BROCCOLI GROWTH, ANTIOXIDANT ACTIVITY AND HEALTH TISSUES RESPONSES TO ASCORBIC ACID UNDER DEFICIT IRRIGATION

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ABSTRACT

Two field experiments were conducted during 2013/2014 and 2014/2015 in a private Farm, Ibshway district, Fayoum, Egypt to study deficit irrigation (60, 80 and 100% ETc) and ascorbic acid foliar application (0, 200 and 400 mg l^{-1}) on growth, relative water content (RWC), membrane stability index (MSI), electrolyte leakage (EL), leaf photosynthetic pigments (LPS), osmoprotectants (OS) and DPPH radical-scavenging activity of broccoli plants. The obtained results clarified that, irrigation amount at 80% or 100% ETc, significantly, resulted in higher mean values of stem length and diameter, number of leaves $plant^{-1}$, leaf MSI, RWC, carotenoids, DPPH-radical-scavenging and total free amino acids content than irrigation amount at 60% ETc. On other side, irrigation amounts, irrespective the level used, did not reflect any noticeable impact on number of branches $plant^{-1}$ and head contents of ascorbic acid. Increasing irrigation water from 60% up to 100% ETc decreased the contents of leaf EL, chlorophyll A, B, A + B, anthocyanin, free proline and TSS. Exogenous application of ascorbic acid at concentrations of 200 and 400 mg l^{-1}, significantly improved most studied growth parameters, RWC, MSI, anthocyanin, DPPH, total free amino acids and endogenous ascorbic acid however, leaf EL decreased gradually. Moreover, the impact of ascorbic acid, irrespective of the concentration used, on leaf photosynthetic pigment, free proline and total soluble sugars contents was at par.

Key Words: Deficit irrigation, Ascorbic acid, Antioxidant activity, Broccoli growth, RWC, MIS, EL, leaf photosynthetic pigments, osmoprotectants and DPPH radical-scavenging activity**.**

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica*) belongs to family Brassicacea which includes cabbage, cauliflower, chinese cabbage, sprouting broccoli, brussels sprouts and kohlrabi (Thompson and Kelly, 1957). The edible portions of broccoli are central and lateral heads, fleshy peduncles and pedicels whereas, flower part represents a very small part of the head. Broccoli has enormous nutritional and medicinal values due to its high content of vitamins, minerals and antioxidant substances (Rozek and Wojciechowska,2005; Wojciechowska *et al.*, 2005) which prevent the formation of cancer causing agents (Beecher, 1994). In controlled study, a strong association between increased broccoli consumption and the protection against cancer (Verhoeven *et al.,* 1996).The complement of phytochemicals in broccoli including vitamins C and E, flavonoids, carotenoids and glucosinolates found to be the reason of this protective effect (Podsędek, 2007).

The amount and frequency of water for broccoli crop depends upon the soil type, environmental conditions, location and maturity. Broccoli crop requires plenty of water to maintain high water uptake for good performance (Nonnecke, 1989). It requires between 300 to 400 mm of water ha^{-1} or 125 to 170 mm of water fed^{-1} (Ludong, 2008). Recently, the available amount of water to agriculture is decline worldwide due to rapid population growth and incidence of drought caused by climate change and different human activities (World Bank, 2006). Successful management of available limited water depends on better agricultural practices and enhanced understanding of water productivity (Jones, 2004). Deficit irrigation i.e. irrigation below the optimum crop water requirements is a strategy for water-saving by which crops are subjected to a certain level of water stress either during a particular period or throughout the entire growing season (Pereira *et al.*, 2002).

Ascorbic acid ($C_6H_8O_6$) is a small molecular soluble in water and insoluble in other solvents as chloroform, benzene and fats. Being a powerful antioxidant, it scavenges and controls the concentration of H_2O_2 in plants (Sairam *et al*., 1998) with the help of an enzyme ascorbate peroxidase. This enzyme transfer electrons from ascorbate to H_2O_2 producing dehydro-ascorbate and water as products (Raven, 2002). Ascorbic acid participates in a variety of processes including photosynthesis, cell wall growth and cell expansion, resistance to environmental stresses and synthesis of ethylene, gibberellins, anthocyanin and hydroxyl proline (Smirnoff *et al.,* 2000). Application of acorbic acid have been found to protect numerous crop species under drought stress (Amin *et al.,* 2009; Dolatabadian *et al*., 2010; Hussein and Khursheed, 2014).

Accordingly, the current investigation was proposed in order to examine the effect of deficit irrigation and foliar application of ascorbic acid on morphological characters and tissue health of broccoli plants.

MATERIALS AND METHODS

Two field experiments were conducted during 2013/2014 and 2014/2015 seasons in a private Farm, Ibshway district, Fayoum, Egypt. The objective of this work is to assess the impact of surface irrigation amounts (60, 80 and 100% ETc) and foliar application of ascorbic acid (0, 200 and 400 mg l^{-1}) on morphological characters, leaf tissue health measurements, photosynthetic pigments, osmoprotectants, antioxidant activity of broccoli plants (*Brassica oleracea* L. var. *italica*). Preceding the initiation of each experiment, soil samples to 25 cm depth were collected and analyzed in Soil Testing Laboratory, Faculty of Agriculture, Fayoum University according to the standard published procedures (Wilde *et al*., 1985). Results of soil samples analysis are presented in Table 1.

Table (1): Physical and chemical characteristics of the experimental site during the seasons of 2013/2014 and 2014/2015.

Property	2013/2014	2014/2015
Physical properties		
Clay %	49.47	48.21
Silt %	21.61	22.75
Fine sand %	23.17	23.11
Coarse sand %	5.75	5.93
Soil texture	Clay	Clay
Chemical properties		
pH (1 : 2.5)	7.41	7.48
CEC (meq/100 g soil)	37.22	36.86
ECe (ds m)	2.83	2.88
Organic matter (%)	1.81	1.73
Ca CO_3 (%)	4.31	4.23
N (%)	0.59	0.56
Available elements (mg kg^{-1} soil):		
K	652	703
P	562	523

Imported broccoli hybrid seeds cv. Groene Calabrese (Seminis-Peto seed Company, USA) were hand sown in speedling trays filled with a mixture of growth media {50% peat moss + 50% vermiculite (v/v)} on September 18th, 2013 and September 11th, 2014. After thirty days of seed sowing, seedlings were transplanted into the field at in-rows spacing of 40cm. The devoted area of each experimental unit was 12 m^2 including five rows of 4 m long and 60 cm wide. The experimental layout was a split-plot system based on Randomized Complete Blocks design with three replications. Levels of irrigation amount and concentrations of ascorbic acid were randomly distributed in the main and sub- plots, orderly. Each two adjacent experimental unites was separated by 2.0 m. alley to protect against side effects.

2.1 Irrigation water application (IWA)

Three irrigation treatments were applied as a percentage of the crop evapotranspiration (ETc) representing one of the following:

I_1 =100% of ETc, I_2 =80% of ETc and I_3 = 60% of ETc.

The daily ETo was computed by equation (1) according to Doorenbos and Pruitt (1992):

$$\text{ETo} = \text{Kpan} \times \text{Epan} \ldots\ldots\ldots (1)$$

Where:

E*pan*	Is evaporation from the Class A pan (mm/day).
K*pan*	Is the pan evaporation coefficient.

Monthly mean weather data for a 16-year (January 1997 - December 2012) were obtained from Attsa weather station, Fayoum Governorate. Monthly mean relative humidity, wind speed and class A pan evaporation for Attsa weather station are shown in Figure 1. The average daily Epan was 7.65, 7.51, 7.07 and 6.33 mmd^{-1} for October, November, and December months, respectively.

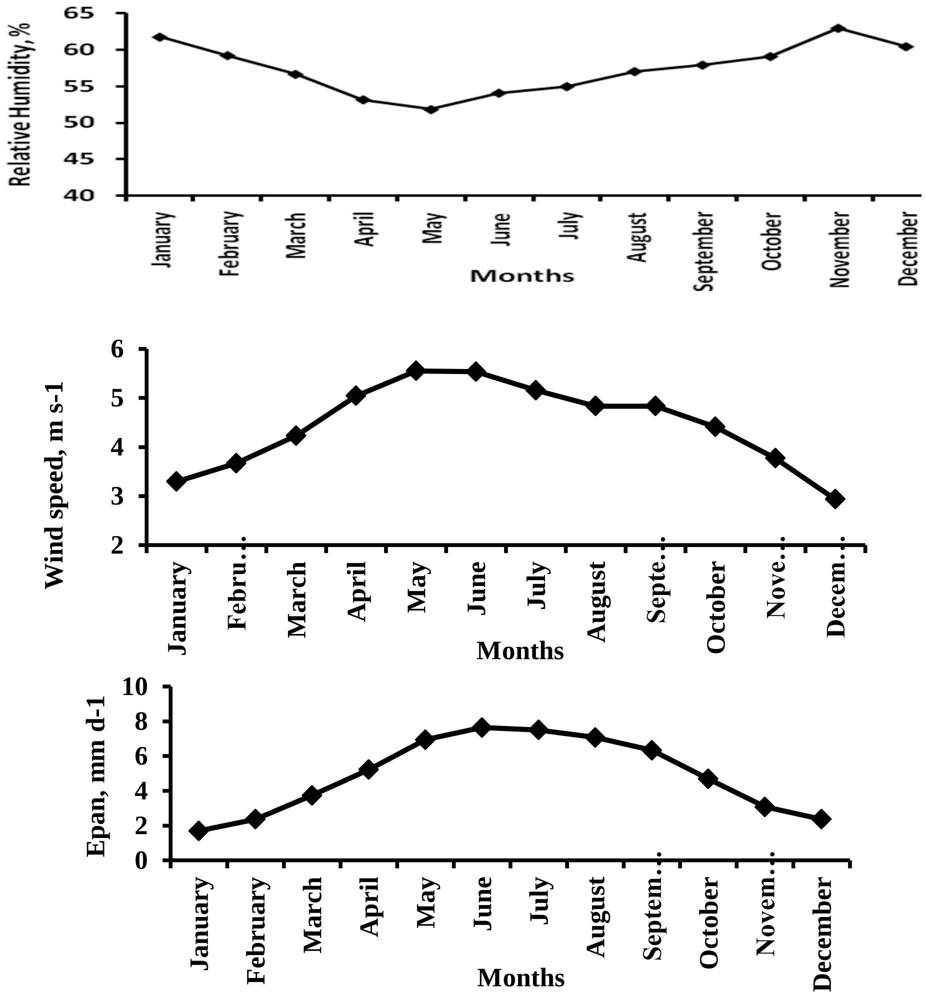

Figure 1. Monthly mean relative humidity, wind speed and class A pan evaporation for Attsa Station.

The crop water requirements (ETc) were estimated using the crop coefficient according to the following equation:

$$ETc = ETo \times Kc$$

Where:

Etc = crop water requirements (mm d^{-1}).
Kc = crop coefficient.

The lengths of the different crop growth stages were 35, 45, 40, and 15 days for initial, crop development, mid-season and late season stages, respectively. The crop coefficients (Kc) of initial, mid and end stages were 0.70, 1.05 and 0.95 respectively according to Allen *et al.* (1998).

The broccoli plants were irrigated every fifteen days intervals by different amounts of water. The amount of irrigation water applied to each plot during the irrigation regime was determined by using the equation given below:

$$IWA = \frac{A \times ETc \times Ii}{Ea \times 1000} + LR$$

Where:

IWA = irrigation water applied (m^3).
A = plot area (m^2).
ETc = crop water requirements (mm d^{-1}).
Ii = irrigation intervals (d).
Ea = application efficiency (%).
LR = leaching requirements (m^3).

The amount of irrigation water applied (IWA) was controlled through plastic pipe (spiles) of 50 mm diameter. One spile per plot was used to convey water for each plot. The amount of water delivered through a plastic pipe was calculated using equation (3) according to Israelson and Hansen (1962).

$$Q = CA\sqrt{2gh} \times 10^{-3} \ldots\ldots\ldots (3)$$

Where:

Q = discharge of irrigation water, (l. sec^{-1}),
C = coefficient of discharge,
A = cross section area of irrigation pipe, (cm^2),
G = gravity acceleration, (cm. sec^{-2}),
H = average effective head of water, (cm).

Irrigation treatments were isolated with 2 m fallow land to avoid the lateral movement of water during irrigation. Irrigation treatments were started after the first irrigation.

2.2 Ascorbic acid application

Ascorbic acid concentrations (0, 200 and 400 mg l^{-1}) were foliar sprayed, to run off, three times; 30, 40 and 50 days after transplanting. All experimental unites received N, P_2O_5 and K_2O at rates of 33, 15 and 24 kg fed^{-1}, respectively. During soil preparation, phosphorus fertilizer was broadcasted and N and K fertilizers were side banded at two equal portions; 3 and 6 weeks after transplanting. Recommended agro-management practices were performed for the commercial production of broccoli.

2.3 Plant sampling

Randomly plants were chosen in each experimental unit to measure morphological characters and leaf RWC, MSI, EL, photosynthetic pigments, osmoprotectants and antioxidant activity.

2.4 Data Recorded

2.4.1 Morphological characters

After 70 days of transplanting (about 25% of the total number of plants in each experimental unit started flowering), four randomly plants, in each experimental unit, were chosen, carefully cut off at the ground level and immediately transferred to the laboratory. Plant samples were separated into leaf-blades and stems. The following morphological characters were measured:

2.4.1.1 Stem length (cm); measured starting from the ground level to the epical meristem of the stem.

2.4.1.2 Stem diameter (cm); measured by using Sealy So707-Digital Electronic Vernier Caliper 0-150 mm/0-6″ at ground level.

2.4.1.3 Number of branches and leaves plant^{-1} was counted.

2.4.1.4 Total leaf area plant^{-1} (m^2); mathematically calculated using leaf area-leaf weight relationship from leaf disks obtained by a cork borer Wallace and Munger (1965).

2.4.1.5 Leaf area leaf^{-1} (dm^2); mathematically calculated using the following formula:

$$\text{Leaf area leaf}^{-1} = \frac{\text{Leaves area plant}^{-1}}{\text{Number of leaves plant}^{-1}}$$

2.4.1.6 Leaves and stems dry weights plant^{-1} (g); gained by drying at 70°C in a forced-air oven till the weight became constant.

2.4.1.7 Canopy dry weight plant^{-1} (g); mathematically calculated by summation oven dried leaves and stems.

2.4.2 Tissue health measurements

Leaves of four randomly plants, in each experimental unit, after 70 days from transplanting, were collected, washed with tap water, rinsed three times with distilled water. The following parameters were considered:

2.4.2.1 Leaf relative water content (RWC)

Twenty discs from fully expanded fresh leaf (2 cm diameter disc^{-1}) after excluding the midrib. Fresh leaf discs were weighed (FM) and immediately floated on double-distilled water in a Petri dish for 24 h, in the dark, to saturate them with water and the turgid mass (TM) was weighed. The dry mass (DM) was recorded after dehydrating the discs at 70 °C for 48 h. The percentage of RWC was calculated using the formula introduced by Hayat *et al.* (2007):

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100$$

2.4.2.2 Leaf membrane stability index (MSI)

Duplicate samples of fully-expanded leaves tissue, each weighed 0.2 g. The 1st leaf sample was placed in a test tube containing 10 ml of double-distilled water and heated till 40 °C in a water bath for 30 min. Electric conductivity of the solution for test tube (EC_1) was measured by a conductivity bridge (Starlac Industries, Ambala, Haryana, India). The same previously steps were performed with the 2nd leaf sample but, heated till 100 ^oC for 10 min and EC_2 was measured. Membrane stability index was calculated according to the following formula mentioned by Sairam (1994): $MSI\ (\%) = [1 - (EC_1 / EC_2)] \times 100$

2.4.2.3 Leaf electrolyte leakage (EL)

The percentage of total leakage of inorganic ions (LE) from fully-expanded leaves was determined using the method of Sullivan and Ross (1979). Twenty leaf discs (2 cm in diameter) were placed in a boiling tube containing 10 ml deionised water and the electrical conductivity (EC_1) was recorded. The contents were then heated to 45º – 55ºC in a water bath for 30 min and the electrical conductivity (EC_2) was recorded. The sample was then boiled at 100ºC for 10 min and the electrical conductivity (EC_3) was recorded. Electrolyte leakage was calculated using the formula: $EL\ (\%) = [(EC_2 - EC_1) / EC_3] \times 100$

2.4.3 Leaf photosynthetic pigments

2.4.3.1 Leaf chlorophyll A , B and carotenoid

Leaf chlorophyll A, B, A + B and carotenoid contents (mg g^{-1} fresh weight) were spectrophotometrically measured (Bauschand Lomb-2000 Spectronic 21 spectrophotometer) at wave lengths 663, 645 and 470 nm (Arnon,1949)

2.4.3.2 Leaf anthocyanin

Leaf anthocyanin content (mg g^{-1} dry weight) was spectrophotometrically measured using Bauschand Lomb-2000 Spectronic 21 Spectrophotometer at wave lengths 510 and 700 nm as outlined by (Meyers *et al.*, 2003).

.4.4 Osmoprotectants and antioxidant activity

2.4.4.1 Leaf free proline

Leaf free proline content (µg g^{-1} leaf dry weight) was spectrophotometrically measured by apparatus Bauschand Lomb-2000 Spectronic 21 Spectrophotometer at wave length 520 nm as described by Bates *et al.* (1973).

2.4.4.2 Leaf DPPH·radical-scavenging activity

Leaf antioxidant activity was spectrophotometrically measured at wave length 515 nm and DPPH radical-scavenging activity was calculated according to the following formula: DPPH radical-scavenging activity (%) = $[(A_{control} -$

A_{sample})] / ($A_{control}$)] × 100, where A is the absorbance at 515 nm (Lee *et al*., 2003).

2.4.4.3 Leaf total soluble sugar (mg g^{-1} DW)

Leaf total soluble sugars (TSS) were measured spectrophotometrically at wave length 625 nm using a Bauschand Lomb-2000 Spectronic Spectrophotometer as described by Irigoyen *et al.* (1992).

2.4.4.4 Leaf total free amino acids (mg $100g^{-1}$ DW)

Leaf total free amino acids were measured spectrophotometrically at wave length 570 nm according to the modified method of Dubey and Rani (1989).

2.4.4.5 Head ascorbic acid content (mg $100g^{-1}$ head FW)

Head ascorbic acid content was determined using 2,6-dichloro-indophenol method (Helrich, 1990).

2.4 Statistical analysis.

Appropriate analysis of variance was performed on results of each experiment. Comparisons among means of different treatments were performed using the Least Significant Difference procedure (L.S.D.) at $P = 0.05$ level as illustrated by Snedecor and Cochran (1980).

3. Results

3.1 Morphological Characters

Tables 2-4 showed that irrigation amount at 80% ETc was pioneer and significantly resulted in higher mean values of stem length, and number of leaves plant^{-1}, in two seasons. Unlikely, the leaf area leaf^{-1} which gives the highest value with the amount at 100% ETc, in both years. Application of broccoli plants with 60% ETc of irrigation water reflected negative significant influences on stem diameter, total leaf area plant^{-1} and canopy dry weight and its components in comparison with the irrigation water amounts 80% or 100% ETc, while increasing irrigation water amount from 80% to 100% ETc was at par in both seasons. Exception the stem diameter in the first year the amount of 100% ETc showed a not significant difference of 60% ETc. On other side, number of branches plant^{-1} did not show any appreciable response to any applied amount of irrigation water, in both seasons.

Tables 2-4 showed that irrigation amount at 80% ETc, significantly, recorded higher mean values of stem length and number of leaves plant^{-1} than irrigation amounts at 60 and 100% ETc, in the two experimental seasons. Generally, increasing irrigation amount from 60 to 80 and furtherly to 100% ETc accompanied with increased leaf area leaf^{-1} and leaf area plant^{-1}. Stem, leaves and canopy dry weights, significantly, attained higher mean values at 80 and 100 than 60% ETc, in both seasons. However, irrigation (irrespective of the amount of water used) did reflect any significant effect on number of branches plant^{-1}.

Foliar application of ascorbic acid at 200 and 400 ppm, generally, reflected, significant, increments in stem height, leaf area and canopy dry weight and their components plant^{-1} compared to the control (untreated plants) treatment, in the two in quested seasons. Meanwhile, number of branches plant^{-1} did not seem to be affected. At 200 mg l^{-1} ascorbic acid, stem diameter was, significantly, the thickest.

The interactions between the two studied variables (i.e., irrigation water and ascorbic acid) reflected significant effects on stem length and diameter, number of branches, leaves area and canopy dry weight plant^{-1} and their components, in both seasons. The best results achieved with the combination of 80 and/or 100% ETc together with 200 and/or 400 mg l^{-1} ascorbic acid.

Table (2): Effect of foliar application of ascorbic acid on stem height, stem diameter and number of branches plant^{-1} of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season								
	2013/2014					2014/2015			
	Ascorbic acid (mgl^{-1})								
	0	**200**	**400**	**Mean**		**0**	**200**	**400**	**Mean**
	Stem length (cm)								
60	31.7^{cd}	33.2^{bcd}	34.3^{bc}	**33.1^{B*}**		35.9e	38.5cde	41.3bc	**38.6B**
80	33.2^{bcd}	38.3^{a}	41.1^{a}	**37.5^{A}**		39.9cd	44.7ab	45.7a	**43.4A**
100	31.3^{d}	35.3^{b}	34.6^{bc}	**33.7^{B}**		36.9de	42.2abc	39.3cde	**39.5B**
Mean	**32.0^{B}**	**35.6^{A}**	**36.7^{A}**			**37.6B**	**41.8A**	**42.1A**	
	Stem diameter (cm)								
60	3.3d	3.4cd	3.4cd	**3.4B**		3.2c	3.3c	3.3c	**3.3B**
80	3.6cd	4.3a	4.0ab	**4.0A**		3.5bc	4.1a	3.9a	**3.8A**
100	3.5cd	4.0ab	3.7bc	**3.7AB**		3.4c	4.1a	3.8ab	**3.7A**
Mean	**3.4B**	**3.9A**	**3.7AB**			**3.4B**	**3.8A**	**3.6AB**	
	Number of branches plant^{-1}								
60	8.3abc	7.9abc	6.8c	**7.6A**		9.4a	8.9ab	6.4c	**8.2A**
80	7.5bc	9.2a	9.1a	**8.6A**		8.6ab	9.4a	8.6ab	**8.9A**
100	7.5bc	7.7abc	9.1a	**8.1A**		7.6bc	8.4ab	9.6a	**8.5A**
Mean	**7.8A**	**8.3A**	**8.3A**			**8.5A**	**8.9A**	**8.2A**	

*Values marked with the same letter(s) within the main and interaction effects are statistically similar using Revised LSD test at P = 0.05. Uppercase letter(s) indicate differences between main effects, and lowercase letter(s) indicate differences within interaction of each character.

Table (3): Effect of foliar application of ascorbic acid on leaf area and its components plant^{-1} of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season								
	2013/2014				2014/2015				
	Ascorbic acid (mgl^{-1})								
	0	200	400	Mean		0	200	400	Mean
	Number of leaves plant^{-1}								
60	13.3d	15.2bc	15.6ab	**14.7B***		15.2b	15.1b	15.3b	**15.2B**
80	13.8cd	17.0a	17.0a	**15.9A**		15.9b	18.8a	18.2a	**17.6A**
100	13.7cd	14.1bcd	14.2bcd	**14.0B**		15.7b	15.8b	16.3b	**15.9B**
Mean	**13.6B**	**15.4A**	**15.6A**			**15.6B**	**16.6A**	**16.6A**	
	Total leaf area plant^{-1} (m^2)								
60	1.79c	1.89bc	1.86bc	**1.85C**		1.72b	1.85b	1.86b	**1.81B**
80	1.91bc	2.30a	2.18ab	**2.13B**		1.95b	2.33a	2.21a	**2.16A**
100	1.92bc	2.37a	2.41a	**2.24A**		1.93b	2.37a	2.35a	**2.22A**
Mean	**1.88B**	**2.19A**	**2.15A**			**1.86B**	**2.18A**	**2.14A**	
	Leaf area leaf^{-1} (dm^2)								
60	13.5bc	12.5bc	12.0c	**12.6C**		11.3b	12.3b	12.1b	**11.9B**
80	13.9b	13.5bc	12.8bc	**13.4B**		12.3b	12.5b	12.1b	**12.3B**
100	14.0b	16.9a	17.0a	**16.0A**		12.3b	15.0a	14.4a	**13.9A**
Mean	**13.8A**	**14.3A**	**13.9A**			**12.0B**	**13.3A**	**12.9A**	

Table (4): Effect of foliar application of ascorbic acid on canopy dry weight and its components plant^{-1} of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season								
	2013/2014				2014/2015				
	Ascorbic acid (mgl^{-1})								
	0	200	400	Mean		0	200	400	Mean
	Leaves dry weight plant^{-1} (g)								
60	81c	86c	86c	**84B**		86c	88c	87c	**87B**
80	91bc	108a	102a	**101A**		95bc	117a	110a	**107A**
100	89c	107a	101ab	**99A**		94bc	112a	108ab	**105A**
Mean	**87B**	**100A**	**96A**			**92B**	**105A**	**102A**	
	Stems dry weight plant^{-1} (g)								
60	120d	116d	112d	**116B**		123c	120c	122c	**122B**
80	145bc	159a	150ab	**151A**		152b	171a	155b	**159A**
100	135c	153ab	160a	**149A**		145b	160ab	172a	**159A**
Mean	**133B**	**142A**	**141A**			**140B**	**150A**	**150A**	
	Canopy dry weight plant^{-1} (g)								
60	201d	202d	198d	**200B**		209d	208d	209d	**209B**
80	236bc	267a	252ab	**252A**		248bc	287a	265abc	**267A**
100	223c	260a	261a	**248A**		239c	272ab	280a	**264A**
Mean	**220B**	**243A**	**237A**			**232B**	**256A**	**252A**	

*Values marked with the same letter(s) within the main and interaction effects are statistically similar using Revised LSD test at P = 0.05. Uppercase letter(s) indicate differences between main effects, and lowercase letter(s) indicate differences within interaction of each character.

3.2 Tissue health measurements

Results of statistical analysis in Table 5 displayed that irrigation amount at 80 and 100% ETc, significantly, resulted in higher mean values of leaf relative water (RWC) and membrane stability index (MSI) than irrigation amount at 60% ETc, in the two experimental years. Nevertheless, increasing the amount of water irrigation up to 100% ETc, significantly and progressively, decreased leaf electrolyte leakage, through the two seasons.

Table (5): Effect of foliar application of ascorbic acid on leaf RWC, MSI and EL of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season								
	2013/2014				2014/2015				
	Ascorbic acid (mgl^{-1})								
	0	**200**	**400**	**Mean**		**0**	**200**	**400**	**Mean**
	RWC %								
60	72.3e	75.4de	79.7c	**75.8B***		75.4d	77.2cd	77.8cd	**76.8B**
80	78.4cd	84.6ab	84.2ab	**82.4A**		78.4cd	82.5abc	85.2ab	**82.0A**
100	81.9bc	86.5a	88.5a	**85.6A**		81.0bcd	86.7ab	88.1a	**85.2A**
Mean	**77.5B**	**82.2A**	**84.1A**			**78.3B**	**82.1A**	**83.7A**	
	MSI %								
60	80.9f	82.3ef	83.3e	**82.2B**		82.0e	84.0de	85.3cd	**83.8B**
80	86.3d	89.0bc	90.5b	**88.6A**		87.5bc	90.0ab	91.0a	**89.5A**
100	87.4cd	90.8b	92.6a	**90.3A**		90.2ab	91.4a	92.4a	**91.3A**
Mean	**84.9B**	**87.4A**	**88.8A**			**86.6B**	**88.5A**	**89.6A**	
	EL %								
60	14.8a	14.2ab	13.8b	**14.3A**		16.7a	14.7b	13.8bc	**15.1A**
80	14.0b	13.0c	12.3d	**13.1B**		14.6b	13.9bc	13.2cd	**13.9B**
100	13.0c	12.2d	11.5e	**12.3C**		13.1cd	12.7cd	12.3d	**12.7C**
Mean	**13.9A**	**13.1B**	**12.6C**			**14.8A**	**13.8B**	**13.1C**	

*Values marked with the same letter(s) within the main and interaction effects are statistically similar using Revised LSD test at P = 0.05. Uppercase letter(s) indicate differences between main effects, and lowercase letter(s) indicate differences within interaction of each character.

In both two experimental seasons, spraying ascorbic acid at 200 or 400 mg L^{-1} reflected, significant, increments in leaf RWC and MSI % compared with the check treatment. Meanwhile, increasing the concentration of ascorbic acid up to 400 ppm, leaf EL%, significantly, declined, in two seasons.

At any concentration of ascorbic acid, increasing irrigation water amount increased MSI% and RWC%, and decreased EL%, similarly, at any amount of irrigation water, increasing ascorbic acid concentration increased MSI% and RWC%, and decreased EL%, in the two growing seasons.

At any irrigation amount; increasing ascorbic acid concentration increased MSI% and RWC%, and decreased EL%. Similarly, at any ascorbic acid concentration; increasing irrigation water amount increased MSI% and RWC%, and decreased EL%. Therefore, the best combined treatment for RWC% and MSI% was irrigation amount at 80% or/and 100% ETc and foliar ascorbic acid at 200 or/and 400 mg l^{-1} whilst, the best combined treatment for EL% was 60% ETc irrigation amount and ascorbic acid control (untreated plant), in both seasons.

3.3 Leaf photosynthetic pigments content

Table 6 shows that increasing irrigation amount from 60% up to 100% ETc, significantly, accompanied with a progressive decline of chlorophyll A, B, A + B and anthocyanin contents. On the other hand, irrigation amount at 80% ETc, significantly, resulted in the highest value of carotenoids content, whereas, not significant increases were detected between the amounts 60% and 100% ETc and the trend was the same in two seasons.

Table (6): Effect of foliar application of ascorbic acid on leaf photosynthetic pigments of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season							
	2013/2014				2014/2015			
	Ascorbic acid (mgl^{-1})							
	0	**200**	**400**	**Mean**	**0**	**200**	**400**	**Mean**
	Chlorophyll A (mg g^{-1} FW)							
60	1.38a	1.31a	1.27ab	**1.32A***	1.23a	1.20a	1.18a	**1.21A**
80	1.03de	1.09cd	1.17bc	**1.10B**	1.03cd	1.07bc	1.14ab	**1.08B**
100	0.95e	1.02de	1.09cd	**1.02C**	0.90e	0.95de	0.97cde	**0.94C**
Mean	**1.12A**	**1.14A**	**1.18A**		**1.05A**	**1.07A**	**1.10A**	
	Chlorophyll B (mg g^{-1} FW)							
60	0.83a	0.79a	0.76abc	**0.79A**	0.82a	0.72ab	0.66b	**0.74A**
80	0.67cd	0.69cd	0.72bc	**0.69B**	0.63b	0.66b	0.70ab	**0.66B**
100	0.62d	0.65cd	0.66cd	**0.64C**	0.60b	0.62b	0.64b	**0.62C**
Mean	**0.71A**	**0.71A**	**0.71A**		**0.68A**	**0.67A**	**0.67A**	
	Chlorophyll A + B (mg g^{-1} FW)							
60	2.21a	2.11ab	2.03b	**2.11A**	2.06a	1.92ab	1.84bc	**1.94A**
80	1.70de	1.78cd	1.89c	**1.79B**	1.66cde	1.73bcd	1.83bc	**1.74B**
100	1.59e	1.69de	1.78cd	**1.68C**	1.50e	1.57de	1.61de	**1.56C**
Mean	**1.83A**	**1.86A**	**1.90A**		**1.74A**	**1.74A**	**1.76A**	
	Carotenoids (mg g^{-1} FW)							
60	0.29c	0.32c	0.31c	**0.30B**	0.23bcde	0.22cde	0.20e	**0.22B**
80	0.33bc	0.37ab	0.39a	**0.36A**	0.25bc	0.26b	0.30a	**0.27A**
100	0.32c	0.30c	0.32c	**0.31B**	0.21de	0.24bcd	0.24bcd	**0.23B**
Mean	**0.31A**	**0.33A**	**0.34A**		**0.23A**	**0.24A**	**0.25A**	
	Anthocyanin (mg g^{-1} DW)							
60	0.54bc	0.58ab	0.60a	**0.57A**	0.57b	0.61a	0.63a	**0.60A**
80	0.47e	0.52cd	0.55bc	**0.51B**	0.48d	0.53c	0.57b	**0.53B**
100	0.41f	0.46e	0.48de	**0.45C**	0.43e	0.46de	0.49d	**0.46C**
Mean	**0.47B**	**0.52A**	**0.54A**		**0.49B**	**0.54A**	**0.56A**	

*Values marked with the same letter(s) within the main and interaction effects are statistically similar using Revised LSD test at P = 0.05. Uppercase letter(s) indicate differences between main effects, and lowercase letter(s) indicate differences within interaction of each character.

Foliar application of ascorbic acid, irrespective of the concentration used, appeared to be not effective on chlorophyll A, B, A + B and carotenoid contents, in the two experimental seasons. Conversely, spraying the foliage with the various concentrations of ascorbic acid 200 and 400 mg l^{-1} reflected positive significant influences on leaf anthocyanin contents in comparison with the control, in both seasons. Differences between ascorbic acid concentration 200 and 400 mg l^{-1} were not true, in the two years.

The combined treatment of 60% ETc and 0 mg l^{-1} irrigation amount-ascorbic acid concentration, orderly, significantly, attained the highest mean values of chlorophyll A, B, A + B and anthocyanin contents whereas, the interaction treatment of 80% ETc and 400 mg l^{-1} irrigation amount and ascorbic acid concentration, orderly, significantly, achieved the highest mean value of carotenoids content.

3.4 Osmoprotectants, antioxidant activity

3.4.1 Leaf free proline and DPPH radical-scavenging activity

Data shown in Tables 7 revealed that a reversal relationship between irrigation amount and free proline content however, irrigation amount at 80 and 100 % ETc, significantly, recorded higher mean values of leaf DPPH radical-scavenging activity than irrigation amount at 60% ETc.

Foliar application of ascorbic acid at 200 mg l^{-1}, significantly, resulted in higher leaf DPPH radical-scavenging activity than 0 and 400 mg l^{-1}. Meanwhile, foliar application of ascorbic acid, irrespective the concentration used, did not reflect any appreciable effect on DPPH radical-scavenging activity.

The combination treatment of irrigation amount together ascorbic acid at 80 %ETc and 200 mg l^{-1}, respectively recorded, significantly, the highest DPPH radical-scavenging activity.

Table (7): Effect of foliar application of ascorbic acid on leaf free proline content and DPPH radical-scavenging activity of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season								
	2013/2014					2014/2015			
	Ascorbic acid (mgl^{-1})								
	0	200	400	Mean		0	200	400	Mean
	Free proline (µg g^{-1} DW)								
60	29.9a	32.6a	32.9a	**31.8A***		29.0a	31.3a	31.9a	**30.7A**
80	21.2a	24.6a	25.6a	**23.8B**		19.5a	22.9a	23.3a	**21.9B**
100	16.6a	15.6a	14.6a	**15.6C**		17.4a	17.3a	17.0a	**17.2C**
Mean	**22.6A**	**24.3A**	**24.4A**			**22.0A**	**23.8A**	**24.0A**	
	DPPH radical-scavenging activity (%)								
60	26.8d	29.7d	31.4d	**29.3B**		22.2f	24.5ef	26.1ef	**24.3B**
80	38.5c	59.5a	47.6b	**48.5A**		32.4de	60.7a	49.3bc	**47.5A**
100	44.5b	54.2a	46.3b	**48.3A**		41.3cd	56.5ab	41.7c	**46.5A**
Mean	**36.6C**	**47.8A**	**41.8B**			**32.0C**	**47.3A**	**39.0B**	

*Values marked with the same letter(s) within the main and interaction effects are statistically similar using Revised LSD test at P = 0.05. Uppercase letter(s) indicate differences between main effects, and lowercase letter(s) indicate differences within interaction of each character.

3.4.2 Leaf TSS, total free amino acids and head ascorbic acid contents

Increasing irrigation amount up to 100% ETc, significantly and progreesivly, decreased leaf TSS but, head ascorbic acid content was not affected (Table 8). Nevertheless, irrigation amount at 80% and 100% ETc, significantly, augmented total free amino acids compared to irrigation amount at 60% ETc, in 2013/2014 and 2014/2015.

Raising the concentration of ascorbic acid up to 400 mg l^{-1} associated with augmentation of ascorbic acid content whilst, foliar spray of ascorbic acid did not affect leaf TSS. Meanwhile, application of head ascorbic acid at 200 and 400 mg l^{-1}, significantly, resulted in higher total free amino acids content, in both seasons.

The interaction between the two studied factors did not reflect any significant influence on TSS and total free amino acids but, the treatment combination of irrigation amount at 60% ETc together with 400 mg l^{-1} ascorbic acid was pioneer on ascorbic acid content, in both seasons.

Table (8): Effect of foliar application of ascorbic acid on leaf TSS, free amino acid and head ascorbic acid contents of broccoli grown under deficit irrigation during 2013/2014 and 2014/2015.

Irrigation (ETc%)	Season								
	2013/2014				2014/2015				
	Ascorbic acid (mgl^{-1})								
	0	**200**	**400**	**Mean**		**0**	**200**	**400**	**Mean**
	TSS (mg g^{-1} DW)								
60	3.33a	3.23a	3.18a	**3.25A***		3.59a	3.55a	3.51a	**3.55A**
80	2.62a	2.56a	2.37a	**2.52B**		2.81a	2.67a	2.49a	**2.66B**
100	2.27a	2.15a	2.05a	**2.16C**		2.23a	2.19a	2.12a	**2.18C**
Mean	**2.74A**	**2.64A**	**2.53A**			**2.88A**	**2.80A**	**2.71A**	
	Total free amino acids (mg 100g^{-1} DW)								
60	82.6a	87.6a	94.3a	**88.1B**		80.7a	87.5a	95.6a	**87.9B**
80	86.8a	96.2a	99.9a	**94.3A**		90.7a	98.7a	98.4a	**95.9A**
100	87.3a	97.7a	98.9a	**94.7A**		84.1a	94.4a	100.9a	**93.2A**
Mean	**85.6B**	**93.8A**	**97.7A**			**85.2B**	**93.6A**	**98.3A**	
	Ascorbic acid (mg 100g^{-1} head FW)								
60	78.2e	105.3b	119.9a	**101.1A**		78.5d	103.4bc	125.4a	**102.4A**
80	84.7de	98.0bc	107.2b	**96.6A**		84.2d	96.5c	108.1b	**96.2A**
100	83.9de	93.2cd	99.0bc	**92.0A**		83.7d	95.1c	101.5bc	**93.4A**
Mean	**82.2C**	**98.8B**	**108.7A**			**82.1C**	**98.3B**	**111.7A**	

*Values marked with the same letter(s) within the main and interaction effects are statistically similar using Revised LSD test at P = 0.05. Uppercase letter(s) indicate differences between main effects, and lowercase letter(s) indicate differences within interaction of each character.

4. Discussion

4.1 Morphological Characters

Irrigation of growing broccoli plants with 60% ETc led to a negative significant effect on all plant growth parameters (stem length and diameter, number of branches and number of leaves plant^{-1}, total leaf area plant^{-1}, leaf area leaf^{-1}, and leaves, stem and canopy dry weights plant^{-1}) compared to irrigation amount at 80% or 100% ETc, in two seasons. This may be due to the treatment of irrigation amount at 60% ETc led to severe damage in the growth of the plant broccoli. It is well known that water stress conditions cause a multitude of molecular, biochemical and physiological changes, thereby affecting plant growth and development (Boutraa, 2010). A decline response of growth due to water stress might be explained on the bases of suppression cell elongation, cell turgor, volume and eventually growth. Water-stress caused a marked suppression in plant photosynthetic capacity and efficiency, mainly due to closing stomata and inhibition of (Rubisco) enzyme (Lawlor and Cornic, 2002). The depressive effect of water stress with 60% ETc in this study on growth parameters of broccoli plants may also be attributed to a drop in membrane stability index (MSI), relative water content (RWC), radical-scavenging activity (DPPH) and increase in electrolyte leakage (EL) compared to 80 or 100% ETc as indicated in Tables 5 and 7. In addition, a reduction in leaf total free amino acids content (Table 8) which affect negatively on the rate of cell division and enlargement. Water stress also reduced the excessive accumulation of intermediate compounds such as reactive oxygen species ROS (Yazdanpanah *et al.*, 2011; Razaji *et al*., 2014) which cause oxidative damage to DNA, lipid and proteins and consequently a decrease in plant growth. As well as, water stress leads to increase abscisic acid caused an inhibition of the growth (Abdalla, 2011). Many other reports support our obtained results in broccoli plants such as Latimer (1990); Zaicovski *et al.* (2008); Khan *et al.* (2010); Erken and Oztokat (2010).

The promoting effect of applied irrigation amount at 80% ETc on morphological characters of broccoli plants may can be avoid stress by deep rooting, allowing access to soil moisture lower in the soil profile so, plant reduces its osmotic potential in order to absorb water and maintain turgor. Enhancing MSI, RWC, DPPH and decrease EL under irrigation at 80% ETc (Tables 5 and 7) caused accumulation of a variety of inorganic and organic osmotica substances including compatible solutes such as free proline, total free amino acids and TSS contents in broccoli plant (Tables 7 and 8) to adjust osmotic pressure. Likwise, increasing leaf carotenoids and anthocyanin contents compared to 100% ETc amount (Table 6) which their functions as antioxidants enable tissues to react with free radicals, specially peroxyl radicals and singlet oxygen (Sies and Stahl, 1995). Therefore, application irrigation amount at the rate of 80% ETc increase the morphological characters (Tables 2 - 4), in the two growing seasons of 2013/2014 and 2014/2015. Numerous investigators reported similar results on different crops as Pasakdee *et al.* (2006) on broccoli; Bakry *et al.*(201٣) on wheat; Abd El-Mageed and Semida (2015) on cucumber.

The beneficial effects of foliar ascorbic acid application on the studied morphological characters can be discussed on the ground that ascorbic acid has auxinic effect and protect plant cells against free radicals that are responsible for plant senescence (Prusky, 1988; Elade, 1992). In addition, ascorbic acid might regulate cell wall expansion, cell division, and cell elongation through its action in cell vacuolization (Arrigoni, 1994; Navas and Gomez-Diaz, 1995; Cordoba-Pedregosa *et al*., 1996). Ascorbic acid was found to improve absorption of phenolic compounds, which lead to save the growing tissues from toxic effects of the oxidized phenols (Gupta *et al*., 1980). Moreover, ascorbic acid seemed to be enhance biosynthesis of free amino acids (Table 8) which are vital steps in stepping up plant tissues. Under stress, the amount of ascorbic acid increases in plants that plays a significant role in regulation of mechanisms of photosynthesis and defense against oxidative stress (Amin *et al.,* 2009; Dolatabadian *et al.,* 2009, 2010; Khalil *et al.,* 2010). In addition, ascobic acid probably reduced osmotic potential and maintain turgor as well as increase MSI, RWC, DPPH and decrease EL (Tables 5 and 7) which are responsible for plant growth to go forwad. Likewise, the leaf content of anthocyanin and head content of ascorbic acid were increased compared to control in Tables 6 and 8, it could the removal of H_2O_2 as a substrate of ascorbate peroxidase, directly reduces O^-_2, quench $^1O_2$ and regenerate reduced α-tocopherol (Foyer, 1993). Previous report of Khalil *et al.* (2010), Razaji *et al.* (2014), Shafiq *et al.* (2014), Amira and Qados (2014), Noman *et al.* (2015) and Malik *et al.* (2015) they indicated that the application of ascorbic acid showed significant increases in all growth parameters under different soil moisture levels. In addition, Osman and Tolba (2009); Osman (2010); Osman and El-Shatoury (2014) they observed that the foliar-applied ascorbic acid improved vegetative growth under different stress.

4.2 Leaf tissue health measurements

Leaf RWC, MSI and EL were the best criteria for plant water status. Leaf RWC has been used as an indicator of plant water balance. In most crop species, the wilting range is between RWC 60% to 70% (Barrs and Whetherley, 1962). The degree of cell membrane injury induced by water stress may be easily estimated through measurements of MSI (Hasheminasab *et al.,* 2012). Electrolyte leakage is one of the best physiological components of drought tolerance in plants (Xu *et al.,* 2008). The three parameters (MSI, RWC and EL) are greatly interrelated. Soil water potential decreases under water deficit conditions, so plant reduces its osmotic potential or osmotic adjustment in order to absorb water and maintain turgor through accumulation of a variety of inorganic and organic osmotica including compatible solutes such as proline, total free amino acids and TSS and is referred to as osmotic adjustment (Tables 7 and 8). Therefore, the obtained results in Table 5 showed that absence of any significant difference in RWC or MSI between irrigation amounts 80% and 100% ETc but, there was a positive significant difference between them and 60% ETc of irrigation amount. Meanwhile, EL decline in 80% or 100% ETc of irrigation amount compared to 60% ETc. These results are in agreement with the findings of Ludong (2008) on broccoli; Wu *et al.* (2012); Hadi and Fuller (2013) on cauliflower; Alam *et al.* (2013) on mustard; Aldesuquy and Ghanem (2015); Malik *et al.* (2015) on wheat; Abd El-Mageed and Semida (2015) on cucumber.

Foliar application of ascorbic acid increased RWC, MSI and reduced EL (Table 5). This indicates that foliar application of ascorbic acid probably reflected positive influence of water uptake or reduced water loss, more accumulation of compatible osmolytes such as total free amino acids, enhance head ascorbic acid content and increase DPPH radical-scavenging activity (Tables 7 and 8) which consequently causes increase in leaf water potential. Hence, it could be concluded that the beneficial effect of ascorbic acid on growth parameters of broccoli plants has been related to the efficiency of their water uptake and utilization also its role in accumulation of osmolytes. Many studies have reported that exogenous application of ascorbic acid when used with optimal concentration, exhibited beneficial effect on growth parameters of some crop plants grown under drought stress (Darvishan *et al.,* 2013; El-Sayed and El-Sayed, 2013; Alam *et al.,* 2014; Amira and Qados, 2014; Malik *et al.,* 2015).

5.3 Leaf photosynthetic pigments content

A reversal relationship between leaf chlorophyll A, B, A + B and anthocyanin contents and irrigation amount. The reversal relationship may be attributed to increasing irrigation amount led to increased leaf RWC, MSI and increased EL (Table 5), so the chlorophyll A, B, A+ B and anthocyanin content decreased. Meanwhile, foliar application of ascorbic acid reflected positive significant influences on leaf anthocyanin content. The proposed mechanism behind anthocyanin-enhanced drought resistance is that anthocyanin is able to stabilize water potential (Tahkokorpi, 2010). Syvacy and Sokmen (2004) mentioned that, anthocyanin acts as scavengers of free radicals and other oxidative species through their antioxidant potential. The beneficial effect of irrigation amount at 80% ETc on carotenoids content (Table 6) can be due to α-carotene, an effective antioxidant pigment, plays a unique role in protecting chlorophyll (Burton and Ingold, 1984). The main protective role of α-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and therefore avoids oxidative stress completely. In this respect, some investigators reported that the drought resulted in a massive increase significantly in the anthocyanin and β-carotene content of plants (Aldesuquy and Ghanem, 2015; Halimeh *et al.*, 2013). Whilst, Shafiq *et al.* (2014) confirmed that foliar applied ascorbic acid (50, 100 and 150 mg L^{-1}) had no significant effect in improving chlorophyll *a* and *b* contents of both canola (*Brassica napus* L.) cultivars Dunkeld and Cyclone.

5.4 Osmoprotectants and antioxidant activity

Plants can partially protect themselves against mild drought stress by accumulating osmolytes such as proline, soluble sugars, total free amino acids and ascorbic acid. Proline is one of the most common compatible osmolytes in drought stressed plants. In Table 7, leaf free proline content increased under drought stress in broccoli plants. These findings are in agreement with some earlier studies in which enhanced accumulation of proline under drought was observed by Erken *et al.* (2013) on broccoli; Alam *et al.* (2013) on mustard; Razaji *et al.* (2014) on rapeseed. The accumulation of proline in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress (Routley, 1966). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al.* 2002). On other side, in Table 7, proline non-significant effect of foliar-applied ascorbic acid, in this connection, Shafiq *et al.* (2014) confirmed that foliar-applied ascorbic acid had no significant effect in proline contents of canola (*Brassica napus* L.).

The reduction in value of DPPH in broccoli leaves grown under 60% ETc may be attributed to exposure to severe drought stress compared to broccoli plants grown under 80% or 100% ETc of irrigation amount. On other side, increased value of DPPH sprayed by ascorbic acid may be attributed to increasing in leaf ascorbic acid and total free amino acids content (Table 8).

Soluble sugars accumulated in plants for osmotic adjustment in response to drought stress and caused the protection of macromolecules and DNA structures (Juan *et al.* 2005). Therefore, in this study showed that drought stress increased total soluble sugars in Table 8, so that the resulting osmotic pressure

could keep the plant from losing water. The osmotic pressure occurs in cells as a result of decomposition of insoluble sugars, therefore, imposing drought stress increases soluble sugars while it decreases insoluble sugars (Halimeh *et al.,* 2013). Drought stress in spinach leaves led to an increase in starch decomposition and accumulation of soluble sugars (Gossett *et al.,* 1999). It is also reported that in plants adapted to drought stress conditions, sugar accumulation may increase photosynthesis (Sato *et al.*, 2004). Soluble sugar contents in shoots and roots of the control, there are various levels of drought stress increased, and this agrees with the findings of (Amirjani and M. Mahdiyeh, 2013; Kabiri *et al.*, 2014).
Increasing the amount of irrigation water from 60 to 80% ETc (Table 8), significantly, increased leaf total free amino acids content and above that level leaf total free amino acids content was not affected. This result was in line with Amira and Qados (2014) who mentioned that increasing the irrigation amount from 40 to 100% ETc caused increases in seed total amino acids of soybean. The obtained results, also, showed that spraying 200 and/or 400 mg l^{-1} ascorbic acid significantly augment leaf total amino acids than untreated control and the same conclusion was reported by El-Sayed and El-Sayed (2013).

Ascorbic acid is a key nonenzymatic antioxidant that participates in redox regulation in different cell compartments to protect plant cells from oxidative stress (Chen *et al.,* 2007). Ascorbic acid reduces O_2 and regenerates reduced α-tocopherol (Bartoli *et al.,* 1999). In addition, ascorbic acid suppressed free radicals by the formation of ascorbyl radicals (Yamaguchi *et al.* 1999). The ene-diol structure plays an effective role in scavenging free radicals (Pavet *et al.* 2005). Therefore, spraying broccoli plants by ascorbic acid increased ascorbic acid content in heads, this led to increased ability of plants to tolerate drought stress, especially for plants grown under 80% ETc of irrigation water amount (Table 8). Such results are agreement with this obtained by El-Sayed and El-Sayed (2013).in tomato; Osman and El-Shatoury (2014) in pumpkin; Alam *et al*, (2014) in *Brassica napus*.

5. Conclusions

Within the experimental conditions studied, it has been concluded that this work provided evidence to the role of ascorbic acid foliar application, especially at the concentration of 200 or 400 mg l^{-1} enhancing morphological characters, leaf tissue health measurements (RWC, MIS and EL), photosynthetic pigments, osmoprotectants, antioxidant activity of broccoli (*Brassica oleracea* L. var. *italica*) cv. Groene Calabrese grown under deficit irrigation amount, especially at 80% ETc under conditions of Fayoum Governorate and other similar regions.

6-REFERENCES

Abd El-Mageed, T.A., Semida, W.M., 2015. Organo mineral fertilizer can mitigate water stress for cucumber production (*Cucumis sativus* L.). Agric. Water Manag. 159, 1–10. http://dx.doi.org/10.1016/j.agwat.2015.05.020

Abdalla, M.M., 2011. Beneficial effects of diatomite on the growth, the biochemical contents and polymorphic DNA in *Lupinus albus* plants grown under water stress. Agric. Biol. JN Am 2, 207–220. doi:10.5251/abjna.2011.2.2.207.220

Alam, M.M., Hasanuzzaman, M., Nahar, K., Fujita, M., 2013. Exogenous salicylic acid ameliorates short-term drought stress in mustard (*Brassica juncea* L.) seedlings by up-regulating the antioxidant defense and glyoxalase system. Aust. J. Crop Sci. 7, 1053–1063.

Alam, M.M., Nahar, K., Hasanuzzaman, M., Fujita, M., 2014. Alleviation of osmotic stress in *Brassica napus, B. campestri*s and *B. juncea* by ascorbic acid application. Biol. Plant. 58, 697–708. doi:10.1007/s10535-014-0447-0

Aldesuquy, H., Ghanem, H., 2015. Exogenous salicylic acid and trehalose ameliorate short term drought stress in wheat cultivars by up-regulating membrane characteristics and antioxidant defense system. J. Hortic. 2: 1-10 http://dx.doi.org/10.4172/2376-0354.1000139.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation and Drainage Paper 56. FAO, Rome 300 pp.

Amin, B., Mahleghah, G., Mahmood, H.M.R., Hossein, M., others, 2009. Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on some of physiological and biochemical parameters in okra (*Hibiscus esculentus* L.). Res J Biol Sci 4, 380–387.

Amira, M.S., Qados, A., 2014. Effect of ascorbic acid antioxidant on soybean (*Glycine max* L.) plants grown under water stress conditions. Int. J. Adv. Res. Biol. Sci. 1, 189–205.

Amirjani, M.R., Mahdiyeh, M., 2013. Antioxidative and biochemical responses of wheat to drought stress. ARPN J. Agric. Biol. Sci. 8, 291–300.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris* L. Plant Physiol. 24, 1-5.

Arrigoni, O., 1994. Ascorbate system in plant development. J. Bioenerg. Biomembr. 26, 407–419. doi:10.1007/BF00762782

Bakry, A.B., Abdelraouf, R.E., Ahmed, M.A., El Karamany, M.F., 2013. Effect of drought stress and ascorbic acid foliar application on productivity and irrigation water use efficiency of wheat under newly reclaimed sandy soil. J. Appl. Sci. Res 8, 4552–4558.

Barrs, H.D., Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15, 413–428.

Bartoli, C.G., Simontacchi, M., Tambussi, E., Beltrano, J., Montaldi, E., Puntarulo, S., 1999. Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. J. Exp. Bot. 50, 375–383. doi:10.1093/jxb/50.332.375

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39, 205–207. doi:10.1007/BF00018060

Beecher, C.W., 1994. Cancer preventive properties of varieties of *Brassica oleracea*: a review. Am. J. Clin. Nutr. 59, 1166 – 1170.

Boutraa, T., 2010. Improvement of water use efficiency in irrigated agriculture: A Review. J. Agron. 9, 1–8. doi:10.3923/ja.2010.1.8

Burton, G.W., Ingold, K.U., 1984. Beta-Carotene: an unusual type of lipid antioxidant. Science 224, 569–573. doi:10.1126/science.6710156

Chen, K.M., Gong, H.J., Wang, S.M., Zhang, C.L., 2007. Antioxidant defense system in *Phragmites communis* Trin. Ecotypes. Biol. Plant. 51, 754–758. doi:10.1007/s10535-007-0154-1

Cordoba-Pedregosa, M., Gonzalez-Reyes, J.A., Canadillas, M., Navas, P., Cordoba, F., 1996. Role of apoplastic and cell-wall peroxidases on the stimulation of root elongation by ascorbate. Plant Physiol. 112, 1119–1125.

Darvishan, M., Tohidi-moghadam, H.R., Zahedi, H., 2013. The effects of foliar application of ascorbic acid (vitamin C) on physiological and biochemical changes of corn (*Zea mays* L) under irrigation withholding in different growth stages. Maydica Maydica electronic publication, 58, 195-200. ww.maydica.org/articles/58_195.pdf

Dolatabadian, A., Ali, S., Modarres, M., Asilan, K.S., 2010. Effect of ascorbic acid foliar application on yield, yield component and several morphological traits of grain corn under water deficit stress conditions. Not. Sci. Biol. 2, 45–50.

Dolatabadian, A., Modarres Sanavy, S.M., Sharifi, M. 2009. Alleviation of water deficit stress effects by foliar application of ascorbic acid on *Zea mays* L. J Agron Crop Sci. 195, 347–355. doi:10.1111/ j.1439-037X.2009.00382.x

Doorenbos, J., Pruitt, W.O., 1992. Crop water requirements. FAO Irrigation and Drainage. Paper, No. 24, FAO, Rome, Italy, 144 pp.

Dubey, R.S., Rani, M., 1989. Influence of NaCl Salinity on Growth and Metabolic Status of Protein and Amino Acids in Rice Seedlings. J. Agron. Crop Sci. 162, 97–106.

Elade, Y., 1992. The use of antioxidants (free radical scavengers) to control grey mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiomm*) in various crops. Plant Pathol. 41, 417–426.

El-Sayed, H.E.A., El Sayed, A., 2013. Exogenous application of ascorbic acid for improve germination, growth, water relations, organic and inorganic components in tomato (*Lycopersicon esculentum* Mill.) plant under salt-stress. New York Sci. J., 6, 123–139.

Erken O., C. O. Kuzucu, R. Cakir, 2013. Impact of different water supply levels on yield and biochemical ingredients in broccoli. Bulgarian Journal of Agricultural Science, 19 (5): 1009-1017

Erken, O., Oztokat, C., 2010. Effects of water stress on yield and some quality parameters of broccoli. In: 2nd International Symposium on Sustainable Development. science_book_p231-p237. http://eprints.ibu.edu.ba/id/eprint/410

Foyer, C.H. 1993. Ascorbic acid. In. R.G.Alscher and J.L.Hess (eds.) Antioxidants in higher plants. pp 31-58. CRC Press, Inc. Florida.

Gossett, S.W. Banks and M.C. Lucas, D.R., 1999. Antioxidant responses to osmotic stress in cotton. Plant physiol 12, 805–809.

Gupta, P.K., Nadgir, A.L., Mascarenhas, A.F., Jagannathan, V., 1980. Tissue culture of forest trees: clonal multiplication of *Tectona grandis* L.(teak) by tissue culture. Plant Sci. Lett. 17, 259–268.

Hadi, F., Fuller, M.P., 2013. Chemically Induced Mutants of *Brassica oleracea* var. *botrytis* Maintained Stable Resistance to Drought and Salt Stress after Regeneration and Micropropagation. Am. J. Plant Sci. 4, 498–507. doi:10.4236/ajps.2013.

Halimeh, R., Mahlagh, G., Maryam, P., Pazoki, A., 2013. Effect of drought interactions with ascorbate on some biochemical parameters and antioxidant enzymes activities in *Dracocephalum moldavica* L. Middle-East J. Sci. Res. 13, 522–531.

Hasheminasab, H., Taghi Assad, M., Aliakbari, A., Rasoul Sahhafi, S., 2012. Influence of Drought Stress on Oxidative Damage and Antioxidant Defense Systems in Tolerant and Susceptible Wheat Genotypes. J. Agric. Sci. 4, 20–30. doi:10.5539/jas.v4n8p20

Hayat, S., Ali, B., Aiman Hasan, S., Ahmad, A., 2007. Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ. Exp. Bot. 60, 33–41. doi:10.1016/j.envexpbot.2006.06.002

Helrich, K.C., 1990. Official Methods of Analysis. Vitamin C (Ascorbic Acid). 15th Edition. Association of Official Analytical Chemists. Benjamin Franklin Station, Washington, DC, USA. 1058 – 1059.

Hussein, Z.K., Khursheed, M.Q., 2014. Effect of foliar application of ascorbic acid on growth, yield components and some chemical constituents of wheat under water stress conditions. Jordan J. Agric. Sci. 10, 1-15.

Irigoyen, J.J., Einerich, D.W., Sánchez-Diaz, M., 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativd*) plants. Physiol. Plant. 84, 55–60.

Israelsen, O.W., Hansen, V.E., 1962. Irrigation principles and practices. Soil Sci. 95, 218.

Jones, H.G., 2004. What is water use efficiency? In: Bacon, M.A. (Ed.), Water Use Efficiency in Plant Biology. Blackwell Publishing, Oxford, UK, pp. 27–41.

Juan, M., Rivero, R.M., Romero, L., Ruiz, J.M., 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. Environ. Exp. Bot. 54, 193–201. doi:10.1016/j.envexpbot.2004.07.004

Kabiri, R., Nasibi, F., Farahbakhsh, H., 2014. Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in Nigella sativa plant under hydroponic culture. Plant Prot. Sci. 50, 43–51.

Khalil, S.E., Nahed, G., Azizi, A., Bedour, L.A.H., 2010. Effect of water stress and ascorbic acid on some morphological and biochemical composition of *Ocimum basilicum* plant. J. Am. Sci. 6, 33–44.

Khan, M.A.M., Ulrichs, C., Mewis, I., 2010. Influence of water stress on the glucosinolate profile of *Brassica oleracea* var. *italica* and the performance of *Brevicoryne brassicae* and *Myzus persicae*. Entomol. Exp. Appl. 137, 229–236. doi:10.1111/j.1570-7458.2010.01059.x

Latimer, J.G., 1990. Drought or mechanical stress affects broccoli transplant growth and establishment but not yield. HortScience 25, 1233–1235.

Lawlor, D.W., Cornic, G., 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell Environ. 25, 275–294. doi:10.1046/j.0016-8025.2001.00814.x

Lee, S.C., Kim, J.H., Jeong, S.M., Kim, D.R., Ha, J.U., Nam, K.C., Ahn, D.U., 2003. Effect of far-infrared radiation on the antioxidant activity of rice hulls. J. Agric. Food Chem. 51, 4400–4403.

Ludong, D. P. M. 2008. Effects of irrigation rate on the growth, yield, nutritive value, and water use efficiency of Carrot (*Daucus carota*) and Broccoli (*Brasiola oleracea*). Ms. Thesis, Fac. of Agric. Curtin University of Technology, Australia.

Maggio, A., Miyazaki, S., Veronese, P., Fujita, T., Ibeas, J.I., Damsz, B., Narasimhan, M.L., Hasegawa, P.M., Joly, R.J., Bressan, R.A., 2002. Does proline accumulation play an active role in stress-induced growth reduction? Plant J. 31, 699–712. doi:10.1046/j.1365-313X.2002.01389.x

Malik, S., Ashraf, M., Arshad, M., Malik, T.A., 2015. Effect of Ascorbic Acid Application on Physiology of Wheat Under Drought Stress. Pak. J. Agri. Sci 52, 209–217.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H., 2003. Antioxidant and antiproliferative activities of strawberries. J. Agric. Food Chem. 51, 6887–6892.

Navas, P., Gomezdiaz, C., 1995. Ascorbate free radical and its role in growth control. Protoplasma 184, 8–13.

Noman, A., Ali, S., Naheed, F., Ali, Q., Farid, M., Rizwan, M., Irshad, M.K., 2015. Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (*Zea mays* L.) cultivars under drought stress. Arch. Agron. Soil Sci. 61, 1659–1672. doi:10.1080/03650340.2015.1028379

Nonnecke, I.L., 1989. Vegetable Production. Van Nostrand Reinhold, New York: Springer Science & Business Media. 657 pp.

Osman, A.Sh., 2010. Efficiency of growth and productivity of leek plants as affected by organic manure and ascorbic acid under saline reclaimed soil conditions. Egypt. J. Hort., 37, 65–83.

Osman, A.Sh., El-Shatoury, R.S., 2014. Stimulation growth and productivity of *Cucurbita moschata* under reclaimed saline soil condition by using sulphur soil application and ascorbic acid foliar spray. J. Plant Production, Mansoura Univ., 5, 1703- 1715.

Osman, A.Sh., Tolba, M.S., 2009. Alleviate the harmful effects of saline reclaimed soil conditions on growth and productivity of onion plants using ascorbic acid and organic fertilizer. Fayoum J. Agric. Res. Dev., 23, 10–27.

Pasakdee, S., Banuelos, G., Shennan, C., Cheng, W.X., 2006. Organic N fertilizers and irrigation influence organic broccoli production in two regions of California. J. Veg. Sci., 12, 24–46. doi:10.1300/J484v12n04

Pavet, V., Olmos, E., Kiddle, G., Mowla, S., Kumar, S., Antoniw, J., Alvarez, M.E., Foyer, C.H., 2005. Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol., 139, 1291–1303. doi:pp.105.067686/r10.1104/pp.105.067686

Pereira, L.S., Oweis, T., Zairi, A., 2002. Irrigation management under water scarcity. Agric. Water Manage. 57, 175 –206.

Podsedek, A., 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT-Food Sci. Technol. 40, 1–11.

Prusky, D., 1988. The use of antioxidants to delay the onset of anthracnose and stem end decay in avocado fruits after harvest. Plant Dis. 72, 381 – 384.

Raven, E.L., 2002. Peroxidase-catalyzed oxidation of ascorbate structural, spectroscopic and mechanistic correlations in ascorbate peroxidase, in: Enzyme-Catalyzed Electron and Radical Transfer Subcellular Biochemistry, 35, 317–349.

Razaji, A., Farzanian, M., Sayfzadeh, S., 2014. The effects of seed priming by ascorbic acid on some morphological and biochemical aspects of rapeseed (*Brassica napus* L.) under drought stress condition. Int J Biosci. 4, 432–442. http://dx.doi.org/10.12692/ijb/4.1.432-442

Routley, D.G., 1966. Proline Accumulation in Wilted Ladino Clover Leaves1. Crop Sci. 6, 358-361. doi:10.2135/cropsci1966.0011183X000600040019x

Rozek, S., Wojciechowska, R., 2005. Effect of urea foliar application and different levels of nitrogen in soil on broccoli head yield and its quality in autumn growing cycle. Sci. Work. Lith. Inst. Hortic. Lith. Univ. Agric. Hortic. Veg. Grow. 24, 291–301.

Sairam, R., 1994. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. Indian J. Exp. Biol. 32, 584–592.

Sairam, R.K., Deshmukh, P.S., Saxena, D.C., 1998. Role of antioxidant systems in wheat genotypes tolerance to water stress. Biol. Plant. 41, 387–394.

Sato, F., Yoshioka, H., Fujiwara, T., Higashio, H., Uragami, A., Tokuda, S., 2004. Physiological responses of cabbage plug seedlings to water stress during lowtemperature storage in darkness. Sci. Hortic. (Amsterdam). 101, 349–357. doi:10.1016/j.scienta.2003.11.018

Shafiq, S., Akram, N.A., Ashraf, M., Arshad, A., 2014. Synergistic effects of drought and ascorbic acid on growth, mineral nutrients and oxidative defense system in canola (*Brassica napus* L.) plants. Acta Physiol. Plant. 36, 1539–1553. doi:10.1007/s11738-014-1530-z

Sies, H., Stahl, W., 1995. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am. J. Clin. Nutr. 62, 1315–1321.

Smirnoff, N., Wheeler, G.L., 2000. Ascorbic acid in plants: biosynthesis and function. Crit. Rev. Biochem. Mol. Biol. 35, 291–314. doi:10.1080/07352680091139231

Snedecor, G.W., Cochran, W.G., 1980. Statistical Methods. 7th ed., Iowa State Univ. Press, Ames, Iowa, USA.

Sullivan, C.Y., Ross, W.M., 1979. Selecting for drought and heat resistance in grain sorghum. Stress Physiol. Crop Plants 263–281.

Syvacy, A., Sokmen, M., 2004. Seasonal changes in antioxidant activity, total phenolic and anthocyanin constituent of the stems of two Morus species (*Morus alba* L. and *Morus nigra* L.). Plant Growth Regul. 44, 251–256. doi:10.1007/s10725-004-4500-4

Tahkokorpi, M., 2010. Anthocyanins under drought and drought-related stresses in bilberry (*Vaccinium myrtillus* L.). Acta Univ. Ouluensis A Sci. Rerum Nat. 556, 1-46.

Thompson, H. C., Kelly, W. C. 1957. Vegetable Crops. 5th Ed. Mc Grew Hill Book Company, Inc. New York, 611 pp.

Verhoeven, D.T., Goldbohm, R.A., van Poppel, G., Verhagen, H., van den Brandt, P.A., 1996. Epidemiological studies on Brassica vegetables and cancer risk. Cancer Epidemiol. Biomarkers Prev. 5, 733–748.

Wallace, D.H., Munger, H.M., 1965. Studies of the physiological basis for yield differences. I. Growth analysis of six dry bean varieties. Crop Sci. 5, 343-348. doi:10.2135/cropsci1965.0011183X000500040018x

Wilde, S.A., Corey, R.B., Lyer, J.G., Voight, G.K., 1985. Soil and Plant Analysis for Tree culture. Oxford and IBM Publishers. New Delhi. India. 3rd ed. 93 – 106 pp.

Wojciechowska, R., Ŝ, S.R., Rydz, A., 2005. Broccoli yield and its quality in spring growing cycle as dependent on nitrogen fertilization. Folia Hort 17, 141–152.

World Bank, 2006. Directions in Development. Reengaging in Agricultural Water Management: Challenges and Options. The International Bank for Reconstruction and Development/The World Bank, Washington, DC, pp. 218.

Wu, H., Wu, X., Li, Z., Duan, L., Zhang, M., 2012. Physiological evaluation of drought stress tolerance and recovery in cauliflower (*Brassica oleracea* L.) seedlings treated with Methyl Jasmonate and Coronatine. J. Plant Growth Regul. 31, 113–123. doi:10.1007/s00344-011-9224-x

Xu, P.L., Guo, Y.K., Bai, J.G., Shang, L., Wang, X.J., 2008. Effects of long-term chilling on ultrastructure and antioxidant activity in leaves of two cucumber cultivars under low light. Physiol. Plant. 132, 467–478.

Yamaguchi, F., Yoshimura, Y., Nakazawa, H., Ariga, T., 1999. Free radical scavenging activity of grape seed extract and antioxidants by electron spin resonance spectrometry in an H_2O_2/NaOH/DMSO system. J. Agric. Food Chem. 47, 2544–2548.

Yazdanpanah, S., Baghizadeh, A., Abbassi, F., 2011. The interaction between drought stress and salicylic and ascorbic acids on some biochemical characteristics of *Satureja hortensis*. African J. Agric. Res. 6, 798–807. doi:10.5897/AJAR10.405

Zaicovski, C.B., Zimmerman, T., Nora, L., Nora, F.R., Silva, J.A., Rombaldi, C.V., 2008. Water stress increases cytokinin biosynthesis and delays postharvest yellowing of broccoli florets. Postharvest Biol. Technol. 49, 436–439. doi:10.1016/j.postharvbio.2008.02.001

استجابة نمو، ونشاط مضادات الأكسدة وصحة أنسجة البروكلى لحمض الأسكوربيك تحت نظام الرى المتناقص

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أجرى هذا البحث خلال موسمين متتاليين (2013/2014 و 2014/2015) بمزرعة خاصة بمركز أبشواى، الفيوم، مصر لدراسة تأثير الرش الورقى لحمض الأسكوربيك (٠ و ٢٠٠ و ٤٠٠ مجم/ لتر ماء) على الصفات المورفولوجية، والقياسات الفسيولوجية لسلامة الخلايا (محتوى الماء النسبى للخلايا RWC، ودليل ثبات الأغشية MIS، وفقد الأيونات EL)، وصباغات البناء الضوئى وOsmoprotectants، ونشاط مضادات الأكسدة (DPPH) لنباتات البروكلى (*Brassica oleracea* L. var. *italica*; cv. Groene Calabrese) النامية تحت نظام الرى السطحى المتناقص (٦٠ و ٨٠ و ١٠٠٪ ETc) تحت ظروف أراضى الفيوم.

النتائج المتحصل عليها يمكن تلخيصها فى الآتى:

معدل الرى ٨٠٪ أو ١٠٠٪ ETc أعطى أعلى قيم متحصل عليها لطول وسمك الساق، عدد الأوراق / نبات، والمساحة الورقية الكلية / نبات ومكوناتها، الوزن الجاف الكلى / نبات ومكوناته، ومحتوى الماء النسبى للخلايا RWC، ودليل ثبات الأغشية MIS، ومحتوى الأوراق من الكاروتينات، وقيم DPPH والأحماض الأمينية الكلية ولم يكن هناك فرق معنوى بينهم فى كلا الموسمين، فى حين لم يتأثر عدد الأفرع / نبات، ومحتوى حمض الاسكوربيك للرؤوس بمعدلات الرى الثلاثة. ومن ناحية أخرى أدت زيادة معدلات الرى المستخدمة من ٦٠٪ إلى ١٠٠٪ ETc، أدت إلى حدوث نقص تنازلى فى قيم EL، محتوى الأوراق من كلوروفيل A، B، A + B و الأنثوسيانين، ومحتوى الأوراق من البرولين والسكريات الذائبة الكلية فى كلا الموسمين.

الرش الورقى بحمض الأسكوربيك بغض النظر عن التركيز المستخدم، عكس زيادة كبيرة فى طول وقطر الساق، ومساحة الأوراق ومكوناتها، والوزن الجاف/ نبات ومكوناته، وقيم RWC و MSI،

محتوى الأوراق من الأنثوسيانين، محتوى الرؤوس من حمض الأسكوربيك، وقيم DPPH، ومحتوى الأوراق من الأحماض الأمينية الكلية مقارنة بالنباتات غير المعاملة (الكنترول)، فى حين لم يتأثر عدد الأفرع / نبات فى كلا الموسمين. وعلى العكس من ذلك أدى زيادة تركيز حامض الاسكوربيك من صفر حتى ٤٠٠ مجم/لتر ماء إلى خفض فى قيم EL، لكلا الموسمين. فى حين لم يؤثر الرش بحمض الأسكوربيك بغض النظر عن التركيز المستخدم على عدد الأفرع / نبات، متوسط مساحة الورقة، ومحتوى الأوراق من الكلوروفيل A، B، A + B والكاروتنديات والبرولين والسكريات الذائبة الكلية في موسمى التجربة.

التداخل بين عاملى الدراسة (معدلات الرى وتركيزات حمض الأسكوربيك) عكس تأثير معنوى على الصفات المورفولوجية المدروسة (طول وقطر الساق، عدد الأوراق / نبات، مساحة الأوراق الكلية ومتوسط مساحة الورقة والوزن الجاف للنبات للسيقان والأوراق)، وقيم RWC و MSI، محتوى الأوراق من الكاروتنيدات وقيم DPPH فى كلا موسمين الزراعة. وكانت أفضل النتائج المتحصل عليها من التداخل بين معدل الرى ٨٠% أو ١٠٠% ETc مع ٢٠٠ أو ٤٠٠ مجم/لتر ماء من حمض الأسكوربيك. وعلى العكس من ذلك أدت إلى حدوث نقص فى قيم EL، ومحتوى الأوراق من الكلوروفيل A، B، A + B والأنثوسيانين. فى حين أعطى التداخل بين مياه الري بمعدل ٦٠٪ ETc وحامض الاسكوربيك بتركيز ٤٠٠ مجم/لتر ماء القيم الأعلى من محتوى الرؤوس من حامض الاسكوربيك فى كلا الموسمين. بينما لم يكن للتداخل بين عاملى الدراسة أى تأثير على محتوى الأوراق من البرولين، والسكريات الذائبة الكلية، والأحماض الأمينية الكلية.

في ضوء النتائج السابقة يمكن الاستنتاج أن الرش الورقى لحمض الأسكوربيك خاصة بتركيز ٢٠٠ أو ٤٠٠ مجم/لتر ماء دور إيجابيا على الصفات المورفولوجية، RWC، MIS، EL، وصباغات البناء الضوئى، Osmoprotectants، ونشاط مضادات الأكسدة (DPPH) لنباتات البروكلى النامية تحت نظام الرى السطحى المتناقص خاصة عند معدل رى ٨٠% Etc تحت الظروف البيئية السائدة بمحافظة الفيوم والمناطق المشابهة الأخرى.