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Influencing of Water Stress and Micronutrients on Physio-Chemical Attributes, Yield and Anatomical Features of Common Bean Plants (*Phaseolus vulgaris* L.)

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WATER stress is the most destructive agent which extreme yield productivity of many crops such as common bean. Foliar applications with micronutrients are needed to alleviate the effects of water stress. Pots experiments were carried out during the two growing summer seasons of 2015 and 2016 under greenhouse conditions at the Experimental Station of the Agricultural Botany Department, Fac. Agric., Zagazig Univ., Sharkia Governorate, Egypt to investigate the effect of foliar application of micronutrients as Fe and Zn on plant vegetative criteria, some physiological and biochemical properties, anatomical structure of leaf blade as well as yield and its components of common bean plants (*Phaseolus vulgaris* L.) cv. Giza 3 grown under water stress conditions. The results reported that, all vegetative growth characters expressed as plant height (cm), number of both leaves and branches/plant, leaf area (cm^2) and dry weight of shoot (g) were significantly decreased under water stress.While, the foliar application with Fe appeared topartly overcome the hurtful effects of water stress on the aforementioned growth criteria of common bean plants.There was significant decrease in photosynthetic pigments, nitrogen, crude protein, phosphorus and potassium in leaves was noticed due to drought stress treatments; *i.e.,* 18 days irrigation (W3) compared with control plants; *i.e.,* 6 days irrigation (W1). In reverse,water stress increased significantly the Malondialdehyde (MDA) and phenolic compounds in leaves, where as yield and its components as well as anatomical features of leaflet blade; *i.e.,* blade thickness, palisade and spongy tissue thickness, length and width midvein, phloem and xylem tissue thickness and diameter of xylem vessel were significantly decreased by water stress and the highest values were obtained under control (W1) followed by; *i.e.,* 12 days irrigation (W2) and the lowest were obtained under (W3), although spraying plants with Fe improved plant yield and all above mentioned anatomical characters by partly overcome the harmful effects of water stress.

Keywords: Common bean plants, Iron and zinc sulphate, Water stress, Biochemical analysis, Leaf anatomy.

Introduction

Commonbean (*Phaseolus vulgaris* L.) is one of the most important legumes, which have the important role in the human diet, contains protein, vitamins and fibers that increased food value and contribute an important part of human requirement of protein (Sadeghipour & Aghaei, 2012 and Akhshi et al., 2014). The amount of grain protein in legumes is about 2-4 times of cereal and 10-20 times of tuber plants (Alireza et al., 2014).

Water stress is lead to osmotic stress: it causes a primary decrease of cell water and reduction of cell osmotic potential. The decline of cell water content under water stress is due to water deficit in the soil (Vahdati & Leslie, 2013). Plants require to maintain internal water potential here under that of the soil to save turgor and water uptake for growth. This requires an increment in osmotic by uptake of soil solutes. The accumulating of soil solutes in response to some stresses is a greatly distributed phenomenon in the plants and considered as a basic for protection and survival of plants under abiotic stress (Zhonghua et al., 2007).

Water stress on vegetable production may be defined as one or more environmental factors that affect the plant and confine its growing causing a decrease in production. Water stress has counteractive effects on many plant processes such as transpiration, photosynthesis, allocation and accumulation of assimilate substances (Ohashi et al., 2006).

Water stress is effect on physiological characteristics of plants. In this respect, Sadeghipour & Aghaei (2012) illustrated that drought stress conditions reduced significantly the leaf relative water content of bean plants. They demonstrated that relative water content is related to root water

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absorption and loss of the water by transpiration. Exposure of plants under drought stress decreased the leaf water potential consequently leaf relative water content was decreased considerably since that both of these conditions increase the leaf temperature. Upkeep capacity of photosynthetic and chlorophyll content of leaf considered under drought stress as the physiological parameters involved in drought tolerance and cultivars have greater drought resistance showing higher chlorophyll content. Drought stress leads to increase production of reactive oxygen species in plants resulted in decreasing of chlorophyll content, indicating the extent of the oxidative damages. This decrease may be also due to chlorophyll biosynthesis pathway inhibition (Lalinia et al., 2012). Increasing irrigation intervals and drought stress conditions significantly decreased leaf chlorophyll and carotenoid in mungbean (Thalooth et al., 2006).

Under drought stress conditions due to reduction of soil water content and nutrient reduction distribution in soil, roots nutrient uptake decreased, consequently the nutrients transfer from roots to shoots will be reduced. The reason of this loss is injury active carriers and loss flexibility root cell membrane (Alexieva et al., 2001). So that the nutrients deficiency is one of the most important factors, limiting plant growth under water stress. Plants have enough nutrition can be raising level of plants tolerance against a variety of environmental stresses and in this regard, iron and zinc are the most important essential micronutrients in plant nutrition (Baybordy & Mamedov, 2010). In this respect Akbari et al. (2013) indicated that micronutrients fertilizer could increase plant resistance to drought stress. Metal ions such as iron, copper, manganese, zinc, and magnesium as a cofactor share in creation of many antioxidant enzymes.Cakmak et al. (2010) showed that, deficiency of micronutrients decrease antioxidant enzyme activities and thus increase the sensitivity of plants to environmental stresses. Thalooth et al. (2006) illustrated that exogenous application of zinc sulfate under water stress conditions enhanced growth, yield and its components of mung bean plant. Experimental results of Odeley & Animashaun (2007) also showed that foliar application of micronutrients increased the soybean yield, quality, resistance to drought stress. They indicated that micronutrients plays an important role in growth and development of plants although the need of plants to these nutrients is very little. So that the micronutrients such as copper, boron, iron, zinc and manganese have many contributions in cell wall formation and plant resistance to an environmental stresses.

The aim of this study was to evaluate the effects of foliar application of iron and zinc sulfate, individually on vegetative criteria, some physiological and biochemical properties, anatomical structure of leaf blade as well as yield components of common bean plants grown under different irrigation intervals.

Matrials and Methods

Pots experiments were conducted during the two successive growing summer seasons of 2015 and 2016, under greenhouse conditions at the Experimental Station, Fac. Agric., Zagazig Univ., Sharkia Governorate, Egypt, to study the effect of foliar application with Fe as $FeSO_4$ and Zn as $ZnSO_4$ on plant vegetative criteria, some physiological and biochemical properties, leaf blade anatomical structure as well as yield and its components of common bean plants (*Phaseolus vulgaris* L.) cv. Giza 3 grown under water stress conditions (irrigation intervals).

Common bean seeds were obtained from Vegetative Research Section, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt. Seeds were sown on the 2nd March in both seasons in plastic pots 45 cm inner diameter and 30 cm in depth. Each pot contained 13 kg of air dried clay soil. Physical and chemical properties in clay soil are presented in (Table 1).

TABLE 1. Physical and chemical properties of the investigated soil

Mechanical analyses			Chemical analyses											
			Cations mg/100g soil				Anions mg/100g soil							
Coarse sand %	Silt %	Clay %	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO3^{-}$	$HCO3^{-}$	Cl^{-}	$SO4^{-}$	E.C^{a+} 25c ds/m (mmhos/cm)	PH Soil reaction		WHC
45.38	28.75	25.87	2.98	1.7	2.5	0.1	0.00	0.5	1.15	5.68	2.88	7.62		33.54

This experiment included nine treatments (5 pots for each treatment), which were the combination between three irrigation intervals and three foliar spraying treatments. These treatments were distributed in split plot design with 3 replicates. Irrigation intervals were considered as the main plot with three levels including 6 days irrigation (W1), 12 days irrigation (W2) and 18 days irrigation (W3) during the growth stage, and the sub plot included three treatments of micronutrients; i.e., spraying with distilled water (control treatment), spraying with 500 ppm $FeSO_4$ and spraying with 500 ppm $ZnSO_4$. Eight seeds/pot for at equal distances and depths were sown. After two weeks from sowing, seedlings were thinned leaving two seedlings /pot.

Iron Sulfate ($FeSO_4$) and Zinc Sulfate ($ZnSO_4$)were obtained from Sigma chemical Co. USA.

The recommended agricultural practices of growing common bean plants were applied where phosphorus fertilizer in the form of calcium super phosphate (15.5% P_2O_5) was mixed with the soil before planting at the rate of 1.8 g/pot. While, potassium and nitrogen fertilizers were in the form of potassium sulphate (48% K_2O) and urea (46% N) added individually with water irrigation after thinning at the rate of 1.3 g/ pot for both.

Foliar applications of $FeSO_4$ and $ZnSO_4$were carried out three times using hand atomizer and wetting agent after 25, 35 and 45 days from sowing under W1, W2 and W3 water stress conditions. Control plants were sprayed with distilled water and the volume of the spraying solution of the tested micronutrients were maintained just to cover completely the plant foliage till drip.

Data recorded

A random sample of three plants was taken from each treatment at 55 days old at flowering stage during each growing season to record plant vegetative characters and physiological properties.

Plant vegetative criteria

Plant height (cm), number of both leaves and branches/plant, leaf area (cm^2) and dry weight of shoot (g) were recorded. Plant samples were dried using an electric oven with drift fan at 70°C for 48 h till a constant weight was reached.

Physiological and biochemical properties

Photosynthetic pigments

Disk samples from the fourth upper leaf were taken at 55 days after sowing to determine chlorophyll a, b and total chlorophyll (a+b) as well as carotenoid contents according to the method described by Wettestein (1957) then calculated as mg/g fresh weight.

Phenolic compounds

Free, bound and total phenols were determined in common bean fresh leaves using the colorimetric method described by Snell & Snell (1953).

Malondialdehyde (MDA) analysis

The method defined by Lutts et al. (1996) was employed for measuring the amount of (MDA), which is produced as a result of lipid per oxidation causing damage to cellular membrane. MDA concentration was determined by using "extinction" coefficient, which is 155 mM/cm^3, in mmol/g fresh weight. The following equation was used in the calculation:

MDA= (A 523 - A 600) x volume of the extract (ml) / (155 mM/cm^3 x sample amount).

Macroelements and protein concentrations

Nitrogen, phosphorous and potassium concentrations were determined in common bean dried shoots (70ºC till constant weight) according to AOAC (2002). Crude protein was calculated based on total Nmultiplying by 6.25.

Yield and its components

At harvesting stage, three random plants per treatment were taken to record the values of yield and its components; *i.e.,* number of seeds /pod, number of pods / plant, number of seeds / plant, 100 seed weight (g) and seed yield /plant (g).

Leaf anatomy

From the findings in the first season (2015) regarding the growth, physiological and yield characters of common bean plants, it was found that the foliar spray with iron sulfate gave higher increases in the studied characters compared to zinc sulfate, consequently iron was chosen for studying the anatomical structure of leaf during the second season (2016). Specimens of selected treatments after 55 days from sowing (flowering stage) during the second season (2016) were taken from the terminal leaflet of the corresponding leaf.

Microtechnique procedures given by Nassar & El-Sahhar (1998) were followed. These specimens were killed and fixed for at least 48 h in FAA (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series, embedded in paraffin wax of 56^{o}C melting point, Sectioned to a thickness of 20 microns, double stained with safranin and light green, cleared in xylene and mounted in canada balsam. Sections were examined to detect histological manifestations of the chosen treatments and photomicrographed.

Statistical analysis

Data of the present work were statically analyzed and the differences between the means of the treatments were considered significant when they were more than the least significant differences (L.S.D) at the 5% level by using computer program of Statistix version 9 (Analytical software, 2008).

Results and Discussion

Growth characters

Results in Table 2 indicate that the effect of water stress and foliar application of micronutrients on all investigated growth criteria; plant height, number of both leaves and branches/plant, leaf area/ plant and dry weight of shoots of common bean plants were significant. All the studied characters were significantly decreased with increasing the irrigation intervals, especially when plants were irrigated every 18 days (W3) as compared to control plants which irrigated every 6 days (W1).

Generally, foliar application of both micronutrients (iron and zinc sulfate) gave the maximum values compared to control plants (distilled water). Iron confirmed to be more effective than zinc to reduce the hurtful effect of water stress with respect to the interaction, the most efficient treatment existed among irrigation every 6 days and foliar spray with iron recorded the best values (34.65cm, 16.67, 8.67, 74.93cm^2 and 9.48g) of plant height, number of leaves and branches/plant, leaf area/ plantand dry weight of shoots, respectively. While, the minimum values (18.28 cm, 5.67, 3.33, 37.36 cm^2 and 4.21 g), respectively were registered with plants irrigation every 18 days combined with foliar spray with distilled water.The enhancement of growth of common bean plants in response to foliar application of iron and zinc sulfate may result in improving quantity and quality of pods such as increased protein and chlorophyll content.

These results could be ascribed to the effective role of iron and zinc sulfate in controlling different enzymes activities, photosynthetic pigments formation and increased protein, consequently affecting plant growth. Katyal & Randhawa (1983) reported that the corroborative effect of iron could be articulated as Fe plays an important role in several basic processes in plant like photosynthesis and consequently affected plant growth. As well as, zinc is known to be directly complicated in biosynthesis on IAA hormone which stimulates cell division and elongation. The stimulating effect of Fe on dry weight/ plant could be imputed to that iron is a constituent of many enzymes connected with energy transfer, lignin formation and nitrogen reduction and fixation. Iron is connected with sulfur in plants to shape compounds that stimulate other reactions (Mortvedt et al., 1991). Srivastava & Goupta (1996) found that the effect of zinc on plant growth characters colud be due to its major role in some important metallic functions such as photosynthesis, respiration, a component of some enzymes, transport of carbohydrates, energy production, regulation of meristematic activity and protein metabolism. All functions would directly or indirectly participate to increase the plant growth.

The nutrients failure is one of the principal factors, limiting plant growth under drought. Plant have sufficient nutrition can be elevating level of plants toleration against different environmental stresses and in this concern, Fe and Zn are the most important vital micronutrients in plant nutrition (Baybordy & Mamedov, 2010). On mung bean plant, the foliar application of zinc sulfate under drought conditions enhanced growth (Thalooth et al., 2006).

Photosynthetic pigments

Statistical analysis of results revealed that the concentrations of photosynthetic pigments (chlorophyll a, b and total chlorophyll (a+b) and carotenoids) of common bean leaves tissues were significantly influenced by water stress levels and foliar application of micronutrients single or in combinations (Table 3). Irrigation of common bean every six days gave the highest values of chlorophyll a, b and total chlorophyll (a+b) and carotenoids in leaf tissues. Foliar application of micronutrients gave the highest values compared to control plants treated by distilled water. The results indicated that the highest concentrations of chlorophyll a, b, (a+b) and carotenoids in leaves tissues were recorded under irrigation every 6 days incorporated with iron foliar spray where, it recorded 1.685, 0.480, 2.165 and 1.722 mg/g FW, respectively, while plants irrigated every 18 days and foliar spray with distilled water gave the lowest results; 1.209, 0.302, 1.511 and 1.069 mg/g FW, respectively. In addition, these results indicated that spraying plants with iron and zinc sulfate diminished the harmful effect of drought on all above mentioned photosynthetic pigments. Water stress resulted in increment production of reactive oxygen radicals in plants leading to a decrease amount of chlorophyll contents, pointing out the degree of the oxidative damages. This reduction may be also caused by chlorophyll biosynthesis route prevention (Lalinia et al., 2012). In mung bean plant, increment irrigation intervals and water stress condition significantly reduced leaf chlorophyll and carotenoid (Thalooth et al., 2006). On common bean plants, Sadeghipour & Aghaei (2012) found that reduction of chlorophyll concentration is the major reason of reduced photosynthetic capacity under water stress. At the long-term of drought, dehydration of tissues leads to increment of oxidative processes that cause damaging of chloroplast structure, decreased chlorophyll, and finally minimize the photosynthetic activity (Singh, 2007).

TABLE 2. Effect of water stress, foliar application with micronutrients and their interactions on growth characters of common bean plants grown at 55 days from sowing (average of the two seasons of 2015 and 2016|).

Treatments	Characters	Plant height (cm)	No. of leaves	No. of branches	Leaf area (cm²)	Dry weight of shoot (g)
Effect of water stress						
W1 (6 days irrigation)		31.91 a	**16.11 a**	**8.33 a**	**74.33 a**	**9.12 a**
W2 (12 days irrigation)		25.94 b	**12.67 b**	**6.44 b**	**60.83 b**	**6.86 b**
W3 (18 days irrigation)		21.31 c	**7.44 c**	**4.11 c**	**40.93 c**	**4.39 c**
L.S.D. (0.05)		1.41	**0.91**	**0.40**	**0.40**	**0.33**
Effect of foliar spray with micronutrients						
Distilled water		24.12 c	10.89 c	5.44 b	56.33 c	6.40 c
Zn(500 ppm)		26.64 b	12.33 b	6.44 a	59.61 b	6.88 b
Fe (500 ppm)		28.39 a	13.00 a	7.00 a	60.15 a	7.08 a
L.S.D. (0.05)		0.78	0.61	0.66	0.45	0.13
Water stress	**Foliar spray**	**Effect of interaction**				
W1 (6 days irrigation)	Distilled water (control)	29.70 c	15.33 b	7.67 ab	73.48 b	8.74 c
	Zn (500ppm)	31.36 b	16.33 ab	8.67 a	74.58 a	9.14 b
	Fe (500ppm)	34.65 a	16.67 a	8.67 a	74.93 a	9.48 a
W2 (12 days irrigation)	Distilled water	24.38 e	11.67d	5.33 c	58.14 d	6.26 e
	Zn (500ppm)	26.17 d	12.67 cd	6.67 b	61.84 c	7.06 d
	Fe (500ppm)	27.29 d	13.67 c	7.33 b	62.50 c	7.27 d
W3 (18 days irrigation)	Distilled water	18.28 g	5.67 f	3.33 e	37.36 f	4.21 g
	Zn (500ppm)	22.40 f	8.00 e	4.00 de	42.41 e	4.45 f
	Fe (500ppm)	23.23 ef	8.67 e	5.00 cd	43.03 e	4.51 f
L.S.D. (0.05)		1.77	1.24	1.00	0.74	0.38

TABLE 3. Effect of water stress, foliar application with micronutrients and their interactions on photosynthetic pigments (mg/g fresh weight) of common bean plants at 55 days from sowing (average of the two seasons of 2015 and 2016).

Treatments \ Characters		Chl. a	Chl. b	Chl. (a+b)	carotenoids
Effect of water stress					
W1 (6 days irrigation)		1.65 a	0.47 a	2.12 a	1.68 a
W2 (12 days irrigation)		1.49 b	0.44 a	1.92 b	1.48 b
W3 (18 days irrigation)		1.28 c	0.36 b	1.64 c	1.20 c
L.S.D. (0.05)		0.110	0.042	0.153	0.153
Effect of foliar spray with micronutrients					
Distilled water		1.42 c	0.40 b	1.81 b	1.37 b
Zn (500ppm)		1.49 b	0.43 a	1.93 a	1.49 a
Fe (500ppm)		1.50 a	0.44 a	1.94a	1.50 a
L.S.D. (0.05)		0.010	0.021	0.025	0.025
Water stress	**Foliar spray**	**Effect of interaction**			
W1 (6 days irrigation)	Distilled water (control)	1.601 c	0.459 a	2.060 b	1.618 b
	Zn (500ppm)	1.665 b	0.476 a	2.141 a	1.698 a
	Fe (500ppm)	1.685 a	0.480 a	2.165 a	1.722 a
W2 (12 days irrigation)	Distilled water	1.443 d	0.430 ab	1.873 c	1.431 c
	Zn (500ppm)	1.507 c	0.435 ab	1.942 b	1.504 b
	Fe (500ppm)	1.511 c	0.442 ab	1.952 b	1.506 b
W3 (18 days irrigation)	Distilled water	1.209 f	0.302 c	1.5110 e	1.069 e
	Zn (500ppm)	1.308 e	0.391 b	1.698 d	1.256 d
	Fe (500ppm)	1.318 e	0.393 b	1.710 d	1.268 d
L.S.D. (0.05)		0.111	0.051	0.156	0.157

Yadavi et al. (2014) reported that Fe and Fe+Zn foliar application raised the quantity of total chlorophyll content compared to control in common bean leaves. These increases would be ascribed to the functional role of Fe and Zn in activation of enzymes that complicated in chlorophyll biosynthesis route and some antioxidant enzymes as glutathione reductase and ascorbate peroxidase in the pathway protection of chlorophyll reduction by the free active oxygen radicals (Ayad et al., 2010 and Zayed et al., 2011).

Macroelements and protein concentration

Results indicatedthat the effect of different irrigation intervales and exogenous application with micronutrients on macroelements and protein concentrations of common bean plant leaves were significant (Table 4). The concentrationsof N, P, K and crude protein were gradually decreased with increasing the drought stress, whereas, plants irrigation every 18 days gave the lowest values as compared to plants which irrigated every 6 days.On the other hand, these parameters of common bean plants leaves were increased after foliar application of iron and zinc sulfate compared to control plants (distilled water). The highest concentrations of N, P, K and crude protein were recorded when used the combination between (W1) and foliar with iron were (1.99, 2.493, 0.45 and 12.42%, respectively). While, irrigated plants every 18 days (W3) and foliar sprayed with distilled water gained the minimum concentrations of N, P, K and crude protein (1.15, 1.563, 0.273 and 7.21%, respectively).

TABLE 4. Effect of water stress, foliar application with micronutrients and their interactions on chemical constituents (%) of common bean leaves at 55 days from sowing (average of the two seasons of 2015 and 2016).

Treatments \ Characters		N	P	K	Crude protein
Effect of water stress					
W1 (6 days irrigation)		1.93 a	2.44 a	0.43 a	12.04 a
W2 (12 days irrigation)		1.66 b	2.02 b	0.34 b	10.39 b
W3 (18 days irrigation)		1.25 c	1.68 c	0.29 c	7.82 c
L.S.D. (0.05)		0.06	0.08	0.01	0.37
Effect of foliar spray with micronutrients					
Distilled water		1.53 c	1.96 c	0.34 b	9.56 c
Zn (500ppm)		1.62 b	2.06 b	0.36 a	10.12 b
Fe (500ppm)		1.69 a	2.12 a	0.37 a	10.58 a
L.S.D. (0.05)		0.05	0.04	0.02	0.29
Water stress	**Foliar spray**	**Effect of interaction**			
W1 (6 days irrigation)	Distilled water (control)	1.88 b	2.370 b	0.416 b	11.75 b
	Zn (500ppm)	1.91 ab	2.457 a	0.434 ab	11.96 ab
	Fe (500ppm)	1.99 a	2.493 a	0.453 a	12.42 a
W2 (12 days irrigation)	Distilled water	1.55 d	1.950 d	0.322 d	9.71 d
	Zn (500ppm)	1.68 c	2.030 c	0.349 c	10.50 c
	Fe (500ppm)	1.75 c	2.080 c	0.357 c	10.96 c
W3 (18 days irrigation)	Distilled water	1.15 f	1.563 g	0.273 f	7.21 f
	Zn (500ppm)	1.26 e	1.697 f	0.294 df	7.90 e
	Fe (500ppm)	1.34 e	1.783 e	0.306 de	8.35 e
L.S.D. (0.05)		0.09	0.077	0.026	0.55

Alam (1999) illustrated that water stress decreased both nutrients uptake by roots that transfered from roots to shoots, due to limited transpiration rates and damaged membrane permeability and active transport. The applied Zn improves NPK concentrations in leaves of the treated plants. Also, it might protect plasma membrane and its linked transporter enzymes against the harmful effects of oxidative stresses (Marschner, 1998). Because of that, it improves its transportation functions for other elements and solutes. Srivastava & Gupta (1996) reported that zinc is a component of some enzymes like proteinase, dehydrogenase, phosphohydrolase and peptidases important for metabolism of protein and phosphate. Drought stress inhibits cell amplification more than cell division. It minimizes plant growth by affecting different physiological and biochemical processes, such as nutrient metabolism and ions uptake (Farooq et al., 2008). El-Tayeb (2005) found that the free amino acids and soluble protein content in shoot were reduced in stress conditions. Spraying pea and garlic plants with Zn or Fe significantly incrementmineral content and uptake of N, P and K compared with the control (Mohammed et al., 1998 and Abou El-khair et al., 2011).

Malondialdehyde (MDA) and phenolic compounds

Results presented in Table 5 illustrate the effect of foliar application with micronutrients on malondialdehyde (MDA) and phenolic compounds of common bean plants grown under water stress. Irrigation intervals, foliar spray with micronutrients and interactions had no significant effect on phenolic compounds in leaves of common bean. Spraying plants with iron and zinc sulfate under different levels of drought showed significant differences for MDA, but wasn’t significant for phenolic compounds. The obtained results found that the maximum value of MDA (2.42µmol/g) recorded under drought stress level when plants irrigation every 18 days and spraying with distilled water. The lowest value of MDA (0.66μmol/g) was registered under irrigation every 6 days incorporated with iron sulfate foliar spray. Moreover, iron was more effective than zinc to reduce the harmful effectof drought, whereas iron decreased MDA over zinc compared to control plants (foliar sprayed by distilled water).

TABLE 5. Effect of water stress, foliar application with micronutrients and their interactions on malondialdehyde (MDAμmol/g) and phenolic compounds (mg/gm F. wt.) of common bean leaves at 55 days from sowing (average of the two seasonsof 2015 and 2016).

Treatments \ Characters		MDA	Free phenols	Bound phenols	Total phenols
		Effect of water stress			
W1 (6 days irrigation)		0.71 c	1.08	0.72	1.80
W2 (12 days irrigation)		1.55 b	1.23	0.80	2.02
W3 (18 days irrigation)		2.21 a	1.35	0.88	2.23
L.S.D. (0.05)		0.04	0.35	0.21	0.45
		Effect of foliar spray with micronutrients			
Distilled water		1.62 a	1.25	0.85	2.10
Zn (500ppm)		1.46 b	1.22	0.77	1.99
Fe (500ppm)		1.38 c	1.19	0.77	1.96
L.S.D. (0.05)		0.05	0.08	0.09	0.17
Water stress	**Foliar spray**	**Effect of interaction**			
W1 (6 days irrigation)	Distilled water	0.76 f	1.11	0.73	1.84
	Zn (500ppm)	0.70 fg	1.10	0.70	1.80
	Fe (500ppm)	0.66 g	1.05	0.71	1.76
W2 (12 days irrigation)	Distilled water	1.69 c	1.25	0.89	2.14
	Zn (500ppm)	1.56 d	1.23	0.75	1.98
	Fe (500ppm)	1.41 e	1.21	0.74	1.95
W3 (18 days irrigation)	Distilled water	2.42 a	1.40	0.93	2.33
	Zn (500ppm)	2.14 b	1.35	0.84	2.19
	Fe (500ppm)	2.06 b	1.31	0.86	2.17
L.S.D. (0.05)		0.07	0.51	0.32	0.55

Ahanger et al. (2014) illustrated that the production and accumulation of free radicals under stress conditions raised manifold and these free radicals trigger the peroxidation of unsaturated lipid component resulting in the decline of membrane integrity which finally leads to leakage and desiccation. Hura et al. (2006) found that water stress increased phenolic compounds in leaves. Phenolics modify optical properties of leaves and have possibility to defend photosynthetic apparatus during water stress.

Yield and its components

Analysis of results showed that the effect of the interaction between different levels of water stress and foliar application with iron and zinc sulfate were significant on yield and its components of common bean plants (Table 6). Data indicate that yield and its components; number of pods/plant, number of seeds/pod, number of seeds/plant, 100 seed weight and seed yield/ plant of common bean plants were significantly decreased with increasing drought level. The lowest values (5.00, 3.00, 15.00, 40.13 g and 6.02 g) were recorded with treatment of (W3) and foliar spray by distilled water for above mentioned characters, respectively. The foliar spray by micronutrients reduced the harmful effect of drought and gave (6.00, 4.00, 24.00, 42.57g and 10.21g, respectively) when foliar stressed plants grown under drought (W3) that sprayed with iron sulfate. Also, the maximum values were obtained among treatment (W1) and foliar sprayed with iron (15.67, 8.33, 130.33, 54.69g and 71.29g, respectively). These increments would be attributed to the major role of the applied microelements in photosynthesis activation, pigments formation and carbohydrates assimilation converted to the grains which optimize the economic part of the plant (Hilman & Asandhi, 1987). Farooq et al. (2009) reported that water stress leads to severe decrease in yield components of crop plants probably by disrupting leaf gas interchange properties which not only restricted the size of the source and sink tissue show ever the phloem loading, assimilate translocation and dry matter distribution are also impaired.Thalooth et al. (2006) illustrated that the foliar spray with zinc sulfate under drought condition have the positive effect on yield and its components of mungbean plant. On soybean plants, Heidarian et al. (2011) found an increase of seed yield by foliar application of iron and zinc through the effect on No. of seeds/plant and seed weight. Experimental result of Odeley & Animashaun (2007) also showed that foliar application of micronutrients increased the soybean yield, quality and resistance to drought stress.

TABLE 6. Effect of water stress, foliar application with micronutrients and their interactions on yield and its components of common bean plants at 55 days from sowing (average of the two seasons of 2015 and 2016).

Treatments	characters	No. of pods / plant	No. of seeds / pod	No. of seeds / plant	100 seed weight (g)	Seed yield/ plant (g)
Effect of water stress						
W1 (6 days irrigation)		15.00 a	7.78 a	118.33 a	54.01 a	65.07 a
W2 (12 days irrigation)		12.33 b	5.67 b	70.56 b	44.92 b	31.88 b
W3 (18 days irrigation)		5.56 c	3.56 c	19.89 c	41.42 c	8.27 c
L.S.D. (0.05)		1.01	1.23	12.16	0.91	6.39
Effect of foliar spray with micronutrients						
Distilled water		9.89 c	5.11 c	58.78 c	45.57 c	28.99 c
Zn (500ppm)		11.11 b	5.67 b	69.00 b	46.88 b	35.33 b
Fe (500ppm)		11.89 a	6.22 a	81.00 a	47.90 a	40.90 a
L.S.D. (0.05)		0.46	0.50	5.40	0.82	1.87
Water stress	**Foliar spray**	**Effect of interaction**				
W1 (6 days irrigation)	Distilled water (control)	14.33 b	7.33 b	109.67 b	53.57 a	58.77 c
	Zn (500ppm)	15.00 ab	7.67 ab	115.00 b	53.77 a	65.15 b
	Fe (500ppm)	15.67 a	8.33 a	130.33 a	54.69 a	71.29 a
W2 (12 days irrigation)	Distilled water	10.33 d	5.00 de	51.67 e	43.02 c	22.19 f
	Zn (500ppm)	12.67 c	5.67 cd	71.33 d	45.31 b	32.25 e
	Fe (500ppm)	14.00 b	6.33 bc	88.67 c	46.43 b	41.19 d
W3 (18 days irrigation)	Distilled water	5.00 f	3.00 f	15.00 f	40.13 d	6.02 h
	Zn (500ppm)	5.67 ef	3.67 ef	20.67 f	41.56 c	8.59 gh
	Fe (500ppm)	6.00 e	4.00 e	24.00 f	42.57 c	10.21 g

Leaf anatomy

From the aforementioned findings in this work, regarding the growth, physiological and yield characters of common bean plants in the first growing season (2015), it was found that foliar spray with iron sulfate gave high increase in the studied characters compared to zinc sulfate, consequently iron was chosen for studying the anatomical structure of common bean plant leaf.

Results in Table 7 and Fig. 1 indicated that common bean plants irrigated every 18 days (W1) and foliar sprayed with iron increased all anatomical features of leaves. The increment percentages more than the control were 25.0, 42.86, 11.11, 35.0, 17.35, 25.0, 100.0 and 80.0% for blade thickness, palisade thickness, spongy thickness, length and width midvein, phloem thickness, xylem thickness and diameter of vessel average, respectively.

TABLE 7. Effect of water stress, foliar application with micronutrients and their interactions on counts and measurements of certain anatomical features in transverse sections through the leaflet blade of the fourth upper compound leaf on the main stem of common bean plants at 55days from sowing during the second growing season (2016).

Treatments \ Characters		Blade Thic.(µ)	Palisade Thic. (µ)	Spongy Thic. (µ)	Midveinlenth (µ)	Midvein width (µ)	Phloem Thic. (µ)	Xylem Thic. (µ)	Diameter of vessel average (µ)
Effect of water stress									
W1 (6 days irrigation)		239.40	90.44	101.08	1250.20	1133.16	191.52	239.40	33.32
W2 (12 days irrigation)		175.56	58.52	74.48	973.56	798.00	143.64	143.64	23.80
± % to control		-26.67	-35.29	-26.32	-22.13	-29.58	-25.00	-40.00	-28.57
W3 (18 days irrigation)		164.92	47.88	69.16	899.08	750.12	122.36	122.36	20.23
± % to control		-31.11	-47.06	-31.58	-28.09	-33.80	-36.11	-48.89	-39.29
Effect of foliar spray with micronutrients									
Distilled water		166.69	56.75	74.48	893.76	801.55	134.77	131.23	20.63
Fe (500ppm)		+219.89	+74.48	+88.67	+1188.13	+985.97	+170.24	+205.71	+30.94
± % to control		31.91	31.25	19.05	32.94	23.01	26.32	56.76	50.00
Water stress	**Foliar spray**	**Effect of interaction**							
W1 (6 days irrigation)	Distilled water (Control)	212.8	74.48	95.76	1064	1042.72	170.24	159.6	23.8
	Fe (500ppm)	266	106.4	106.4	1436.4	1223.6	212.8	319.2	42.84
	± % to control	25.00	42.86	11.11	35.00	17.35	25.00	100.00	80.00
W2 (12 days irrigation)	Distilled water	159.6	53.2	74.48	872.48	744.8	127.68	127.68	21.42
	± % to control	-25.00	-28.57	-22.22	-18.00	-28.57	-25.00	-20.00	-10.00
	Fe (500ppm)	191.52	63.84	74.48	1074.64	851.2	159.6	159.6	26.18
	± % to control	-10.00	-14.29	-22.22	1.00	-18.37	-6.25	0.00	10.00
W3 (18 days irrigation)	Distilled water	127.68	42.56	53.2	744.8	617.12	106.4	106.4	16.66
	± % to control	-40.00	-42.86	-44.44	-30.00	-40.82	-37.50	-33.33	-30.00
	Fe (500ppm)	202.16	53.2	85.12	1053.36	883.12	138.32	138.32	23.8
	± % to control	-5.00	-28.57	-11.11	-1.00	-15.31	-18.75	-13.33	0.00

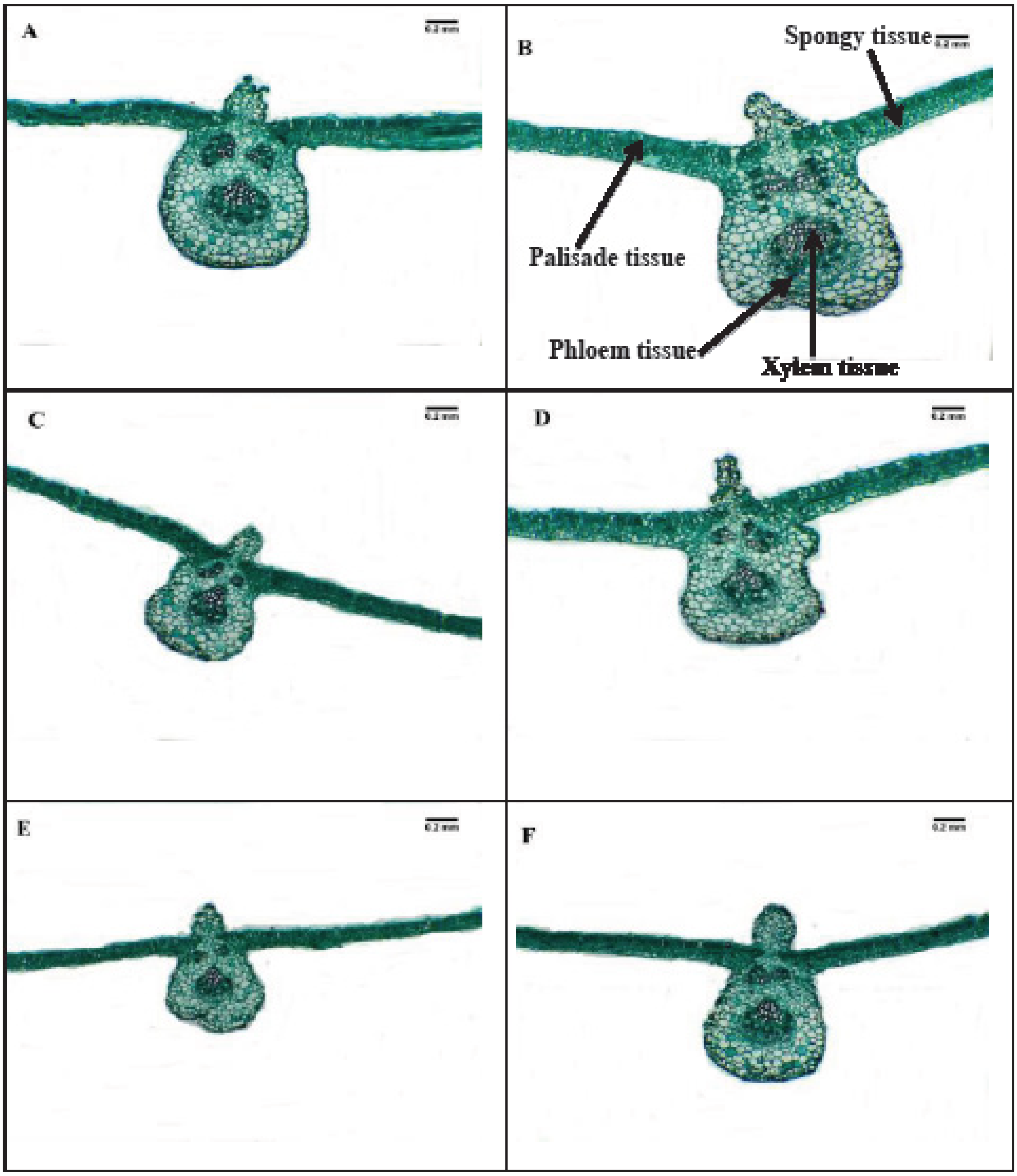

Fig. 1.Transverse section through the leaflet blade of common bean plants affected with the interaction treatments between foliar application with Fe and three levels of water stress during the second growing season (2016).

A: Irrigation every 6 days (W1) spraying with distilled water; B: W1 spraying with Fe; C: Irrigation every 12 days (W2) spraying with distilled water; D: W2 spraying with Fe; E: Irrigation every 18 days (W3) spraying with distilled water; F: W3 spraying with Fe.

Data also revealed that spraying common bean plants with iron was effective in reducing the negative effects of water stress (W2). The percentage of reduction in leaf features irrigation every 12 days (W2) without application iron were (25.0%) blade thickness, (28.57%) palisade thickness, (22.22%) spongy thickness, (18.0%)midvein length, (28.57%) midvein width, (25.00%) phloem thickness, (20.0%) xylem thickness and (10.0%) diameter of vessel average. But, in the same interval of irrigation every 12 days (W2) and sprayed with iron reached to 10.0, 14.29, 22.22, 18.37 and 6.25% less than control for blade thickness, palisade thickness, spongy thickness, midvein length and phloem thickness, respectively, while the midvein width and diameter of vessel average were 1.0 and 10.0% more than control, respectively.

It is also clear from the data that the effect of water stress (W3) had considerable decrease on common bean plant leaflet blade thickness (40.0%), palisade thickness (42.86%), spongy tissue (11.11%), midvein length (35.0%), midvein width (40.82%), phloem thickness (37.50%), xylem thickness (33.33%) and diameter of vessel average (30%). Meanwhile, when foliar stressed plants (W3) with iron sulfate was reduced the harmful effect of drought in all abovementioned characters and gave (5.00, 28.57, 11.11, 1.00, 15.31, 18.75, 13.33 and zero%, respectively) less than the control.

On wheat plants, Ibrahim (2012) found that water stress may have a repressing effect on the activity of the different initial cells forming the leaf blade with concerning to cell enlargement and division. The decline in mesophyll tissue, phloem and xylem cause a slow rate in the translocation of photoassimlates for the developing seeds. Furthermore, the decline in the diameter of vascular bundle in the leaf blade lead to lowering the accumulation of essential water needed for photosynthesis. Akram et al. (2016) showed that drought stress caused a significant decrease in thickness of leaf midvein, the area of vascular bundle and parenchyma cell and the number of vascular bundles in leaf of radish plants. On broad bean plants (*Vicia faba* L.), Ali et al.(2009) reported that foliar application with zinc registered favorable anatomical changes in leaf due to the effect of micronutrients.

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تأثير الإجهاد المائي والعناصر الصغري على الخصائص الفسيوكيمائية والمحصول وكذلك التركيب التشريحي لنباتات الفاصوليا

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يعتبر الإجهاد المائى أحد أهم العوامل التى تؤثر على إنتاجية العديد من المحاصيل مثل الفاصوليا ، ولتخفيف الأثار الضارة للإجهاد المائي يمكن إستخدام الرش الورقى بالعناصر الصغرى .

أجريت تجربة أصص خلال موسمي الزراعة 2015 ، 2016 بصوبة كلية الزراعة جامعة الزقازيق بمحافظة الشرقية لدراسة تأثير الرش الورقى بكلاً من عنصرى الحديد والزنك على صفات النمو الخضرى وبعض الصفات الفسيولوجية والبيوكيمائية والتركيب التشريحي لنصل الورقة وكذلك المحصول ومكوناته لنبات الفاصوليا صنف جيزة 3 تحت تأثير 3 فترات من الرى (كل 6 ، 12 ، 18 يوم).

أظهرت النتائج المتحصل عليها أن جميع صفات النمو مثل (إرتفاع النبات ، عدد الأوراق والأفرع ، مساحة الورقة ، الوزن الجاف للمجموع الخضري) إنخفضت معنوياً تحت ظروف الاجهاد المائي بينما أدي تطبيق الرش الورقى بعنصر الحديد إلي تخفيف الأثار الضارة للإجهاد المائي على الصفات المذكورة مسبقاً لنبات الفاصوليا.

كما لوحظ أيضاً إنخفاض معنوى فى صبغات التمثيل الضوئي والصفات البيوكيمائية مثل (نسبة البروتين ، النيتروجين ، الفسفور ، البوتاسيوم) والمحصول ومكوناته وكذلك الصفات التشريحية للورقة فى النباتات المعاملة بالاجهاد المائى تحت مستوى ري (18 يوم) مقارنة بالكنترول تحت مستوى رى (6 ايام)، وعلى العكس حدثت زيادة معنوية للمركبات الفينولية ومركبات المالونديالدهيد. من جهة أخرى أدي الرش الورقى بعنصر الحديد إلى تحسين الصفات الفسيولوجية والبيوكيمائية والتشريحية وصفات المحصول تحت ظروف الإجهاد المائي، وكانت أفضل مستويات الري المستوى الأول (6 ايام) يليه المستوى الثانى (12 يوم) يليه المستوى الثالث (18 يوم). وبالتالى نجد أن الرش بعنصر الحديد حسن محصول كمية النبات وكذلك الصفات التشريحية وذلك بالتغلب جزئيا على الأثار الضارة للإجهاد المائي .