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Full Length Research Paper

Effects of foliar micronutrient application on osmotic adjustments, grain yield and yield components in sunflower (Alstar cultivar) under water stress at three stages

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In order to study the effects of foliar micronutrient application under water stress, at three stages of growth, on prolinee and carbohydrate concentration, grain yield and yield components of sunflower (Alstar cultivar), a field experimental in split plot design with three replications was conducted in 2007. Alstar cultivar was comprised under water stress at three stages of growth (heading, flowering and grain filling) as main plot and seven micronutrient treatments, Fe, Zn, Mn, Fe+Zn, Fe+Mn, Zn+Mn and Fe+Zn+Mn, as sub-plots. Results showed that water stress at three stages of growth significantly decreased grain yield, biological yield, 1000 weight seeds, cap diameter and cap weight of sunflower (Alstar cultivar). The impact of water stress was more pronounced when applied at grain filling. Use of foliar micronutrient increased grain yield in water stress on the other hand, use of Mn foliar application had the highest positive effect on yield components and grain yield. Free prolinee and total soluble carbohydrate concentration were increased under water stress, at all of the three stages of growth. The highest concentration of these two components was found on the flowering stage.

Key words: Water stress, micronutrient, grain yield, osmotic adjustment, sunflower.

INTRODUCTION

Water is the most important and vital commodity on which whole life depends. It constitutes 80 to 90% of living protoplasm and covers 75% area of the earth. Agricultural productivity is solely dependent upon water and it is essential at every stage of plant growth, from seed germination to plant maturation (Turner, 1991). Due to water deficits, the physiology of crop is disturbed which causes a large number of changes in morphology and anatomy of plant. These changes have an extensive effect on growth and thus ultimate yield of the crop (Ashraf and O'Leary, 1996; Reisdorph and Koster, 1999). Among various factors responsible for the low yield, the water requirement for the crop is the most important because water has a direct relationship with the yield of crop as reported by Karam (1978) that, increase in the irrigation interval reduced seed yield, plant height, head diameter, seed index, and seed oil content and also increased the percentage of unfilled seeds.

Reddy et al. (1995) reported that, low yielding genotypes showed the least reduction in leaf area per plant, seed yield and total dry matter production due to moisture stress. Anwar (1995) stated that, all the yield components were affected by the number of irrigations. Soriano et al. (1994) concluded that sunflower seed yield was the most sensitive to water stress after anthesis. He also emphasized the need of irrigation management under limited water supply, especially during the reproductive period. Foliar application of various macro

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Table 1. Chemical analysis of soil of experiment.

Mn (Mg L^{-1})	Zn (Mg L^{-1})	Fe (Mg L^{-1})	Ca (Meq L^{-1})	P(Meq L^{-1})	K (Meq L^{-1})	N (Meq L^{-1})	EC (Ds m^{-1})	pH
0.32	1.615	0.03	12.1	1.56	317	0.027	1.8	7.2

and micro nutrients has been proved beneficial, foliar feeding is a relatively new and controversial technique of feeding plants by applying liquid fertilizer directly to their leaves. Six micronutrients that is Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants (Welch, 1995). Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants (Halvin et al., 1999). Manganese is involved in the enzyme systems related to carbohydrate and nitrogen fixation in legumes, zinc is essential for sugar regulation and enzymes that control plant growth (Halvin et al., 1999). Macro and micronutrients deficiencies have been reported for different soils and crops (Hussain et al., 2006; Jahiruddin et al., 1995). Soylu et al. (2005) and Kenbaev and Sade (2002) reported significant increase in number of spikes m^{-2} in wheat with foliar application of different micronutrients individually or in combination. Guenis et al. (2003) and Soleimani (2006) reported marked increase in number of grains spike^{-1} of wheat for foliar application of boron and zinc, respectively. Soleimani (2006) reported increase in biological yield for foliar application of zinc.

The results also agreed with Torun et al. (2001) and Grewal et al. (1997) who reported increased dry matter production for application of micronutrients over control. Torun et al. (2001) and Grewal et al. (1997) reported increased wheat production with application of zinc and boron over control. The deficiency of micronutrients may either be primary, due to their low total contents or secondary, caused by soil factors that reduce their availability to plants (Sharma and Chaudhary, 2007). Even on the world scale, it is estimated that Fe and Zn deficiencies are widespread occurring in about 30 and 50% respectively of cultivated soils (Cakmak, 2002). While B deficiency have also been reported in over 80 countries and on 132 crops (Shorrocks, 1997). Iron and boron deficiency exhibited in citrus, deciduous fruits, groundnuts and many other crops (Tariq et al., 2004). They further stated that the makeup of mineral nutrients, improves the crop quality and increases resistance in plants against biotic and abiotic stresses.

Deficiencies of various micronutrients are related to soil types, crops and even to various cultivars. Most of micronutrients, for example Fe and Mn are readily fixed in soil having alkaline pH. Plant roots are unable to absorb these nutrients adequately from dry topsoil (Graham et al., 1992 and Foth and Ellis, 1996). Thus, the application of macro and micronutrients fertilizer in the cultivation zone may not be meeting the crop requirement for root growth and nutrient use. The alternative approach is to apply these micronutrients as foliar sprays. The introduction of new high yielding hybrids or cultivars capable of demanding a higher level of soil fertility has further accentuated the incidence of micronutrients deficiencies. Soil and plant analysis showed that > 50% of the cultivated soils of the country are unable to supply sufficient Zn and B to meet the needs of many crops (Khattak, 1995).

MATERIALS AND METHODS

This experiment was conducted in 2007 cropping at Agriculture Research Center of Zabol University. The site lies at longitude 61°29 , and latitude 31°2 and the altitude of the area is 487 m above sea level. It has a warm dry climate with the mean minimum, mean maximum, and average air temperatures of 18, 41 and 29°C, respectively. The soil characteristics of Agriculture Research Center is sandy-loam in texture, pH = 7.4 and EC = 1.8 ds.m^{-1} (The soil properties prior to the experiment is shown in Table 1). The experimental design was split plot, using randomized complete block design with tree replication. The treatment were comprised of three levels of irrigation water (W1= no irrigation in cap appearance stage, W2= no irrigation in flowering stage and W3= no irrigation in grain filling stage) in main plot and seven levels foliar application (F1=Fe, F2=Zn, F3=Mn, F4=Fe + Zn, F5=Fe + Mn, F6=Zn + Mn and F7=Fe + Zn + Mn) in sub- plot. Before performing the experiment according to soil chemical analysis results, nitrogen at a rate of 250 kg/ha was applied, in the form of urea, phosphate at a rate of 200 kg/ha was applied, in the form of triple super phosphate and potassium at a rate of 150 kg/ha was applied, in the form of potassium sulfate.

Nitrogen fertilizer was incorporated to soil in three stages (1/3 before sowing 1/3 in stage 4 leaves and 1/3 in stage 8 leaves) and phosphate and potassium fertilizers were incorporated in soil before planting. Experiment plots were seeded with Alstar cultivar at 25 kg/ha with 60 cm row to row distance and 15 cm between plants. Sunflower was planted manually in January 2006. Seeds were sown 4 cm deep and 3 cm apart within rows. Two seeds were sown in each position and the plots thinned to the desired plant population when the seedlings reached the first leaf fully emerged stage. Weeds were removed by hand. After planting, irrigation was applied as required during the growing season. The sunflower was harvested in January 2006. Data collected (obtained by combining the five center rows at each experiment unit) included: seed yield, biological yield, weight and diameter of cap, weight of (100) seeds and harvest index. Amounts of two osmotic regulators of proline and carbohydrate measured in youngest leaves. Soluble carbohydrates extract and measured by ethanol and according to sulphoric acid method and proline amount was determined according to the modified method of Bates et al. (1973) . 0.2 g of leaf tissues from control and treated plants were weighed. In 2 ml of 3% sulphosalicylic acid, 0.2 g of leaf samples were homogenized with glass-glass homogenizer, leaf samples were homogenized with mortar and pestle.

Homogenates were transferred into eppendorf tubes and centrifuged with Thermo IEC Micromax RF micro centrifuge at 14000 rpm at 4°C for 5 min. Into an eppendorf tube, 0.2 ml acid ninhydrin (0.31 g ninhydrin, 7.5 ml acetic acid, 5 ml 6 M phosphoric

Table 2. Analysis of variance for yield and yield components, proline and carbohydrates concentration.

S.O.V	df	Seed yield	Biological yield	1000 seed weight	Cap weight	Cap diameter	Proline concentration	Carbohydrates concentration
				Mean square				
Replication	2	235.2^{ns}	551.8^{ns}	14.4^{ns}	4^{ns}	0.41^{ns}	70.2^{ns}	0.05^{ns}
Water stress	2	5398.5**	16553.2**	160.5**	907.7**	5.7**	132.6**	26.9**
Error a	4	764.9	2643.1	22.6	70.9	0.1	26.2	0.3
Micronutrient	6	525.2^{ns}	998.2^{ns}	16.4^{ns}	161.8**	0.72**	128.1**	1.6**
Interaction	12	1185.7**	2039.1^{ns}	24^{ns}	541.8**	1.42**	216.2**	1.8**
Error b	36	428	2469.9	12	38.1	0.19	24.1	0.2
CV	-	19.5	19.5	12.7	12	5.09	12	4.2

*, ** significant at the 5% and 1% levels of probability respectively and n.s (non-significant).

Table 3. Mean comparison of interaction effects yield and yield components, proline and carbohydrates concentration.

Treatment	Seed yield (g/m^2)	Biological yield (g/m^2)	1000 seed weight (g)	Cap weight (g)	Cap diameter (cm)	Proline concentration (µMol/g)	Carbohydrates concentration (µMol/g)
Water stress							
Cap appearance	126.04 a	280.13 a	29 a	55.36 a	9.21 a	38.62 b	10.14 c
flowering	97.34 b	259.18 ab	28.8 a	54.82 a	8.89 b	43.47 a	12.41 a
Grain filing	95.34 b	224.54 b	24.5 b	43.71 b	8.19 c	39.91 ab	11.12 b
Micronutrient							
Fe	115.52 a	272.9 a	26.86 ab	51.98 ab	8.91 ab	39.75 dc	11.42 ab
Zn	104.41 a	252.1 a	29.37 a	53.38 ab	8.81 bc	45.72 a	11.52 ab
Mn	112.4 a	248.5 a	28.68 a	53.42 ab	8.88 ab	42.94 abc	11.32 ab
Fe + Zn	97.87 a	240.9 a	26.38 ab	44.8 c	8.35 c	36.45 d	10.36 c
Fe + Mn	106.18 a	240.9 a	26.63 ab	49.91 bc	8.54 bc	36.55 d	11.03 b
Zn + Mn	94.01 a	259.9 a	25.53 b	57.79 a	9.23 a	44.56 ab	11.24 ab
Fe + Zn + Mn	108.68 a	260.2 a	27.64 ab	47.8 bc	8.64 bc	38.68dc	11.66 a

Mean followed by similar letters in each column, are not significant at the 5% level of probability.

acid), 0.2 ml phosphoric acid, and 0.1 ml 3% sulphosalicilic acid were put. 0.1 ml supernatant from the homogenate was added into the eppendorf tube. This was done for each sample and eppendorf tubes were incubated at 96°C for 1h for complete hydrolysis of the proteins. Tubes were vortexed and centrifuged at 14000 rpm for 5 min.1 ml of toluene was added to each tube. Upper phase was transferred into the quartz cuvette and absorbance was measured at 520 nm wavelength in Schimadzu double beam spectrophotometer. The data were analyzed using MSTATC software; mean comparison was done using Duncan Multiple Comparison at 5% probability level.

RESULTS AND DISCUSSION

Seed yield

These results show that exertion of water stress in three stages of plant growth had significant effect on seed yield of sunflower (Alstar cultivar). Since in every stages of appearance cap, flowering and seed filling form special parts of seed yield components, so water stress had a different effect on every stage. Flagella et al. (2002) showed that in water stress condition, seed yield of sunflower decreased. Mean comparison (Table 3) showed that water stress in appearance cap and seed filling stage had minimum and maximum effect on sunflower seed yield (Alstar cultivar) respectively. Result showed about 24/3% decrease in amount seed yield when water stress was exerted in seed filling stage, in comparison to exerted water stress in appearance cap stage. Use of micro–nutritious elements separately and mixed could change seed yield, although there was no significant difference between seed yield, in using different micro-nutrients (Table 2). But when using elements separately, yield was more than when using mixed micro-nutrients (Table 3).

However, micro-nutrient had a significant effect on seed yield in exertion conditions of water stress in every growth stage (appearance of cap, flowering, and seed filling) (Table 2). Such w1b3 treatment (using manganese under water stress in cap appearance stage) with average 155/9 and w3b3 treatment (manganese element and water stress in seed filling stage) with average 66/3 gr/m^2 had maximum and minimum seed yield, respectively. Wilson et al. (1982) determined that, manganese has an important effect in photosynthesis process. They expressed that manganese has an effect on O_2 releasing during water photolysis process, carbohydrate synthesis and lipid metabolism. Also, manganese is a necessary element for the durability of chloroplast and some of proteins synthesis. In this experiment, exertion of water stress had a significant effect on all components of sunflower seed yield (Alstar cultivar) such as biological yield, harvest index, (100) seeds weight and diameter of cap (Table 2).

Comparison of characteristics average in different water stress time indicate that, maximum and minimum amount loss of this characteristics in result of water stress for all seed yield components except harvest index, at flowering stage, were observed in seed filling and cap appearance stages, respectively. In seed filling stage, water stress decreases the biological yield at a rate of 24/7%. Amount of loss in 100 seeds weight, cap weight, cap diameter and harvest index was 28/2, 26/6, 12/4, and 20/3%, respectively at flowering stage (Table 3). In this experiment, micro–nutrients only had significant effect on weight and diameter of cap (Table 2). Sing et al. (1996) reported similar results in sunflower. Manganese had maximum and minimum effect on seed yield components in cap appearance stage (w¬1b3) and seed filling stage (w3b3), respectively. After manganese in cap appearance stage, using iron and manganese foliar application had second rank for weight and diameter of cap and had maximum effect on seed yield components during water stress. Wilson et al. (1982) concluded that manganese has an important role in increasing seed yield of soybean, because, this element has a main effect on carbohydrates metabolism, synthesis of some proteins and nitrates. Chen (1989) stated that, manganese had significant effect on 100 seed weight and increased seed yield in wheat.

Osmotic adjustment

Variance analysis results in Table 2 indicate that, exertion of water stress has significant effect on aggregating amount, two osmotic adjustments carbohydrates and proline of sunflower, Alstar cultivar in every stage of cap appearance, flowering and seed filling. In the meanwhile density of proline is about three fold carbohydrate in the three stages of exertion of water stress. Maximum amount of carbohydrate with average 12/4 µmol/g and proline with average 43/4 µmol/g, obtained in exertion of water stress at flowering stage (Table 3). The amino acid proline is the most widely distributed compatible osmolyte. In organisms, from bacteria to plants, there is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effect of high environmental salinity. Many other environmental stresses have also been reported to increase the level of proline in plants.

The compound has been attributed to a variety of functions, such as an osmoticum, protective agent for cytoplasmic enzymes, a reservoir of nitrogen and carbon source, a stabilizer of membranes and the machinery of protein synthesis, a scavenger of free radicals and a sink for energy to regulate redox potential (Rout and Shaw, 1998). Heuer (1995) concluded that proline aggregation increased in all plant organs during water stress; however, its aggregation on leaves was more than in other organs. The amino acid proline is stored in the cytoplasm and probably has an efficient role in the protection of intra–cell macromolecules structure during water stress. Good and zapachiniski (1994) reported that, aggregation of combinations such as proline and amino acids in green tissue of rape seed in water stress condition, could provide water absorption from root environment for plant, but reliance of plants on these organic combinations is too costly for them and this results in a decrease in crop products. Micro–nutritious elements had significant effect on synthesis and aggregating of carbohydrate and proline in this cultivar of sunflower (Table 2). In the meanwhile, fertilizer treats b2 (zinc) and b7 (iron and zinc and manganese) had maximum effect on synthesis and aggregating of proline and carbohydrate, respectively (Table 3).

Marshner (1995) stated that iron, zinc and manganese have a main effect on photosynthesis process and carbohydrate production. In this experiment, proline in w2b1 treatment (water stress in flowering stage and foliar application of iron) and carbohydrate in w2b7 treatment (water stress in flowering stage and foliar application of iron + zinc + manganese) have maximum density in tissue of green leaves. Proline made by two important ways, totally, the glutamate way, that its enzymes place in cytoplasm and the urentein way, that its enzymes place in mitochondria. The glutamate way is important in plants (Reddy et al., 2004). Probably, the key enzymes of this way have a positive reaction to iron and zinc foliar application. Hemantranjan (1994) stated that, zinc has a very important role in protein and carbohydrates synthesis, cell metabolism, protection of membrane against oxygen free radicals. Marshner (1995) reported the role of manganese and iron in carbohydrate production and Wilson et al. (1982), also reported the role of manganese.

Conclusion

From results of this experiment, we could infer totally that, occurrence of dryness tension in every stage of growth: cap appearance, flowering and seed filling could make some changes in production or seed yield of sunflower Alstar cultivar, in the meanwhile, occurrence of water stress had maximum effect on the seed filling stage and in comparison with cap appearance and flowering stages, it causes more loss in seed yield. Use of micro–nutritious elements especially manganese or combination zinc and manganese in these conditions had positive effect on yield components and then increased production seed yield. Though during water stress condition density of osmotic adjustment such as carbohydrate and proline increased but use of this metabolism by plant has proven costly for them and decreased plant production.

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