                                       |

#### *Mineral Composition of Leaves*

Table (4) shows that nitrogen, phosphorus, potassium, calcium and magnesium content of leaves were significantly decreased with increasing the irrigation intervals from (3, 7, 14 and 21) days except 14 days irrigation intervals for phosphorus, potassium and 21days irrigation intervals for calcium whereas application of AsA causing significant increases in the values of the minerals except 14 days irrigation intervals for nitrogen. The lowest values were 34.55, 0.706, 27.61, 25.87 and 0.570 mg g<sup>-1</sup> obtained at 21 days irrigation intervals for nitrogen, phosphorus, potassium, calcium and magnesium respectively which were significantly increased to 36.95, 1.241, 31.93, 28.13 and 0.896 mg g<sup>-1</sup> respectively by application of 200ppm AsA. Sodium and chloride content were significantly increased by increasing irrigation intervals except 14 days irrigation intervals for sodium, the higher values were 1.482 and 3.137 mg g<sup>-1</sup> for sodium and chloride which obtained at 21days irrigation intervals

respectively. Application of 200ppm AsA led to significant increase in both sodium and chloride as compared with their corresponding controls when interacted with 14 and 21 days irrigation intervals except 21days irrigation intervals for sodium, the values of sodium was 1.553 mg g<sup>-1</sup> for 14 days irrigation interval plus 200ppm AsA compared with 0.915mg g<sup>-1</sup> obtained by 14 days irrigation interval only. Although the values of chloride was 2.293 and 3.984 mg g<sup>-1</sup> for 14 and 21 days irrigation interval plus 200ppm AsA respectively, compared with 1.931 and 3.137 mg g<sup>-1</sup> obtained at 14 and 21 days irrigation intervals only.

#### *Photosynthetic Pigment*

Table (5) shows that chlorophyll a decreased significantly with increasing irrigation intervals to 14 and 21days, this reduction was recovered by addition of 200ppm AsA which caused significant increase in chlorophyll a compared to their corresponding controls, the lower value was 0.721 mg g<sup>-1</sup> F.wt. obtained at

21days irrigation interval which was increased to 0.800 mg g<sup>-1</sup> F.wt. by application of 200ppm AsA. Although chlorophyll b decreased significantly by increasing irrigation intervals to 14 and 21days the lower value

(0.342 mg g<sup>-1</sup> F.wt.) was recorded with 21 days irrigation interval. Moreover, spraying the well watered plants with 200ppm AsA caused significant increase in total carotenoids compared with control.

**Table 4. Combination effect of irrigation intervals and foliar application of ascorbic acid (AsA) on mineral composition (mg g<sup>-1</sup>) in the leaves of wheat cv. Rizgary after 85days from sowing.**

| Treatments   | Irrigation interval (Days) | Nitrogen mg g <sup>-1</sup> | Phosphorus mg g <sup>-1</sup> | Potassium mg g <sup>-1</sup> | Calcium mg g <sup>-1</sup> | Magnesium mg g <sup>-1</sup> | Sodium mg g <sup>-1</sup> | Chloride mg g <sup>-1</sup> |
|--------------|----------------------------|-----------------------------|-------------------------------|------------------------------|----------------------------|------------------------------|---------------------------|-----------------------------|
| Controls     | 3                          | 42.71                       | 1.596                         | 31.44                        | 25.09                      | 0.846                        | 1.234                     | 1.690                       |
|              | 7                          | 40.38                       | 1.173                         | 29.57                        | 27.06                      | 0.845                        | 1.482                     | 2.173                       |
|              | 14                         | 37.28                       | 1.112                         | 29.38                        | 29.52                      | 0.580                        | 0.915                     | 1.931                       |
|              | 21                         | 34.55                       | 0.706                         | 27.61                        | 25.87                      | 0.570                        | 1.482                     | 3.137                       |
| 200ppm AsA + | 3                          | 44.71                       | 2.205                         | 32.23                        | 28.43                      | 0.980                        | 1.127                     | 1.811                       |
|              | 7                          | 42.71                       | 2.012                         | 30.65                        | 29.81                      | 1.193                        | 1.234                     | 2.293                       |
|              | 14                         | 38.83                       | 1.935                         | 29.97                        | 29.42                      | 0.925                        | 1.553                     | 2.293                       |
|              | 21                         | 36.95                       | 1.241                         | 31.93                        | 28.13                      | 0.896                        | 1.587                     | 3.984                       |
| L.S.D 0.01   |                            | 1.62                        | 0.373                         | 0.494                        | 1.45                       | 0.245                        | 0.275                     | 0.313                       |

#### **Biochemical Composition**

Table (6) shows that proline content was significantly increased by increasing interval days of irrigation to 14 and 21 days compared to control. The higher value of proline was 0.245 µg Kg<sup>-1</sup> F.wt. obtained at 21days water regime. Application of 200ppm AsA caused significant increase in proline content in plants irrigated every 3 and 7 days compared to their corresponding control. Increasing water stress caused more AsA relative to the fresh leaves, in addition exogenous application of AsA caused significant increase in endogenous AsA, the maximum value was 5.45 µg Kg<sup>-1</sup> F.wt. which obtained by 21days irrigation interval plus 200ppm AsA compared to 4.79 µg Kg<sup>-1</sup> F.wt at 21days irrigation interval only. However, the same table shows that soluble sugars were increased significantly only at 7 days irrigation interval (198.8 mg g<sup>-1</sup>). On the other hand,

addition of 200ppm AsA increased significantly soluble sugar content in leaves as compared with their controls except in 7 days irrigation interval, the lower value was 162.3 mg g<sup>-1</sup> obtained by 21days irrigation interval plus 200ppm AsA compared to 147.4 mg g<sup>-1</sup> obtained by 21days water regime without AsA. Spraying the plants with 200ppm AsA caused significant increases in carbohydrate content in leaves of the plants grown under 3, 7, 14, and 21days irrigation interval compared to their controls.

The data recorded in the same table indicated that protein content was gradually significant decreased with increasing days of irrigation interval (7, 14 and 21 days). The treatments with AsA under the various levels of water stress (3, 7, 14 and 21) days irrigation interval resulted in significant increases in the contents of protein as compared to their corresponding controls, the lowest

value was 210.61 mg g<sup>-1</sup> obtained by 21days irrigation interval plus 200ppm AsA compared to 196.93 mg g<sup>-1</sup> obtained by 21days irrigation interval only.

**Table 5. Combination effect of irrigation intervals and foliar application of ascorbic acid (AsA) on chlorophyll a, chlorophyll b and carotenoid pigments (mg g<sup>-1</sup> F.Wt.) in the leaves of wheat cv. Rizgary after 85days from sowing.**

| Treatments   | Irrigation interval (Days) | Chlorophyll a (mg g <sup>-1</sup> F.Wt.) | Chlorophyll b (mg g <sup>-1</sup> F.Wt.) | Total carotenoids (mg g <sup>-1</sup> F.Wt.) |
|--------------|----------------------------|--|--|--|
| Controls     | 3                          | 0.867                                    | 0.465                                    | 0.542  |
|              | 7                          | 0.814                                    | 0.404                                    | 0.534  |
|              | 14                         | 0.718                                    | 0.362                                    | 0.526  |
|              | 21                         | 0.721                                    | 0.342                                    | 0.482  |
| 200ppm AsA + | 3                          | 0.986                                    | 0.533                                    | 0.602  |
|              | 7                          | 0.972                                    | 0.432                                    | 0.565  |
|              | 14                         | 0.936                                    | 0.414                                    | 0.526  |
|              | 21                         | 0.800                                    | 0.410                                    | 0.497  |
| L.S.D 0.01   |                            | 0.056                                    | 0.079                                    | 0.056  |

**Table 6. Combination effect of irrigation intervals and foliar application of ascorbic acid (AsA) on proline, AsA, soluble sugars, total carbohydrates and protein content in the leaves of wheat cv. Rizgary after 85days from sowing.**

| Treatments   | Irrigation interval (Days) | Proline (µg g <sup>-1</sup> F. wt.) | Ascorbic acid (µg Kg <sup>-1</sup> F. wt.) | Soluble sugars (mg g <sup>-1</sup> ) | Total carbohydrates (mg g <sup>-1</sup> ) | Protein (mg g <sup>-1</sup> ) |
|--------------|----------------------------|-------------------------------------|--|--------------------------------------|---|-------------------------------|
| Controls     | 3                          | 0.207                               | 4.29                                       | 154.6                                | 329.1                                     | 243.44                        |
|              | 7                          | 0.216                               | 4.66                                       | 198.8                                | 334.3                                     | 230.16                        |
|              | 14                         | 0.235                               | 4.70                                       | 148.8                                | 322.7                                     | 212.49                        |
|              | 21                         | 0.245                               | 4.79                                       | 147.4                                | 317.3                                     | 196.93                        |
| 200ppm AsA + | 3                          | 0.237                               | 4.92                                       | 214.1                                | 389.1                                     | 254.84                        |
|              | 7                          | 0.245                               | 5.20                                       | 179.2                                | 380.9                                     | 243.44                        |
|              | 14                         | 0.253                               | 5.26                                       | 167.2                                | 355.2                                     | 221.33                        |
|              | 21                         | 0.260                               | 5.45                                       | 162.3                                | 353.7                                     | 210.61                        |
| L.S.D 0.01   |                            | 0.027                               | 0.36                                       | 13.2                                 | 15.3                                      | 9.23                          |

### **Yield Components**

The results in (Table 7), clearly demonstrate that

both 14 and 21days irrigation interval caused significant decrease in spike length, number of spikes, weight of

spikes, grain numbers, 100-grains D.wt. and grain weight per plant. Number of spikes, weight of spikes and grain numbers were also decreased significantly under 7days irrigation interval compared with control. Concerning the combination between (3, 7 and 21) days irrigation interval with 200ppm AsA and (3, 14 and 21) days irrigation interval with 200ppm AsA, it was found that these treatments increased spike length and number of spikes significantly respectively, compared with their corresponding controls. Spike weight under 14days

irrigation interval plus 200ppm AsA was also increased significantly compared with control. While application of AsA caused significant increase in grain numbers under 3, 7 and 14 days irrigation interval as compared with their controls. Whereas application AsA caused no significant difference in 100-grains D.wt. On the other hand AsA treatment was increased significantly grain yield of well watered plant compared with 3 days interval only.

**Table 7. Combination effect of irrigation intervals and foliar application of ascorbic acid (AsA) on yield and its components of wheat cv. Rizgari.**

| Treatments   | Irrigation interval (Days) | Spikes length (cm) | Number of spikes Plant <sup>-1</sup> | Weight of spikes Plant <sup>-1</sup> | Grain numbers plant <sup>-1</sup> | 100-grains D.wt.(g) | Grain weight (g plant <sup>-1</sup> ) |
|--------------|----------------------------|--------------------|--------------------------------------|--------------------------------------|-----------------------------------|---------------------|---------------------------------------|
| Controls     | 3                          | 10.82              | 5.32                                 | 7.69                                 | 109.00                            | 4.46                | 4.99                                  |
|              | 7                          | 10.32              | 3.32                                 | 6.67                                 | 82.00                             | 4.40                | 4.69                                  |
|              | 14                         | 9.82               | 2.00                                 | 4.27                                 | 61.66                             | 4.18                | 3.86                                  |
|              | 21                         | 7.32               | 1.32                                 | 1.31                                 | 23.00                             | 3.42                | 0.80                                  |
| 200ppm AsA + | 3                          | 11.35              | 6.32                                 | 8.05                                 | 135.60                            | 4.64                | 5.74                                  |
|              | 7                          | 11.00              | 3.65                                 | 7.32                                 | 106.60                            | 4.58                | 4.09                                  |
|              | 14                         | 9.65               | 3.32                                 | 2.81                                 | 92.33                             | 3.98                | 2.34                                  |
|              | 21                         | 8.00               | 2.32                                 | 1.73                                 | 23.32                             | 3.45                | 0.78                                  |
| L.S.D 0.05   |                            | 0.61               | 0.55                                 | 0.45                                 | 5.62                              | 0.21                | 0.34                                  |

### DISCUSSION

The present study revealed the harm effect of water stress on vegetative growth and biomass characters of wheat. It shows that water stress decreased plant height, flag leaf area and shoots dry weight as compared with the well watered plants (table 3). These results partially agreed with those obtained by (Hamada, 2000; Al-Hakimi and Hamada, 2001; Hamad and Hamada, 2004). This reduction probably due to the ABA action which it

is produced in the cells under water stress condition, is a stress signal, inhibit cell division and/or DNA synthesis as well as ABA accumulation induces stomatal closure and inhibits transpiration (Martinez *et al.*, 2004). The relative increase in root dry matter over that of shoot may be because at water potentials that completely inhibit shoot growth, the primary root continues to grow. Translocation of N from shoot to root has been reported, could cause the root to grow more than the shoots and

lead to an increased root: shoot ratio in drought stressed plants. Water content was decreased by water deficiency in soil, as well as the root might assimilate lower water amount/ volume with increase the period under water stress (Taiz, and Zeiger, 2006). Furthermore this reduction probably due to stomatal closure; reduce water absorption and transpiration rate, especially in dry environments (Verslues *et al.*, 2006). In higher plants the oxygen toxicity is more serious under condition of water-deficit conditions, water stress causes stomatal closure, which reduces the CO<sub>2</sub>/O<sub>2</sub> ratio in leaves and inhibits photosynthesis. These conditions increase the rate of reactive oxygen species (ROS) like superoxide radical (O<sup>-2</sup>) hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radical (OH) particularly in chloroplast and mitochondria (Mittler, 2002), via enhanced leakage of electrons to oxygen. The hydroxyl radical, one of the most reactive oxygen species, is responsible for oxygen toxicity *in vivo*, causing damage to DNA, protein, lipids, chlorophyll and almost every other organic constituent of the living cell. Plants protect cellular and sub-cellular system from the cytotoxic effects of active oxygen radicals with anti-oxidative enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) as well as metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Alscher *et al.*, 2002).

The results showed that nitrogen, phosphorus, potassium and magnesium decreased significantly with increasing water stress compared to control, while foliar application of AsA increase the concentration of mentioned above mineral, on the other hand calcium, chloride and sodium increased significantly as compared to the control, in addition application of AsA caused increase in calcium, chloride and sodium significantly compared to their controls (table 4), these results partially agreed with those obtained by (Kaya *et al.*, 2006) on maize. They reported that under drought stress

maize leaves contained approx. 50% less calcium than control plants, the decrease in Ca<sup>++</sup> concentration in plant cells is harmful because this element plays an essential role in maintaining the structural and functional integrity of plant membranes and regulation of their permeability and selectivity. The ability of plants to maintain membrane stability is a crucial trait of stress resistance. Disruption of ion homeostasis may result from reduced K<sup>+</sup> concentrations in water-stressed plants. Potassium plays an important role in processes involving osmotic adjustment and its adequate level in plants may improve water stress tolerance. The decreasing in the N content under water stress may be due to a decreased transpiration rate to transport N from roots to shoots and reduced the N content in plants, water improved development and arrangement of root at normal moisture contents in the soil, hence increased overall absorbing capability of the root, in addition water being a universal medium for ion diffusion from the soil solution and absorbing complex of the soil toward root hairs so moisture deficiency upset the functioning of enzyme system, caused hydrolysis and degradation of organic substance, sharply reduce the photosynthesis rate, and brings plant growth to a half (Yagodin, 1982). It is generally accepted that the uptake of P by crop plants is reduced in dry-soil conditions and that may be explained that the translocation of P to the shoots is severely restricted even under relatively mild drought stress (Rasnick, 1970). Furthermore, the availability of K<sup>+</sup> to the plant decreases with decreasing soil water content, due to the decreasing mobility of K<sup>+</sup> under these conditions (Asch *et al.*, 2000). Moreover, application of AsA caused better resistance to water stress by increasing ions content in the organs of the stressed plants through their role in increasing osmotolerance and/or through regulating various processes including absorption of nutrients from soil solution (El-Bassiouny

*et al.*, 2005).

Chlorophyll a, b decreased with increasing water stress, while AsA application caused significant increase in chlorophyll a compared to control (table 5), these results partially agreed with those obtained by (Hamad and Hamada, 2004). The reduction in photosynthesis pigment may be attributed to the decreasing the availability of nutrients to plant which are necessary to formation the photosynthetic pigment under water stress condition such as N, P, Mg, Fe and so on or may be related to decline the level of some enzyme such as nitrate reductases and other enzymes which are essential for construction the chlorophyll structure (Galmes *et al.*, 2007). Moreover, (Choudhury *et al.*, 1993) suggested that the positive effect of AsA on photosynthesis attributed to stabilizing and protecting the photosynthetic pigments and photosynthetic apparatus from oxidization.

Application of AsA caused increase in proline content, soluble sugars, total carbohydrate, endogenous AsA and protein (table 6). These results partially agreed with those obtained by (Amin *et al.*, 2008), although the result of proline agreed with those obtained by (Hamada, 2000) on wheat and of AsA agreed with those obtained by (Dolatabadian *et al.*, 2009) on maize. Mukherjee and Choudhuri (1993) reported an enhancement in AsA content of *Vigna* seedling when subjected to severe water stress. Drought stress increase the production of free radicals, causes cellular membrane damage, enzyme inactivation and leakage of ions from plant cells, disturb osmotic adjustment and therefore proline accumulation occurs normally in the cytosol when it contributes substantially to the cytoplasmic osmotic adjustment (Ketchum *et al.*, 1991). The decreasing in soluble sugars

and carbohydrate may be attributed to the decreasing of chlorophyll content because of increasing the production of oxygen radicals that cause peroxidation of these pigments and chemical analyzing them and finally lead to decreasing sugar and carbohydrate content (Wise and Naylor, 1987). There was a general decreasing trend in total proteins in plant leaves due to water deficit. This reduction is due to possible increase of the proteases enzyme activity, on which promotes the breakdown of the proteins and consequently decrease the protein amount presents in the plant under water stress conditions (Wagner *et al.*, 2004).

The present experiments revealed a decrease in spike length, number of spikes plant<sup>-1</sup> weight of spikes plant<sup>-1</sup>, grain numbers plant<sup>-1</sup>, 100-grains D.wt. and grain weight with increasing drought stress as compared to the control (table 7). These results were partially agreed with those obtained by (Hamada, 2000; Hamad and Hamada, 2004). The deficiency of water leads to severe decline in yield traits of crop plants probably by disrupting leaf gas exchange properties which not only limited the size of the source and sink tissues but the phloem loading, assimilate translocation and dry matter partitioning are also impaired (Farooq *et al.*, 2009).

## CONCLUSION

According to results, it can be concluded that increasing the endogenous AsA of wheat plant by foliar application of AsA mitigates the adverse effects of drought stress through corrects the nutritional disorders, biosynthesis of photosynthetic pigments, water conservation, increasing amounts of carbohydrates and proline.

## REFERENCES

- A.O.A.C. 1995. Official Methods of Analysis, *Association of Official Analytical Chemists*, 16<sup>th</sup> ed, K Ulrich Alrington, Virginia, pp.1058-1059.
- Abdel-Hameed, A.M., Sarhan S.H., Abdel-Salam H.Z. 2004. Evaluation of some organic acid as foliar application on growth, yield and some nutrient contents of wheat (*Triticum aestivum* L.), *J. Agric. Sci., Mansoura Univ.*, 20(5): 2476-2481.
- Al-Hakimi, A.M.A., Hamada A.M. 2001. Counteraction of salinity stress on wheat (*Triticum aestivum* L.) plants by grain soaking in ascorbic acid, thiamin or sodium salicylate, *Biologia Plantarum*, 44(2): 253- 261.
- Alscher, R.G., Erturk, N. Heath, L.S. 2002, Role of superoxide dismutase (SODs) in controlling oxidative stress in plants, *Journal of Experimental Botany*, 53: 133-141.
- Amin, A.A., El-Sh. M. Rashad, F.A.E. Gharib, 2008, Changes in morphological, physiological and reproductive characters of wheat plants as affected by foliar application with salicylic acid and ascorbic acid, *Australian J. Basic and Appl. Scis.*, 2(2): 252-261.
- Asch, F., M. Dingkuhn, K. Miezian, K. Dorffling. 2000. Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice, *Euphytica*, 113: 109-118.
- Bates, L.S., Waldren R.P. Teare I.D. 1973. Rapid determination of free proline for water stress studies, *Plant Soil*, 39(1): 205-207.
- Choudhury, N. K., Cho T. H., Huffaker R. C. 1993. Ascorbate induced Zeaxanthin formation in wheat leaves and photoprotection of pigment and photochemical activities during aging of chloroplasts in light, *Plant Physiology*, 141: 551-556.
- Dolatabadian, A., S. A. M. Sanavy, M. Sharifi, 2009, Alleviation of Water Deficit Stress Effects by Foliar Application of Ascorbic Acid on *Zea mays* L., *J. Agro. Sci.*, 195: 347-355.
- El-Bassiouny, H.M.S., M.E. Gobarah, A.A. Ramadan, 2005, Effect of antioxidants on growth, yield, savism causative agents in seeds of *Vicia faba* L. plants grown under reclaimed sandy soils, *International Journal of Agriculture and Biology*, 4: 653-659.
- Fang Q.X., Y.H.Chen, Q. Q. Li, S.Z.Yu, Y. Luo, Z. Ouyang, 2006a, Effects of soil moisture on radiation utilization during late growth stages and water use efficiency of winter wheat, *Acta Agron. Sin.* 32: 861-866.
- Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, M. Basra, 2009, Plant drought stress effects, mechanisms and management, *Agron. Sustain Dev.*, 29: 185-212.
- Galmes, J., Medrano, H. Flexas, J. 2007. Photosynthesis and photoinhibition in response to drought in a pubescent (var. *minor*) and aglabrous (var. *palau*) variety of *Digitalis minor*, *Environment Experimental Botany*, 60:105-111.
- Gill, P.K., Sharma, A.D. Singh, P. Bhullar, S.S. 2003. Changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various a biotic stresses, *Plant Growth Regulation*, 40: 157-162.
- Hamad, A.M. H., Hamada A.M. 2004. Grain soaking presowing in ascorbic acid or thiamin versus the adverse effects of combined salinity and drought on wheat seedlings, Assiut University, Egypt, S15-005.
- Hamada, A.M., 2000, Amelioration of drought stress by ascorbic acid, thiamin or aspirin in wheat plants, *India J. Plant Physiology*, 4(4): 263-269.
- Hanlon, E.A., 1992, Determination of Potassium, *Calcium and Magnesium in plant by atomic absorption techniques*, C.O Plank (E.d), pp. 33-36.
- Hanson, A.D., 1980, Interpreting the metabolic response of plants to water stress, *Am. Soc. Hort. Sci.*, V, 15, pp. 623-629.
- Horemans, N., Foyer, C.H. Potters, Asard, G. H. 2000, Ascorbate function and associated transport systems in

- plants, *Plant Physiology*, 38: 531–540.
- Kaya, C., Tuna, L. Higg, D. 2006. Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions, *Plant Nutrition*, 29:1469-1480.
- Ketchum, R.E.B., Warren, R.C. Klima, L. J. F. Lopez-Gutierrez, M.W. Nabors, 1991, The mechanism and regulation of proline accumulation in suspension cultures of the halophytic grass *Distichlis spicata* L., *Plant Physiology*, V. 137, pp. 368-374.
- Liang, Z., Zhang F., Shao M., Zhang J. 2002. The relations of stomatal conductance, water consumption, growth rate to leaf water potential during soil drying and rewatering cycle of wheat (*Triticum aestivum*), *Bot. Bull. Acad. Sin.*, 43: 187-192.
- Lichtenthaler, H., 1987, Chlorophylls and carotenoids: Pigments of Photosynthetic Biomembrane, *Methods of Enzymology*, 148: 350-382.
- Luvaha, E., Netondo, G.W. Ouma, G. 2008. Effect of water deficit on the physiological and morphological characteristics of mango (*Manigifera indica*) rootstock seedlings, *Am. J. Plant Physiology*, 3(1): 1-15.
- Martinez, J.P., Lutts, S. Schanck, A. Bajji, M. Kinet, J.M. 2004, Is osmotic adjustment required for water stress resistance in the mediterranean shrub *Atriplex halimus* L., *Plant Physiology*, 161(9): 1041-1051.
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance, *Trends Plant Sci.*, 7: 405–410.
- Mukherjee, S.P., Choudhuri, M.A. 1993, Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings, *Physiologia Plantarum*, 58: 166-170.
- Munns, R. 2002. Comparative physiology of salt and water stress, *Plant Cell and Environment*, 25(2): 239–250
- Pandey, O.P., Bajpai, D.N. Giri S. 2000. Practical Chemistry, S.Chand Company LTD., pp.117-120.
- Rasnick, M. 1970. Effect of mannitol and polyethylene glycol on phosphorus uptake by Maize plants, *Annals Botany*, 34: 497-502.
- Ryan, J., Estefon G., Rashid A. 2001. Soil and plant analysis laboratory manual, 2<sup>nd</sup> ed, National Agriculture Research Center (NARC) Islamabad, Pakistan. Sabdariffa L seedlings. *J.Bull. Fac. Sci.*, 31( 2-D): 295-303.
- Sadasivam, S., Manickam A. 1992, *Biochemical Methods of Agricultural Sciences*, Wiley Eastern Ltd., New Delhi, pp.189-191.
- Shao, H.B., Chu, L. Y., Lu, Z. H., Kang, C.M. 2008, Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells, *International J. of Biology Science*, 4: 8–14.
- Smirnoff, N. Wheeler G.L. 2000. Ascorbic acid in plants: biosynthesis and function, *Critical Reviews in Biochemistry and Molecular Biology*, 35: 291–314.
- Snedecor, G.W., Cochran W.G. 1989. Statistical methods, 7<sup>th</sup> ed, The Iowa State University Press USA, pp. 215-298.
- Strain, D. F., Fletcher J. 2003. Plant ascorbic: acid chemistry, function, metabolism, bioavailability and effects of processing, *J. Sci. Food and Agri.*, 80, pp. 825-850.
- Taiz, L., Zeiger E. 2006. *Plant Physiology*, 4<sup>th</sup> ed, Sinauer Associates, Inc. Publ., USA, pp.671-677.
- Talebi, R., Fayaz F., Naji, A.M. 2009. Effective selection criteria for assessing drought stress tolerance in durum wheat (*Triticum durum* desf.). *General and applied plant physiology*, 35(1–2): 64–74.
- Tatar, O., Gevrek, M.N. 2008. Influence of water stress on proline accumulation and water content of wheat (*Triticum aestivum* L.), *Asian J. Plant Sci.*, 12(1): 23-28
- Temminghoff, E.J.M., Houba E. V. J.G. 2004. *Plant analysis Procedures*, 2<sup>nd</sup> ed, Kluwer Academic Publishers, London, p.p.157-163.
- Vendruscolo, A.C., Schuster, I. Pileggi, M. Vieira, C. 2007, Stress- induced synthesis of proline confers tolerance to water deficit in wheat, *Plant Physiology*, 164(10): 1367-1376.
- Verslues, P. E., M. Agarwal, S. Katiyar-Agarwal, J. Zhu, J.K. Zhu, 2006, Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses

- that affect plant water status, *The plant J.*, 45(4): 523-539.
- Wagner, D., D. Przybyla, R. Camp, C Kim, F. Landgraf, K.P. Lee, M. Wursch, C. Laloi, M. Nater, E. Hideg, K. Apel, 2004, The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*, *Science*, 306: 1183-1185.
- Wahid A., Close, T.J. 2007. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves, *Biol. Plant*, 51: 104–109.
- Wise, R.R., Naylor, A.W. 1987. Chilling-enhanced photooxidation: evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants, *Plant Physiology*, 83: 278-282.
- Yagodin, B.A. 1982. Agricultural chemistry, 1<sup>st</sup> ed, Mirpublisher Moscow, Russia, pp. 90-92.

## تأثير الرش الورقي بحامض الأسكوربيك في النمو ومكونات الحاصل وفي بعض المكونات الكيميائية للحنطة تحت ظروف الإجهاد المائي

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### ملخص

اجريت هذه الدراسة في البيت الزجاجي للفترة من 26 تشرين الأول لعام 2009 الى 15 ايار 2010 وفيها استخدمت بذور الحنطة الشتوية (*Triticum aestivum* L.) وصنف رزكاري لدراسة تأثير الرش بحامض الأسكوربيك بتركيز (200) جزء في المليون في خصائص النمو الخضري والكتلة الحيوية في مراحل مختلفة من النمو وفي بعض مكونات الكيمياء وبالبايوكيميائية للنبات، حيث تم وضع النباتات تحت ظروف إجهاد نقص الماء من خلال الري لفترات (3 ، 7 ، 14 ، 21) يوماً و لمدة 90 يوماً. انخفضت خصائص النمو الخضري ومكونات الحاصل تحت ظروف عجز الماء فضلاً عن التغيرات في مستوى الأيونات المعدنية والمكونات الكيميائية في الأوراق . وانخفض ارتفاع النبات والوزن الجاف للمجموع الخضري والمحتوى المائي والنمو النسبي للورقة بشكل معنوي مع ازدياد فترة الجفاف، بينما زادت وزن الجاف للجذر ونسبة الوزن الجاف للجذر على الوزن الجاف للمجموع الخضري. وأما عن بعض المحتويات الكيميائية والبايوكيميائية للورقة؛ فقد انخفض محتوى النايتروجين والفسفور والبوتاسيوم والمغنسيوم وزاد البرولين وحامض الأسكوربيك بشكل واضح مع إجهاد الجفاف المتزايد وكانت اعلى قيمة حصل عليها (21) يوماً، أما السكريات الذائبة والكاربوهيدرات الكلية والبروتين وكلوروفيل a و b والكاروتينات الكلية للورقة، فقد نقصت وكانت أدنى قيمة لهذه المقاييس (21) يوماً. وكان طول السنبل وعدد السنابل ووزن السنابل والوزن الجاف لـ(100) حبة وعدد الحبوب وحاصل الحبوب قد انخفض أيضاً، بينما معاملة النبات بحامض الاسكوربيك خففت التأثير التثبيطي للجفاف من خلال تحسين الصفات المذكورة أعلاه .

الكلمات الدالة: حامض الاسوربيك، الإجهاد المائي، الحنطة.

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