African Journal of Agricultural Research Vol. 6(15), pp. 3526-3531, 4 August, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.1142
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Effect of water stress and micronutrients (Fe, Zn and Mn) on chlorophyll fluorescence, leaf chlorophyll content and sunflower nutrient uptake in Sistan region

Mahdi. Babaeian[1]*, Issa Piri[2], Abolfazl. Tavassoli[2], Yasser. Esmaeilian[1] and Hossein. Gholami[1]

[1]Departmant of Agriculture, Shirvan Branch, Islamic Azad University, Shirvan, Iran.
[2]Departmant of Agriculture, Payame Noor University, P. O. Box 19395-4697, IR, Iran.

Accepted 26 June, 2011

In order to study the effect of water stress and micronutrients (Fe, Zn and Mn) on chlorophyll fluorescence, leaf chlorophyll content and sunflower nutrient uptake in Sistan region, a field experimental in split plot design with three replications was conducted in 2007. Alstar cultivar was comprised of water stress at two stages of growth (control, flowering and grain filling stage) as main plot and seven micronutrient foliar application treatments, Fe, Zn, Mn, Fe+Zn, Fe+Mn, Zn+Mn and Fe+Zn+Mn, as sub plots. Results showed, exertion of water stress in flowering and grain filling stage decreased grain yield of sunflower significantly. 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 grain yield. Chlorophyll a fluorescence was increased under water stress at the two stages of growth. The highest chlorophyll a fluorescence was recorded in B_1 (foliar application of Fe), and maximum amount of chlorophyll content was observed in B_5 (foliar application of Fe+Mn). The highest seed contents of Fe and Mn were measured in the control treatment.

Key words: Water stress, micronutrient, chlorophyll fluorescence, nutrient uptake, sunflower.

INTRODUCTION

Water is essential at every stage of plant growth and 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). Drought stress is one of the most important abiotic stress factors which are generally accompanied by heat stress in dry season (Dash and Mohanty, 2001). Due to water deficits, the physiology of crop is disturbed which causes a large number of changes in morphology and anatomy of plant. Water stress causes deceleration of cell enlargement and thus reduces stem length by inhibiting inter nodal elongation and also checks the tillering capacity of plants (Ashraf and O'Leary, 1996). Drought also lowers leaf area by inhibiting leaf expansion (Reisdorph et al., 1999). 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. Drought tolerant crop can be characterized by growth response, changes in water relations of tissues exposed to low water potential, stomata conductance, ion accumulation and changes in fluorescence induction parameters under water stress (Blum, 1988). 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). Deficiencies of various micronutrients are related to soil types, crops and even to various cultivars. Most micronutrients, for example Fe and Mn are readily fixed in soil having alkaline pH. Plant roots are unable to absorb these nutrients adequately from the dry topsoil (Graham et al., 1992; Foth and Ellis, 1996).

*Corresponding author: E-mail: mahdi_bbn@yahoo.com.

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	7.4

Although, micronutrient elements are needed in relatively very small quantities for adequate plant growth and production, their deficiency may cause great disturbance in the physiological and metabolic processes involved in the plant. Thus, the application of 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. 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 (Havlin et al., 1999). Manganese is involved in the enzyme systems related to carbohydrate and nitrogen fixation in legumes and Zinc is essential for sugar regulation and enzymes that control plant growth (Havlin 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) 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. This study was conducted to determine the role of micronutrients (Fe, Zn and Mn) effect, on growth of sunflower in water stress condition in Sistan region.

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 are shown in Table 1). The experimental design was split plot, using randomized complete block design with tree replication. The treatment was comprised of three levels of irrigation water (W_1 = control, W_2 = no irrigation in flowering stage and W_3 = no irrigation in grain filling stage) in main plot and seven levels of foliar application (F_1 = Fe, F_2 = Zn, F_3 = Mn, F_4 = Fe + Zn, F_5 = Fe + Mn, F_6 = Zn + Mn and F_7 = Fe + Zn + Mn) in sub plot. Before performing 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 the 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. The yield (g/m^2) was recorded at harvest time at Juan 2006. The fluorescence measurements were made with PAM-2000 fluorometer. For measuring seed nutrient content (Fe, Mn and Zn) leaf samples were collected after foliar application of micronutrient before harvesting time (90 to 180 cm above the ground) then washed, oven dried, ground and extracted with wet acid digestion method and analyzed for elemental content of Fe, Mn and Zn by Atomic Absorption Spectrophotometer, model-2380 (Jones and Case, 1990). The data were analyzed using MSTATC software, mean comparison was done using Duncan Multiple Comparison at 5% probability level.

RESULTS

Grain yield

Results showed that exertion of water stress had significant effect on seed yield of sunflower (Alstar cultivar). Maximum amount of grain yield was measured in control treatment with average of 126.04 and minimum amount of it was obtained in water stress exertion in grain filling stage (96.34) as shown in Table 3. Seed yield was decreased 24/3% when water stress was exerted in grain filling stage in comparison to control. Flagella et al. (2002) showed that in water stress condition, seed yield of sunflower was decreased. Micronutrient had a significant effect on grain yield of sunflower (Alstar cultivar) (Table 2). w_1b_3 treatment (foliar application of Mn + Control) with average of 155/9 and w_3b_3 treatment (Mn foliar application and water stress in grain filling stage) with average of 66/3 g/m^2 had maximum and minimum amount of grain yield, respectively. Wilson et al. (1982) determined that manganese has an important effect in photosynthesis process. Further, they expressed that manganese has an effect on O_2 releasing during water photolysis process, carbohydrate synthesis and lipids metabolisms.

Table 2. Analysis of variance on grain yield, chlorophyll, chlorophyll fluorescence and seed elements.

Treatment	df	Mn	Zn	Fe	Fv/Fm	Chlorophyll	Grain yield (g.m^{-2})
		mg/kg				SPAD	
Replication	2	281.6^{ns}	222.3^{ns}	1149.7^{ns}	0.002^{ns}	1.6^{ns}	235.2^{ns}
Water stress	2	1687.9*	54797.9**	7368.4*	0.02**	14.9**	5398.5**
Error a	4	1235.1	10643.7	672.2	0.0007	0.76	764.9
Micronutrient	6	1180.3*	19880.2**	11491.9**	0.0053*	13.3**	525.2^{ns}
Water stress×Micronutrient	12	796.1^{ns}	15261.8**	16024.8**	0.0057**	7.04**	1185.7**
Error b	36	473.4	3393.8	1460.2	0.0017	1.27	428
%CV		7.24	7.09	10.4	5.8	7.5	19.5

ns, * and **, Non-significant, significant at 5% and 1% probability levels, respectively.

Table 3. Mean comparison for grain yield, chlorophyll, chlorophyll fluorescence and seed elements.

Treatment	Mn	Zn	Fe	Fv/Fm	Chlorophyll	Grain yield
	mg/Kg				SPAD	(g/m^2)
Water stress						
Control	310.7^a	770.1^b	387.4^a	0.71^b	14.07^b	126.04^a
Flowering	296.9^a	821.9^b	354.6^b	0.75^a	15.4^a	97.34^b
Grain filling	293.9^a	872.2^a	355.4^b	0.69^b	15.6^a	95.34^b
Micronutrient						
Fe	291.9b^c	786.1^b	377.6^{ab}	0.74^a	14.4^b	115.52^a
Zn	315.5^a	798.3^b	412.6^a	0.71^a	13.13^c	104.41^a
Mn	298.2^{abc}	795.7^b	385.5^{ab}	0.73^a	15.9^a	112.4^a
Fe+Zn	311.1^{ab}	792.4^b	315.9^c	0.72^a	14.7^b	97.87^a
Fe+Mn	285.2^c	797.5^b	321.3^c	0.71^a	16.4^a	106.18^a
Zn+Mn	291.9^{bc}	884.3^a	360.9^b	0.66^b	16.2^a	94.01^a
Fe+Zn+Mn	309.1^{ab}	895.2^a	386.9^{ab}	0.71^a	14.2^{ab}	108.68^a

Test mean followed by similar letters in each column, are not significantly different at the 5% level of probability.

Chlorophyll fluorescence (F_V/F_M) and leaf chlorophyll content

Water stress and foliar application of macronutrient and their interaction had significant effect on, chlorophyll content and chlorophyll fluorescence of sunflower leaf (Alstar cultivar) as shown in Table 4. The most amount of chlorophyll fluorescence was recorded in B_2 (water stress in flowering stage) treatment and the lowest of it was observed in B_3 (water stress in grain filling stage) treatment (Figure 1). Water stress induced reduction in photosynthesis may be due to stomatal limitations, metabolic limitations or altered chlorophyll fluorescence or combination of these factors (Athar and Ashraf, 2005). Result showed maximum amount of leaf chlorophyll and minimum amount of it achieved in control (Table 4) treatment (Figure 2).

Murillo-Amadot et al. (2002) reported increasing of chlorophyl content in different genotypes of cowpea leafs under salinity stress. In another research, Zhao et al. (2007) stated that salinity cause loss of leaf chlorophyl content in oat leafs. Result in Table 4 showed that the maximum amount of leaf chlorophyll content was obtained in B_5 (foliar application Zn and Mn), B_3 (foliar application Mn) and B_2 (foliar application Zn) respectely. This result showed that manganese and zinc have a main efffect on chlorophyll making in plant. Marschner (1995) stated that manganese is important in chlorophyll making. Maximum amount of chlorophyll fluorescence (fv/fm) was observed in B_1 (foliar application of Fe) and the minimum of it was achieved in B_6 (foliar application Zn + Mn) (Table 3). content achieved in W_3 (water stress in grain filling stage).

Micronutrient (Fe, Zn and Mn) content in seed of sunflower

Variance analysis results in Table 2 indicate that exertion of water stress and micronutrient foliar application had

Table 4. Correlation coefficients between grain yield, chlorophyll, chlorophyll fluorescence and seed elements.

Character	1	2	3	4	5	6	7	8
Grain yield	1							
Chlorophyll fluorescence	-0.082 ns	1						
Chlorophyll	0.182 ns	-0.044 ns	1					
Carbohydrate	-0.27*	0.23*	0.074 ns	0.14 ns	1			
Fe	0.27*	-0.116 ns	0.17*	0.08 ns	0.06 ns	1		
Zn	0.23*	-0.16 ns	0.142 ns	0.31*	0.14 ns	-0.02 ns	1	
Mn	0.25*	-0.02ns	0.24*	0.039ns	0.095 ns	0.15 ns	-0.07 ns	1

ns ,* and ** Non-significant, significant at 5% and 1% probability levels, respectively.

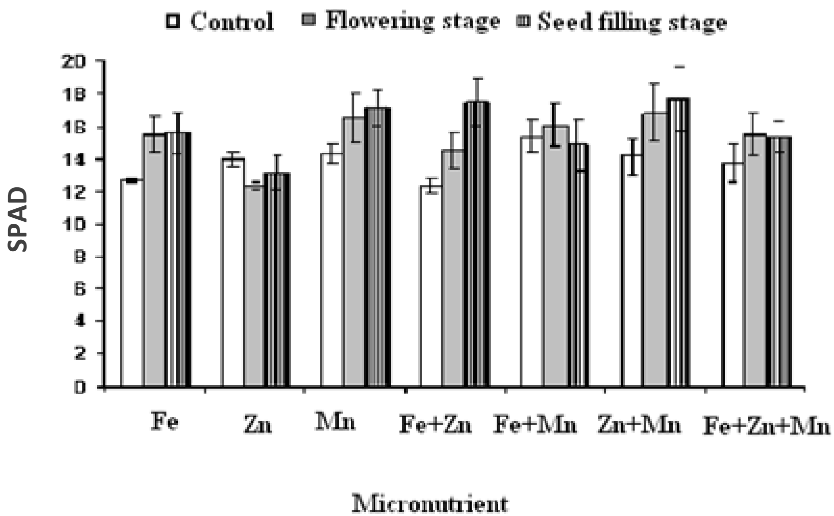

Figure 1. Interaction between water stress and micronutrient on chlorophyll fluorescence.

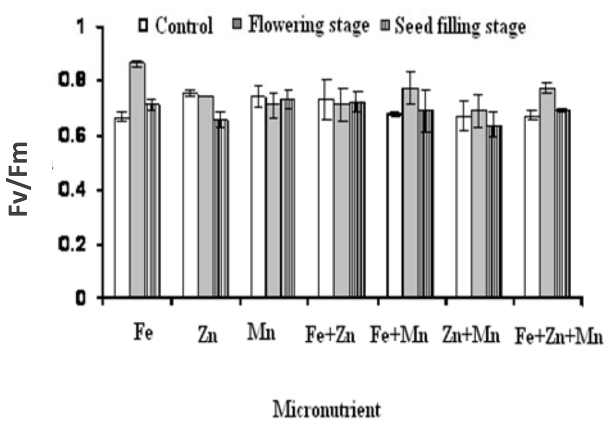

Figure 2. Interaction between water stress and micronutrient on chlorophyll content.

significant effect on concentration of iron, zinc and manganese in seeds of sunflower (Alstar cultivar). The result of statistical analysis, showed that interaction of water stress and micronutrient was significant on Fe and Zn concentration of sunflowers seeds (Table 3). Result in Table 4 show that the highest level of Fe and Zn concentrations were observed in control and water stress exertion in filling stage treatments (Figures 3 and 4). Similar to this experiment result, Movuhdi et al. (2001) reported increasing concentration of seeds zinc with exertion of water stress in seed filling stage in rap seed. Use of micronutrient elements separately or mixly increased the amount of Fe, Zn and Mn in sunflower seeds (Alstar cultivar). Maximum amount of Fe in sunflower seeds was recorded in b_1 (foliar application of Fe), maximum amount of Zn in sunflower seeds was observed in b_7 (foliar application of Zn) and maximum amount of Mn in sunflower seeds was recorded in b_3 (foliar application of Mn) (Table 4).

Combined treatment of Fe+Zn+Mn cause increase of seeds Zn concentration 10/8% more than from foliar application of zinc solely tritement. According to this

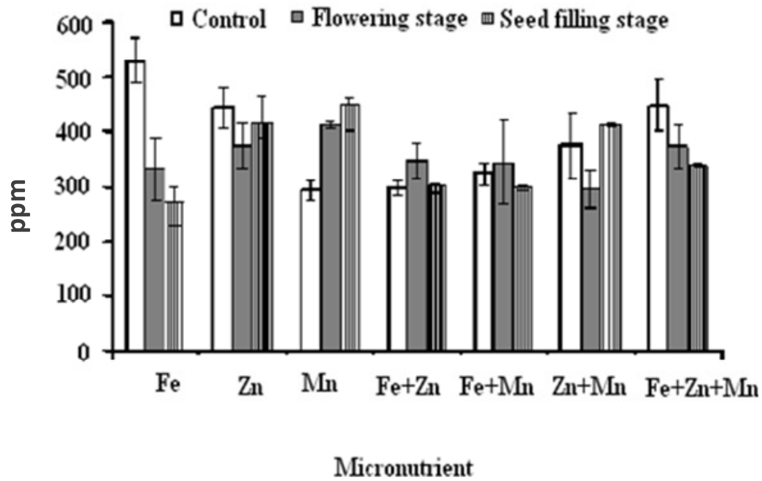

Figure 3. Interaction between water stress and micronutrient on Fe content in seed.

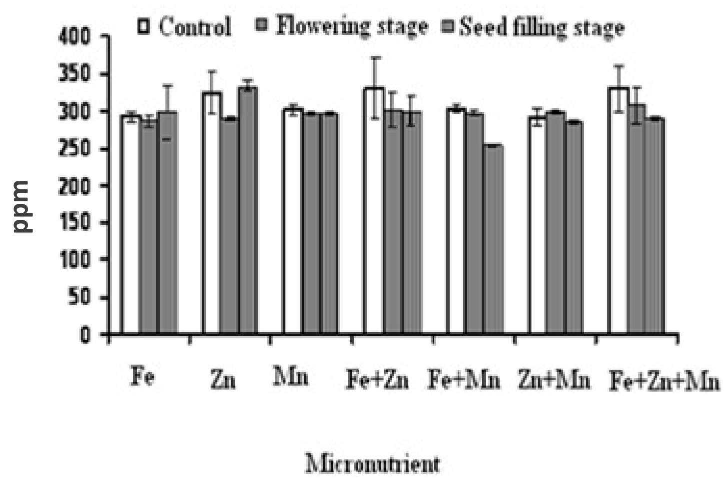

Figure 4. Interaction between water stress and micronutrient on Zn content in seed.

result, these three elements (Fe, Zn and Mn) have a synergic effect on zinc absorption and its transfer to sunflower seeds. The interaction of water stress and foliar application had significant effect concentration on Fe and Mn (P < 5%) (Table 2). In this experiment, amount of Fe in w_1b_1 (control in water stress treatment and foliar application of Fe) and Mn in W_3b_2 (water stress in seed filling stage and foliar application of Mn) was maximum when compared to other treatments (Figures 4 and 5). According to correlation coefficients results in Table 4, the micronutrients (Fe, Zn and Mn) had a significant and positive correlation with seed yield. These elements also, by decreasing chlorophyll fluorescence (negative correlation), caused the increase of photosynthesis efficiency in plants. Marschner (1995) stated that micronutrients elements have positive effect on photosynthesis in plants.

Conclusion

Result in this experiment showed occurrence of water stress in every stage of flowering, and seed filling decreased grain yield of sunflower (Alstar cultivar) in the meanwhile, occurrence of water stress in grain filling stage had maximum effect on yield and yield components. Use of micronutrient elements, especially manganese or a combination of zinc and manganese had positive effect on yield components of sunflower. Micronutrient had a significant effect on seed yield in exertion conditions of water stress in every growth stage. The most amount of chlorophyll fluorescence was observed in exertion of water stress in flowering stage and the lowest of it was recorded in exertion of water stress in seed filling stage. Maximum leaf chlorophyll content was measured under water stress in seed filling stage treatment.

REFERENCES

Anwar M, Rehman S, Khan S, Quarishi Z (1995). Response of sunflower varieties to different irrigation regimes during Kharif season in Peshawar Valley. Sarhad J. Agric., 11:273-278.

Ashraf M, O'Leary JW (1996). Effect of drought stress on growth, water relations and gas exchange of two lines of sunflower differing in degree of salt tolerance. Int. J. Plant Sci., 157: 729-732.

Athar H, Ashraf M (2005). Photosynthesis under drought stress. In: Hand Book Photosynthesis, 2nd (ed.) by M. Pessarakli. C. R. C. Press, New York, USA, pp. 795-810.

Blum A (1988). Plant breeding for stress environments. Boca Raton, Florida: CRC Press.

Dash S, Mohanty N (2001). Evaluation of assays for the analysis of thermo tolerance and recovery potentials of seedlings of wheat (*Triticum aestivum* L.) cultivars. J. Plant Phys., 158: 1153-165.

Flagella Z, Pastore D, Campanile RG, Fonzo NDI (1995). The quantum yield or photosynthetic electron transport evaluated by cholophyll fluorescence as indicator of drought tolerance in durum wheat. J. Agric. Sci. Cambridge, 125(3): 325-329.

Foth HD, Ellis BG (1996). Soil fertility. 2nd Ed. Lewis Pub. New York.

Graham RD, Ascher JS, Hynes SC (1992). Selecting zinc-efficient genotypes for soils of low zinc status. Plant and Soil, 146: 241-250.

Grewal HS, Zhonggu L, Graham RD (1997). Influence of subsoil zinc on dry matter production, seed yield and distribution of zinc in oilseed rape genotypes differing in zinc efficiency. Plant and Soil, 192(2): 181-189.

Guenis A, Alpaslan M, Unal A (2003). Effects of Boron Fertilization on the Yield and Some Yield Components of Bread and Durum Wheat. Turk. J. Agric., 27: 329-335.

Havlin JL, Beaton JD, Tisdale SL, Nelson WL (1999). Soil Fertility and Fertilizers – An introduction to nutrient management 6th Ed. Prentice Hall, New Jersey.

Hussain MZ, Rehman N, Khan Roohullah MA, Ahmed SR (2006). Micronutrients status of Bannu basen soils. Sarhad J. Agric., 22(2): 283-285.

Jahiruddin M, Ali MS, Hossain MA, Ahmed MU, Hoque MM (1995). Effect of Boron on grain set, yield and some others of wheat cultivars. Bangladesh J. Agric. Sci., 22: 179-184.

Jones JB, Case VW (1990). Sampling, Handling and Analysis Plant Tissue Sample. In: Soil Testing and Plant Analysis. Westermann RL (Ed.), 3rd., 1990. Soil Sci. Soc. Am. Book Series No. 3, Madison, WI., pp: 389-427.

Kenbaev B, Sade B (2002). Response of field-grown barley cultivars grown on zinc-deficient soil to zinc application. Comm. Soil Sci. Plant Anal., 33(3-4): 533-5544.

Marschner H (1995). Mineral Nutrition of Higher Plants. 2nd Academic Press. Ltd. London.

Murillo Amadot BER, Troyo-Dieguez A, Lopez-Aguilar CL, Lopez-Cortes H, Tinoco-Ojanguri G, Kaya C (2002). Matching physiological traits and ion concentrations associated with salt stress in cowpea genotypes. Aust. J. Agric. Res., 53: 1243-1255.

Reddy YAN, Shaoker RU, Virupakshappa K (1995). Studies on sunflower genotypes under moisture stress conditions. Banglore, India. 'JAVK, pp. 560-065.

Reisdorph NA, Koster K1 (1999). Progressive loss of desiccation tolerance in germinating pea (*Pisum sativum*) seeds. Physiol. Plant., 105: 266-271.

Sharma JC, Chaudhary SK, (2007). Vertical distribution of micronutrient actions in relation to soil characteristics in lower shivaliks of Solan district in North-West Himalayas. J. Ind. Soc. Soil Sci., 55: 40-44.

Soriano MA, Villalobos FJ, Fereres E, Orgaz F, Borin M, Sattin M (1994). Response of sunflower grain yield to water stress applied during different phonological stages, Abano-Padovo, Italy, pp.18-22.

Soleimani R (2006). The effects of integrated application of micronutrient on wheat in low organic carbon conditions of alkaline soils of western Iran. 18th World Congress of Soil Science.

Soylu S, Sade, B Topalv A, Akgun N, Gezgin S (2005). Responses of Irrigated Durum and Bread Wheat Cultivars to Boron Application in Low Boron Calcareous Soil. Turk. J. Agric., 29: 275-286.

Torun A, Itekin IGÃ, Kalayci M, Yilmaz A, Eker S, Cakmak I (2001). Effects of zinc fertilization on grain yield and shoot concentrations of zinc, boron, and phosphorus of 25 wheat cultivars grown on a zinc-deficient and boron-toxic soil. J. Plant Nut., 24(11): 1817-1829.

Turner LB, (1991). The effect of water stress on the vegetative growth of white clover (*Trifolium repens* L.): Comparison of long-term water deficit and short-term developing water stress. J. Exp. Bot., 42: 311-316.

Welch RM, Allaway WH, House WA, Kubota J (1991). Geographic distribution of trace element problems. In: Mortvedt J.J., ed. Micronutrients in agriculture, 2nd Ed. Madison, Wisconsin: SSSA Book Ser. 4. SSSA, pp. 31-57.

Wilson DO, Boswell FC, Ohki K, Parker MB, Shuman LM, Jellum MD (1982). Change in soybean seed oil and protein as influenced by manganese nutrition. Crop Sci., 22: 948-952.

Zhao GQ, Ma BL, Ren CZ (2007). Growth, gas exchange, chlorophyll fluorescence and ion content of Naked oat in response to salinity. Crop Sci., 47: 123-131.