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Soybean leaf physiological responses to drought stress improved via enhanced seed zinc and iron concentrations

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Abstract:

To study the effects of both drought stress and seed zinc and iron concentrations on the photosynthesis, chlorophyll fluorescence, and proline and carbohydrate accumulations in soybean (*Glycine max* (L.) Merr. cv. M9) leaf, a split plot experiment was carried out in 2012 based on a randomized complete block design with three replications in greenhouse conditions at Yasouj University. Drought stress, based on 70% depletion of the available soil water, was designated as the main-plot that included drought stress at the flowering, pod filling, and seed filling stages. Zinc and iron concentrations were designated as sub-plot factors that included high zinc, iron and zinc+iron contents of the seed, and control as well as seed soaking in distilled water, 3% zinc sulfate, 3% iron sulfate, and 3% zinc+3% iron sulfate solutions. Results showed that photosynthesis characteristics, net photosynthesis rates, stomatal conductance, and transpiration rates decreased under the drought stress conditions. Drought stress also led to decreased water use efficiency (WUEi). Moreover, leaf proline and soluble carbohydrate contents increased in response to drought stress. While PSII quantum yield reduced under drought stress, it increased under high seed zinc and iron concentrations. It was concluded that enhanced seed zinc and iron concentration was capable of ameliorating the impacts of drought stress on leaf proline and carbohydrate concentrations.

Keywords: Chlorophyll fluorescence, Iron, Photosynthesis, Soybean, Zinc.

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Introduction:

Optimized use of water is of special importance in agricultural production, particularly in such (semi-) arid regions as Iran, that affects plant growth and development (Tohidloo *et al*, 2005). Soybean *(Glycine max* (L.) Merr.) is one of the most important oilseed crops with a relative sensitivity to drought stress (Tarumingkeng and Coto, 2003). The maintenance of the closely related photosynthetic characteristics (i.e., plant growth and yield) is of great significance to the sustainable production of crops. Photosynthesis inhibition leading to reduced crop growth due to drought conditions has been extensively investigated in the literature (Kim *et al*., 2000; Zlatev and Yorandanov, 2004; Wang *et al*., 2009). Generally, the first response of plant cell metabolism to root zone water depletion can be seen in its disrupted photosynthetic activities as a result of stomatal and non-stomatal limitations (Flexas *et al*., 2008). Low intercellular CO_2 concentrations might inhibit not only the RUBISCO and some other photosynthetic proteins (Haupt-Herting and Fok, 2000) but also the activities of ATP synthase enzymes (Noges and Baker, 2000).

Zinc is known to play important roles either as a metal component of enzymes or as a functional, structural, or regulatory cofactor in a large number of enzymes (Grotz and Guerinot, 2006). It also plays an important role in the production of biomass (Cakmak, 2008). Furthermore, zinc takes part in chlorophyll production, pollen function, and fertilization (Pandey *et al*., 2006). As an activator of Cu-Zn or Mn-SOD, either zinc or manganese is involved in membrane protection against oxidative damages through the detoxification of reactive oxygen species (Marschner, 1995). A high seed Zn content plays very important physiological roles during both seed germination and early seedling growth (Cakmak, 2008). On the other hand, zinc deficiency affects both the photochemical processes of thylakoids and photosynthesis (HajiBoland and Beiramzadhe, 2010), reduces the chlorophyll content, and is likely to cause severe damages to the chloroplast structure (Chen *et al*., 2007). Studies have reported on reduced stomatal conductance due to zinc deficiency in some plants such as maize (Wang and Jin, 2005) and rice (Hajiboland and Beiramzadhe, 2008).

Iron plays a pivotal role in the different photosynthetic processes of plants. It is, indeed, a key element in CO_2 fixation and biomass production (Briat *et al*., 2010) while it is also involved in O_2 transport, activation, and detoxification as well as in nitrogen fixation and several photosynthetic reactions. Fe not only contributes to many biological functions and, by altering the ligands to which it is coordinated, has access to a wide range of redox potentials but can also participate in many electron transfer reactions, spanning the standard redox potential (Cairo *et al*, 2002). In order

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to perform its multiple tasks, iron must be incorporated in the heme moieties of hemoglobin, myoglobin, and cytochromes; alternatively, it may bind to enzymes in the form of nonheme moieties or Fe–S motifs (such as those associated with several mitochondrial enzymes) (Cairo *et al*., 2002).

Accumulated proline, known to be a compatible solute, both decreases the cell osmotic potential and helps a sustained water uptake by plants (Hoekstra *et al*., 2001). Proline reportedly accumulates under a wide range of osmotic stresses (Verbruggen and Hermans, 2008) and its accumulation under drought stress has been well documented in various plant species such as chickpea (Mafakheri, 2010), corn (Serraj and Sinclair, 2002), and peanut (Smith *et al*., 2002). Accumulation of soluble sugars has also been reported in plants subjected to osmotic stresses despite the fact that the overall plant CO_2 fixation is significantly decreases under osmotic stresses (Murakeozy *et al*., 2003). Soluble carbohydrates in drought-stressed cells serve such functions as osmotic adjustment, cell protection, carbon reservation, and detoxification (Parvaiz and Satyawati, 2008).

Water use efficiency is defined as the ratio of dry matter accumulation to the amount of water used during the whole growing season, or alternatively, as the ratio of assimilation (A) to transpiration (E) in the short term. This second definition has come to be known as the 'intrinsic water use efficiency' (WUEi) (Hessini *et al*., 2009). Soil water depletion would cause roots to produce ABA and to translocate it via the xylem to the leaves, thereby decreasing stomatal conductivity (Behera *et al*., 2002; Liu *et al*., 2005). Reduced conductivity might in some cases lead to higher levels of WUEi. Variations in the parameters Fv/Fm (maximum quantum yield of PSII) and Fo (initial/minimal fluorescence) are still accepted as reliable diagnostic indicators of the photo-inhibition mechanism. Drought stress has been reported to cause a decline in the maximum quantum yield (Fv/Fm) in wheat (Paknezhad *et al*., 2007). Adams *et al*. (2000) maintained that there is an indirect relationship among nutrients (e.g., zinc), chlorophyll synthesis, and electron transfer.

The objectives of this study were to identify the effects of seed zinc and iron concentrations on the photosynthesis, fluorescence parameters, proline content, and total soluble sugars in soybean leaves under drought stress.

Materials and Methods:

A pot experiment was conducted as a split-plot in a randomized complete-block design with three replications. The experiment was carried out in 2012 under greenhouse conditions with a partial shade canopy at Yasouj University,Yasouj, Iran. Drought stress was designated as the main plot with the four levels of well-watered and drought-stressed treatments at the flowering, pod formation, and seed filling stages. Whole-plot factors were randomly assigned to three blocks. The sub-plot factors consisted of seed nutrient contents with the following eight levels: high seed zinc (78.25 mg kg^{-1}), seed iron (95.57 mg kg^{-1}), seed zinc (77.96 mg kg^{-1})+iron (94.13 mg kg^{-1}), and the control (Zn 61.02 mg $kg^{-1}$and Fe 77.1mg kg^{-1}) as well as seed soaking in distilled water, in 3% zinc sulfate, in 3% iron sulfate, and in 3% zinc+3% iron sulfate solutions. Drought stress was scheduled based on the maximum allowable depletion (MAD) of the available soil water (asw) (i.e., between –0.03 to 1.5 Mpa). Drought stress at each developmental (flowering, pod filling, and seed filling) stage was imposed by irrigation after depletion of 70% of available soil water determined by the weight of each pot (Liu and Stutzel, 2004). The control treatment was irrigated after depletion of only 40% of the available soil water. Seeds with high and low zinc and/or iron contents were obtained from foliar application of zinc and/or iron sulfate in a previous field experiment (Jalil, 2011). Six seeds of the soybean M9 cultivar were sown at a depth of 4 cm in a 25 cm (diameter) × 35 cm (height) pot containing 7 kg of soil. The plants were subsequently thinned to 3 plants per pot at V_3 stage. The soil had a ratio of 2:1:1 of clay, fine sand, and organic manure, respectively. After five re-irrigation events in each drought stress treatment (drought stress at the flowering, pod filling, and seed filling stages), a portable gas exchange system (LCA4, ADC Bioscientific Ltd) was used to measure the photosynthetic parameters including the net photosynthesis rate (Pn), transpiration (E), stomatal conductance to the CO_2 (Gs), and substomatal CO_2 concentration (Ci) using three healthy leaves picked from each pot. Measurements were accomplished in the morning (at 10:00 to 12:00 am) at a temperature of 24 to 35 °C. Chlorophyll fluorescence parameters, including the maximum efficiency of PSII (Fm-Fo/Fm), were measured on three fully expanded young (dark-adapted for 30 min) leaves using a pulse amplitude modulation chlorophyll fluorometer (OS1-FL). The proline content and total carbohydrate sugars of the soybean leaves were measured using the methods described in Paquine and Lechasseure (1979) and Irigoyen and *et al*. (1992), respectively. The average value of each trait obtained for each pot was calculated and used in the subsequent statistical analysis using the GLM procedure of SAS. Treatment means were compared using Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).

Results:

Photosynthetic parameters: The effect of drought stress and seed nutrient content interaction was found to be significant ($P \leq 0.05$) for all the measured traits, except in the case of Fv/Fm (Table 1). Drought stress severely decreased the value of Pn (Table 2). Seed nutrient content increased Pn at all levels of drought stress. In the non-stress treatment, the highest increase in Pn was observed with the high seed zinc+iron content

TABLE 1 Analysis of variances for plant characteristics of soybean in the drought stress treatments

Source of variation	df	Pn	E	Ci	Gs	WUEi	FV/FM	Leaf proline content	Leaf Carbohydrate content
Replication	2	0.020^{ns}	0.22^{ns}	2.65^{ns}	0.00004^{ns}	0.118ns	0.00007^{ns}	0.227^{ns}	213.6240^{ns}
Drought stress	3	380.80**	120.34^{**}	79204.02**	0.016**	2.60**	0.0673**	144.197**	1817.3217**
Error a	6	0.248	0.1385	78.72	0.00004	0.050	0.0005	0.6517	92.9888
Element	7	16.81**	7.17**	151353.05**	0.00043**	1.37*	0.0005**	10.54**	1335.1031**
Drought * element	21	6.28**	3.31**	7603.29**	0.00046**	1.26**	0.0002^{ns}	12.107**	1575.3616**
Error b	56	0.26	0.18	63.2	0.000052	0.074	0.00023	1.52	144.1959
CV		13.49	14.9	6.42	21	21.5	1.99	20.9	0.203

***, **, Significant at the 0.05 and 0.01 levels of probability, respectively. ns, not significant.**

(116%) compared to the normal seed zinc and iron content (Table 2). The maximum value of Pn in the drought stress treatment at the flowering stage belonged to the ones with a high seed zinc+iron content and a high seed zinc content. In the drought stress treatment at the pod filling stage, the highest increase in Pn was obtained for the high seed zinc content (401%) and for the seed soaking in iron solution (368%) compared to the normal seed zinc and iron content. At the seed filling stage, the maximum value of Pn under drought stress belonged to the treatment containing a high seed zinc content followed by that of seed soaking in the zinc solution and the zinc+iron solution. In this experiment, the lowest value of Pn was observed with the normal seed zinc and iron content treatment.

Values of transpiration rate, E, exhibited a significant decline for all the experimental levels of drought stress. However, the extent of the decline differed among the treatments, with the greatest observed in the drought stress treatments at the seed filling stage and with the seed soaking in iron solution (Table 2). In the non-stress treatment, E was observed to increase in the treatment with a high seed zinc content (75%) and that with a high seed zinc+iron content (66%) compared to the one with a normal seed zinc and iron content (Table 2). Drought stress at the flowering stage and that at the pod filling stage led to increments in E values in leaves with a high seed zinc+iron content (11%) and those from seeds soaked in the iron solution (588%), respectively. Under the drought stress at the seed filling stage, the maximum value of E was observed with both seed soaking in a zinc solution and the high seed zinc and iron content. Zinc and iron concentrations had different effects on the photosynthetic characteristics at each drought stress level. The reduced transpiration and photosynthesis rates were mainly associated with the closure of leaf stomata.

Substomatal CO_2 concentration (Ci) increased in all the drought stress treatments (Table 2). In the non-stress treatments, the lowest and of the highest Ci were observed with seed soaking in iron as well as seed soaking in distilled water and normal seed zinc and iron contents, respectively (Table 2). In the drought stress treatment at the flowering stage, Ci decreased with a high seed zinc content (522%) and with the seed soaking in a zinc solution (394%) compared to those in the normal seed zinc and iron content. In the drought stress treatments at both the pod filling and the seed filling stages, Ci decreased with seed soaking in the zinc and iron solution (101%) and with the high seed zinc content (50%) compared to the normal seed zinc and iron content.

Drought stress severely decreased the value of Gs (Table 2). In the non-stress treatment, Gs increased with the high seed zinc+iron content (107%). In drought stress imposed at the flowering stage, Gs increased with a high seed zinc content (106%) anda high seed zinc+iron content (44%) as well as the seed soaking in a zinc solution (44%) and in a zinc+iron solution (44%) as compared to plants with a normal seed zinc and iron content (Table 2). In the drought stress treatment at the flowering stage, the maximum value of Gs was observed with the high seed zinc content, the high seed zinc + iron content, and the seed soaking in both zinc and zinc + iron solutions. In the drought stress treatment at the pod filling stage and the one at the seed filling stage, maximum values of Gs were observed with both the seed soaking in an iron solution and that in a zinc+iron solution and in distilled water.

WUEi decreased under drought stress (Table 2). In the non-stress treatment, seed soaking in the zinc solution, in the iron solution, and in the zinc+iron solution increased WUEi values by 93%, 92%, and 88%, respectively, compared to those with a normal seed zinc and iron content (Table 2). WUEi increased in the drought stress treatments at the three flowering, pod filling, and seed filling stages with the high seed zinc and iron content (407%), the seed soaking in water (2%), and the seed soaking in iron (887%) compared to the normal seed zinc and iron content.

Drought stress led to reduced maximum quantum yield (Fv/Fm) (Fig. 1). The maximum value of quantum yield was observed in the non-stress treatment (Fig. 1). Maximum values of Fv/Fm were also observed with the high seed zinc content, the high seed zinc + iron content, and the seed soaking in a zinc +iron solution (Fig. 2).

Leaf proline and carbohydrate concentrationDrought stress strongly increased leaf proline concentration (Table 2). In the non-stress treatment, the minimum leaf proline content was observed in the seed soaking in a

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TABLE 2 Mean comparisons of the interaction effect s of seed mineral content and drought stress by LSD method

Drought stress	Element	Pn (μmol m^{-2}s^{-1})	E (mmol m^{-2}s^{-1})	Ci (μmol mol^{-1})	Gs (mol m^{-2}s^{-1})	WUEi (μmol mol^{-1})	WUEi (μmol mol^{-1})	Leaf proline content (μmol gr^{-1})	Leaf Carbohydrate content (mg gr^{-1})
Non stress	Normal seed zinc and iron content	6.21^{e}	5.27^{c}	124.16^{a}	0.053^{d}	1.18^{b}	1.18^{b}	2.31^{ab}	50.27^{bc}
	High seed zinc content	12.22^{b}	9.25^{a}	7.26d^{e}	0.096^{b}	1.32^{b}	1.32^{b}	2.66^{ab}	33.1^{cd}
	High seed iron content	9.41^{d}	7.11^{b}	31.33^{b}	0.073^{c}	1.34^{b}	1.34^{b}	3.74^{a}	31.8^{cd}
	High seed zinc and iron content	13.44^{a}	8.80^{a}	18.5^{bcd}	0.110^{a}	1.52^{b}	1.52^{b}	3.49^{a}	34.5^{cd}
	Seed soaking in water	5.10^{f}	5.14^{cd}	125.96^{a}	0.053^{d}	0.99^{c}	0.99^{c}	2.11^{ab}	43.6^{bc}
	Seed soaking in zinc solution	12.26^{b}	5.48^{c}	20.80^{bc}	0.073^{c}	2.28^{a}	2.28^{a}	2.62^{ab}	24.0^{d}
	Seed soaking in iron solution	9.17^{d}	4.06^{e}	5.48^{e}	0.073^{c}	2.27^{a}	2.27^{a}	2.15^{ab}	56.7^{b}
	Seed soaking in zinc and iron solution	10.46^{c}	4.69^{de}	12.83^{cde}	0.056^{d}	2.23^{a}	2.23^{a}	0.67^{b}	76.6^{a}
Drought stress at flowering stage	Normal seed zinc and iron content	0.48^{c}	1.82^{cd}	245.3^{a}	0.016^{b}	0.26^{d}	0.26^{d}	4.68^{f}	26.6^{c}
	High seed zinc content	2.94^{a}	2.59^{b}	39.41^{f}	0.033^{a}	1.12^{ab}	1.12^{ab}	8.50^{cd}	37.9^{bc}
	High seed iron content	1.47^{b}	2.07^{bc}	79.33^{d}	0.013^{b}	0. 70^{bcd}	0. 70^{bcd}	6.21^{ef}	30.7^{c}
	High seed zinc and iron content	3.75^{a}	2.88^{a}	68.30^{e}	0.023^{a}	1.32^{a}	1.32^{a}	12.32^{a}	56.4^{ab}
	Seed soaking in water	1.46^{b}	1.34^{d}	143.43^{c}	0.016^{b}	1.10^{ab}	1.10^{ab}	7.00^{de}	59.4^{a}
	Seed soaking in zinc solution	1.66^{b}	1.66^{cd}	49/60^{f}	0.023^{a}	1.00^{abc}	1.00^{abc}	9.04^{bc}	57.7^{a}
	Seed soaking in iron solution	0.92^{bc}	1.50^{cd}	156^{c}	0.013^{b}	0.62^{cd}	0.62^{cd}	7.51^{cde}	43.8^{abc}
	Seed soaking in zinc and iron solution	1.44^{b}	1.61^{cd}	178.50^{b}	0.023^{a}	0.88^{abc}	0.88^{abc}	10.66^{ab}	40.5^{abc}
Drought stress at pod filling stage	Normal seed zinc and iron content	0.6^{bc}	0.42^{d}	208.63^{a}	0.020^{bc}	1.49^{a}	1.49^{a}	4.60^{bc}	38.0^{b}
	High seed zinc content	3.01^{a}	2.24^{ab}	159.73^{d}	0.030^{ab}	1.33^{ab}	1.33^{ab}	4.09^{bc}	39.9^{b}
	High seed iron content	0.42^{c}	0.49^{cd}	191.33^{b}	0.010^{c}	0.87^{c}	0.87^{c}	8.47^{a}	40.8^{b}
	High seed zinc and iron content	0.95^{bc}	1.59^{bc}	143.33^{e}	0.013^{c}	0.62^{c}	0.62^{c}	10.06^{a}	47.1^{b}
	Seed soaking in water	2.29^{b}	1.54^{bc}	176.70^{c}	0.013^{c}	1.52^{a}	1.52^{a}	5.92^{b}	54.1^{b}
	Seed soaking in zinc solution	1.43^{b}	1.52^{c}	125.70^{f}	0.016^{c}	0.94^{bc}	0.94^{bc}	3.73^{c}	56.8^{a}
	Seed soaking in iron solution	2.81^{a}	2.89^{a}	111.33^{g}	0.033^{a}	0.95^{bc}	0.95^{bc}	8.48^{a}	47.9^{b}
	Seed soaking in zinc and iron solution	1.29^{b}	0.92^{cd}	103.36^{g}	0.010^{c}	1.38^{ab}	1.38^{ab}	3.46^{c}	41.1^{b}
Drought stress at seed filling stage	Normal seed zinc and iron content	0.56^{e}	1.33^{c}	151.70^{d}	0.026^{ab}	0.41^{c}	0.41^{c}	5.73^{b}	49.5^{e}
	High seed zinc content	3.11^{a}	2.9^{a}	101.20^{e}	0.020^{b}	1.06^{b}	1.06^{b}	8.46^{a}	151.0^{a}
	High seed iron content	2.36^{abc}	2.11^{b}	149.70^{d}	0.020^{b}	1.11^{b}	1.11^{b}	4.73^{b}	100.5^{c}
	High seed zinc and iron content	2.09^{bc}	1.79^{bc}	232^{b}	0.020^{b}	1.17^{b}	1.17^{b}	4.93^{b}	139.9^{ab}
	Seed soaking in water	1.64^{cd}	1.46^{bc}	189.40^{c}	0.033^{a}	1.17^{b}	1.17^{b}	7.44^{ab}	90.9^{cd}
	Seed soaking in zinc solution	3.70^{a}	3.57^{a}	154.66^{d}	0.026^{ab}	1.03^{b}	1.03^{b}	6.22^{b}	131.2^{b}
	Seed soaking in iron solution	1.1d^{e}	0.28^{d}	291.05^{a}	0.013^{b}	4.05^{a}	4.05^{a}	7.45^{ab}	50.9^{e}
	Seed soaking in zinc and iron solution	2.46^{ab}	2.13^{b}	147.86^{d}	0.036^{a}	1.15^{b}	1.15^{b}	8.37^{a}	80.1^{d}

Means within each column followed by the same latter are not significantly different (α=0.05)

zinc+iron solution. In the drought stress treatment at the flowering stage, leaf proline concentration increased with the high seed soaking in a zinc+iron solution (163%) compared to the normal seed zinc and iron content. In the drought stress treatment at the pod filling stage, leaf proline concentration increased with the high seed zinc+iron content (119%), seed soaking in an iron solution (84%), and the high seed iron content (83%) compared to the normal seed zinc and iron content. In the drought stress treatment at the seed filling stage, leaf proline concentration increased with the high seed zinc content (57%) and the seed soaking in a zinc+iron solution (46%) compared to the treatment with a normal seed zinc and iron content.

Overall, drought stress increased severely the soluble carbohydrate content of the leaves (Table 2). In the non-stress treatment, at the flowering, pod filling, and seed filling stages, the carbohydrate content increased with seed soaking in a zinc+iron solution (52%), seed soaking in distilled water (123%), seed soaking in a zinc solution (49%), and in those with a high seed zinc content (205%) relative to the normal seed zinc and iron content, respectively (Table 2). Also, in the drought stress treatment at the seed filling stage, an increase was observed in the leaf soluble carbohydrate content.

Discussion:

In this experiment, transpiration rate and stomatal conductance decreased under drought stress conditions at all the reproductive stages of soybean. Zinc and iron concentrations had different effects on the photosynthetic characteristics at each level of the drought stress conditions. The results of this experiment showed that high levels of zinc and zinc+iron contents in seeds led to identical enhancements in stomatal conductance and transpiration rates under drought conditions at the flowering stage. Seed soaking in the iron sulfate solution and a high seed zinc concentration increased stomatal conductance in the drought stress treatment at the pod filling stage. Wang *et al.* (2009) reported that zinc deficiency in corn reduced the photosynthesic rate, stomatal conductance, and transpiration rate under drought conditions. Zinc may play an important role in increasing stomata openings due to the translocation of large volumes of K^+ into guard cells. Furthermore, the enhanced Pn of well-watered soybean plants might be associated with increasing stomatal conductance which, as suggested by Sharma *et al.* (1995), increases intercellular CO_2 concentrations. On the other hand, it may be claimed that the carbonic anhydrase activity reduced both stomatal conductance and photosynthesis under a zinc deficiency (Hacisalihoglu *et al.*, 2003). It has been shown that adequate zinc nutrition has a protective effect against oxidative reaction caused by reactive oxygen species (Wang and Jin, 2005). Molassiotis *et al.* (2006) reported that, besides zinc, iron plays an important role in the stomatal opening process. Zinc plays a complex role in various processes and has a close relationship with carbon fixation. In this study, zinc and zinc+iron were observed to have positive effects on the photosynthetic efficiency, even under non-stress conditions. These results are clearly inconsistent with those reported elsewhere (Hajiboland and Amirzadeh, 2010; Zhao and Shen Zhang, 2006; Chuyning *et al.*, 2005; Yin *et al.*, 2006; Felexas *et al.*, 2008).

The present study (Table 2) revealed that Ci increased in the drought stress. Treatment; this might be associated with the low photosynthetic rate. These results suggest that, in addition to the stomatal factors, other non-stomatal factors also affect photosynthetic activities in soybean leaf. The increasing amount of Ci under stress indicates that the metabolic processes involved in carbon fixation are impaired so that cells fail to use efficiently the intracellular carbon. Moradi and Ehsanzadeh (2015) reported that a decline in Pn associated with an increase in Ci has been frequently interpreted as a direct effect of stress on plant photosynthetic capacity.

Zinc and iron nutrients were found to lead to reduced Ci under drought conditions. In general, the lowest Ci was achieved with a high seed zinc concentration, seed soaking in a zinc+iron solution, or seed soaking in a zinc solution in all the three drought stress treatments. This is evidence of the fact that zinc and iron play their protective and structural roles in drought stress conditions to improve upon or maintain photosynthesis. Zinc deficiency has a strong effect on reducing the leaf chlorophyll content; as such, it causes severe damages to the chloroplast structures, especially in drought stress conditions (Chen *et al.*, 2007). Iron is an essential element in the electron transport of photosystem II and is required for chlorophyll synthesis (Ahmadi, 2010). Our results showed that stomatal conductance has a greater effected on Pn than on Ci. Chavez (2004), however, reported that Gs had the greatest effect on Pn under drought stress conditions. Thus, it could be claimed that non-stomatal factors had greater effects on the photosynthesis processes than the stomatal factors did under the drought stress conditions in this experiment. Zinc and iron, especially zinc, were able to enhance photosynthesis under drought stress viatheir effect on stomatal (stomata opening) and non-stomatal (detoxification properties, enabling photosynthetic enzyme activation, etc.) factors.

Another important aspect of drought stress regarding the soybean performance is the intrinsic water use efficiency (WUEi), which is defined as the ratio of photosynthetic rate to transpiration rate (Hessini *et al.*, 2009). The partial stomatal closure under the drought stress conditions led to a greater reduction in transpiration rate than in photosynthesis rate, which resulted in a higher WUEi. The full closure of stomata or damages inflicted on the photosynthesis system would, however, decrease WUEi due to the reduced photosynthesis activities. Zinc and iron nutrition had positive effects on WUEi in soybean plants. This is while the drought stress treatments in the present experiment led to reduced WUEi values in soybean although many authors have reported on improved

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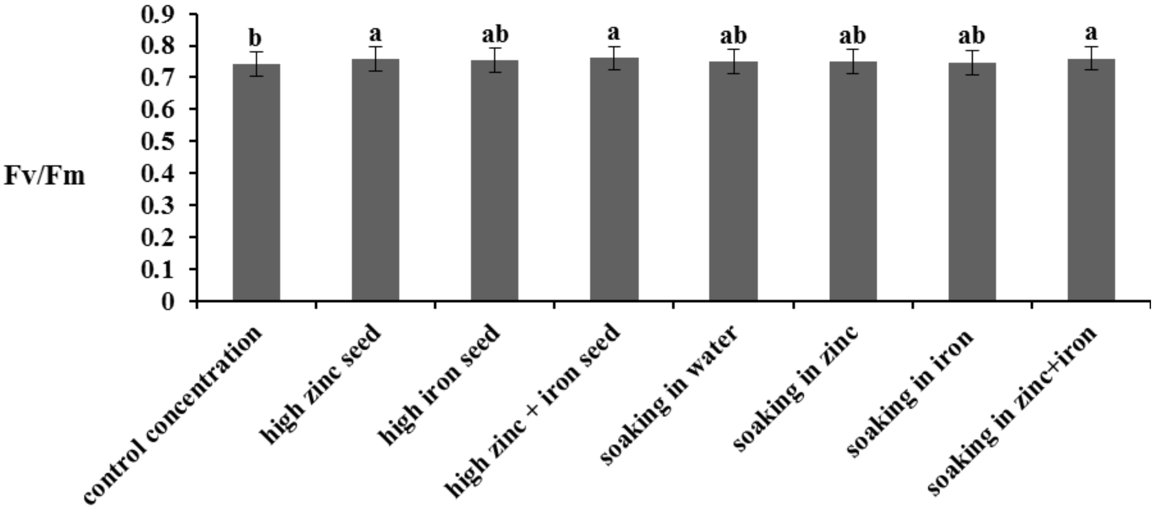

Figure 1. Mean comparison of Fv/Fm as affected by drought stress at the different developmental stages of soybean. Means within each column followed by the same latter are not significantly different (α=0.05).

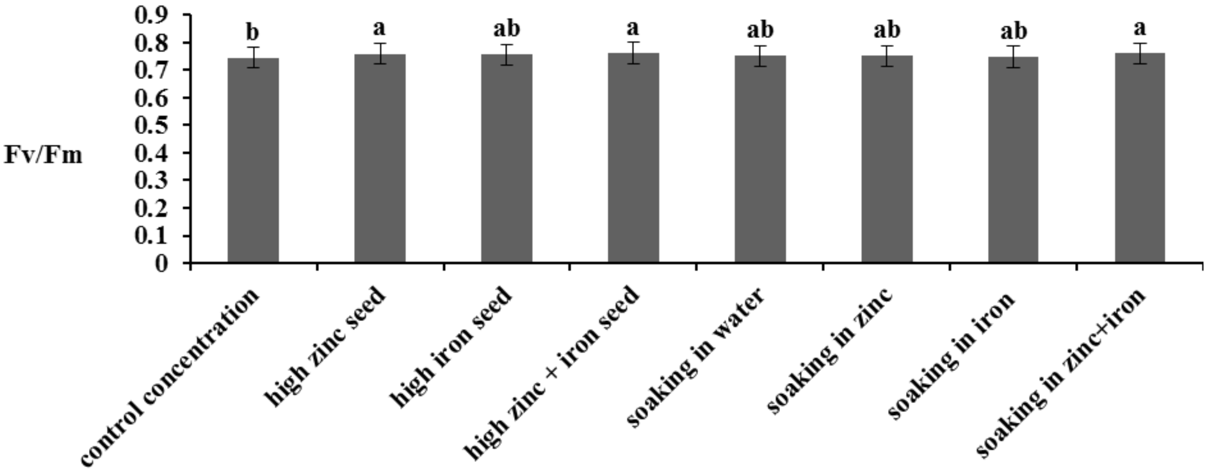

Figure 2. Mean comparison of Fv/Fm as affected by the different seed zinc and iron concentrations in soybean. Means within each column followed by the same latter are not significantly different (α=0.05).

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experiment led to reduced WUEi values in soybean although many authors have reported on improved WUEi under water limitation (Hessini *et al.,* 2009; Liu *et al.,* 2005).

Maximum quantum yield (Fv/Fm) reduced under drought stress (Fig 1). The lowest quantum yield was observed in the drought stress treatment at the pod filling stage while its highest value was obtained with high seed zinc+iron concentrations and with seeds soaked in the zinc+iron solution. One reason for the reduced quantum yield or the light inhibition observed is the reduction of leaf chlorophyll content because of a leaf zinc deficiency. Pätsikkä *et al.* (2002) reported that the total leaf chlorophyll content played an essential role in the imposition of light inhibition in leaves, so that leaves with low levels of chlorophyll were more sensitive to light inhibition. Pn depends on quantum yield so thatquantum yield increases with increasing net photosynthesis rate and the reduction in carbon dioxide fixation during water stress has been reported to depend on stomatal closure (De Souza *et al.*, 2005).

Drought stress was found to increase proline accumulation in leaves. Maximum proline accumulation was observed with the high seed zinc+iron concentration and with seeds soaked in the zinc+iron solution under drought stress at the flowering and grain filling stages. Proline accumulation helps plants tolerate drought stress (Jaleel *et al.,* 2007; Verbruggen and Hermans, 2008). Askari and Ehsanzadeh (2015) reported that drought stress in fennel (*Foeniculum vulgare* Mill) led to increased proline and carbohydrate concentrations. The high zinc and iron concentrations were found to have different effects on the leaf soluble sugars in the different drought stress treatments. Seed soaking in a zinc+iron solution in the non-stress treatment led to maximum accumulation of soluble carbohydrates. Generally, seed soaking in each of the elements, compared to the high seed zinc and/or iron concentration(s), was found more effective in accumulating soluble sugars. Emam Aziz *et al.* (2010) reported that foliar application of the zinc+iron solution led to maximum accumulation of soluble carbohydrates in the Lemongrass plant. Moreover, foliar application of zinc has been reported to increase soluble carbohydrates in safflower under drought stress (Movahhedi Dehnavi, 2004). It was observed in the present study that the higher the soluble carbohydrate content, the less was the Pn and that the reduced soluble carbohydrate activity led to an enhanced photosynthetic rate (Pego *et al.*, 2000). Research has shown that the concentration of such soluble carbohydrates as glucose and fructose increase the activity of invertase in cells exposed to drought stress (Pinheiro *et al.*, 2001; Trouverie *et al.,* 2003).

Conclusion:

Based on our findings, it might be suggested that increasing seed zinc and/or iron content by foliar application of their solution (s) on the maternal plant or by soaking seeds in the proper solution is a useful method for increasing the photosynthetic parameters in the resulting soybean plants, especially when the plants are exposed to drought stress conditions.

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