

Physiological responses of sesame (*Sesamum indicum* L.) to foliar application of boron and zinc under drought stress

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Abstract:

Micronutrient deficiency is characteristic of drought stress conditions and the remedy lies in the administration of nutrients with boron and zinc supplements for the crops to tolerate and survive drought conditions. This study evaluated the effects of zinc and boron foliar application on the physiological traits of sesame under different irrigation regimes. The experiment was conducted in 2013 as a split plot in a randomized complete block design with three replications at Fars Research Center for Agriculture and Natural Resources. The main factors included three irrigation levels (irrigation after 75, 110, and 145 mm evaporation levels from class A evaporation pan) and the sub-factors included foliar sprays of water, zinc sulphate, boric acid, and the mixture of zinc sulphate and boric acid. Results showed that leaf proline content increased significantly as a result of foliar application of boric acid in the 75-mm evaporation treatment and by boric acid and the mixture of zinc sulphate and boric acid in the 110- and 145-mm evaporation treatments. Maximum glycinebetaine content was attained with the foliar application of zinc sulphate at all the three irrigation levels. Foliar application of zinc sulphate and boric acid led to the highest leaf relative water content as compared with that of the control. Maximum seed zinc content in the 75-mm evaporation treatment was obtained when either zinc sulphate or the mixture of zinc sulphate and boric acid were applied. Generally, results showed that foliar application of zinc sulphate and boric acid improved both the physiological traits of sesame and its tolerance to drought stress.

Keywords: Chlorophyll, Malondialdehyde, Proline, Relative water content.

Introduction:

Sesame (*Sesamum indicum* L.) is one of the most important oil crops widely grown in different parts of the world. For many centuries, sesame seeds have been used as a source of oil, protein, vitamins, and minerals for human and animal nutrition (Weiss, 2000). Sesame oil is a very stable one due to its content of such antioxidants as sesamin, sesamol, and sesamol (Suja *et al.*, 2004). The seed is not only rich in oil (42–45%) but also in proteins (20%) and carbohydrates (14–20%). The micronutrient content of sesame seeds generally follows the order Fe>Cu>Zn>Mn (Suresh *et al.*, 2013).

Iran is mostly characterized by arid and semi-arid climates. Drought stress in this region is, therefore, one of the most important environmental factors reducing the growth and yield of many crops (Reddy *et al.*, 2004). Having an average annual precipitation of 250 mm, Iran receives less than one third of the global average precipitation (750 mm). To this must be added the problem of uneven distribution of precipitation (Khorasanizadeh, 2012) so that water shortage is a common feature of both irrigated and rainfed farms in Iran.

Plant responses to abiotic stresses, especially drought, include the production of reactive oxygen species (ROS) and other toxic substances (Xiong *et al.*,

2002) that cause fatty acid peroxidation and damage to cell membranes, which in turn create small hydrocarbon segments such as Malondialdehyde (MDA) (Moussa and Aziz, 2008). Increased Malondialdehyde content is a sign of drought impact on plant cells.

Another common response of plants to abiotic stresses is the production of various types of organic solvents including proline (Szabados and Savouré, 2010; Mohamed and Abdel-Hamid, 2013). Proline is a compatible solution that helps the regulation of osmotic potential in the cytoplasm (Caballero, 2005) and serves as a physiological marker under osmotic stress. Oraki *et al.* (2012) found that increased free proline occurred as a result of water deficit so that its concentration was higher in stress-tolerant than in stress-sensitive sunflower plants. This is because proline has positive effects on enzyme and membrane integrity while it is also part of the stress signaling system influencing plant adaptive responses (Szabados and Savouré, 2010).

Relative water content (RWC) is an important physiological trait that determines plant's tolerance to drought stress (Sánchez-Blanco *et al.*, 2002). Velu and Palanisami (2002) reported that drought stress reduced RWC in sunflower. Plants that maintain a higher RWC are obviously more drought-tolerant.

Leaf chlorophyll content is an important

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physiological trait that will change under stress. A reason for changes in chlorophyll content as affected by water deficit is the production of reactive oxygen species (ROS) under drought stress that might lead to lipid peroxidation and, consequently, to chlorophyll destruction (Mirnoff, 1993). Zarco-Tejada *et al* (2000) considered leaf chlorophyll as one of the most important factors representing environmental pressure on plants. The authors maintained that the amount of chlorophyll in plants reduces under most stresses to decrease total light absorption by the plant.

Nutrient uptake through the roots is also reduced under drought conditions due to the reduced soil moisture, which decreases nutrient transfer from plant roots to branches. One reason for this decrease is the deteriorating flexibility of cell membranes that adversely affects their active uptake of nutrients (Hu *et al.*, 2007). This ultimately leads to nutrient deficiency as one of the main factors limiting plant growth under drought stress. Moreover, water stress has been reported to cause changes in mineral absorption rates in the plant root system and its flow in the stem (Sween *et al.*, 2003).

In arid and semi-arid regions like Iran, foliar application of nutrients, as compared with soil fertilization, is a more suitable option when roots fail to provide the necessary nutrients. Foliar application of liquid fertilizers and foliar feeding form the two main effective approaches which rapidly and directly supply the missing nutrients required in plant branches, leaves, and fruits (Ling and Silberbush, 2002). Mn, Fe, Cu, Zn, B, and Mo are the six essential micronutrients required by all higher plants (Welch, 1991). Sufficient amounts of such micronutrients as zinc play an important role in increasing crop tolerance to environmental stresses (Baybordi and Mamedov, 2010). Zinc also has a role to play in some metabolic processes of plants, such as protein synthesis and membrane integrity (Cakmak and Marschner 1988) as well as nitrogen, phosphorus, and potassium uptake and metabolism (Li *et al.*, 2009).

Karami *et al* (2016) reported that water and zinc deficiency lead to severely reduced chlorophyll *a*, *b*, and total chlorophyll. Savitskaya (1976) stated that proline and chlorophyll in higher plants are synthesized by glutamate. It seems that when plants obtain enough Zn through foliar application, a major portion of glutamate interferes with chlorophyll biosynthesis to decrease its amounts needed in the proline biosynthesis pathway (Karami *et al.*, 2016).

Boron is also one of the most common micronutrients whose deficiency in plants mainly arises from alkaline soils poor in boron. This element is necessary for protein synthesis, seed and cell wall formation, pollen germination, and pollen tube growth (Dordas, 2006). Bellaloui *et al* (2013) reported the likely effects of foliar application of boron in soybean plants under drought stress. The higher demand for boron during the flowering and seeding stages has been reported even in soils with apparently adequate boron

contents.

Fahad *et al* (2014) reported an enhanced leaf chlorophyll content as a result of foliar application of the micronutrients B, Zn, and Fe. Increasing leaf chlorophyll content has also been reported to be directly related to the availability of proper proportions of nutrients at the right time (Fahad *et al.*, 2014).

Considering the importance of sesame as a source of plant oil, the present study was designed and implemented to determine the effects of foliar application of boron and zinc on seed and leaf zinc and boron contents and on the physiological traits of this plant under drought stress.

Materials and Methods:

Plant material and treatments: This experiment was carried out in 2013 on a research farm in Zarghan (52° 43' longitude, 29°46' latitude at an altitude of 1604 m) located 25 km northeast of Shiraz, Iran. The experiment was conducted as a split plot in a randomized complete block design with three replications. The main factors included drought stress levels as delayed irrigation after 75, 110, and 145 mm of evaporation from the class A pan. The sub-plots consisted of spraying only water and foliar applications of zinc sulphate, boric acid, and the mixture of zinc sulphate and boric acid. Foliar applications of zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 99% , 3 gr L⁻¹) and boric acid (H_3BO_3 99.8%, 2 gr L⁻¹) were carried out in two stages: the first at the eight-leaf stage, and the repetition three weeks after the first application (Movahhedy-Dehnavy *et al.*, 2009).

Prior to the experiment, soil samples were obtained with an auger from soil depths of 0–30 cm and 30–60 cm to determine their physical and chemical properties (Table 1). In order to supply the required levels of nitrogen and phosphorus as determined by soil tests, 80 kg ha⁻¹ of triple super-phosphate was applied to the land at sowing in addition to 180 kg ha⁻¹ of urea split in two stages, before planting and four weeks after the first application. Planting was performed on July 6 in rows distanced 50 cm from each other with a seed distance of 5 cm in rows. Darab 1 cultivar, widely grown in Shiraz, was used in this experiment. Before planting, the seeds were disinfected with carboxyinthiram. Thinning was performed after emergence and plant establishment. Weed control was performed manually during development. The first irrigation was accomplished after planting and recorded as the planting date.

Sample preparation: Leaf samples collected from the youngest fully expanded leaves on the main stem were prepared for the determination of proline, glycine betaine, and Malondialdehyde (MDA) contents as well as the SPAD index and RWC immediately before irrigating the drought-stressed treatments after two cycles of irrigation had been already accomplished. Leaf zinc and boron contents were also determined at the beginning of capsule formation.

Determination of proline: Proline content was determined as described in Paquine and Lechasseur

Table 1-Physico-chemical properties of the experimental field soil

pH	EC (dS/m)	Humidity (%)	Lime (%)	Organic carbon (%)	Total N (%)	Available P (mg/kg)
7.92	0.48	56	45.5	0.10	0.95	8
Available K (mg/kg)	Sand (%)	Silt (%)	Clay (%)	Soil texture	Zn (mg/kg)	Boron (mg/kg)
496.5	16	50	34	Silty clay loam	0.6	0.56

(1979). Briefly, approximately 0.5 gr of leaf was homogenized in 5 ml of 70% ethanol and the homogenate was centrifuged for 15 min at 15,000 rpm. One ml of the supernatant was used for the measurement of proline content. The reaction mixture consisted of 5 ml of acid ninhydrin and 5 ml of glacial acetic acid (99.9%), which was boiled for 45 minutes in a boiling water bath. The samples were then cooled before 10 ml of benzene was added to each and left to rest for 30 min when the absorbance of the samples was read at 515 nm using a spectrophotometer (Lambda EZ 210). A standard curve was constructed for proline to determine proline concentration in each sample.

Determination of Glycinebetaine: Glycinebetaine was determined using the method described by Grattan and Grieve (1999). Briefly, dried, finely powdered leaves (0.5 g) were shaken in 20 ml of deionized water for 48 h at 25 °C. The extracts were diluted 1:1 with 2N H₂SO₄. Aliquots of 0.5 ml were placed in test tubes and cooled in ice water for 1 h before a cold KI-I₂ reagent (0.2 ml) was added. The tubes were then stored at 0–4 °C for 16 h and centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was aspirated and the periodite crystals were dissolved in 9 ml of 1,2-dichloroethane. After 2–2.5 h, the absorbance was measured at 365 nm.

Determination of leaf RWC: RWC was estimated by recording the turgid weight of fresh leaf samples and keeping them in water for 6 h followed by drying in the hot air oven until a constant weight was reached. The following formula was used to determine RWC values expressed in percent:

$$RWC = ((FW - DW) / (TW - DW)) \times 100$$

where, FW is fresh weight, TW is the turgid weight of hydrating samples enclosed in a black envelope at about 25 °C for 6 h, and DW is the dry weight of leaves after oven-drying the samples at 85 °C for 4 h (Levit, 1980).

Determination of SPAD index: Leaf chlorophyll index was measured using a hand-held dual-wavelength chlorophyll meter (SPAD 502; Minolta) after two water stress periods had been exercised.

Determination of Malondialdehyde (MDA) content: Oxidative damage to lipids was estimated by measuring leaf MDA content. For this purpose, leaf segments (0.2 g) were homogenized in 3 ml of 10% trichloroacetic acid (TCA). Then, 1 ml of 0.5% thiobarbituric acid (TBA) in 10% TCA was added to an aliquot of 1 ml from the supernatant. The mixture was heated in a boiling water bath for 30 min before it was cooled to determine absorbance at 532 and 600 nm using a spectrophotometer (Lambda EZ 210). MDA content was calculated as described by Heath and

Pacher (1968).

Determination of leaf and seed zinc contents: One gram of leaf powder was ashed in an oven at 500 °C for 7 hours. To the ash thus obtained was added 5 ml of 2N HCl and placed on the heater. Finally, the sample volume was made to 50 ml using double distilled water. Absorption values were determined using an atomic absorption spectrophotometer (Lambda EZ 210).

Determination of leaf and seed boron contents: A standard boron solution was prepared by dissolving 0.114 g of sodium borate in 700 ml of water and diluted to 1.0 L with water. The solutions contained 20 µg ml⁻¹ boron. The reagent solution was prepared by dissolving 0.45 g of 4-methoxy-azomethine-H in 100 ml of ascorbic acid. A buffer solution was prepared by dissolving 250 g of ammonium acetate and 15 g Ethylenediaminetetraacetic acid (EDTA) in about 400 ml of water. Afterwards, 125 ml of acetic acid was added to the solution and pH was adjusted with diluted ammonia solution using a pH-meter. Then, 1.0 ml of the blank, the sample extracts, and standard series was transferred into a 10 ml plastic tube before 2 ml of the buffer solution and 2 ml of 4-methoxy-azomethine-H were added sequentially. Absorbance was measured at 420 nm after 30 min.

Statistical analysis: Statistical analysis was carried out using SAS 9.1 software. The mean values obtained for the main effects of the experimental factors were determined using the Least Significant Difference (LSD) test at 5% when the interaction of factors was significant. This was followed by slicing and comparison of means was carried out according to the L.S. Means procedure.

Results:

Leaf Proline: Interaction of irrigation and foliar application was significant ($P \leq 0.01$) for proline (Table 2). Comparison of the means showed that foliar application of boron in the irrigation treatment after 75 mm of evaporation increased leaf proline content significantly (Table 3). The highest proline contents were achieved in the irrigation treatments after 110 and 145 mm evaporation with foliar application of boron and the mixture of zinc and boron (Table 3).

Glycinebetaine: Interaction of irrigation and foliar application was significant ($P \leq 0.01$) for glycinebetaine (Table 2). Comparison of means (Table 3) showed that foliar application of zinc yielded the highest glycinebetaine content in all the irrigation treatments, which was significantly different from the other three foliar applications.

Relative Water Content (RWC): tments had

Table 2- Analysis of variance of the effect of irrigation and foliar application on measured traits for sesame

Source of variability	df	Proline	Glycine betain	RWC	SPAD	MDA	Leaf zinc content	Seed zinc content	Leaf boron content	Seed boron content
Rep	2	0.06 ^{ns}	0.03 ^{ns}	24.05 ^{ns}	48 ^{ns}	6.49 ^{ns}	37.5 ^{ns}	1.58 ^{ns}	17.02 ^{ns}	3.33 ^{ns}
Irrigation	2	2.51 ^{**}	27 ^{**}	207 [*]	79.3 ^{ns}	871 ^{**}	399 [*]	8547 ^{**}	0.63 ^{ns}	9.57 ^{ns}
Error a	4	0.04	0.29	17.9	38.2	19.7	32.5	54.1	6.64	2.82
Foliar application	3	2.73 ^{**}	80.0 ^{**}	141 ^{**}	132 ^{**}	117 [*]	35081 ^{**}	60.97 ^{**}	415.22 ^{**}	19.32 ^{**}
Irrig×Foliar	6	0.68 ^{**}	8.82 ^{**}	8.68 ^{ns}	25.0 ^{ns}	51.0 ^{ns}	428 ^{**}	2845 ^{**}	36.08 ^{ns}	8.69 ^{ns}
Error b	18	0.07	0.18	11.1	11	26.2	106	126	15.78	3.62
Cv (%)		12.9	15.7	4.53	7.32	16.4	9.74	20.5	23.43	20.90

* and **: Significant at the 5 and 1% probability level, respectively and ^{ns}: non-significant

Table 3- Mean comparison of interaction of irrigation and foliar application for some of the sesame traits.

Irrigation	Foliar application	Proline μmol g ⁻¹ fw	Glycinebetain (mg g ⁻¹ dw)	Leaf zinc content (μg g ⁻¹)	Seed zinc content (μg g ⁻¹)
Irrigation after 75 mm evaporation	Water	1.26 ^b	0.36 ^b	43.5 ^c	31.6 ^b
	Boric acid	2.50 ^a	1.00 ^b	97.8 ^b	34.8 ^b
	Zinc sulfate	1.40 ^b	4.00 ^a	155 ^a	147 ^a
	Combined of zinc and boric acid	1.33 ^b	0.7 ^b	152 ^a	128 ^a
Irrigation after 110 mm evaporation	Water	2.06 ^b	0.6 ^b	21.6 ^c	43.1 ^a
	Boric acid	2.50 ^a	0.7 ^b	80.3 ^b	30.8 ^a
	Zinc sulfate	2.10 ^b	7.06 ^a	155 ^a	51.6 ^a
	mixture of zinc and boric acid	2.90 ^a	0.83 ^b	159 ^a	43.5 ^a
Irrigation after 145 mm evaporation	Water	1.66 ^b	0.7 ^c	25.6 ^c	30.8 ^b
	Boric acid	3.23 ^a	1.06 ^c	58.6 ^b	26 ^b
	Zinc sulfate	1.60 ^b	10.0 ^a	164 ^a	53.1 ^a
	mixture of zinc and boric acid	3.26 ^a	5.83 ^b	155 ^a	35.3 ^{ab}

In each column and in each irrigation level, means with at least one common letter are not significantly different based on L.S.Means procedure.

Table 4- Mean comparison of the effects of irrigation on RWC and MDA of sesame leaves

Irrigation	(%) RWC	MDA (nmol g ⁻¹ fw)
Irrigation after 75 mm evaporation	78.4 ^a	22.6 ^c
Irrigation after 110 mm evaporation	72.2 ^b	31.0 ^b
Irrigation after 145 mm evaporation	70.5 ^b	39.6 ^a

In each column, means with at least one common letter are not significantly different based on LSD test (P≤0.05).

Table 5- Mean comparison of the effects of foliar application on some physiological traits of sesame

Foliar applications	RWC (%)	SPAD	MDA (nmol g ⁻¹ fw)	Leaf boron conc. (μg g ⁻¹)	Seed boron conc. (μg g ⁻¹)
Water	68.2 ^c	39.9 ^b	35.8 ^a	11.1 ^c	7.03 ^b
Boric acid	77.4 ^a	45.8 ^a	30.8 ^b	25.9 ^a	9.38 ^a
Zinc sulfate	75.5 ^{ab}	49.0 ^a	27.0 ^b	12.3 ^c	9.53 ^a
Mixture of zinc and boric acid	73.6 ^b	46.2 ^a	30.7 ^b	18.3 ^b	10.4 ^a

In each column, means with at least one common letter are not significantly different based on LSD test.

significant effects (P≤0.05 and P≤0.01, respectively) on RWC (Table 2). Comparison of means revealed that delayed irrigation, irrigation after 110 and 145 mm evaporation, led to significant decreases in RWC relative to the value obtained with irrigation after 75 mm evaporation (Table 4). Compared to the control, all foliar applications increased RWC significantly (Table 5).

SPAD index: Foliar applications were observed to have significant (P≤0.01) effects on SPAD index (Table 2). Comparison of means showed that all the foliar applications, as compared with water only application, led to significant increases in SPAD (Table 5).

Malondialdehyde (MDA): Irrigation treatments and foliar applications were found to have significant (P≤0.01 and P≤0.05, respectively) effects on MDA (Table 2). Comparison of means indicated that delayed irrigation and the resulting enhanced drought stress increased MDA content (Table 4), with the highest obtained with irrigation after 145 mm evaporation. Compared to water treatment, foliar applications led to significant reductions in malondialdehyde, indicating the positive impact of micronutrients on reducing malondialdehyde content (Table 5).

Leaf zinc content: Interaction of irrigation and foliar application was significant (P≤0.01) for leaf zinc

content (Table 2). In all the three irrigation treatments, foliar applications of zinc and combined zinc and boron significantly increased leaf zinc content compared to those of water only and boron (Table 3).

Seed zinc content: Interaction effect of irrigation and foliar application was significant ($P \leq 0.01$) on seed zinc content (Table 2). In the irrigation treatment after 75 mm evaporation, foliar applications of only zinc and the mixture of zinc and boron led to significant increases in seed zinc content (Table 3). Similar results were obtained with the irrigation treatment after 145 mm evaporation.

Leaf boron content: Effect of foliar application was significant ($P \leq 0.01$) on leaf boron content (Table 2). Foliar application of boron resulted in enhanced leaf boron content followed by foliar application of zinc and boron mixture. Foliar application of water only yielded the lowest leaf boron content (Table 5).

Seed boron content: Effect of foliar treatment was significant ($P \leq 0.01$) on seed boron content (Table 2). Compared to water application, all foliar treatments increased seed boron content significantly (Table 5).

Discussion:

Proline content was shown to increase as a result of delayed irrigation (Table 3). This is confirmed by the findings of other researchers who reported drought stress played a direct role in raising leaf proline concentration (Sinclair *et al.*, 2007). Aspinall and Paleg (1981) observed accumulated proline during drought stress as an avoidance mechanism to counteract the declining pressure potential in various plants due to water deficiency. Proline plays a significant role in scavenging free radicals and in stabilizing biological membranes to regulate cell metabolism and growth in response to drought stress (Verbruggen and Hermans, 2008). Foliar applications of boron and zinc-boron mixture in this study were found to increase proline content (Table 3). Nasf *et al.* (2006) reported that the effect of foliar application of boron might be related to the role of this element in basic metabolic reactions (i.e., increased production of proline). Boron is also involved in a number of metabolic pathways, such as the metabolism of carbohydrates, RNA, and phenol. Moreover, foliar application of zinc and boron, due to their effect on increasing proline production, contributes to plant tolerance to drought stress and helps maintain the cell osmotic pressure.

Glycinebetaine, as the most common compatible osmolyte accumulating in various microorganisms, higher plants, and even animals, was also found to increase as a result of delayed irrigation (Table 3). The increased glycinebetaine content in plant organs led to a higher drought tolerance in the plant. This may be explained by the fact that being one of the many known quadruple ammonium compounds, glycinebetaine is the most abundant compound in plants that accumulates in response to dehydration stress (Mohanty *et al.*, 2002; Yang *et al.*, 2003). By maintaining cell water potential

under water deficiency stress, these compounds maintain cell turgor (Reddy *et al.*, 2013) that is one of the most important mechanisms to sustain the physiological activities of plants under water stress conditions (Kumar *et al.*, 2003; Farooq *et al.*, 2008). Glycinebetaine also supports the functions of proteins, enzymes, and lipids of the photosynthetic apparatus and keeps the flow of electrons through the thylakoid membrane (Xing and Rajashekar, 1999). As zinc plays roles in the production of glycinebetaine and osmotic adjustment, it may contribute to plant resistance to drought stress. Misagh *et al.* (2016) showed that zinc foliar application led to a significant increase in sesame grain yield while boron application's effect was insignificant. It may, therefore, be concluded that zinc and boron enhance drought tolerance in sesame.

Relative water content (RWC) in sesame was observed to decrease under drought conditions (Table 4), and as such it has been identified as an indicator of drought-tolerance in crops (Jones, 2007; Terzi *et al.*, 2013). WRC also establishes a close relationship with plant water potential (Ober *et al.*, 2005) such that decreasing RWC can be attributed to the imbalance between re-watering and water losses due to evapotranspiration from the canopy (Jones, 2007). In their study of potato, Kawakami *et al.* (2006) reported similar results.

Foliar application of boron and zinc in the present study was observed to enhance RWC (Table 5). Boron plays a key role in the transfer of water and nutrients from the roots to the shoots. Moreover, application of boron is reported to lead to a significant enhancement in leaf area (Nasf *et al.*, 2006). In a study of sunflower, BaniAbbas *et al.* (2012) found that treatments supplied with more zinc sulfate recorded higher values of leaf RWC. According to Weisany *et al.* (2011), zinc plays an important role in the regulation of stomata opening; the authors claimed this was because zinc is involved in the maintenance of potassium in the stomata guard cells, which increases leaf RWC by reducing water loss. They also observed that plants not treated with zinc exhibited reduced potassium contents in their stomata guard cells. They attributed the loss of potassium to the increased potassium leakage through cellular membranes in the absence of zinc to reduce membrane integrity and leaf RWC.

SPAD index was observed to increase in all the foliar treatments (Table 5). Although zinc is not directly involved in chlorophyll synthesis, it affects the concentrations of nutrients involved in chlorophyll biosynthesis or in the availability of such other elements as nitrogen and magnesium which serve as constituents in the molecular structure of chlorophyll (Kaya and Higs, 2002). Moreover, zinc plays a crucial role in triggering some of the chlorophyll biosynthetic pathway enzymes and antioxidant ones such as ascorbate peroxidase and glutathione reductase (Ayad *et al.*, 2010). To this may be added the advantage that it prevents the destruction of chlorophyll by reactive

oxygen species. Besides zinc, boron is reported to lead to significant enhancements in plant chlorophyll content and leaf photosynthetic rates (Nasef *et al.*, 2006).

The destruction of cell membrane and the production of malondialdehyde under the influence of water deficiency serve as good measures of plant response to drought stress. MDA was observed in the present study to increase as a result of delayed irrigation (Table 4). Drought stress leads to rising MDA content, indicating the effect of water stress on membrane lipid peroxidation through reactive oxygen species (ROS) (Sairam *et al.*, 2000). This is counteracted by zinc which protects membranes against the activity of free oxygen radicals (Marshnr, 1995) as already mentioned above on the roles played by zinc and boron in reducing malondialdehyde content (Table 5).

As was shown in this study, foliar application of zinc and the combined zinc and boron led to significant enhancements in leaf and seed zinc contents (Table 3). Hong and Ji Yan (2007) reported that zinc application enhanced root, stem, and leaf concentrations of zinc in corn with a higher zinc content observed in the shoots, rather than the roots. Compared to soil application of zinc, foliar treatment with zinc sulfate in rapeseed has been shown to yield the highest zinc concentration in plant leaf (Baybordi and Mamedov, 2010). Wissuwa *et al* (2008) reported that foliar application of zinc sulfate increased zinc concentration in rice seed. These results have been confirmed by the findings of Cakmak (2009) who maintained that foliar application of zinc was effective in increasing seed zinc concentration.

Elevated drought stress in the present study was observed to reduce seed zinc concentration. This is while zinc mobility in soil declines with reducing soil moisture content. Thus, the restricted root growth causes the plant to face a severe zinc deficiency (Bagci *et al.*, 2007). Finally, increased drought stress not only causes photosynthetic rates to reduce but makes it difficult for nutrients to be transported to the seeds as well (Lawlor and Cornic, 2002).

Foliar applications of boron and the zinc-boron mixture were found to increase leaf boron content; not

only that, the highest seed boron content was achieved with foliar treatments (Table 5). Sindoni *et al* (1994) reported that boron concentrations in sesame leaves, stems, and pods declined significantly after soil boron concentration was reduced in the treatments. Tariq and Mott (2006) reported that increasing soil boron increased tomato leaf boron content but decreased its manganese content. Bellaloui *et al* (2013) showed that foliar application of boron increased leaf and seed boron contents in soybean under both well-watered and water-stressed conditions. Soybean leaves and seeds were observed to respond positively to boron supply under water stress conditions, suggesting that, even when adequate born was present in soil, soybean plants grown under drought stress would need additional foliar boron fertilizer supplies in order to increase and maintain their leaf and seed boron concentrations high enough (Bellaloui *et al.*, 2013).

Conclusion:

Delayed irrigation increases malondialdehyde content. This can be compensated for with foliar application of zinc and boron to protect cell membranes against reactive oxygen radicals. Moreover, by increasing proline and glycine betaine (organic solutes), foliar application of zinc and boron leads to osmotic adjustment, reduction of oxidative damage, and maintenance of cell turgor. The observed increase in relative water content as a result of foliar application of zinc and boron in drought stress conditions also confirms the positive effects of boron and zinc on the uptake of water and nutrients and their capacity in maintaining cell turgor. Foliar application of zinc and boron was also observed to increase zinc and boron contents in sesame leaves and seeds, which both improved metabolism of the nutrients involved in chlorophyll biosynthesis and enhanced photosynthesis. It may be generally claimed that foliar application of boron and zinc under different irrigation treatments was observed in this study to improve the physiological characteristics of sesame and to enhance the plant's drought-tolerance.

References:

- Aspinall, D. and Paleg L. G. (1981) Proline accumulation, physiological aspects. In: Paleg LG, Aspinall D, editors. *The Physiology and Biochemistry of Drought Resistance in Plants*. New York, NY, USA: Academic Press. pp. 206–240.
- Ateyyeh, A. F. and Shatat, F. A. (2006) Effect of foliar boron application on fruit set of olive (*Olea europaea* L.) cultivar 'Rasie'. *Olivbioteq1* 223–229.
- Ayad, H. S., Reda, F. and Abdalla, M. S. A. (2010) Effect of putrescine and zinc on vegetative growth, photosynthetic pigments, lipid peroxidation and essential oil content of geranium (*Pelargonium graveolens* L.). *World Journal of Agricultural Sciences* 6: 601- 608.
- Bagci, S. A., Ekiz, H., Yilmaz, A. and Cakmak, I. (2007) Effect of zinc deficiency and drought on grain yield of field grown wheat cultivars in central Anatolia. *Journal of Agronomy Crop Science* 193: 189-206.
- Baniabbass, Z., Zamani, G. and Sayyari, M. (2012) Effect of drought stress and zinc sulfate on the yield and some physiological characteristics of sunflower (*Helianthus annuus* L.). *Environmental Biology* 6: 518-525.
- Baybordi, A. and Mamedov, G. (2010) Evaluation of application methods of zinc and iron for canola (*Brassica napus* L.). *Notulae Scientia Biologicae* 2: 94- 103.

- Bellaloui, N., Hu, Y., Mengistu, A., Kassem, M. A. and Abel, C. A. (2013) Effects of foliar boron application on seed composition, cell wall boron, and seed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopes in water-stressed soybean plants. *Frontiers in Plant Science, Plant Nutrition* 4:1-12.
- Caballero, J. I., Verduzco, C. V., Galan, J. and Jimenez, E. S. D. (2005) Proline accumulation as a symptom of drought stress in maize: A tissue differentiation requirement. *Journal of Experimental Botany* 39: 889-897.
- Cakmak, I. (2009) Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. *Journal of Trace Elements in Medicine and Biology* 23: 281-298.
- Cakmak, I. and Marschner, H. (1988) Increase in membrane permeability and exudation of roots of zinc deficient plants. *Journal of Plant physiology* 132: 356-361.
- Dordas, C. (2006) Foliar boron application improves seed set, seed yield, and seed quality of alfalfa. *Agronomy Journal* 98(4): 907-913.
- Fahad, S., Masood Ahmad, K. h., Akbar Anjum, M. and Hussain, S. (2014) The Effect of Micronutrients (B, Zn and Fe) Foliar Application on the Growth, Flowering and Corm Production of *Gladiolus* (*Gladiolus grandiflorus* L.) in Calcareous Soils. *Journal of Agricultural Science and Technology* 16: 1671-1682.
- Farooq, M., Basra, S. M. A., Wahid, A., Cheema, Z. A., Cheema, M. A. and Khaliq, A. (2008) Physiological role of exogenously applied glycinebetaine in improving drought tolerance of fine grain aromatic rice (*Oryza sativa* L.). *Journal of Agronomy Crop Science* 194: 325-333.
- Grattan, S. R. and Grieve, C. M., (1999) Salinity mineral nutrient relations in horticultural crops. *Journal of Scientia Horticulturae* 78: 127-157.
- Heath, R. L. and Pacher, L. (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125: 189-198.
- Hong, W. and Ji-Yun, J. (2007) Effects of zinc deficiency and drought on plant growth and metabolism of reactive oxygen species in maize (*Zea mays* L.). *Agricultural Sciences in China* 6: 988-995.
- Hu, Y., Burucs, Z., Von Tucher, S. and Schmidhalter, U. (2007) Short-term effects of drought and salinity on mineral nutrient distribution along growing leaves maize seedlings. *Environmental and Experimental Botany* 60: 268- 275.
- Jones, H. G. (2007) Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany* 58: 119-130.
- Karami, S., Modarres Sanavy, S. A. M., Sanam Ghanehpour, S. and Keshavarz, H. (2016). Effect of foliar Zinc application on yield, physiological traits and seed vigor of two soybean cultivars under water deficit. *Notule Science of Biology* 8:181-191.
- Kawakami, J., Iwama K. and Jitsuyama, Y. (2006) Soil water stress and the growth and yield of potato plants grown from microtubers and conventional seed tubers. *Field Crops Research* 95: 89-96.
- Kaya, C. and Higgs, M. (2002) Response of tomato (*Lycopersicon esculentum* L.) cultivars to foliar application of zinc when grown in sand culture at low zinc. *Science Horticulturae* 93: 53-64.
- Khorasanizade, Sh. (2012) Water scarcity iv Iran. International Conference on Climate, Water and Policy (ICCWP) Busan, Republic of Korea 1-2.
- Kumar, S. G., Mattareddy, A. and Sudhakar, C. (2003) NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Science* 165: 1245-1251.
- Lawlor, D. W. and Cornic, G. (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environment* 25: 275-294.
- Levit, J. (1980) Responses of plants to environmental stresses. Water, Radiation, Salt and Other Stresses, Vol. II. Academic Press, New York.
- Li, D. D., Tai, F. J., Zhang, Z. T., Li, Y., Zheng, Y., Wu, Y. F. and Li, X. B. (2009) A cotton gene encodes a tonoplast aquaporin that is involved in cell tolerance to cold stress. *Gene* 438: 26-32.
- Ling, F. and Silberbus, M. (2002) Response of maize to foliar vs. soil application of nitrogen-phosphorus-potassium fertilizers. *Journal of Plant Nutrition* 25: 2333-2342.
- Marschner, H. (1995) "Mineral Nutrition of Higher Plants," 2nd Edition, Academic Press, San Diego.
- Misagh, M., Movahhedi Dehnavi M. Yadavi A. and Khademhamzeh, H. (2016) Improvement of yield, oil and protein percentage of sesame (*Sesamum indicum* L.) under drought stress by foliar application of zinc and boron. *Journal of crop Production* 9: 163-180.
- Mirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* 125: 27-58.
- Mohamed, H. I. and Abdel-Hamid, A. M. E. (2013) Molecular and biochemical studies for heat tolerance on four cotton genotypes. *Romanian Biotechnol Letters* 18: 7223-7231.
- Mohanty, A., Kathuria, H., Ferjani, A., Sakamoto, A., Mohanty, P., Murata, N. and Tyagi, A. K. (2002) Transgenics of an elite indica rice variety Pusa Basmati1 harbouring the *codA* gene are highly tolerant to salt stress. *Theoretical and Applied Genetics* 106: 51-57.

- Moussa, H. and Abdel-Aziz, S. M. (2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Science* 1:31-36.
- Movahhedy-Dehnavy, M., Modarres-Sanavy, S. A. M. and Mokhtassi-Bidgoli A. (2009) Foliar application of zinc and manganese improves seed yield and quality of safflower (*Carthamus tinctorius* L.) grown under water deficit stress. *Industrial Crops and Products* 30:82-92.
- Nasef, M. A., Badran, N. M. and Abd El-Hamide, A. F. (2006) Response of peanut to foliar spray with boron and/or rhizobium inoculation. *Journal of Applied Sciences Research* 2: 1330-1337.
- Ober, E. S., Bloa, M. L., Clark, C. J. A., Royal, A., Jaggardand, K. W. and Pidgeon, J. D. (2005) Evaluation of physiological traits as indirect selection criteria for drought tolerance in sugarbeet. *Field Crops Research* 91(2): 231-249.
- Oraki, H., Parhizkar Khanjani, F. and Aghaalikhna, M. (2012) Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (*Helianthus annuus* L.) hybrids. *African Journal of Biotechnology* 11: 164-168.
- Paquine, R. and Lechasseur, P. (1979) Observations sur une methode dosage la libre dans les de plantes. *Canadian Journal of Botany* 57: 1851-1854.
- Reddy, A. R., Chaitanya, K. V. and Vivekanandan, M. (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology* 161: 1189-1202.
- Reddy, K. R., Henry, W. B., Seepaul, R., Lokhande, S., Gajanayake, B. and Brand, D. (2013) Exogenous application of glycinebetaine facilitates maize (*Zea mays* L.) growth under water deficit conditions. *American Journal of Experimental Agriculture* 3: 1-13.
- Sairam, R. K., Srivastava, G. C. and Saxena, D. C. (2000) Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. *Biological Plant* 43: 245-251.
- Sánchez-Blanco, M. J., Rodríguez, P., Morales, M. A., Ortuño, M. F. and Torrecillas, A. (2002) Comparative growth and water relation of *Cistus albidus* and *Cistus monspeliensis* plants during water deficit conditions and recovery. *Plant Science* 162: 107-113.
- SAS Institute Inc. (2002) The SAS System for Windows, Release 9.0. Statistical Analysis Systems Institute, Cary, NC, USA.
- Savitskaya, N. N. (1976). On the physiological role of the proline in plants. *Biologos Nauki (Moscow)* 19:49-61.
- Sindoni, M., Zamora, J. and Ramirez, R. (1994) Symptoms of boron deficiency and dry matter production in sesame. *Agronomic Tropical (Maracay)* 4: 135-150.
- Sinclair, T. R., Purcell, L. C. and Vadez, V. (2007) Drought tolerance and yield increase of soybean resulting from improved symbiotic N₂ fixation. *Field Crops Research* 101: 68-71.
- Suja, K. P., Abraham, J. T., Thamizh, S. N., Jayalekshmy, A. and Arumughan, C. (2004) Antioxidant efficacy of sesame cake extract in vegetable oil protection. *Food Chemistry* 84: 393-400.
- Suresh, G., Murthy, I. Y. L. N., Sudhakara Babu, S. N. and Varaprasad, K. S. (2013) An overview of Zn use and its management in oilseed crops. *Journal of SAT Agricultural Research* 11: 1-11.
- Sween, D. W., Long, J. H. and Kirkham, M. B. (2003) A single irrigation to improve early maturing soybean yield and quality. *Soil Science Society of America Journal* 167:235-240.
- Szabados, L. and Savouré, A. (2010) Proline: a multifunctional amino acid. *Trends Plant Science* 15: 89-97.
- Tariq, M. and Mott, C. J. B. (2006) Effect of boron supply on the uptake of micronutrients by radish (*Raphanus sativus* L.). *Journal of Agricultural and Biological Science* 1: 1-8.
- Terzi, R., Saruhan Güler, N., Kutlu Çalıřkan, N. and Kadioğlu, A. (2013) Lignification response for rolled leaves of *Ctenanthesetosa* under long-term drought stress. *Turkish Journal of Biology* 37: 614-619.
- Velu, G. and Palanisami, K. (2002) Impact of moisture stress on growth and yield of sunflower. *Madras Agriculture Journal* 88: 660-665.
- Verbruggen, N. and Hermans, C. (2008) Proline accumulation in plants: a review. *Amino Acids* 35: 753-759.
- Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A. and Ghasemi-Golezani, K. (2011) Physiological response of soybean (*Glycine max* L.) to zinc application under salinity stress. *Australian Journal of Crop Science* 5: 1441-1447.
- Weiss, E. A. (2000) *Oilseed Crops*, second edition. Blackwell Science LTD, United Kingdom. 355 pp.
- Welch, R. M., Allaway, W. H., House, W. A. and Kubota, J. (1991) Geographic distribution of trace element problems. In: *Micronutrients in agriculture*, 2nd Ed. Madison, Wisconsin 31-57.
- Wissuwa, M., Ismail, A. and Graham, R. (2008) Rice grain zinc concentration as affected by genotype native soil- zinc availability, and zinc fertilization. *Plant and Soil* 306: 37-48.
- Xing, W. and Rajashekar, C. B. (1999) Alleviation of water stress in beans by exogenous glycinebetaine. *Plant Science* 148:185-95.
- Xiong, L., Schumaker, K. S. and Zhu, J. K. (2002) Cell signaling during cold, drought, and salt stress. *Plant Cell* 14:165-183.

- Yang, W. J., Rich, P. J., Axtell, J. D., Wood, K. V., Bonham, C. C., Ejeta, G., Mickelbart, M. V. and Rhodes, D. (2003) Genotypic variation for glycine betaine in sorghum. *Crop Science* 43: 162–169.
- Zarco-Tejada, P. J., Miller, J. R., Mohammad, G. H., Noland, T. L. and Sampson, P. H. (2000) Chlorophyll fluorescence effects on vegetation apparent reflectance: II Laboratory and airborne level measurements with hyperspectral data. *Remote Sensing of Environment* 74: 596-608.