Effect of microelements on some physiological traits and yield of soybean (*Glycine max* L.) under water deficit stress conditions

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Abstract

In order to investigate the effect of foliar application of iron (Fe), zinc (Zn), and boron (B) on grain yield and some physiological characteristics of soybeans under water stress, two separate experiments were conducted in a randomized complete block design with eight treatments in three replications in Aleshtar, Lorestan, during 2013-2014 and 2014-2015. In the first and second experiment, irrigation was performed after 50 mm and 100 mm of evaporation from the Class A evaporation pan, respectively. Experimental treatments included spraying of pure water, Zn, Fe, B, Zn + Fe, Zn + B, Fe + B, and Zn + Fe + B. The results of variance analysis showed that the effects of irrigation and foliar application were significant on grain yield, proline content, soluble sugars, chlorophyll a, chlorophyll b, PWC, and anthocyanin. Water stress resulted in an increase in proline content compared with normal irrigation. Water stress also led to decreased grain yield (30%). The maximum grain yield was related to normal irrigation treatment (2070.5 kg. ha^{-1}) and foliar application of Zn + Fe + B (2200.1 kg. ha^{-1}).

Keywords: Soybean, Photosynthetic pigments, Proline, Soluble sugars, grain yield

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Introduction

Drought stress is one of the most important and common nonbiological environmental stresses that limits agricultural production (Tiqah al-Islami et al., 2008; Vahdi et al., 2015). Iran is a country located in arid and semi-arid geographical areas. Plants in these areas are exposed to drought stress at different stages of their growth. On the other hand, nutrients can play an important role in plant resistance against various environmental stresses (Abedi Baba Arabi et al., 2011).

Despite an annual production of 271 thousand tons of oilseeds in the country, most of the oil consumed is supplied from foreign sources. Therefore, the development of oilseed cultivation is of great importance. Soybean oil is used in preparation of vegetable oil and human nutrition. Soybean meal, which contains a large amount of favorable protein, is used in animal husbandry, especially poultry (Younes Sinki, 2008). The

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positive effect of micronutrients on dry matter yield may be due to the increased auxin biosynthesis, increased chlorophyll concentration, increased phosphoenol pyruvate carboxylase and ribulose bisphosphate carboxylase, decreased sodium accumulation in plant tissues, and increased nitrogen and phosphorus uptake efficiency in the presence of zinc. Boron plays an important role in cell division and iron in the formation of chlorophyll in plants (Sharafi et al., 2011; Ravi et al., 2008).

Micronutrients are required for normal plant growth. In addition to participating in the structure of some organs, they are involved in many biochemical reactions of plants (Ravi et al., 2008). Deficiency of these elements can sometimes limit absorption of other nutrients and growth, and this highlights the need to pay more attention to their use. Foliar application of micronutrients can improve the growth status of the plant (Movahhedy-dehnavy et al., 2009). Repeated foliar application of micronutrients while eliminating their deficiency, increases the quantitative and qualitative yield of the plant (Whity and Chambliss, 2005). In addition to increasing the quantity and quality of agricultural products, micronutrients also have a significant impact on human and animal health (Sharma et al., 2005). Pooladvand et al. (2012) suggested that FeSO4 increased seed production, nodule formation, and number of pods and leaves of Glycine *max* L. They also reported higher concentrations of FeSO4 reduced nodules and leaf numbers.

Considering the response of plants against drought stress, there are two main mechanisms: avoidance and tolerance in contrast to water stress depends on the existence of compatible materials in root and stem architecture and plant morphology (Aspinal and Paleg, 2003), but osmotic regulation is considered as an important part of the mechanism of drought tolerance in plants (Zhang, 2010). According to Blum (2007), osmotic regulation is a decrease in cell potential due to an increase in intracellular solutes, not a decrease in cell water content. Plants accumulate low-molecular-weight solutes, commonly called compatible solutes, under different environmental conditions, which contain amino acids, sugars, and betaine. In addition, some inorganic solutes are an important part of intracellular osmotic solutes (Bajji, 2011). Osmotic regulation is a physiological phenomenon in which low molecular weight soluble substances, called compatible solutes, accumulate in plants, maintaining the inflammatory pressure of cells, and the stability of membranes and macromolecules, long cell proliferation, stomatal cleavage, and continued photosynthesis are rooted in survival and further proliferation. During drought stress, the activity of enzymes such as chlorophilase and peroxidase enhances and the activity of enzymes responsible for chlorophyll synthesis is disrupted, reducing chlorophyll and consequently, reducing photosynthesis (Smirnoff, 2007). Arndt et al. (2008) reported that when plants are exposed to drought and salinity stress, they begin to make and accumulate solutions such as amino acids (e.g., proline and aspartic acid), proteins, sugars (e.g., sucrose, glucose, and mannitol), alcoholic compounds (e.g., glycine betaine and alanine betaine), cyclitols (e.g., pinitol, cybracitol, and quercitol), and organic acids in their cells.

Zinc, iron, and boron have meaningful functions in plant metabolism; therefore, this study was conducted to determine the effects of these micronutrients on important physiological traits of soybean under water stress condition.

Materials and Methods

This research was carried out in Lorestan province, Aleshtar, on a land area of 1500 m^2, located at 33° 49' N, 48° 15' E, and 1500 m above sea level. This region is considered as one of the semi-arid regions of the country with cold and wet winters and hot and dry summers.

Two years before planting, a composite sample of 0.0-30 cm depth was collected from the soil of the project and sent to a soil laboratory. Results of the soil test analysis are shown in Table 1.

In order to investigate the physiological reactions of soybeans to water restriction and application of micro-zinc, iron, and boron elements, two separate experiments were carried out in the form of a randomized complete block design with eight

Table 1
Physicochemical properties of the soil collected (0-30 cm depth) from the study site

Soil texture	Sand (%)	Silt (%)	Clay (%)	B mg/kg	Fe mg/kg	Zn mg/kg	K mg/kg	P mg/kg	Total N (%)	Organic matter (%)	Lime (%)	pH	EC (ds/m)	Soil properties
clay-loam	24	35	41	0.25	1.73	0.75	308	11.2	0.5	0.85	21.06	6.8	0.95	1st year
clay-loam	24	35	41	0.23	1.76	0.70	275	10.0	0.8	0.90	19.8	6.8	0.95	2nd year

treatments in three replications during two cropping years 2013-2014 and 2014-2015. Irrigations in the two experiments were performed after 50 mm (normal irrigation) and 100 mm (drought stress) evaporation from Class A pan, respectively. The examined factor was micronutrients, including pure water (control), foliar application of zinc, iron, boron, zinc + iron, zinc + boron, iron + boron, and zinc + iron + boron. The studied traits included proline, soluble sugars, chlorophyll a, chlorophyll b, anthocyanin, oil yield and grain yield. land preparation operation consisted of plowing to a depth of 30 cm using a reversible plow and then two vertical discs were performed. After leveling the ground with a leveler, furrows were made at a distance of 50 cm from each other. Leakage irrigation operation was adjusted by siphon and by transferring water from the main canal into the grooves based on the statistics received from the evaporation pan of Aleshtar meteorological station with 50 mm and 100 mm evaporation from the irrigation pan. Soybean seeds, M9 line, were prepared from the oilseeds section of Jihad-e-Keshavarzi of Aleshtar, Lorestan province. For sowing operations, grooves were first made on the ridges by tilting the shovel, and seeds were planted at a distance of 7 cm. Seed sowing depth was considered 4 cm. The experiment comprised of 48 plots, each plot consisting of five planting lines with a length of 6 m and spacing of 50 cm per row, and two planting lines were considered between planting rows to prevent lateral leakage. In the growing stage, operations such as thinning (creating the desired density) and weeding (three stages) were performed. Foliar application of micronutrients was performed at the flowering stage. Samples were prepared from terminal mature leaves after foliar application (flowering stage) and transferred to the laboratory for physiological chemical analysis.

Proline measurement

To measure proline, test tubes with a volume of at least 30 ml were first prepared according to the number of treatments. In the next step, 1 ml of alcoholic extract was transferred to the tubes and 10 ml of double distilled water was added to each tube. Then, 5 ml of ninhydrin was added to the samples (to prepare ninhydrin per sample, 0.125 g ninhydrin was poured in 2 ml of 6 M phosphoric acid and 3 ml of glacial acetic acid (99.9%) and stirred with a magnet for 16 hours until completely dissolved. Then, 5 ml of glacial acetic acid (99.9%) was added to each sample and the samples were placed in a boiling water bath (Ben Marie) for 45 minutes. The samples were then taken out and cooled to the ambient temperature. Ten (10) ml of benzene was then added to each sample and shaken vigorously to allow proline to enter the benzene phase. After this step, the samples were kept at rest for half an hour and then the light absorption of the samples was read at 515 nm using a spectrophotometer (SHIMADZO 54 A model).

Before performing the above steps, it was necessary to make standards of proline (L-proline) with concentrations of 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.3, 0.4, 0.5, and 0.6 micromoles per milliliter. For this purpose, first the molecular weight of proline was read (molecular weight of L-proline is 115.13 g. mol^{-1}). Then a1 mM proline solution was prepared, which was used as the mother solution. Thus, 115.13 mg of proline (weighing with an accuracy of 0.0001 g) was dissolved in one liter of double distilled water (Bates et al., 1973).

Soluble sugars measurement

To measure soluble sugars, first the number of experimental treatments with closed test tubes

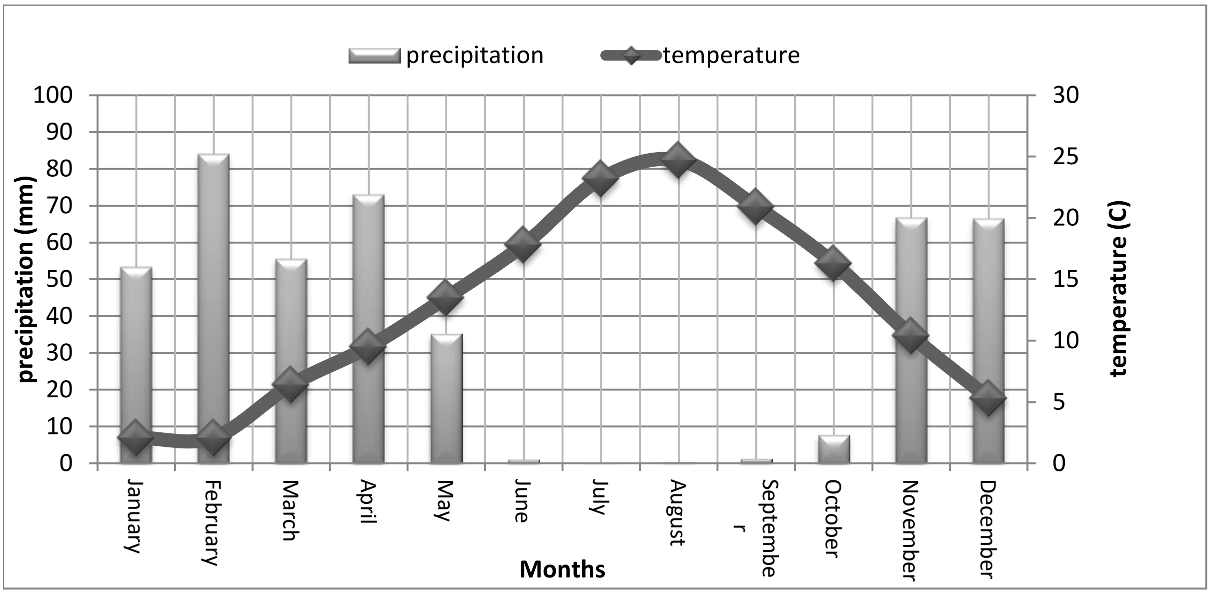

Fig. I. The long-term average temperature and precipitation at Aleshtar station

was selected. Then, 0.1 ml of alcoholic extract was selected and poured into the test tubes. After that, three ml of freshly prepared antron was added to them tubes, which were then placed in a boiling-water bath for 10 minutes. To prepare antron, 150 mg of the antron was dissolved in 100 ml sulfuric acid 72% (w/w). Note that the antron must be prepared fresh each day according to the number of samples. After cooling the samples in the laboratory, the absorbance of the samples at 625 nm was read using a spectrophotometer (SHIMADZO 54 A model). Before performing the above steps, standards of glucose with concentrations of 0, 25, 50, 100, 500, and 1000 ppm were prepared, and all the mentioned steps were performed on them. Then the calibration curve was plotted using the glucose standard, and the amount of soluble sugars in the samples was calculated in m.g^{-1} FLW (fresh leaf weight).

Measurement of photosynthetic pigments

Fresh plant samples were used to measure photosynthetic pigments. For this reason, 0.2 g of fresh plant leaves was removed and ground in a porcelain mortar with 10 ml of 80% acetone, and the solution was transferred to a centrifuge tube. The tubes were centrifuged at 4800 g for 20 minutes until separated. The final volume of the extract was increased to 20 ml with another 10 ml of 80% acetone. At this stage, to calculate the density of chlorophylls a and b, the adsorption of the solution at 645 and 663 nm wavelengths was read using a control (acetone 0.08) in a spectrophotometric device with JENWAY specifications. Finally, the amount of pigments according to the fresh weight of each sample was evaluated in terms of milligrams per fresh weight.

To measure the final grain yield for each plot from the middle row (third) after removing the margin from the sides and 0.5 m from the top and bottom, plants were harvested to a length of 5 m and after harvesting, threshing, and weighing and converted into unit's kg.ha^{-1} was converted and grain yield was calculated.

Statistical Analysis

The obtained data, including physiological and biochemical traits were analyzed based on statistical criteria of two-year combined randomized complete block design using SAS ver. 9.1.3 and mean were compared using Duncan's Multiple Range Test (DMRT). Before analyzing the data, the normality test was performed with MSTAT-C software and after ensuring the normal distribution of the data, they were analyzed. The correlation analysis was done by SPSS ver. 21.

Results

Results of variance analysis showed that the effects of irrigation and foliar application of micronutrients were significant on proline,

Table 2
Analysis of variance (ANOVA) results for studied characteristics

S.O.V	df	proline	soluble sugar	Chl. a	Chl. b	anthocyanin	RWC	grain yield
Year	1	15659.953**	4.307^{ns}	0.783*	0.009*	0.073^{ns}	824.397*	3794.254*
Irrigation	1	276405.44**	723.428**	31.251**	0.239**	39.611**	2300.709**	92337.244**
Year× Irrigation	1	1562.885**	6.962^{ns}	0.002^{ns}	0.002^{ns}	0.009^{ns}	35.973*	1840.214*
Error	8	882.603	6.412	0.121	0.003	0.160	158.065	635.544
Foliar application	7	1736.11**	7.856**	0.122**	0.011**	0.687**	128.964**	7274.401**
Year× Foliar application	7	54.765^{ns}	2.846**	0.072*	0.002**	0.138*	24.778*	222.070^{ns}
Irrigation × Foliar application	7	140.059*	0.344^{ns}	0.007^{ns}	0.004**	0.225^{ns}	14.268^{ns}	217.379^{ns}
Year×Irrigation × Foliar application	7	23.893^{ns}	0.703^{ns}	0.034^{ns}	0.001*	0.146*	8.162^{ns}	124.068^{ns}
Error	56	47.773	0.570	0.022	0.001	0.024	6.666	112.230
CV (%)	-	4.73	6.03	7.57	5.36	5.55	5.83	10.48

ns: non-significant, **and * significant at 1 and 5 %, respectively

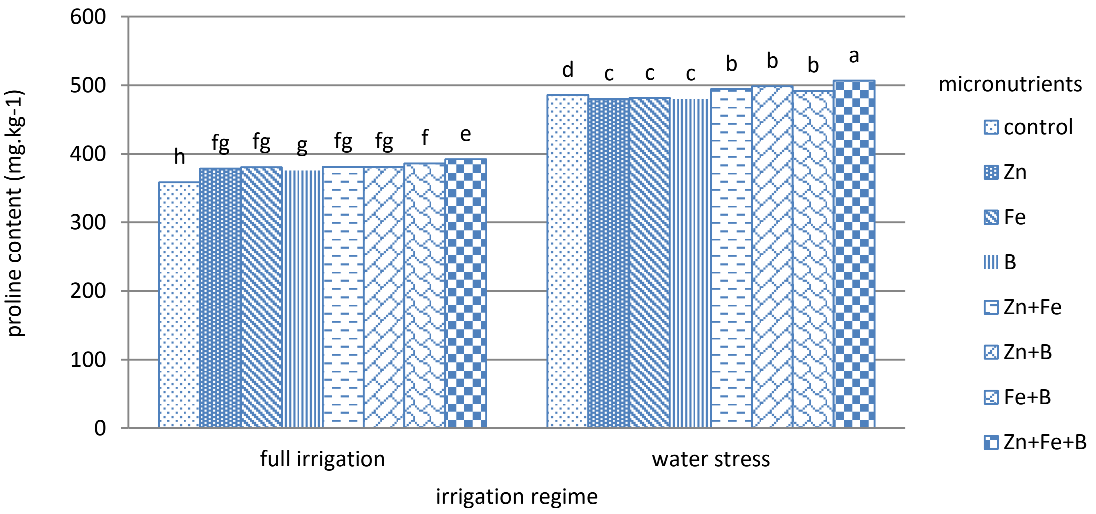

Fig. II. Comparison of mean proline contents under irrigation regime, micronutrients, and their interaction

soluble sugar, chlorophyll a, chlorophyll b, anthocyanins contents, RWC, and grain yield (P≤0.01). Also, the interaction of irrigation × micronutrients had significant effects on proline content (P≤0.05) and chlorophyll b (P≤0.01) (Table 2). Comparison of the mean interaction effects of irrigation and micronutrients on proline showed that the lowest proline content was related to normal irrigation treatment and foliar application of Zn+Fe+B with a mean of 358.5 mg.kg^{-1} while the highest proline content was related to stress and foliar application of zinc, iron, and boron micronutrient fertilizers with a mean of 506.9 mg.kg^{-1} (Fig. II).

Comparison of mean effects of irrigation on soluble sugar revealed that the maximum and minimum soluble sugars were related to water stress and normal irrigation with means of 15.38 and 9.89 mg. g^{-1} FW, respectively. Comparison of mean effects of foliar application of micronutrients indicated the most and least amounts of soluble sugars were related to foliar application of Zn+Fe+B and non-application of micronutrient with a

Table 3
The mean comparison of irrigation and foliar application of micronutrients

Treatments	Soluble sugar (mg.g^{-1} FW)	chlorophyll a (mg.g^{-1} FW)	Anthocyanin (mmol.kg^{-1})	RWC (%)	Grain yield (kg.ha^{-1})
normal irrigation	9.890 b	2.54 a	3.43 a	66.59 a	2070.5 a
Water stress	15.38 a	1.40 b	2.14 b	56.80 b	1449.7 b
Control	1.600 e	1.86 c	2.38 c	55.50 e	1370.1 f
Zn	11.82 b	2.01 b	2.70 b	63.03 bc	1701.0 cd
Fe	13.52 de	1.93 bc	2.73 b	61.22 c	1607.0 de
B	12.20 cd	1.85 c	2.68 b	58.68 d	1607.0 e
Zn+Fe	12.32 bc	2.02 b	3.13 a	64.14 ab	1928.0 b
Zn+B	13.72 ab	2.01 b	2.79 b	62.42 bc	1760.1 c
Fe+B	12.02 de	1.94 bc	2.78 b	62.60 bc	1866.0 b
Zn+Fe+B	13.92 a	2.16 a	3.09 a	65.99 a	2200.1 a

Means within the same column and row factors, followed by same letter are not significantly difference ($p<0.05$).

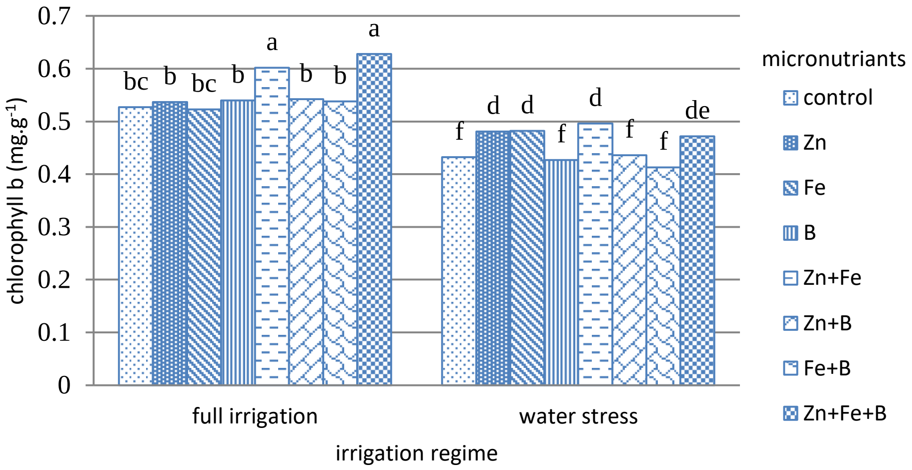

Fig. III. Comparison of mean effects of irrigation regimes and micronutrients interaction on chlorophyll b content

mean of 13.92 and 11.60 mg. g^{-1} FW, respectively (Table 3).

The results of Duncan's test showed that the highest amount of chlorophyll a was related to normal irrigation treatment with a mean of 2.54 mg. g^{-1} fresh leaf weight, and the lowest level was related to stress treatment with a mean of 1.40 mg. g^{-1} fresh leaf weight. The highest amount of chlorophyll a was in the foliar application of zinc, iron, and boron micronutrient fertilizers with a mean of 2.16 mg. g^{-1} of fresh leaf weight, and the lowest amount was in the non-application of boron micronutrient fertilizers with a mean of 1.85 mg. g^{-1} fresh leaf weight. Duncan's test showed that the highest amount of chlorophyll a was related to normal irrigation treatment and foliar application of Zn+Fe+B with a mean of 2.76 mg. g^{-1} FWL, and the lowest amount was related to stress treatment and non-foliar application of micronutrient fertilizers with a mean of 1.24 mg. g^{-1} FWL (Table 3).

Comparison of the mean interaction effect of irrigation × micronutrients indicated that the highest amount of chlorophyll b was related to irrigation treatment after 100 mm of evaporation from the pan and foliar application of zinc, iron, and boron micronutrient fertilizers with a mean of 0.628

Table 4
Correlation coefficients of the characteristics under study

Characteristics	Proline	Soluble sugar	Chlorophyll a	Chlorophyll b	Anthocyanin	RWC	Grain yield
proline	1						
Soluble sugar	0.908**	1					
chlorophyll a	-0.869**	-0.804**	1				
chlorophyll b	-0.698**	-0.634**	0.8**	1			
anthocyanin	-0.768**	-0.728**	0.862**	0.752**	1		
RWC	-0.393**	-0.496**	0.600**	0.565**	0.626**	1	
Grain yield	-0.573**	-0.565**	0.767**	0.704**	0.806**	0.741**	1

** significant at 1%

mg. g^{-1} FW, and the lowest amount was related to the drought treatment with 50 mm of evaporation from the pan and foliar application of Fe+B micronutrient fertilizers with a mean of 0.413 mg. g^{-1} FW (Fig. III).

The highest anthocyanin was related to stress treatment with a mean of 3.43 mM kg^{-1}, and the lowest anthocyanin was related to normal irrigation treatment with a mean of 2.14 mM kg^{-1}. The highest anthocyanin was found in the combined foliar application of zinc and iron micronutrient fertilizers with a mean of 3.13 mM kg-1, which was in the same statistical group as Zn+Fe treatment. The lowest anthocyanin content was related to the non-application of micronutrient fertilizers with a mean of 2.38 mM kg^{-1} (Table 3).

The results of the mean effects of irrigation on RWC showed that the highest and lowest values were related to irrigation treatment after 50 and 100 mm evaporation with averages of 66.59% and 56.80%, respectively. Furthermore, the highest relative water content was recorded in foliar application of Zn+Fe+B with an average of 65.99% while the lowest level of this attribute was observed in non-application of micronutrient fertilizers with an average of 55.50% (Table 3).

Comparison of the mean effects of irrigation on grain yield indicated the highest and lowest grain yield was observed under normal irrigation and stress treatment with means of 2070.5 and 1449.7 kg ha^{-1} respectively. Furthermore, foliar application of Zn+Fe+B resulted in an increase by 38% in the grain yield compared to control. The highest and lowest grain yield was found in the foliar application of Zn+Fe+B and non-application of micronutrient (control) with means of 2200.1 and 1370.1 kg ha^{-1} (Table 3).

Correlation analysis

Results of correlation analysis showed grain yield had positively significant correlations with chlorophyll a, chlorophyll b, anthocyanin contents, and RWC and negatively significant correlations with proline and soluble sugar contents (Table 4).

Discussion

Increased proline concentrations under stress may indicate a possible role for this amino acid in drought stress resistance. Accumulation of proline in plants under abiotic stresses is due to proline synthesis and inactivation of its degradation. Low levels of proline in irrigated plants can result in its combination with oxygen and conversion to glutamic acid and other compounds under stress conditions. Zinc and manganese, especially in drought-resistant cultivars under stress conditions, play an increasing role in yield. They have an osmotic mass (due to an increase in proline or soluble sugars). Proline is generally made through two main pathways: the glutamate pathway, whose enzymes are located in the cytoplasm, and the urethra pathway, whose enzymes are located in the mitochondria. The glutamate pathway is more important in higher plants and key enzymes in this pathway appear to have responded positively to zinc and manganese spraying (Zaifnejad, 2007).

Considering changes in the level of soluble sugar, it appears that increasing the activity of amylase enzyme under drought stress decomposes starch and converts it into smaller units, and this is done to withstand stress through osmotic regulation. The increase in soluble sugars in response to drought stress has been attributed to its slower transfer from leaf to stem and its slower consumption due to the reduced growth and other changes such as starch hydrolysis and lower soluble carbon carbohydrates in leaves in the absence of stress are largely related to the continued consumption of carbohydrates produced at plant growth points. The accumulation of soluble sugars inside the cells plays an important role in osmotic regulation and helps to reduce the water potential of the cell and keep more water to maintain the tourniquet under dehydration stress (Kafi et al., 2009). This mechanism stabilizes biological membranes. Proteins increase photosynthesis and drought resistance (Sato et al., 2004).

In this study, chlorophyll a decreased due to drought stress. This might be attributed to the increased production of oxygen radicals, which cause peroxidation and decomposition of this pigment. In a study on safflower, foliar application of zinc and manganese increased chlorophyll, which may be due to the role of these elements in nitrogen metabolism and chlorophyll production (Movahedi Dehnavi et al., 2004).

Under stress, the amount of chlorophyll b increased because it is a protective and supporting pigment that protects the plant and chlorophyll a against stress. Micronutrients are required for the normal growth of plants and are involved in many plants' biochemical reactions while participating in the structure of some organs. For example, zinc is involved in the production of growth hormones (auxin) and photosynthesis, boron is involved in cell division, and iron is involved in chlorophyll formation (Ravi et al., 2008). In a study on safflower, spraying zinc and manganese increased chlorophyll (Movahedi Dehnavi et al., 2004). This may be due to the role of these elements in nitrogen metabolism and chlorophyll production (Movahedi Dehnavi et al., 2004). Furthermore, Anthocyanins, like flavonoids, are protective pigments that protect plants against stress (Chalker-Scott, 2012). It has been reported that the amount of anthocyanins increases under stress. This increase is due to the light protection role of anthocyanins by direct removal of ROS during oxidative stress (Zhang et al., 2010).

On the other hands, Drought stress reduces dry matter and grain yield in plants, because it decreases leaf area and plant height while increasing transfer of the specific photosynthetic material to the roots compared to the aerial part of the plant. Normally, in suitable moisture conditions, the uptake and transfer of micronutrients such as zinc sulfate in plants is more easily done, and it is natural that in non-stress conditions, the effect of zinc sulfate foliar application on higher yield be wet. Increased yield by spraying fertilizer on zinc can have several causes, including increased auxin biosynthesis in the presence of zinc, increased chlorophyll concentration, increased phosphoinol pyruvate carboxylase and ribosomal phosphate phosphatase ribose, carbohydrate phosphatase ribosome, reduction of sodium accumulation in plant tissues and increase the efficiency of nitrogen and phosphorus uptake in the presence of micronutrients. Iron plays a vital role in chlorophyll production and electron transfer in photosynthesis, and ferroxin is an iron-carrying protein involved in electron transfer (Ahmadi et al., 2005), so it is natural that as leaf iron increases, leaf chlorophyll content also increases. Increase photosynthesis and ultimately increase performance. In general, considering the role of zinc, iron and boron in photosynthesis, it can be said that trace elements increase yield in plants by increasing photosynthetic capacity and photosynthetic rate of plants. Movahedi Dehnavi et al. (2004), Abolhassani and Saeedi (2006) and Naderi Darbaghshahi et al. (2004) also reported a decrease in grain yield during water stress in safflower. In soybean, grain yield was significantly reduced due to drought stress (Kargar et al., 2004). Soybean yield is related to the number of pods producing seeds per unit area, and the number of pods is proportional to the number of flowers that turn into pods. Therefore, seed yield depends on the number of flowers produced and is inversely related to flower or pod shedding. Therefore, drought stress by reducing these factors can reduce the final yield.

The positive correlations between grain yield and chlorophyll a, b, and anthocyanin indicate that it is necessary to have a suitable green growth (dry matter) with good ability to transfer photosynthetic materials from green growth (dry matter) to grain for achieving a high grain yield (Soleymani Fard and Naseri, 2020).

Conclusion

According to the findings, physiological characteristics of plants change in the face of drought stress. Accumulation of soluble substances in response to drought (osmotic regulation) is a way to maintaining turgor and this increases osmolytes such as proline and soluble sugars. Foliar spraying of zinc, iron, and boron can play a role in compensating for at least some of the harmful effects of drought stress during dehydration and helping to return to normal growth conditions after re-irrigation. This way, the cell continues its vital activities. Finally, foliar application of micronutrients in the soybean plants in this study resulted in an acceptable yield under water deficit stress conditions.

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