

EFFECT OF MICRONUTRIENTS FOLIAR APPLICATION ON GRAIN QUALITATIVE CHARACTERISTICS AND SOME PHYSIOLOGICAL TRAITS OF BEAN (*PHASEOLUS VULGARIS* L.) UNDER DROUGHT STRESS

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ABSTRACT

In order to study the interaction of irrigation and micronutrient on yield, qualitative characteristics and some physiological traits of red bean (Akhtar variety), a split plot experiment was carried out in a randomized complete block design with three replications in 2011. Treatments consisted of three levels of irrigation (irrigation after 50, 75 and 100 mm evaporation from class A pan,) as the main plot and foliar application in four levels (Zn, Fe, Zn + Fe and water) as the sub plot. The results showed that the effect of irrigation interval on all studied traits except for grain iron and zinc content was significant. The effect of foliar application was significant on all characteristics except leaf soluble carbohydrates. Interaction of irrigation and foliar application no leaf soluble carbohydrates and grain yield was significant. With delaying in irrigation and increasing of drought stress intensity, individual and combined foliar application of these two elements had more effect on increasing grain yield, So that in irrigation after 75 mm evaporation treatment, the grain yield in Fe + Ze foliar application treatment compared to control increased about 25.3% and in irrigation after 100 mm evaporation treatment, the grain yield in Fe + Ze foliar application treatment compared to control increased about 64.4%. The results of this experiments showed that the consumption of iron and zinc in drought stress condition can be improved physiological characteristics (relative water content, soluble carbohydrates), yield and grain quality of red bean.

Keywords: *Iron, Protein, Soluble Carbohydrates, Water Stress, Zinc*

INTRODUCTION

Beans are one of the most important pulses, which have the major contribution in the human diet and provide an important part of human protein requirement. The amount of grain protein in pulses is about 2-4 times of cereal and 10-20 times of tuber plants. Drought is a danger for the successful production of crops around the world. Drought stress is the most important factors which limiting bean production in the worldwide (Teran and Singh, 2002). Drought stress has negative effects on many plant processes such as photosynthesis, transpiration; accumulation and allocation of assimilate substances (Ohashi *et al.*, 2006).

Several studies were done on the effect of drought stress on physiological characteristics of plants. In one of these studies on bean plant, Sadeghipour and Aghaei (2012) showed that drought stress conditions significantly reduced the leaf relative water content. They explained that relative water content is related to root water absorption and loss of the water by transpiration. Exposure of plants under drought stress reduced the leaf water potential and subsequently leaf relative water content was reduced considerably since that both of these conditions increase the leaf temperature. Maintenance of photosynthetic capacity and leaf chlorophyll content under drought stress is considered as the physiological parameters involved in drought tolerance and cultivars with higher chlorophyll content appear to have greater drought resistance (Lalinia *et al.*, 2012). Drought stress leads to increase reactive oxygen species production in plants resulted in decreasing of chlorophyll content, indicating the extent of the oxidative damages. This decrease may be also due to inhibition of chlorophyll biosynthesis pathway (Lalinia *et al.*, 2012).

Thalooth *et al.*, (2006) showed that by increasing irrigation intervals and drought stress condition exercised, leaf chlorophyll and carotenoid content in mung bean were reduced significantly. Reduces of

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the chlorophyll concentration in leaves of mung bean under drought stress may be due to degradation of chlorophyll more than its biosynthesis. One of the most important factors in maintaining plants against abiotic stresses is assembly osmotic guard and in this regard, can point out the accumulation of proline, soluble carbohydrates and some of the ions (Slama *et al.*, 2006).

Rozrokh *et al.*, (2012) in their experiment on chickpea reported an increase in soluble carbohydrate under drought stress. This increasing attributed to the slowly transition of soluble sugars from leaves to stem and slower use it due to decrease in growth and other changes such as starch hydrolysis. Under drought stress conditions due to decrease soil water content and reduce nutrient distribution in soil, nutrient uptake by roots decreases, beside the transfer of nutrients 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 lack of nutrients is one of the most important factors, limiting plant growth under water stress.

Plant sufficient nutrition have an important role in 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 and Mamedov, 2010). According to Akbari *et al.*, (2013), micronutrients fertilizer could increase plant resistance to drought stress. Metal ions such as iron, zinc, copper, manganese and magnesium as a cofactor participate in construction of many antioxidant enzymes and results of Cakmak *et al.*, (2010) studies showed that under micronutrients deficiency conditions, antioxidant enzyme activities decrease and thus increases the sensitivity of plants to environmental stresses. Thalooh *et al.*, (2006) reported that foliar application of zinc sulfate in water stress conditions had a positive effect on growth, yield and yield component of mung bean plant. Experimental result of Odeley and Animashaun (2007) also showed that foliar application of micronutrients increased the soybean yield, quality, resistance to pests and diseases and drought stress. They reported that although the need of plants to micronutrients is very little but these nutrients play an important role in growth and development of plants. So that the micronutrients such as iron, copper, boron, zinc and manganese have many contributions in cell wall formation and plant resistance to pests and diseases and environmental stresses.

The aim of this study was to evaluate the effects of foliar application of iron and zinc, individually and in combination on physiological characteristics, osmotic adjustment, grain quality traits and yield of red bean in different irrigation intervals.

MATERIALS AND METHODS

Field experiment was carried out during spring and summer of 2011 at an agricultural farm located in Lordegan, Iran (30.55⁰N, 49.55⁰E, and 1700 m above sea level with an average annual rainfall of about 550 mm). Physiochemical properties of soil are given in table 1. This experiment was arranged as split plot base on randomized complete block design with three replications. Main plots included irrigation period with three levels (Irrigation after 50, 75 and 100 mm evaporation from class A pan) and sub plots were treatments of Zn, Fe and Zn+Fe foliar application and control (water foliar application). Ferrous sulfate and zinc sulfate at 3 mgL⁻¹ concentration were used for iron and zinc treatments. Red bean seed (Akhtar variety) were planted on 10 June 2011 in 25 cm row space, 10 cm plant space within row (density of 40 plant m⁻²) in 3×7 m plots. The distance between main plots, sub plots and blocks was consisted of 3, 1 and 3 m respectively. The experimental field received 70 kg ha⁻¹ P₂O₅ (in the form of triple superphosphate) and K₂O (in the form of potassium sulfate) before planting. Nitrogen at rate of 100 kg ha⁻¹ (in the form of urea) was applied in three stages: before planting, eight-leaf and flowering stages. Foliar spraying of plant was performed in two growth stages of red been (four-leaf and flowering stages).

In full flowering stage of plant from each plot, the youngest fully expanded upper leaves were collected for measuring of relative water content, soluble carbohydrates, chlorophyll content and electrolyte leakage. Relative water content was determined according to the method described by Unyayer *et al.*, (2005). Chlorophyll content was determined according to the method described by Arnon (1949). Soluble carbohydrates were determined according to the method described by Irigoyen *et al.*, (1992). Electrolyte leakage was determined according to the method described by Kaya (2003). In harvesting time after

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eliminating the two lateral row and 50 cm from the sides, grain yield was determined from 10 square meters area. The percentage of total nitrogen in seed was measured by Kjeldahl method according to Chapman and Pratt (1961) and the seed protein percentage was calculated by multiplying the total nitrogen percentage by 6.25. Fe and Zn in grain determined with dry ash method by using Atomic Spectrophotometer (Perkin 400 model) according to the method described by Chapman and Pratt (1961). Data analysis performed by GLM procedure using SAS software (SAS, 1996). Mean comparisons of major effects were done using the Least Significant Difference test at 5% probability and in the case of significance of interactions, sliced analysis were carried out and mean comparisons were done using least square means test.

Table 1: Soil physical and chemical properties of experimental location

Soil Texture	Available Iron (mg.kg ⁻¹)	Available Zinc (mg.kg ⁻¹)	Total Nitrogen (%)	Available Potassium (mg.kg ⁻¹)	Available Phosphor (mg.kg ⁻¹)	Organic Carbon (%)	EC (dS.m ⁻¹)	pH
Silty Clay	3.98	0.61	0.052	227	14.1	0.68	5.21	8.01

RESULTS AND DISCUSSION

Relative Water Content (RWC)

Statistical analysis of data for relative water content of leaf showed that the effect of different irrigation levels and foliar application was significant on this trait (Table 2). With delayed in irrigation, relative water content significantly decreased so that the highest (69%) and lowest (52.9%) RWC was seen in treatments of irrigation after 50 and 100 mm evaporation from class A pan, respectively (Table 3). According to the Rahman Khan *et al.*, (2007) reports, plants grown under water stress conditions decrease the intracellular water by increasing of osmotic compounds to absorb water from the soil powerfully. It seems there is a direct relationship between the soil moisture content and relative water content of leaf so that reduction in soil moisture and increasing water stress reduces relative water content of leaf. Fe and Zn foliar application increased relative water content of red bean leaves so that the highest level of RWC (64.6%) was obtained in Zn+Fe treatment (Table 3). To opinion of the Weisany *et al.*, (2011) the zinc element have an important role in the regulation of stomatal opening, because this element plays a important role in maintenance of potassium in stomata guard cells and by reducing of leaves water loss, increases the leaf relative water content. Akbari *et al.*, (2013) also reported RWC in cumin leaves decreased under water stress and the high amount of RWC was shown in Zn+Fe foliar application in both irrigated and non-irrigated condition.

Electrolyte Leakage

The statistical analysis of data indicated that electrolyte leakage significantly affected by both irrigation ($p \leq 0.01$) and foliar application ($p \leq 0.05$) (Table 2). Mean comparison results showed that the highest electrolyte leakage (70.77%) was recorded by irrigation after 100 mm evaporation and on the contrary, the lowest (57.41%) was seen in irrigation after 50 mm evaporation (Table 3). In drought stress condition, carbon dioxide fixation decrease due the stomata closing, while light reactions and electron transfer continue normality. Under such conditions, there will be limited amounts of NADP to accept the electrons and so oxygen can act as an electron receptor, resulting in the production of reactive oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals. Enhancing the reactive oxygen species cause oxidative injuries in many cellular components such as lipids, proteins, carbohydrates and nucleic acids and lead to cell membrane peroxidation and increasing of electrolyte leakage (Jiang and Huang, 2001). In this regard Alexiva *et al.*, (2001) reported that drought and ultra violet stress in pea and wheat plant through amplifying of reactive oxygen species production increased electrolyte leakage.

Mean comparison of the effect of foliar application on electrolyte leakage showed that application of Fe and Zn micronutrient decreased this parameter significantly so that foliar application of Fe, Zn and Fe+Zn compared to water foliar application decreased electrolyte leakage of leaves about 9%, 9.8% and 10.2% respectively (Table 3). As mentioned earlier, leaf electrolyte leakage, indicating cell membrane damage is

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resulting from membrane lipids peroxidation in presence of reactive oxygen species (Jiang and Huang, 2001). According to this, the balance between production and elimination of reactive oxygen species determines the survival of plant systems.

Zago and Oteiza (2001) stated that iron and zinc elements by increasing the activity of antioxidant systems in plants decreased reactive oxygen species injuries and play an important role in membrane. Accordingly, it seems that foliar application of iron and zinc by increasing the production of antioxidant enzymes decreased electrolyte leakage and improved drought tolerance. Such results are in agreement with those recorded by Tavallali *et al.*, (2010) in pistachio seedlings who found that application of Zn decreased electrolyte leakage in salinity stress condition.

Table 2: The analysis of variance of measured traits in experiment

S.O.V	df	Mean Squares							
		RWC	EL	CC	SC	GIC	GZC	GPC	Grain Yield
Replication	2	28.3 ^{ns}	65.5 [*]	0.004 ^{ns}	19.5 ^{ns}	1758 ^{ns}	22.9 ^{ns}	0.1 ^{ns}	167944 ^{**}
Irrigation(A)	2	714 ^{**}	544 ^{**}	0.194 ^{**}	4376 ^{**}	140 ^{ns}	19.0 ^{ns}	106 ^{**}	5997987 ^{**}
orrEr a	4	32.6	8.6	0.001	25.3	1222	4.7	0.3	64535
Foliar Application(B)	3	78.7 [*]	81.7 [*]	0.006 ^{**}	11.3 ^{ns}	8924 ^{**}	466 ^{**}	5.5 [*]	293568 ^{**}
A ×B	6	14.0 ^{**}	9.9 ^{ns}	0.001 ^{ns}	26.2 [*]	420 ^{ns}	7.3 ^{ns}	0.9 ^{ns}	53711 [*]
Error b	18	16.1	17.0	0.001	8.0	496	10.3	1.2	19803
C.V (%)	-	6.6	6.5	5.65	5.1	13.7	10.0	4.8	6.6

*ns, * and ** indicates non-significant, significant at 5% and 1% probability levels, respectively*

RWC: Relative Water content, EL: Electrolyte Leakage, CC: Chlorophyll Content, SC: Soluble Carbohydrate, GIC: Grain Iron Content, GZC: Grain Zinc Content, GPC: Grain Protein Content

Table 3: The means comparison of the simple effects of different levels of irrigation and foliar application on some traits

Experimental Treatments		RWC (%)	EL (%)	CC (mg g LFW ⁻¹)	GIC (mg kg ⁻¹)	GZC (mg kg ⁻¹)	GPC (%)
Irrigation	After 50mm Evaporation	69.05 ^a	57.41 ^c	0.62 ^a	164.6 ^a	33.53 ^a	19.80 ^c
	After 75mm Evaporation	58.89 ^b	62.71 ^b	0.43 ^b	163.82 ^a	32.06 ^{ab}	23.60 ^b
	After 100mm Evaporation	53.92 ^b	70.77 ^a	0.38 ^c	158.33 ^a	31.03 ^b	25.66 ^a
Foliar Application	Control	57.50 ^b	68.13 ^a	0.46 ^b	123.91 ^c	26.40 ^b	22.19 ^b
	Fe	59.88 ^b	62.49 ^b	0.49 ^a	191.85 ^a	25.57 ^b	22.52 ^b
	Zn	60.50 ^b	62.06 ^b	0.46 ^b	149.45 ^b	38.77 ^a	23.67 ^a
	Fe+Zn	64.61 ^a	61.83 ^b	0.51 ^a	183.79 ^a	38.09 ^a	23.70 ^a

In each column, the means with same letters are not significantly different by LSD test.

RWC: Relative Water content, EL: Electrolyte Leakage, CC: Chlorophyll Content, GIC: Grain Iron Content, GZC: Grain Zinc Content, GPC: Grain Protein Content

Chlorophyll Content

Results showed that the effect of irrigation and foliar application on chlorophyll content of leaves was significant ($p \leq 0.01$) (Table 2). Mean comparison of the effect of irrigation showed that mild stress treatment (irrigation after 75 mm evaporation) and severe stress treatment (irrigation after 100 mm evaporation) compared to non stress treatment (irrigation after 50 mm evaporation) decreased the amount of chlorophyll content about 44.18% and 63.15% respectively (Table 3). Sadeghipoor and

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Aghaei (2012) expressed that reduction of chlorophyll content is the main reason of decreased photosynthetic capacity under drought stress. According to the Singh (2007), at the long-term stresses, dehydration of tissues leads to increased of oxidative processes that causes deterioration of chloroplast structure, reduces chlorophyll, and ultimately reduces the photosynthetic activity.

Also results showed that iron and Fe + Zn foliar application, compared to control, increased the amount of total chlorophyll content in leaves about 6.52% and 10.86% respectively (Table 3). These increases could be attributed to the functional role of iron in activation of enzymes that involved in chlorophyll biosynthesis pathway and some antioxidant enzymes such as ascorbate peroxidase and glutathione reductase in the route protection of degradation of chlorophyll by the active oxygen radicals (Zayed *et al.*, 2011). In addition, Report from Ayad *et al.*, (2010) confirmed a positive effect of zinc in chlorophyll content increasing in Pelargonium leaves. They reported that, zinc element in setting up of some chlorophyll biosynthetic pathway enzymes and some antioxidant enzymes such as ascorbate peroxidase and glutathione reductase have a fundamental role and prevent the chlorophyll degradation by reactive oxygen species.

Soluble Carbohydrates

Analysis of data indicated that effect of irrigation ($P \leq 0.01$) and the interaction between irrigation and foliar application ($P \leq 0.05$) on soluble carbohydrates of leaves was significant (Table 2). Mean comparison of interaction showed that in effect of foliar application on soluble carbohydrates in non drought stress (irrigation after 50 mm evaporation) and mild drought stress (irrigation after 75 mm evaporation) treatments was not significant but in high drought stress treatment, the foliar application levels could have a significant effect on this parameter so that compared to control, foliar application of Fe and Fe + Zn increased soluble carbohydrates about 10.8 and 7.9% respectively (Table 4). Result also showed that with delay in irrigation the amount of soluble carbohydrates increased in all of the foliar application treatments (Table 4). Production from non-photosynthesis pathway, lack of assimilation and degradation of insoluble carbohydrates may be the reasons for increasing these compounds.

Soluble carbohydrates increasing could be evaluated as a response to changes in relative water content and leaf water potential, because under drought stress condition this increasing improve leaf water status (Mohsenzadeh *et al.*, 2006). So, it could be argued that soluble carbohydrates accumulation in addition to have a physiologically important function in energy supply and also certain death avoidance, it can reduce osmotic potential of cell and increase drought tolerance. (Babaeian *et al.*, 2011). The iron and zinc element in stress condition have an enhancing role on osmotic adjustment process (due to the increase of soluble carbohydrates). Zinc is an essential and low consumption element, which has an important role in protein, and carbohydrate synthesis, cell metabolism, protection of cell membrane from reactive oxygen species and other processes associated with adaptation of plants to stress. Also iron involved in photosynthesis and carbohydrate production, so that, under drought stress conditions the role of these elements can be seen as a contributor to osmotic regulation, that with intervention in the synthesis of osmotic compounds for compatibility with stress and maintain turgor pressure performed their roles (Akbari *et al.*, 2013).

Grain Iron Content

Variance analysis results in table 2 indicate that foliar application treatments had significant ($P \leq 0.01$) effect on iron concentration of grain in red bean. Mean comparisons showed that the foliar application with Fe could be increasing the concentration of this element in the grain about 54.83% compared to the control treatment (Table 3). Baybordi and Mamedov (2010) by foliar application with iron in canola increased the amount of iron in grain that confirmed the results of this experiment.

Grain Zinc Content

The effect of foliar application on concentration of zinc in grain was significant ($P \leq 0.01$) (Table 2). The maximum amount of zinc in grain (38.77 mg kg^{-1}) was belonged from Zn foliar application treatment that increased 46.85% compared to the control treatment (Table 3). Grain micronutrients concentration depends on their uptake by root during the seed development stage and remobilization from plant tissues to grain through phloem. Amount of remobilization from this way has largely depended on each element

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moves in the phloem and zinc has good remobilization from leaves to the grain. Kazemi Poshtmasari *et al.*, (2008) also increased concentration of this element in bean grain by using of zinc foliar application, which confirmed the results of this experiment.

Grain Protein Content

Results showed that the effect of irrigation levels on grain protein content was significant ($P \leq 0.01$) (Table 2). Delayed in irrigation increased the grain protein content significantly so that in severe drought stress compared to the non-stress treatment, this parameter increased about 29.59% (Table 3). The higher grain protein percentage in this condition can be related to the reduced grain filling period in water restrictions treatment which reduces the ratio of carbohydrates to protein (due to the decreased abundance of starch synthesis enzymes). Thaloonth *et al.*, (2006) were reported that water stress affected the photosynthesis, enzyme activity and protein synthesis, and this leads to change the movement of metabolites to seed. Sadeghipour and Aghaei (2012) also stated that new protein synthesis is the cause of increased bean grain protein under drought stress.

Results also showed that effect of foliar application on grain protein content was significant ($P \leq 0.05$) (Table 2). Fe + Zn foliar application compared to the control treatment could increase the grain protein content about 6.89% (Table 3). Baybordy and Mamedov (2010) explained that Iron and zinc are two important elements in enzymes structure involved in amino acid biosynthesis and because amino acids are the base of protein synthesis, protein content increases in the case of using these micronutrients. The results obtained by Thaloonth *et al.*, (2006) showed that using of zinc sulfate increases grain protein content of mungbean. In addition, iron is involved in the metabolism of nitrogen and increases leaf area and has a direct impact on the process of protein production. So it can be expected that iron foliar application, increased the plant protein production (Cakmak *et al.*, 2010). In the experiment of Farajzadeh *et al.*, (2009) on corn, also foliar application of iron and zinc increased seed protein content significantly.

Table 4: The mean comparison of the interaction of irrigation and foliar application on soluble carbohydrates and grain yield

Irrigation	Foliar Application	SC (mg g LFW ⁻¹)	Grain Yield (kg ha ⁻¹)
After 50 mm Evaporation	Control	40.66 ^a	2809.56 ^a
	Fe	40.76 ^a	2776.66 ^a
	Zn	38.36 ^a	2736.60 ^a
	Fe+Zn	36.33 ^a	2878.56 ^a
After 75 mm Evaporation	Control	48.86 ^a	2005.46 ^c
	Fe	47.86 ^a	2289.33 ^{ab}
	Zn	50.36 ^a	2138.30 ^{bc}
	Fe+Zn	51.23 ^a	2513.13 ^a
After 100 mm Evaporation	Control	72.50 ^b	1062.40 ^c
	Fe	80.36 ^a	1467.03 ^b
	Zn	73.30 ^b	1305.33 ^b
	Fe+Zn	78.23 ^{ab}	1747.10 ^a

In each column and in each irrigation level, the means that have at least the same letters are not significantly different by L.S. Means test.

SC: Soluble Carbohydrates

Grain Yield

The results showed that, the effect of irrigation ($P \leq 0.01$), foliar application ($P \leq 0.01$) and their interactions ($P \leq 0.05$) on the grain yield was significant (Table 2). The general trend of grain yield with delays in irrigation was decreased. According to the report of Babaeian *et al.*, (2011) drought stress by reducing plant growth and damage to flowering and grain filling, reduces the grain yield. The mean comparison showed that the ameliorative effects of foliar application increased with increasing the severity of drought stress. So that compared to the control treatment, the foliar application with a mixture

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of iron and zinc in mild water stress conditions (irrigation treatment after 75 mm evaporation) and in severe water stress condition (irrigation treatment after 100 mm evaporation) increased grain yield about 25.31% and 64.44 % respectively (Table 4).

Thalooth *et al.*, (2006) showed that the zinc sulfate foliar application in drought stress condition have the positive effect on the growth, yield and yield components of mungebean. Baybordy and Mamedov (2010) reported that the using the mixture of iron and zinc resulted in higher 1000 seed weight and grain yield of canola compared to the use of them individually. Increased of grain yield by consumption of iron and zinc in soybean through the effect on number of seeds per plant and grain weight also have been reported by Heidarian *et al.*, (2011).

Conclusion

According to obtained results, it can be concluded that foliar application of Fe and Zn under drought stress could improved physiological characteristics. Hence, the increase of compatible osmolytes (especially soluble carbohydrates) and decrease lipid peroxidation (reducing electrolyte leakage) due to foliar application of these micronutrients can increase RWC and have a positive impact on drought tolerance mechanisms and yield enhancement. Results also showed that especially in terms of water stress, Fe and Zn have a positive function in protein and microelement content of grain bean. Therefore, according to the limitations of soil Fe and Zn in arid and semi-arid area, foliar application is a rational way to rise of micronutrients level in plants which can ameliorate the impact of drought on red bean yield quantity and quality.

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