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Responses of Soybean to Water Restriction and Zinc, Iron and Boron Foliar Application

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Abstract: *Two separated experiments in the form of randomized complete block design were carried out in Aleshtar, Iran during 2013-2014. The first field experiment was based on normal irrigation and second one was based on water stress condition in soybean* (Glycine max *L.). The investigated factors consisted of micronutrients including non foliar application and zinc, iron, boron, zinc$_+$boron, zinc$_+$boron, iron$_+$boron, and zinc$_+$iron$_+$boron foliar application. The results showed that the effect of irrigation on the rate of proline, soluble sugars, chlorophyll a and chlorophyll b was significant at 1% probability level. The effect of foliar application of micronutrients on grain yield,- rate of proline, soluble sugars, chlorophyll b, chlorophyll and rate of proline had significant difference. The highest rate of proline belonged to the treatment with water stress and foliar application of zinc, iron and boron by the mean of 506.9 mg/kg and the lowest rate was obtain in normal irrigation plus zinc, iron, and boron foliar application by 358.5 mg/kg. The highest rate of grain yield belonged in normal irrigation and zinc, iron, and boron foliar application by 2561 kg/ha and the lowest grain yield belonged was obtain in water stress condition and without micronutrients foliar application about 1086 kg/ha.*

Key words*: soybean, proline, soluble sugars, oil and grain yield*

INTRODUCTION

Soybean (*Glycine max* L.) is one the major sources of protein for human and animal nutrition as well as a key source of vegetable oil. However, it requires adequate soil moisture throughout its growth period to attain adequate yield (Silvente et al., 2012).Water is a critical environmental factor that imposes water stress on crops and a major constraint on plant growth and productivity (Rampino et al., 2006). It is the most damaging a biotic stress affecting agro-ecosystem (Zhang et al., 2006). Sometimes the lack of these elements can act as limiting the absorption of other nutrients and growth and this determines the need for their application. Foliar application of micronutrients can improve plant growth (Movahedi dehnavi et al., 2009). In addition to increasing the quality and quantity of agricultural products, micronutrients also have a significant effect on animal and human health (Sharma et al., 2005). In different environmental conditions the plants make accumulate solutes with low molecular weight which are generally called compatible solutes including amino acids, sugars and betaine too. In addition, some soluble minerals also form an important part of active osmotic solutes within the cells (Bajji, 2011). During the water stress, the activity of enzymes such as chlorophylase and peroxidase increases and the activity of the enzymes responsible for chlorophyll synthesis will disrupt which leads to the decrease of chlorophyll and consequently reduction of photosynthesis (Smirnoff, 2007). It is reported that when plants are exposed to water and

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salinity, they begin to build and accumulate soluble substances such as amino acids, proteins, sugars, alcohol compounds, Cyclitols and organic acids in their own cells (Arndt et al., 2008). Since zinc, iron, and boron have important functions in plants' metabolism, this study was carried out to determine the effects of these micronutrients on physiological traits of soybean under water restriction conditions.

MATERIALS AND METHODS

This study was conducted in Aleshtar (latitude 33°49' N and longitude 48°15'E and 1500 m above the sea level), Iran. Soil samples were taken from the depth of 0-30 cm in both years and the soil properties were measured in soil testing laboratory.

To study the -physiological reactions of soybean (*Glycine max* L. cv. Record) to water restrictions and the use of micro elements zinc, iron and boron, two separate experiments were carried out in the form of randomized complete block design with 8 treatments and three replications during 2013-2014. In the first experiment irrigation was done after each 50 mm evaporation from pan A class (normal irrigation) and in the second experiment irrigation was done after 100 mm evaporation from class A pan (water stress). The studied factors consisted of micronutrients including non foliar application by micronutrients and foliar application by micronutrients such as zinc, iron, boron, zinc + iron, zinc + boron, iron + boron, zinc + iron + boron simultaneously. For planting operations, the seeds

Table 1
Physical and chemical properties of farm soil at depth of (0-30cm)

Years	EC (ds/m)	pH	Lime %	OC %	N %	P mg/kg	K mg/kg	Zn mg/kg	Fe mg/kg	B mg/kg	Clay %	Silt %	Sand %	soil texture
2013	0.95	6.8	21.06	0.85	0.5	11/2	308.5	0.75	1.73	0.25	41	35	24	clay- loam
2014	0.95	6.8	19.8	0.90	0.8	10	275	0.70	1.76	0.23	41	35	24	clay- loam

were sown at rows with 7cm longs. Sowing depth of the seeds was 4 cm and each plot was made up of 5 rows as long as 6 m and the distance between the rows was 50 cm. In the growing phase, operations such as thinning and creating desired density and weeding in three steps were done.

Foliar application of micronutrients occurred at pre-flowering stage. Plant Samples were taken 6 weeks after foliar application. The studied and measured traits were included proline, soluble sugars, chlorophyll a, chlorophyll b contents, oil and grain yield (Movahedi dehnavi et al., 2009). The data analyses were done by SAS software and based on statistical criteria of two-year compound analysis of randomized complete block design and the means of treatments were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Proline

The effect of irrigation and foliar application of micronutrients was significant at %1 probability level and the interactive effect of the treatments was significant at %5 probability level results (Table 2).

The results of Duncan multiple test showed that the lowest rate of proline accumulation belonged to the treatment with normal irrigation by the mean of 379.20 mg/kg and the highest rate belonged to the treatment with stress by the mean of 486.52 mg/kg. In addition, the highest rate of proline belonged to the treatment with foliar application of micronutrients zinc, iron and boron by the mean of 449.7 mg/kg and the lowest rate belonged to the treatment without foliar application of micronutrients by the average of 408.6 mg/kg (Table 3).

The results of Duncan's multiple test on the interactive effects showed that the lowest rate of proline belonged to the treatment with normal irrigation and foliar application of micronutrients zinc, iron, and boron by the mean of 358.5 mg/kg and the highest rate of proline belonged to the treatment with stress and foliar application of micronutrients inc, iron, and boron by the mean of 506.9 mg/kg. The increase of proline concentration

Table 2
The mean squares for measured trials in soybean

Sources of variations	*df*	*Mean squares*					
		Proline	*Soluble sugar*	*ChlA*	*Chl B*	*Oil yield*	*Grain yield*
Year	1	15659.953**	4.307ns	0.783*	0.009*	5.525ns	3794.25*
irrigation	1	276405.44**	723.428**	31.251**	0.239**	4722.07**	92337.24**
Year × irrigation	1	1562.885*	6.962ns	0.002ns	0.002ns	21.784ns	1840.214*
Error1	8	882.603	6.412	0.121	0.003	57.397	635.544
Foliar application	7	1736.11**	7.856**	0.122**	0.011**	358.242**	7274.401**
Year × foliar application	7	54.764ns	2.846**	0.072*	0.002**	11.521ns	222.070ns
Irrigation × foliar application	7	140.059*	0.344ns	0.007ns	0.004**	17.063*	217.379ns
Year × Irrigation ×foliar application	7	23.893ns	0.703ns	0.034ns	0.001*	6.441ns	124.068ns
Error 2	56	47.773	0.570	0.022	0.001	6.952	112.230
Coefficient of variations		4.73	6.03	7.57	5.36	8.93	10.48

ns , * , and ** respectively represent non-significant and significant at 5% and 1% levels.

Table 3
Mean comparisons for simple and interaction treatments effects

Sources of variations	*Means*					
	Proline		*Soluble sugar*		*ChlA*	
Year	*2013*	*2014*	*2013*	*2014*	*2013*	*2014*
Normal irrigation	387.9b	379.2b	989b	980b	2.54a	2.45a
Halt irrigation	503.3a	486.5a	1538a	1580a	1.40b	1.32b
Control	418.9d	408.6e	1160e	1225b	1.86c	1.93a
Zinc	442.5c	429.5d	1318b	1394a	2.01b	1.95a
Iron	445.9c	431cd	1181de	1161b	1.93bc	1.84ab
Boron	441.3c	427.9d	1243cd	1253b	1.85c	1.72b
Zinc+iron	448.2b	437.6bcd	1278bc	1270b	2.03b	1.96a
zinc₊boron	455.8b	439.7ab	1334ab	1422a	2.01b	1.9ab
Iron₊boron	452.1b	438.9abc	1208de	1163b	1.94bc	1.79ab
Zinc₊iron₊boron	460.4a	449.7a	1392a	1395a	2.16a	1.98a
Normal irrigation control	364.6h	358.5g	895h	932efg	2.47b	2.64a
Normal irrigation₊ zinc	387.9fg	378.6ef	1012fg	1053def	2.55b	2.44ab
Normal irrigation₊ iron	390.4fg	380.6ef	895h	831g	2.50b	2.37ab
Normal irrigation₊ boron	384g	375.9f	963gh	948efg	2.42b	2.29b
Normal irrigation₊ zince+ iron	388.4fg	381ef	995fg	972efg	2.59ab	2.56ab
Normal irrigation₊ zinc₊boron	390.8fg	380.9ef	1070ef	108de	2.56b	2.38ab
Normal irrigation₊ iron₊boron	396.1ef	385.8ef	949gh	913fg	2.5b	2.32b
Normal irrigation₊ zinc₊iron₊boron	401.2e	392.4e	1137e	1135d	2.76a	2.58ab
Normal irrigation control	473.2d	458.8d	1425d	1517bc	1.24e	1.22cd
Water stress₊ zinc	497.2c	480c	1624cda	1735a	1.48cd	1.45cd
Water stress₊ iron	501.3c	481. 3c	1466bc	1492c	1.36de	1.32cd
Water stress₊ boron	498.5c	480.5c	1523bc	1558bc	1.29d	e1.15d
Water stress₊ zinc+ iron	508b	494.2bc	1561ab	1568bc	1.47cd	1.35cd
Water stress₊ zinc₊boron	520.8b	498.5a	1599ab	1756a	1.44cd	1.41cd
Water stress₊ iron₊boron	508.1b	492.1bc	1466cd	1414c	1.39cde	1.25cd
Water stress₊ zinc₊iron₊boron	519.5a	506.9ab	1468a	1655ab	1.56c	1.38cd

under stress might indicate the probable role of this amino acid in resistance to water stress (Zaifnejad, M, 2007).

Means followed by the same letters in each column are not significantly different (Duncan's multiple range test 5%).

Soluble Sugar

The results showed that the effect of irrigation and foliar application of micronutrients was significant at %1 probability level and the interactive effect of the treatments was significant at %5 probability level (Table 2). The results of Duncan test showed that the highest rate of soluble sugar by the mean of 1538 mg/g of leaf wet weight belonged to the treatment with water stress and the lowest rate of soluble sugar belonged to the treatment with normal irrigation by the mean of 989 mg/g of leaf wet weight. Moreover, the highest rate of soluble sugar belonged to the treatment with foliar application of micronutrients zinc, iron and boron by the mean of 1392 mg/g of leaf wet weight and the lowest rate belonged to the treatment without foliar application of micronutrients by the mean of 1160 mg/g of leaf wet weight (Table 3). The results of Duncan's multiple test on the interactive effects showed that the highest rate of soluble sugar belonged to the treatment with water stress and foliar application of micronutrients zinc, iron, and boron by the mean of 1648 mg/g of leaf wet weight and the lowest rate of soluble sugar belonged to the treatment with normal irrigation and without foliar application of micronutrients zinc, iron, and boron by the mean of 895 mg/g of leaf wet weight. Accumulation of soluble sugars inside cells plays an important role in osmotic adjustment and contributes to the reduction of cellular water potential and thus more water remains inside the cells to keep the turger pressures under water deficit stress (Kafi et al., 2009). This mechanism leads to the stability of biological membranes, proteins, increase of photosynthesis and resistance to water stress (Sato et al., 2004).

Chlorophyll a

The results showed that the effect of irrigation and foliar application of micronutrients was significant at %1 probability level (Table 2) . The results of Duncan test showed that the highest rate of chlorophyll a belonged to the treatment with normal irrigation by the mean of 2.54 mg/g of leaf wet weight and the lowest rate belonged to the treatment with water stress by the mean of 1.40 mg/g of leaf wet weight. Moreover, the highest rate of chlorophyll a belonged to the treatment with foliar application of micronutrients zinc, iron and boron by the mean of 2.16 mg/g of leaf wet weight and the lowest rate belonged to the treatment without foliar application of micronutrient boron by the mean of 1.85 mg/g of leaf wet weight. The results of Duncan's multiple test on the interactive effects showed that the highest rate of chlorophyll a belonged to the treatment with normal irrigation and foliar application of micronutrients zinc, iron, and boron by the mean of 2.76 mg/g of leaf wet weight and the lowest rate belonged to the treatment with water stress and without foliar application of micronutrients by the mean of 1.24 mg/g of leaf wet weight. Reduction of chlorophyll a, maybe was to due to water stress results from the increased production of oxygen radical (Table 3). These free radicals cause peroxidation and consequently degradation of the pigment. In a study on safflower, foliar application of zinc and manganese increased the chlorophyll which could be due to the role of these elements in nitrogen metabolism and production of chlorophyll (Movahedi dehnavi et al., 2004).

Chlorophyll b

The results (Table 2) showed that the effect of irrigation and foliar application of micronutrients was significant at %1 probability level. The results of Duncan test (Table 4) showed that the highest rate of chlorophyll b belonged to the treatment with water stress by the mean of 0.555 mg/g of leaf wet weight and the lowest rate belonged to the treatment with normal irrigation by the mean of 0.455 mg/g of leaf wet weight. Moreover, the highest rate of chlorophyll b belonged to the treatment with foliar application of micronutrients zinc, iron and boron by the mean of 0.551 mg/g of leaf wet weight and the lowest rate belonged to the treatment with foliar application of micronutrients by the mean of 0.479

Table 4
Mean comparisons for simple and interaction treatments effects

Sources of variations	*Means*					
	Chl B		*Oil yield*		*Grain yield*	
Year	*2013*	*2014*	*2013*	*2014*	*2013*	*2014*
Normal irrigation	0.455b	0.450a	418.5a	411.4a	2176a	2070a
Halt irrigation	0.555a	0.541b	268.8b	271.2b	1468b	1449b
Control	0.479c	0.480bc	264.9e	258e	1454f	1370f
Zinc	0.509bc	0.514b	353.9bc	337.c4	1837cd	1701cd
Iron	0.502bc	0.492bc	313.8cd	309.6d	1710de	1648de
Boron	0.483bc	0.466c	298.4de	301.8d	1663e	1607e
Zinc+iron	0.549a	0.556a	388.6ab	384.5b	2012b	1928b
Zinc$_{+}$boron	0.489bc	0.462bc	348.1c	348.8c	1775de	1760c
Iron$_{+}$boron	0.476bc	0.448c	354.7bc	352.9c	1933bc	1866b
Zinc$_{+}$iron$_{+}$boron	0.550a	0.543bc	421.7a	437.8a	2197a	2201a
Normal irrigation control	0.472bc	0.437cd	341.9ef	322g	1831ef	1655f
Normal irrigation zinc	0.413b	0.498bc	435.8bc	418.c1	2200bcd	2027cd
Normal irrigation iron	0.436bc	0.492bc	387.6cde	372.2ef	2000de	1933de
Normal irrigation boron	0.496b	0.404cd	376.5de	378.8def	2081cd1971cd	
Normal irrigation zince+ iron	0.427b	0.511bc	482.8ab	466.1b	2388ab	2246b
Normal irrigation zinc$_{+}$boron	0.482b	0.419bc	404.4cd	410.1cd	2110cd	2066cd
Normal irrigation iron$_{+}$boron	0.481a	0.385de	418.6cd	403.8cde	2245bc	2101c
Normal irrigation zinc$_{+}$iron$_{+}$boron	0.432b	0.454cd	501.6a	520.8a	2559a	2561ai
Halt irrigation control	0.527ab	0.522bc	188i	193.9j	1077i	1086i
Halt irrigation zinc	0.537a	0.530bc	272gh	256.8i	1475g	1374g
Halt irrigation iron	0.523ab	0.491cd	252gh	247.1i	1420gh	1363gh
Halt irrigation boron	0.540ab	0.529bc	220.2h	224.9i	1246hi	1242g
Halt irrigation zince+ iron	0.602a	0.602a	294.5fg	302.9gh	1636fg	1610f
Halt irrigation zinc$_{+}$boron	0.542a	0.506bc	291.8fg	287.4h	1439gh	1454g
Halt irrigation iron$_{+}$boron	0.534ab	0.512cd	290.8fg	301.9gh	1622fg	1630f
Halt irrigation zinc$_{+}$iron$_{+}$boron	0.628a	0.632a	341.7e	354.9ff	1836ef	1839e

mg/g of leaf wet weight. The results of Duncan's multiple test on the interactive effects showed that the highest rate of chlorophyll b, belonged to the treatment with water stress and foliar application of micronutrients zinc, iron, and boron by the mean of 0.628 mg/g of leaf wet weight and the lowest rate belonged to the treatment with normal irrigation and foliar application of micronutrients iron and boron by the mean of 0.413 mg/g of leaf wet weight. In this study, the rate of chlorophyll b, increased due to water stress because it is a protective and auxiliary pigment that protects the plant and chlorophyll a against water stress. In a study on safflower, foliar application of zinc and manganese increased the chlorophyll which could be due to the role of these elements in nitrogen metabolism and production of chlorophylls (Movahedi dehnavi et al., 2004).

Means followed by the same letters in each column are not significantly different (Duncan's multiple range test 5%).

Oil Yield

The results (Table 2) showed that the effect of irrigation and foliar application of micronutrients on oil yield was significant at %1 probability level and the interactive effect of the treatments was significant at %5 probability level. The results of Duncan multiple test (Table 4) showed that the highest oil yield belonged to the treatment with normal irrigation by the mean of 411.4 kg/ha and the lowest oil yield belonged to the treatment with water stress by the mean of 271.2 kg/ha. Moreover, the highest oil yield belonged to the treatment with foliar application of zinc, iron, and boron by the mean of 437.8 kg/ha and the lowest oil yield belonged to the control treatment by the mean of 258 kg/ha. The results of Duncan's multiple test on the interactive effects showed that the highest oil yield belonged to the treatment with normal irrigation and foliar application of micronutrients zinc, iron, and boron by the mean of 520.8 kg/ha and the lowest oil yield belonged to the treatment with water stress and without foliar application of micronutrients by the mean of 193.9 kg/ha.

Grain Yield

The results (Table 2) showed that the effect of irrigation and foliar application of micronutrients and their interactive effects on grain yield were significant at 1% probability level. The interactive effect of irrigation and foliar application of micronutrients was significant at 5% probability level. The results of Duncan test (Table 4) showed that the highest grain yield belonged to the treatment with normal irrigation by the mean of 2176 kg/ha and the lowest grain yield belonged to the treatment with water stress by the mean of 1468 kg/ha. Moreover, the highest grain yield belonged to the treatment with foliar application of micronutrients zinc, iron, and boron by the mean of 2200.1 kg/ha and the lowest grain yield belonged to the treatment without application of micronutrients by the mean of 1370.1 kg/ha. The results of Duncan's multiple test on the interactive effects showed that the highest grain yield belonged to the treatment with normal irrigation and foliar application of micronutrients zinc, iron, and boron by the mean of 2561 kg/ha and the lowest grain yield belonged to the treatment with water stress and without foliar application of micronutrients by the mean of 1086 kg/ha. Grain yield in soybean decreased significantly because of water stress (Kargar et al., 2004).

CONCLUSION

According to the results it can be stated that in facing with water stress, plants make some changes in some of their physiological properties. Accumulation of solutes in response to water stress is a way to keep osmotic adjustment which is done by increasing osmolytes such as proline and soluble sugars that contribute to the maintenance of turgor pressure in plant cells. Nutrition elements such as zinc, iron, and boron play an important role in the synthesis of plant hormones and contribute to the formation of chlorophyll, photosynthesis, and cell division. To compensate for at least some harmful effects of water stress during the shortage of water and to assist the plant to return to normal growth conditions after re-irrigation, the foliar application of such elements can play a crucial role. Thus the cell continues its vital activities and ultimately the soybean has an acceptable yield in such conditions.

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