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Full Length Research Paper

Effects of methanol and some micro-macronutrients foliar applications on maize (*Zea mays* L.) maternal plants on subsequent generation yield and reserved mineral nutrients of the seed

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Tests were done to investigate effects of foliar applications in various combinations on maternal corn plants for reserved mineral nutrients and seed yield in a subsequent generation. The experiment was done as a factorial based on randomized complete block design (RCBD) with three replications during the growing season of 2009 to 2011. Treatments tested in the investigation were as follows: Four growth stages; 8 to 10 leaf, tasseling, seed-filling and at all stages and seven foliar applications of methanol, Zn, B, Mg, N, Mn, a mixture of all combinations and a separate plot as the control. Data analysis showed a significant effect of the combination type of foliar application in different stages on reserves of N, Mg, Zn, Mn, B, and seed yield of a subsequent generation. Detailed results of the study showed that foliar application with a mixture of all combinations in all stages had the highest reserve of N in seeds (1/45%). Results also proved that Mg foliar application at all stages had the highest effect on Mg reserve in seeds (0/172 mg kg^{-1}) and Zn foliar application at the tasseling stage had the highest effect on reserved Zn of seed (44 mg kg^{-1}). Results showed that Mn foliar application at the 8 to 10 leaf stage had the highest effect on reserved Mn of seed (12 mg kg^{-1}) and B foliar application at all stages had the highest effect on reserved B of seed (9/1 mg kg^{-1}). In conclusion a mixture of all combinations at all stages had the highest (1309 g m^{-2}) and at the tasseling stage had the lowest (713 g m^{-2}) seed yield in the subsequent generation.

Key words: Foliar application, methanol, micro-macronutrients, *Zea mays.*

INTRODUCTION

Maize (*Zea mays* L.) is an important crop worldwide (Graham, 2008). Seed development can be affected by many factors (biotic and/or environmental) that have a negative effect on seed quality and adverse consequences for crop yield (Welch, 1995). Genotype, seed size and weight and environmental stresses such as water deficit, temperature extremes, pathogens, deficient nutrient supply, mineral toxicity, salinity, soil acidity and anaerobiosis are all factors that directly affect growth and nourishment of a maternal plant. These adverse conditions have a direct impact on seed development, seed nutrient reserves and ultimate seed quality (Welch, 1986). As environmental conditions affect seed quality during seed formation this also affects seedling establishment at the next growing season (Zakaria et al., 2009).

A shortage of nitrogen (N) restricts the growth of all

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Abbreviations: NFA, Nitrogen **foliar** application; **MgFA,** magnesium foliar application; **ZnFA,** zinc foliar application; **MnFA**, manganese **foliar** application; **BFA,** boron foliar application; **MFA,** methanol foliar application.

plant organs such as roots, stems, leaves, flowers and fruit (including seeds) (Barker and Bryson, 2007). N-Foliar application serves to increase reproductive structures that can increase seed yield (Welch, 1995). As Sawan et al. (1989) reported increasing N in cotton plants from 108 to 216 kg ha^{-1} increased seed viability in terms of germination velocity and total germination as well as seedling vigor.

Magnesium (Mg) has major physiological and molecular roles in plants; it is a component of the chlorophyll molecule, a co-factor for many enzyme processes associated with phosphorylation and the hydrolysis of various compounds, as well as a structural stabilizer for various nucleotides (Merhaut, 2007). Current known reports on research in to the effect of Mg on seed vigor are insufficient. Welch's (1986) studies on barley (*Hordeum vulgare* L.) showed that seedlings grown from a maternal plant, which were fed insufficient amounts of Mg had less chlorophyll in comparison to those grown from sufficiently fed maternal plants.

Zinc (Zn) can be readily transported from vegetative tissue into reproductive tissue according to a plant's capacity. However, the transformation from vegetative tissue into reproductive tissue decreases when a zinc supply is inadequate (Welch, 1995). An increased zinc content of bread wheat grain from 355 ng to 1465 ng grain^{-1} leads to an increased subsequent yield (Yilmaz et al., 1998). Gangloff et al. (2002) found that an application of zinc sulphate in maize plants increased dry matter and zinc accumulation in leaf and grain.

Manganese (Mn) is involved in many biochemical functions. It primarily acts as an activator of enzymes, involved in respiration, amino acid and lignin synthesis and hormone concentrations (Humphries et al., 2007). Mn foliar application (25 mg Mn L^{-1} as MnEDTA) on cotton (*Gossypium barbadense* L.) grown on Mn-poor soil increased seed yield, seed weight, seed viability and seedling vigor in terms of length of hypocotyls, radicle and fresh and dry weights of seedling (Sawan et al., 1993).

Deficiency of boron (B) can cause reductions in crop yield, impair crop quality, or have both of these effects (Gupta, 2007). B deficiency in a maternal plant causes the cotyledon leaves to become yellow, serrated pointed with distinguishing colors such as yellow or tan, as they reach the reproductive stage. Moreover it causes a high proportion of cells in B-deficient seeds to become empty or to collapse (Welch, 1986). However, during seed development the need for B is higher than it is at the growth period (Marschner, 1995). A shortage of B in the maternal base leads to a decrease in viability of the produced seed. In black gram (*Vigna mungo* L.) the concentration of 6 mg kg $^{-1}$ dry weight of seed is considered as the critical level for normal seed viability (Bell et al., 1989).

Methanol spray is used to increase CO_2 fixation in crops (Nadali et al., 2010). Foliar-spray applications of aqueous methanol are reported to increase yield, accelerate maturity and reduce effects of drought stress (Ramirez et al., 2006; Downie et al., 2004). Mirakhori et al. (2009) demonstrated that 21% (v/v) methanol spray poses the greatest impact on yield, and other physiological traits in soybean. Foliar application is the best way to nourish plants that grow in soil with poor quality due to adverse pH (Ishii et al., 2002). In the dry and semi-dry areas of Iran, the absorption of micronutrients is low due to a high pH level of the soil. In order to use chemical fertilizers efficiently, it is essential that fertilizer is applied by foliar-applications. The aim of this study was to investigate the effects of different combinations of foliar applications on corn maternal plants on yield and reserved mineral nutrients in seeds produced from hybrid corn seeds.

MATERIALS AND METHODS

This research was done at the Agricultural Station of Tabriz Islamic Azad University during the two farming years of 2009 to 2011. The station is located 5 km from Tabriz-Iran at 46° and 17′E and 38° and 5′N, at an altitude of 136 meters above sea level. The experiment was conducted as a factorial in a Randomized Complete Block Design in three replications. The experimental factors were; (A) Four growth stages (a_1: 8 to 10 leaf; a_2: tasseling; a_3: seed-filling, and a_4: all stages); (B) the 7 foliar applications were (b_1: Methanol, b_2: N (Urea), b_3: Mg sulfate ($MgSO_4 \cdot H_2O$), b_4: Zn sulfate ($ZnSo_4$), b_5: boric acid (H_3BO_3), b_6: Mn sulfate ($MnSO_4.H_2O$), and b_7: a mixture of all combinations. In order to perform the orthogonal contrast between treatments and the control, there was an untreated control plot applied as a separate plot. The soil used for tests included on average 68% sand, 18% silt and 14% clay. Taking into account the triangular texture of the soil, the experimental texture of the soil was sandy loam. The pH of the soil was low-average alkaline (7/8 to 8/9), which makes it difficult for a plant to absorb micronutrients such as Fe, Mn, Cu, B and Zn (Table 1).

Plant material

B73 was used as the maternal plant and Mo17 as the paternal plant. In each plot, a row of paternal plants was planted around 3 rows of maternal plants (Beck, 2004). As soon as a tassel appeared in the maternal plants, they were cut. The distance between the site of the experiment and other farms was at least 400 m.

Experimental material

The first growing season

In order to prepare the field for planting the corn to produce hybrid seeds from a maternal base, the first growing season (that is, 2009 to 2010), was spent plowing, harrowing, making furrows and plotting. Then pre-planting fertilization was carried out using urea fertilizer at the rate of 150 kg ha^{-1} in furrows. Each plot consisted of 5 rows, 75 cm row spacing and 25 cm plant intervals. Seeds were planted at the depth of 5 to 7 cm in the water strain of each furrow. In order to pollinate from paternal bases, tassels were cut as soon as they appeared in the maternal bases to extract productive seeds from maternal plants. Hybrid seeds were planted at the end of the first growing season.

Mineral treatment: Mineral concentrations of foliar applications were determined in ratios of 5 to 1000. Considering the size of the

Table 1. Soil physical and chemical analysis.

Clay (%)	Silt (%)	Sand (%)	K (ava) (ppm)	B (ava) (ppm)	Zn (ava) (ppm)	N (%)	Ec × 10^3
14	18	68	600	0/93	0/4	0/133	1/57

plots, applications were made with a hand sprayer to facilitate effective foliar application in terms of precision and delicacy. Spraying was done thoroughly until foliar dropped from the plants. Furthermore, Tween80 was used as a surfactant to enable the leaves to absorb nutrient minerals. The control plots were water sprayed consistently to avoid the effects of foliar application used for experimental plots.

Methanol treatment: 10% volume- methanol was used for the MFA. Amounts of 1 g amino acid Glycine and 1 mg Tetrahydrofolate were added to 1 L of methanol.

Measurement of mineral concentration: Mineral concentrations were determined in all treatments. In order to determine exact amounts of extracts of Mg, Mn, B and Zn, the dry ashing procedure was used and then mixed with hydrochloric acid. Dry ashing was done according to the following procedure; a 2 g sample of seeds was put inside a porcelain crucible and then placed in a muffle furnace. The temperature of the furnace was slowly increased to the ashing temperature of up to 550°C for over 2 h and kept for 6 h. After cooling, 10 cc acid hydrochloric (2 mol) was added. This time the temperature was increased up to 80°C. As soon as white steam was observed, the content of the porcelain crucible was filtered through a Whatman No. 40 filter paper. In this way, 100 cc extract was obtained (Karla, 1998). The digestion method was used to produce an extract for measuring N; the digestion method was used, which is performed inside a volumetric flask with sulfuric acid 96%, salicylic acid and hydrogen peroxide 30%. In this method a 0.3 g sample of seeds was put in the volumetric flask. Then the digestion mixture, including 100 cc of sulfuric acid, 6 g salicylic acid and 18 cc de-ionized water were added and were shaken to mix well (Waling et al., 1989). After it had cooled down, 5 drops of hydrogen peroxide were added and heated to 280°c for 5 to 10 min until white steam emerged. When the sample became colorless and cool, 10 cc of water was added (to make 100 cc). Having made the extract, the atomic spectrophotometer absorption was used (Hanlon, 1998).

The second growing season

To prepare the field for planting, hybrid seeds were collected from the mother plant. And preparations were made during the second growing season (that is, 2010-2011), such as plowing, harrowing, making furrows and plotting. Each plot consisted of 5 rows, 75 cm row spacing and 25 cm plant intervals. Plots were irrigated according to the condition of the soil and climate. Field irrigation was done once a week up to the end of growth.

Statistical analysis

Data was checked for analysis of variance, means comparison (based on Least Significant Difference Test) and the correlation between different treatments by MStatc and SPSS17 packages.

RESULTS AND DISCUSSION

Results for analysis of variance showed a significant effect for the combination of types of foliar application at different stages on reserves of N, Mg, Zn, Mn, B ($p<0.01$) and subsequent generation seed yield (Table 2). Furthermore, analysis of variance between treatments and the control showed significant difference (Table 5).

Seed N concentration

Data on comparison of means showed that foliar application with a mixture of all combinations at all stages (1/45% dry matter of seed) had the highest N concentration. Furthermore, N concentration in foliar application treatments with NFA in seed-filling, MgFA in tasseling, ZnFA in seed-filling, BFA at all stages, and MFA at all stages showed no significant difference. The orthogonal contrast of treatments and the control showed significant difference (Table 3). Based on these results, the reserved N showed a 26% increase due to foliar application with all mixtures at all growth stages compared with the control. The results of this study showed that BFA at all stages influenced N absorption, which in turn increased the concentration of N in the seed. Protein and soluble nitrogenous compounds are decreased in boron-deficient plants (Gupta, 2007). Boron deficiency did not substantially affect relative amino acid composition (Dugger, 1983), but it did enhance the proportion of inorganic nitrogen, particularly nitrate, in plant tissues and translocation fluids (Shelp, 1990). A number of researchers have reported increases in nitrate concentrations as well as corresponding decreases in nitrate reductase activity in sugar beet, tomato, sunflower and corn plants due to boron deficiency (Gupta, 2007; Kastori and Petrovic, 1989). The positive effect of nitrogen fertilizer in the mother plant can contribute to its role in delaying the aging cycle and providing enough time to obtain photosynthetic matter and consequently more weight and higher N seed reserve (Delouch, 1980). ZnFA at the seed-filling stage led to increased N accumulation. The role of zinc and magnesium in protein synthesis has been known about for a long time (Storey, 2007; Marschner, 1995). Protein synthesis resumes when zinc is resupplied because zinc is a structural component of ribosomes and responsible for their structural integrity (Storey, 2007). As the major portion of nitrogen in plants is in the form of proteins, it is clear that with an increase of protein synthesis, nitrogen will be stored in the seeds

Table 2. The analysis of variance of measured traits in experiment.

S.O.V	df	N	Mg	Zn	Mn	B	Seed yield
Rep	2	0/460**	0/011**	28/048**	67/58**	14/07**	46011*
FAS	3	0/030**	0/0001**	39/76**	22/22**	77/29**	43179*
FA	6	0/064**	0/001**	69/51**	8/836**	15/34**	105256**
FAS ×FA	18	0/063**	0/0004**	102/008**	25/06**	29/05**	81328**
Error	54	0/005	0/000002	0/048	0/025	0/528	10587
CV		5/79	1/04	0/72	7/28	19/34	9/85

*,**, Significant at 5 and1% respectively; FAS, foliar application stage; FA, foliar application.

Table 3. Orthogonal contrast of between control Vs other treatments.

	N	Mg	Zn	Mn	B	Yield
Ms	0/032*	0/0004**	49/003**	23/843**	20/352**	1378/558*

*, **, Significant at 5 and1%, respectively.

and will lead to an increase of N concentration. Mikkelsen (2000) argued that an increase of Zn affects the production of N and its absorption. Gupta and Singh (1985) reported that an increase in nitrogen absorption from Zn application can be attributed to an increase in shoot biomass. It has also been reported that nitrogen absorption in corn increased from 53/4 mg in the control plant to 206/2 mg due to a treatment of 2/5 mg Zn kg^{-1} soil. Abou-Hussein and Faiyad (1995) reported that application of 60 kg ha Zn and 8 kg ha B resulted in increased N concentration in plants from 1/2 to 2%. A study conducted by Vahedi (2011) showed that the use of B leads to an increase in seed N concentration. Foliar application of methanol can increase the activity of nitrate reductase in leaves (Zbieć et al., 2003). Therefore, MFA may be the cause of an increase of nitrogen assimilation in leaves that consequently promotes N accumulation in seeds. In addition, MgFA at all stages including the seed-filling stage (1/02%) had the lowest reserve of N in seed (Table 4 and Figure 1). Results of this study are incompatible with those of Choudhury and Khanif (2001) reporting that magnesium treatment increased plant accumulation of nitrogen, applied as urea, in rice (*Oryza sativa* L.).

Seed Mg concentration

Data from comparison of means showed that MgFA at all stages (0/172 mg kg^{-1}) had the highest and MgFA at the seed-filling stage and MFA at the 8 to 10 leaf stage (0/121 mg kg^{-1}) had the lowest Mg concentration in seed (Table 4 and Figure 2). The orthogonal contrast of treatments and the control regarding the Mg reserve showed significant difference (Table 3). Based on these results, Mg reserved in the seed showed an average increase of 33% due to MgFA at all growth stages compared with the control. Magnesium is a physiologically mobile within a plant. Therefore, magnesium can be reallocated from other plant parts and transported through the phloem to actively growing sinks (Merhaut, 2007). In this research, foliar application of maternal plants with Mg and the transfer of this element to seeds as an active sink has increased the concentration of seed-Mg. Nitrogen may either inhibit or promote magnesium accumulation in plants, depending on the particular form of nitrogen: with ammonium, magnesium uptake is suppressed and with nitrate, magnesium uptake is increased (Lasa et al., 2000). According to these results, NFA has increased the Mg-seed reserve. Based on results of correlations (Table 6), Mg-seed concentration had a positive and significant correlation with Mn-seed concentration (r = 0.363**). In hydroponically grown poinsettia, Mg concentrations in leaves increased as the proportion of nitrate-nitrogen to ammonium-nitrogen increased, even though all treatments received the same amounts of total nitrogen (Scoggins and Mills, 1998). In cauliflower (*Brassica oleracea* var. botrytis L.), increasing nitrate-nitrogen fertilization from 90 to 270 kg ha^{-1} increased yield as a response to increased Mg fertilization rates (22/5 to 90 kg ha^{-1}) (Batal et al., 1997).

Seed Zn concentration

Comparing means showed that ZnFA at the tasseling stage (44 mg kg^{-1}) had the highest effect on Zn seed reserve, whereas MFA at the tasseling stage (23/5 mg kg^{-1}) showed the least effect (Table 4 and Figure 3). The orthogonal contrast between treatments and the control showed a significant difference in terms of seed Zn concentration (Table 3). Based on these results, Zn reserve in the seed showed an average 83% increase

Table 4. Mean comparison of interaction between foliar application and growth stage based on LSD.

FAS	FA	N (%)	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Yield (g m^{-2})
8-10 leaves	Methanol	1.350^{a-d}	0.1210^{p}	33.00^{g}	7.000^{j}	7.000^{j}	0.8000^{i}	1125/19^{a-e}
	Zn	1.150^{e-jk}	0.1427^{i}	24.00^{V}	10.00^{d}	10.00^{d}	1.800^{i}	777/51^{ijk}
	B	1.250^{b-f}	0.1407^{j}	26.17^{qr}	4.000^{m}	4.000^{m}	4.600^{e-h}	1067/9^{b-h}
	Mg	1.227^{c-g}	0.1400^{j}	26.50^{q}	6.500^{k}	6.500^{k}	0.8000^{i}	885/63^{g-k}
	N	1.200^{d-j}	0.1527^{cd}	29.50^{m}	9.500^{e}	9.500^{e}	4.600^{e-h}	1242/3^{abc}
	Mn	1.093^{h-k}	0.1507^{fgh}	25.00^{T}	12.00^{a}	12.00^{a}	0.8000^{i}	1120/4^{a-e}
	Mix	1.097^{f-k}	0.1517^{def}	33.50^{f}	3.000o	3.000o	1.107^{i}	1301/1^{a}
Tasseling	Methanol	1.383^{ab}	0.1303^{no}	23.50^{w}	6.000^{l}	6.000^{l}	6.600^{cd}	1279/6^{ab}
	Zn	1.200^{d-j}	0.1313^{mn}	44.00^{a}	2.333^{p}	2.333^{p}	3.500^{h}	1002/7^{d-i}
	B	1.220^{c-h}	0.1300^{o}	31.50^{j}	3.500^{n}	3.500^{n}	3.700^{gh}	881/3^{g-k}
	Mg	1.440^{a}	0.1333^{kl}	25.50s	7.500^{i}	7.500^{i}	7.200^{bc}	848^{h-k}
	N	1.210^{c-i}	0.1503^{gh}	25.50s	6.500^{k}	6.500^{k}	4.600^{e-h}	1051/5^{c-h}
	Mn	1.150^{e-k}	0.1537^{c}	30.50^{k}	4.000^{m}	4.000^{m}	1.800^{i}	1069/1 $^{b-h}$
	Mix	1.300^{a-e}	0.1620^{b}	38.00^{c}	8.000^{h}	8.000^{h}	5.700^{cde}	713/9^{k}
Seed-filling	Methanol	1.210^{c-i}	0.1513^{efg}	30.00^{l}	8.500^{g}	8.500^{g}	0.8000^{i}	1106/6^{a-g}
	Zn	1.420^{a}	0.1500^{h}	35.00^{e}	7.000^{j}	7.000^{j}	0.9000^{i}	1138/4^{a-e}
	B	1.120^{f-k}	0.1323^{lm}	27.00^{p}	9.000^{f}	9.000^{f}	0.9000^{i}	1051/54^{c-h}
	Mg	1.020^{k}	0.1210p	30.50^{k}	11.00^{b}	11.00^{b}	3.700^{gh}	869/2^{h-k}
	N	1.400^{ab}	0.1337^{k}	32.00^{i}	4.000^{m}	4.000^{m}	4.600^{e-h}	1136/3^{a-e}
	Mn	1.207^{c-i}	0.1407^{j}	37.00^{d}	6.500^{k}	6.500^{k}	4.100^{fgh}	1169/1^{a-e}
	Mix	1.060^{jk}	0.1410^{j}	26.00r	8.000^{h}	8.000^{h}	0.7000^{i}	1114/5^{a-f}
All stages	Methanol	1.440^{a}	0.1300o	42.50^{b}	10.50^{c}	10.50^{c}	6.500^{cd}	1218/4^{a-d}
	Zn	1.070^{h-k}	0.1317^{m}	32.50^{h}	9.000^{f}	9.000^{f}	6.400^{cd}	1112/4^{a-f}
	B	1.410^{a}	0.1317^{m}	28.00^{n}	6.500^{k}	6.500^{k}	9.100^{a}	1064/7^{b-h}
	Mg	1.020^{k}	0.1723^{a}	27.50^{o}	3.000^{o}	3.000^{o}	5.300^{def}	967/25^{e-j}
	N	1.360^{abc}	0.1520^{de}	24.50^{u}	3.500^{n}	3.500^{n}	8.700^{ab}	889/34^{f-k}
	Mn	1.050^{jk}	0.1513^{efg}	30.00^{l}	8.500^{g}	8.500^{g}	0.8000^{i}	746/77^{jk}
	Mix	1.450^{a}	0.1423^{i}	24.50^{u}	7.500^{i}	7.500^{i}	5.100^{d-g}	1309/1^{a}
Control	-	1.15	0.129	24	4	4	1.1	982.34
LSD%	-	0.154	0.001	0.126	0.0912	0.0912	1.584	42.55

Within each column, means with the same lower case letter superscript are not significantly different ($P > 0.05$).

Table 5. Two-way analysis of variance of measured traits in experiment.

S.O.V	df	N	Mg	Zn	Mn	B	Seed yield
Rep	2	0/447**	0/011**	29/011**	71/391**	13/425**	1710/59*
Treat	28	0/058**	0/1×10^{-4} **	86/483**	21/241**	20/280**	2880/99**
Error	56	0/005	0/2×10^{-5}	0/154	0/248	0/22	367/878
CV		5/97	2/47	1/31	7/36	19/71	9/77

*, **, Significant at 5 and 1%, respectively.

due to ZnFA at the tasseling stage compared with the control. While zinc can be transported from vegetative tissue into reproductive tissue according to plant capacity (Welch, 1995) therefore, because of ZnFA and its transfer to seeds, the zn-seed concentration will increase. These results indicate that MFA at all stages had a positive

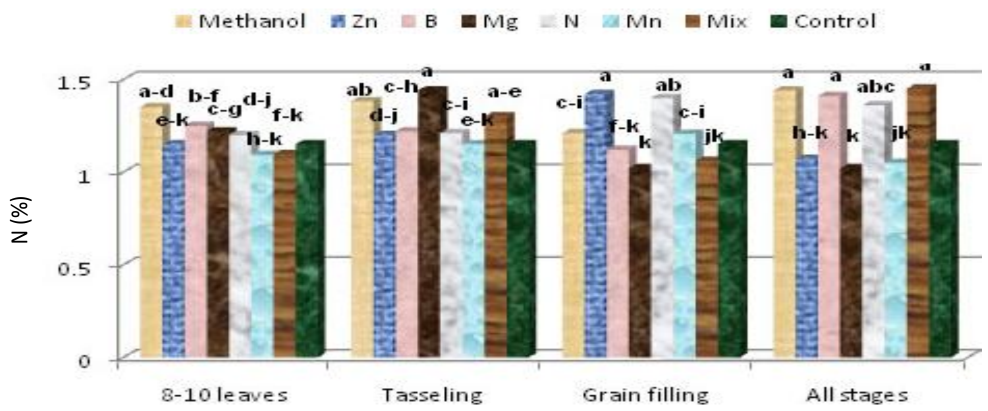

Figure 1. Effect of combination type of foliar application in the different stages on the reserve of N. (LSD5%=0.154)

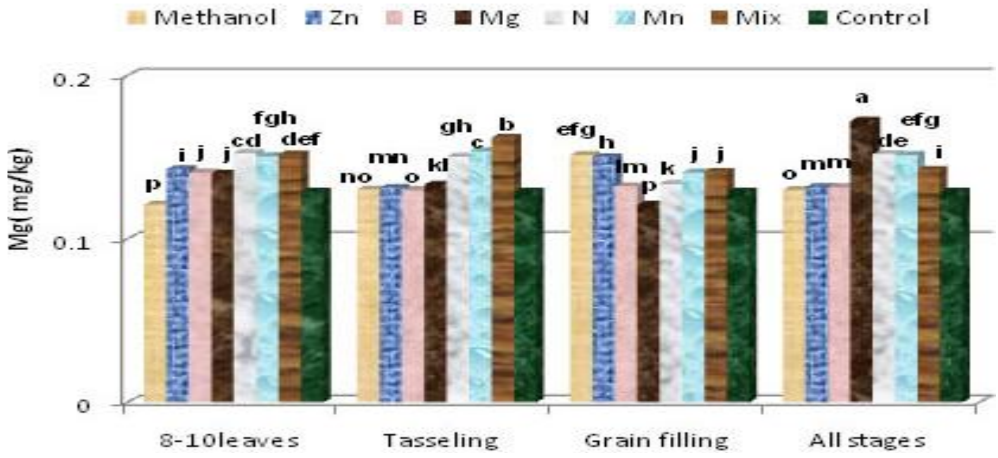

Figure 2. Effect of combination type of foliar application in the different stages on the reserve of Mg. (LSD5%=0.001)

Table 6. Correlation coefficients between measured traits.

	N	Mg	Zn	Mn	B	Seed yield
N	1	0.099	0.052	0.046	0.193*	0.308**
Mg		1	0.042	0.363**	0.098	-0.065
Zn			1	-0.008	0.202*	0.174
Mn				1	-0.048	0.205*
B					1	-0.072
Seed yield						1

*, ** Significant at 5 and 1% respectively.

positive effect and at the 8 to 10 leaf stage it had a negative effect on absorption and accumulation of Zn in the seed. Yilmaz et al. (1998) reported that fertilizer application can increase grain Zn concentration up to three or four-fold. Gangloff et al. (2002) determined that in maize plants and application of zinc sulphate increased dry matter and zinc accumulation in leaf and grain. Similar results have been reported by Maralian (2009).

Seed Mn concentration

Comparison of means showed that MnFA at the 8 to 10 leaf stage (12 mg kg^{-1}) had the highest and ZnFA at the

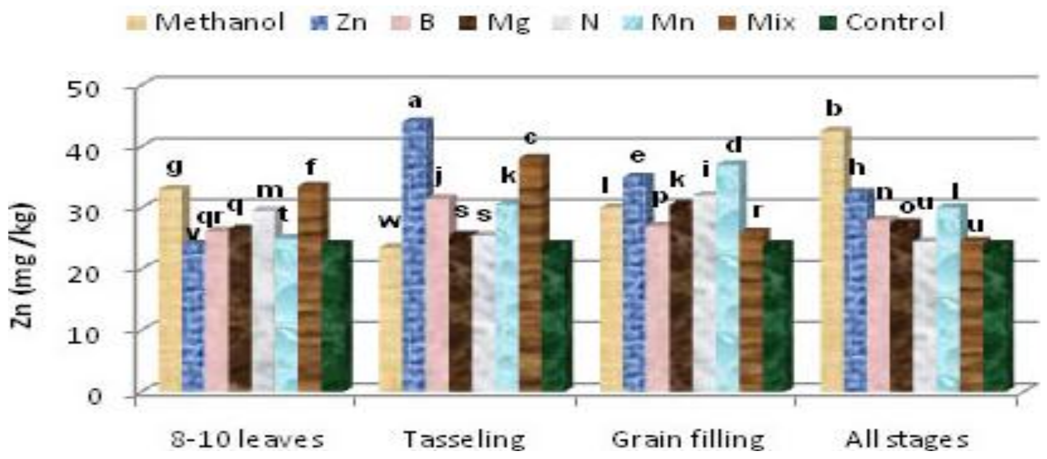

Figure 3. Effect of combination type of foliar application in the different stages on the reserve of Zn. (LSD5%=0.126).

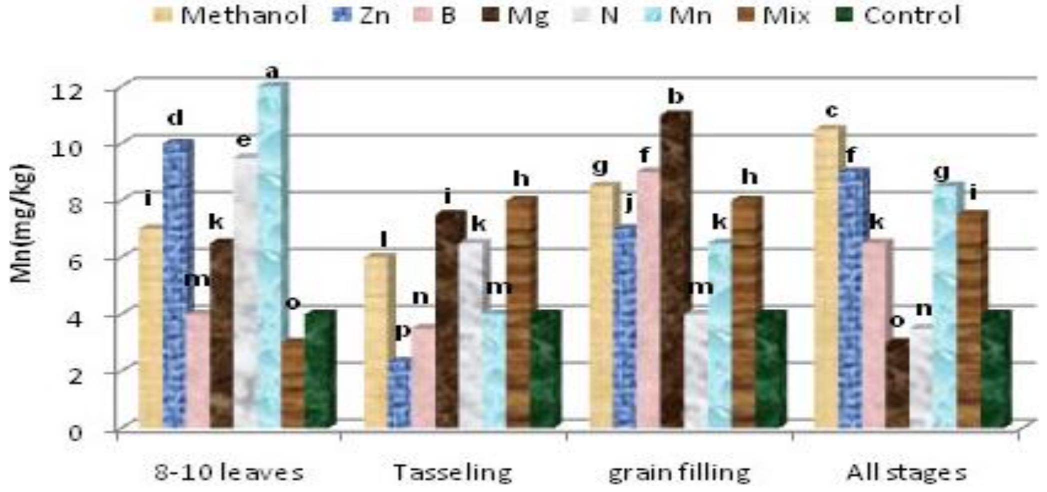

Figure 4. Effect of combination type of foliar application in the different stages on the reserve of Mn. (LSD5%=0.0912).

tasseling stage (2/33 mg kg^{-1}) had the lowest effect on the Mn concentration (Table 4 and Figure 4). The orthogonal contrast between treatments and the control showed a significant difference in terms of seed Mn concentration (Table 3). Mn reserved in the seed showed a 3-fold increase due to MnFA at the 8 to 10-leaf stage compared with the control. Likewise, there was a synergic difference between MnFA and MgFA. In other words, MnFA at the tassel-appearing stage increased the seed concentration of Mg but MgFA at the seed-filling stage increased the concentration of Mn in the seed. This point might be because of the capacity of Mg and Mn as a binding site on the plant's biological membrane (White and Cantor, 1971). A report given by Vahedi (2011) indicated that in soybean plants the highest concentration of Mn was observed in the Zn treatment. In addition, the use of B and Zn increased the Mn reserve in the seed.

Seed B concentration

Comparison of means showed that the BFA at all stages (9/1 mg kg^{-1}) had the highest effect on the seed B concentration from the treatment - mixture of all combinations - and MnFA at all stages (0/7 mg and 0/8 mg kg^{-1}), the MgFA at the 8 to 10 leaf stage (0/8 mg kg^{-1}), the MFA both at the tasseling stage (0/8 mg kg^{-1}), and at all stages (0/8 mg kg^{-1}). The ZnFA and BFA in the seed-filling stage (0/9 and 0/9 mg kg^{-1}) had the lowest effect on seed B reserves (Table 4 and Figure 5). The orthogonal contrast between the treatments and the control showed significant difference in terms of seed B concentration (Table 3). According to these results, the B reserve in the seeds showed 8-fold increase due to BFA at all stages compared with the control. The results of the effect of BFA on N concentration showed that the minerals had a

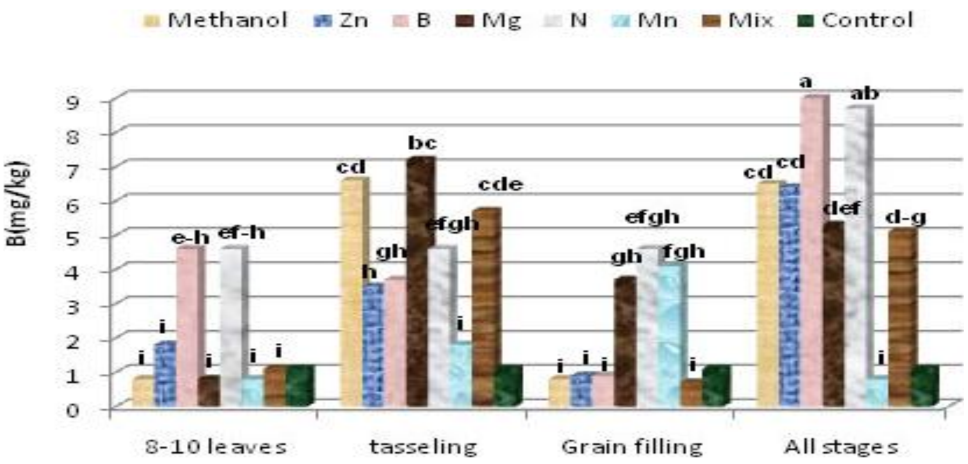

Figure 5. Effect of combination type of foliar application in the different stages on the reserve of B. (LSD5%= 1.584).

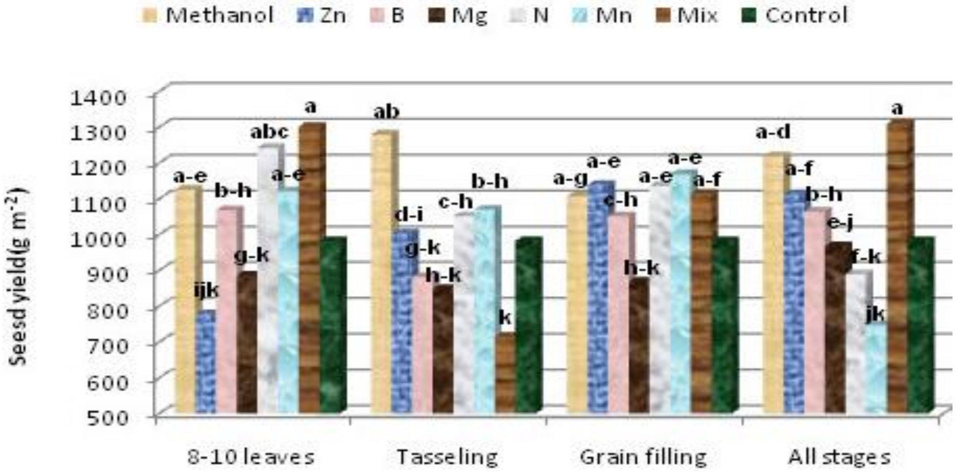

Figure 6. Effect of combination type of foliar application in the different stages on the seed yield. (LSD5%= 42.55).

synergic effect on each other. Boron deficiency in tobacco (*Nicotiana tabacum* L.) resulted in a decrease in leaf N concentration and reduced nitrate reductase activity (Camacho and Gonzales, 1999). Another report Rajaie et al. (2009) determined that Zn application in lime plants contributed to the amount of B in the plant's root. Mikkelsen (2000) in a similar statement refers to the effect of Zn in increasing levels of B and N absorption. A study on the effects of Zn and B on corn showed that high amounts of Zn and B in the soil help to increase seed yield and N absorption. Accordingly Zn-seed concentration had a positive and significant correlation with B-seed concentration (r = 0.202*) (Table 6).

Seed yield

Comparison of means showed that a mixture of all combinations at all stages had the highest effect on seed yield of a subsequent generation (1309 gm^{-2}) (33% increase compared with the control); at the tasseling stage (713 gm^{-2}) it had the lowest effect. This indicates that there was no significant difference between those treatments that had foliar application with a mixture of all combinations at all stages, MFA application at the 8 to 10- leaf stage, the tasseling stage, the seed-filling stage and at all stages, the NFA at the 8 to 10 -leaf stage and the seed-filling stage, the ZnFA at the seed-filling stage and at all stages and a mixture of all combinations at the seed-filling stage (Table 4 and Figure 6). The orthogonal contrast comparing treatments and the control in terms of seed yield showed significant difference (Table 3). This could be interpreted as a result of the positive effect of MFA on seed yield at all four stages. Increasing the amounts of micronutrient minerals stored in seeds and grains of staple food crops increases the yield potential of these crops when they are sown in micronutrient-poor soil. The effect of breeding for micronutrient-dense staple seeds and grains on crop yield has been addressed in a number of recent reviews (Graham et al., 1999). Briefly these reviews have determined that increasing stores of micronutrients in seeds increases seedling vigor and viability, which enhances the performance of seedlings when seeds are planted in a micronutrient-poor soil. This improved seed vigor allows for the production of more and longer roots under micronutrient-deficient conditions, allowing seedlings to scavenge more soil volume for micronutrients and water in early growth, an advantage that can lead to improved yield compared with those seeds with low-micronutrient stores when affected by micronutrient deficiency stress during growth (Welch, 2002). Perry and Harrison (1973) showed that if mother plants undergo high temperature stress, physiological disorders would be induced in seeds that together with delayed germination decrease seedling growth and emergence and produce low yield in the field. Environmental conditions, especially those of soil nitrogen affect a seed's nitrogen content and can increase or decrease yield and yield components (Oskouie and Divsalar, 2011). Yilmaz et al. (1998) showed that an increase in zinc content of bread wheat grain from 355 ng to 1465 ng grain^{-1} leads to increased yields at a subsequent harvest. According to the results of correlations (Table 6), seed yield had a positive and significant correlation with N-seed concentration (r = 0.205**).

Conclusions

As KSC 704 maternal plant treated with foliar applications of Mg and B at all stages, Zn foliar application at tasseling and MN foliar application at 8 to 10 leaf stage, Mg, B, Zn and MN-seed reserves were more than 0/043, 8, 20 and 8 mg kg^{-1} in comparison with the control respectively. Increasing mineral nutrient stores in seeds serves to increase reserves of nutrients in seeds of the subsequent generation. Many countries where micronutrient deficiency is a problem for humans, there are also large areas of micronutrient-poor or deficient soil. Improving seed vigour in terms of its ability to improve micronutrient reserves will be very beneficial to agricultural production in these countries (Welch, 2002).

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