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Differences in the Zinc Efficiency Among and Within Maize Cultivars in a Calcareous Soil

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Abstract: Low availability of Zn in calcareous soils is one of the widest ranging abiotic stresses in world agriculture. Greenhouse experiment were carried out with Four cultivars of maize (301 Single Grass (A), 302 Single Grass (B), 307 Single Grass (C), 400 Single Grass (D), *Zea mays* L.) were used to study the influence of varied zinc (Zn) supply on Zn efficiency, shoot dry matter production, Zn uptake, Chlorophyll content, Leaf Area Index (LAI) and Relative Growth Rate (RGR). Plants were grown in a Zn-deficient calcareous soil under greenhouse conditions with (+Zn = 10 mg/ kg soil) and without (-Zn) Zn supply. Plants were harvested after 40 and 80 days. Zinc efficiency, expressed as the percentage of shoot dry weight produced under conditions of Zn deficiency compared to Zn supply, ranged between 62.3 and 75.5% in first stage and between 63.5 and 81.2% in second stage. Application of Zinc caused increase in shoot dry weight in all cultivars. Zn uptake enhanced with application of zinc and ranged from 100.80 to 231.91 μg/pot in first stage and 458.01 to 858.83 μg/pot among cultivars. Zinc application increased the Chlorophyll content, Leaf Area Index and Relative Growth Rate. Zn efficiency had high and positive relation with Shoot dry weight and Zn uptake in different cultivars.

Key words: Maize, shoot dry weight, Zn efficiency, Zn uptake

INTRODUCTION

Zinc deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries (Alloway, 2004). Low availability of Zn in calcareous soils is one of the widest ranging abiotic stresses in world agriculture. The regions with Zn-deficient soils are also the regions where Zn deficiency in human beings is widespread, for example in India, Pakistan, China, Iran and Turkey (Cakmak *et al.*, 1999; Alloway, 2004; Hotz and Brown, 2004). Zinc deficiency is responsible for many severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development (Hotz and Brown, 2004; Gibson, 2006; Prasad, 2007). Zinc is an important micronutrient. Plant response to Zn deficiency occurs in terms of decrease in membrane integrity, susceptibility to heat stress, decreased synthesis of carbohydrates, cytochromes nucleotide auxin and chlorophyll. Further, Zn-containing enzymes are also inhibited, which include alcohol dehydrogenase, carbonic anhydrase, Cu-Zn-superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase, and RNA polymerase (Marschner, 1995).

Maize, one of important food crops grown in most worlds' area, is sensitive to Zn deficiency. Stunted and chlorotic plants due to Zn deficiency are often observed on early grown maize plants in the field (Liu *et al.*, 1993; Liu, 1996). Differential cultivar responses grown under low soil Zn concentrations have been reported in maize, millet, sorghum, rice and wheat, among others (Brown *et al.*, 1972; Clark, 1978; Cakmak *et al.*, 1999; Fageria, 2001). Zinc efficiency, defined herein as the ability of a plant to grow and yield well under zinc-deficient conditions, varies among cereal species (Graham *et al.*, 1993; Erenoglu *et al.*, 2000). Genotypic differences for zinc use efficiency have been reported for several crops species (Graham *et al.*, 1992; Cakmak *et al.*, 1994; Wiren *et al.*, 1994; Rengel *et al.*, 1995). Physiological mechanism(s) conferring Zn efficiency and their relative significance on low Zn soil/solution culture have been investigated by several workers (Erenoglu *et al.*, 2000; Rengel *et al.*, 2000; Gökhan *et al.*, 2003). However, physiological and biochemical processes that control Zn efficiency, in general, and Zn acquisition by the roots, in particular, is among the less thoroughly studied aspects of plant Zn-nutrition. Graham and Rengel (1993) suggested that more than one mechanism could be responsible for establishing Zn efficiency in a genotype and it is likely that different genotypes subjected to Zn deficiency under different environmental conditions will respond by one or more different efficiency mechanisms. Differences in Zn efficiency have been demonstrated particularly for cereal species in both field and greenhouse experiments

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(Graham *et al*., 1992; Kalayci *et al*., 1999). In recent years, research has been carried out in several different laboratories to elucidate the physiological mechanisms that confer Zn efficiency; however, these mechanisms are still poorly understood. A number of different wheat (*Triticum aestivum*) cultivars have been screened for their response to low Zn in Zn-deficient calcareous soils and significant differences in Zn efficiency among certain wheat cultivars have been consistently found in both field and growth chamber experiments (Cakmak *et al*., 1999; Kalayci *et al*., 1999; Hacisalihoglu *et al*., 2001). Cultivated cereal species, especially wheat, show a large variation in Zn efficiency, i.e., the ability of a cultivar to grow and yield better under Zn-deficient conditions in comparison with other cultivars (Graham *et al*., 1992; Cakmak *et al*., 1999). The aim of this study was to investigate the Zn efficiency and growth indices of maize crop in Zn deficient soil.

MATERIALS AND METHODS

Maize seeds 301 Single Grass (A), 302 Single Grass (B), 307 Single Grass (C), and 400 Single Grass (D), *Zea mays* L.) were grown in a Zn-deficient calcareous soil under greenhouse conditions. The experiments were conducted during the normal winter-summer growing season in greenhouse of university of Tehran. The soil used in the experiments was obtained from a Zn-deficient area. Five seeds were sown in plastic pots containing 4 kg soil with (+Zn = 10 mg Zn. kg soil) and without (-Zn) Zn supply. After emergence the seedlings were thinned to 2 seedlings per pot. The soil had sandy loam texture, pH 8.3, contained 0.9% of organic matter and 6 % of $CaCO_3$ as measured by standard methods given in Jackson (1958). The concentration of plant-available Zn was low, at around 0.4 mg/kg DTPA-extractable Zn, measured by the method of Lindsay and Norvell (1978). Zinc was added to soil in the form of $ZnSO_4$. A basal treatment of 600 mg N kg soil as CO $(NH_2)_2$ and 20 mg P kg soil as $NH_4H_2PO_4$ and 500 mg K kg soil as K_2SO_4 was applied to all plants. All nutrients were mixed homogeneously with soil before sowing. Zinc efficiency (%) was determined by the ratio between shoot dry weights at -Zn to shoot dry weight at +Zn conditions (Peleg *et al*., 2007). To determine dry matter yield and Zn uptake, crops were harvested after 40 (stage 1) and 80 days (stage 2) and dried at 75ºC. Dried crop samples were ground and wet-ashing and Zn concentrations was determined with Shimadzu AA-660 Atomic Absorption Spectrophotometer (Jackson, 1958). Amount of Zn uptake per plant can be calculated as: Plant dry weight (kg) × Zn concentration (mg Zn per kg dry weight). Chlorophyll content (Chl) was measured by Konica Minolta (SPAD-502) model (Fox *et al*., 1994). Leaf Area Index (LAI) and Relative Growth Rate (RGR) were measured by (1) and (2) (Khan *et al*., 2008). LAI and RGR were determined in two stages. StageI (0-40 days) and stage II (40-80 days). All sampling and measurements were carried out by using three replications. All statistical analyses were carried out using statistical package (SAS Institute, 2005). Least Significant Difference (LSD) was used to compare the main treatment and interaction effects at p<0.05.

$$\text{RGR (mg/day)} = (\text{Ln } w_1 - \text{Ln } w_2) / (T_2 - T_1) \quad \text{(T: time w: dry weight)} \qquad (1)$$

$$\text{LAI } (m^2) = \text{Leaf area / Ground covered by plant} \qquad (2)$$

RESULTS

Zn efficiency of maize cultivars: There was a differential response of maize varieties to Zn application in both times of growth (Table1 and 2). The Zn efficiency of different maize varieties was calculated as the ratio of grain yield due to Zn control and Zn addition, multiplied by 100 (Peleg *et al*., 2007). This is also called tolerance index (%).

The cultivars exhibited variation in Zn efficiency (62.3-75.5%) after 40 days and (58.5-81.2%) after 80 days. in stage 1 cultivars D and B showed above 70% Zn efficiency. In stage 2 cultivars D and A showed above 70% Zn efficiency. According to Zn efficiency ratio (%), the cultivars followed the order: D>B>C>A (40 days) and D>A>B>C. Such large variation in Zn efficiency between wheat cultivars was also found in South Australia (Graham *et al*., 1992).

Effect of Zn in shoot dry weight: Shoot dry weight of maize responded significantly to Zn application. The Shoot dry weight increased significantly with increasing Zn rates, as observed in the two stages (Table 1 and 2). In efficient cultivar (D), high Shoot dry weight as obtained with two Zn rates (0, 10 mg/kg) were 4.40-7.40 (in stage1) and 13.9-23.13 gr/pot (in stage 2), respectively. Most of the Zn-efficient cultivars produced higher Shoot dry weight than average, while Shoot dry weight of most Zn-inefficient cultivars were lower than the average, when Zn was not applied. In stage 2, cultivars D and A had both, a high Zn efficiency and high Shoot dry weight under Zn-deficient conditions, and the cultivar B, C had the lowest Zn efficiency and Shoot dry weight. There was no significant difference in Shoot dry weight between cultivars when apply Zinc in both stages. Our results are in agreement with the results obtained with wheat (Graham *et al*., 1992; Cakmak *et al*., 1997), chickpea (Khan *et al*., 1998) and common bean (Hacisalihoglu *et al*., 2004).

Zn uptake: Zinc uptake of cultivars is given in Table 1 and 2. There was positive and significant difference in the Zn uptake between the efficient and in-efficient cultivars at Zn_0 and Zn_{10} in both stages. Under Zn-deficient

Table 1: Effect of varied Zn supply in shoot dry weight (SDW) (g/plant), Zn uptake (µg /pot) and Zn Efficiency (%) in different cultivars after 40 day

	SDW (g/pot)		Zn uptake (µg/pot)		
Cultivar	- Zn	+Zn	-Zn	+Zn	ZnEfficiency (%)
A	3.63 ± 0.9^{d}	7.3 ± 0.2^{a}	39.04 ± 12.9^{f}	188.40 ± 11.9^{b}	62.3
B	4.16 ± 0.5^{c}	6.73 ± 0.6^{b}	76.75 ± 7.5^{e}	160.21 ± 22.5^{c}	73.1
C	3.73 ± 0.3^{d}	7.06 ± 0.2^{a}	56.29 ± 13.7^{f}	165.32 ± 5.2^{c}	65.1
D	4.40 ± 0.2^{c}	7.40 ± 0.5^{a}	100.80 ± 4.6^{d}	231.91 ± 17.6^{a}	75.5

[a]: Values were compared using a LSD multiple range test at the 5% level, Homogeneous groups are denoted with the same letter, Shoot Dry Weight (SDW)

Table 2: Effect of varied Zn supply in shoot dry weight (SDW) (g/plant), Zn uptake (µg /pot) and Zn Efficiency (%) in different cultivars after 80 day

	SDW (g/pot)		Zn uptake (µg/pot)		
Cultivar	- Zn	+Zn	-Zn	+Zn	ZnEfficiency (%)
A	10.03 ± 2.65^{bc}	23.67 ± 2.8^{a}	254.40 ± 83.4^{d}	803.46 ± 6.3^{a}	71.5
B	8.00 ± 1.7^{cd}	23.13 ± 2.7^{a}	152.33 ± 22^{d}	675.10 ± 68.4^{b}	63.5
C	6.70 ± 1.1^{d}	23.80 ± 1.3^{a}	107.94 ± 7.5^{e}	669.22 ± 22.8^{b}	58.5
D	13.90 ± 1.7^{b}	23.13 ± 1.9^{a}	458.01 ± 36.9^{c}	853.83 ± 95.1^{ab}	81.2

[a]: Values were compared using a LSD multiple range test at the 5% level, Homogeneous groups are denoted with the same letter, Shoot Dry Weight (SDW)

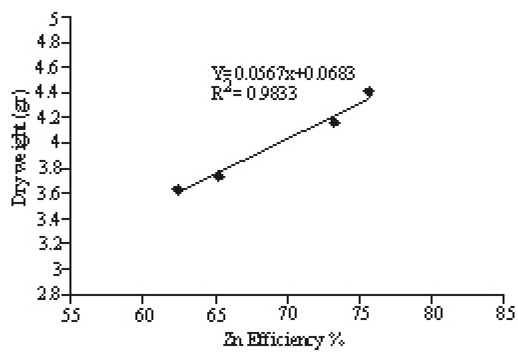

Fig. 1: Relationship between Zn efficiency and Shoot dry weight of different cultivars after 40 day

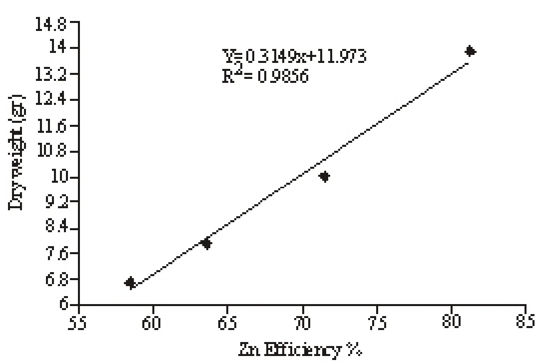

Fig. 2: Relationship between Zn efficiency and Shoot dry weight of different cultivars after 80 day

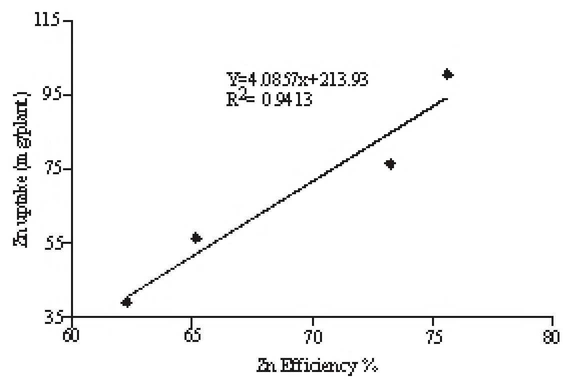

Fig. 3: Relationship between Zn efficiency and Zn uptake of different cultivars after 40 day

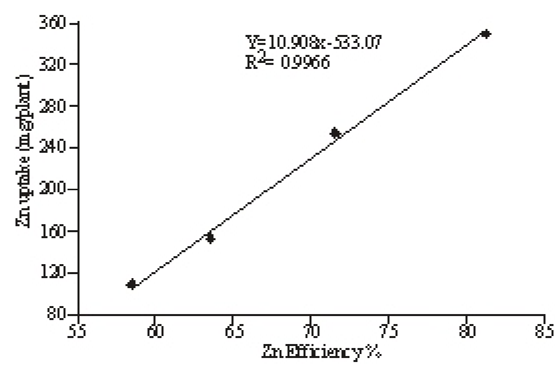

Fig. 4: Relationship between Zn efficiency and Zn uptake of different cultivars after 80 day

conditions, ranging from 100.80-231.91 (in stage 1) and 458.01-858.83 µg/pot (in stage 2). Zinc fertilization increased Zn uptake of all cultivars by 130% in the first and 87.5% in the second stage (Table 1 and 2). Efficient cultivars such as D had high amount of Zn uptake then in-efficient cultivars.

Relationship between Zn efficiency, shoot dry weight and Zn uptake: As presented in Fig. 1 and 2, for all cultivars Zn efficiency ratios appeared to be well related to the Shoot dry weight. However, in the case of Zn efficiency ratios higher than 55% there was a high and

Table 3: Effect of varied Zn supply in chlorophyll content (Chl), Leaf Area Index (LAI) and Relative Growth Rate (RGR) in different cultivars after 40 day

Cultivar	CH1		LAI (cm^2/cm^2)		RGR I (mg/day)	
	- Zn	+Zn	-Zn	+Zn	-Zn	+Zn
A	38.3±0.8[cd]	39.9±1.2[c]	0.18±0.02[d]	0.33±0.02[b]	136.6±4.6[d]	152.7±0.9[b]
B	33.7±0.5[e]	40.7±0.9[c]	0.17±0.01[d]	0.21±0[cd]	139.7±2.5[cd]	150.6±2.1[b]
C	34.1±0.9[de]	36.8±0.8[d]	0.26±0.2[c]	0.3±0.02[b]	137.5±1.7[d]	151.9±0.7[b]
D	43.8±1.7[b]	48±0.5[a]	0.3±0.01[b]	0.42±0.03[a]	141±1.2[c]	159.3±2.4[a]

[a]: Values were compared using a LSD multiple range test at the 5% level. Homogeneous groups are denoted with the same letter. Chlorophyll content (Chl), Leaf Area Index (LAI) and Relative Growth Rate (RGR)

Table 4: Effect of varied Zn supply in chlorophyll content (Chl), Leaf Area Index (LAI) and Relative Growth Rate (RGR) in different cultivars after 80 day

	CH1		LAI (cm^2/cm^2)		RGR II (mg/day)	
Cultivar	- Zn	+Zn	-Zn	+Zn	-Zn	+Zn
A	40.7±2.5[c]	45.5±0.8[b]	0.44±0.02[c]	0.68±0.02[a]	42.3±2.6[bc]	44.9±2.2[b]
B	45.2±0.7[b]	50.1±1[a]	0.36±0.04[cd]	0.7±0.05[a]	36.9±0.68[d]	40.2±3[c]
C	41.9±3.4[c]	46.9±2.1[b]	0.33±0.02[d]	0.51±0.008[b]	36.8±2.02[d]	39.6±0.9[c]
D	45.6±1.1[b]	50.9±1.3[a]	0.42±0.02[c]	0.68±0.03[a]	43.9±2.1[b]	48.1±1.6[a]

[a]: Values were compared using a LSD multiple range test at the 5% level, Homogeneous groups are denoted with the same letter. Chlorophyll content (Chl), Leaf Area Index (LAI) and Relative Growth Rate (RGR)

positive relation between Zn efficiency and Shoot dry weight.

The relation between Zn efficiency and Zn uptake under Zn-deficient conditions for both stages was shown in Fig. 3, 4. Zn efficiency of cultivars was significantly correlated in both stages with Zn uptake per plant (R^2 = 0.94 in first stage, R^2 = 0.99 in second stage). Increasing in Zn efficiency caused enhance in Zn uptake. This result indicates that not only enhanced Zn uptake but also enhanced internal utilization of Zn plays an important role in expression of high Zn efficiency.

Chlorophyll content, leaf area index and relative growth rate: The analysis of Chl, LAI and RGR revealed that significant differences between treatments were established by 40 and 80 day post sowing with each of the zinc applications leading to an increase in Chl, LAI and RGR (Table 3 and 4). Each variable responded significantly to Zn application. Under Zn-deficient condition efficient cultivars such as D had maximum chlorophyll content, Leaf Area Index and Relative Growth Rate in two stages. In first stage, did not observe significant differences between cultivars in Relative Growth Rate but there was positive and significant difference between cultivars of this viewpoint in second stage.

DISCUSSION

Zn nutrition of plants is considered one of major problems in the arid and semi-arid regions (Ekiz *et al.*, 1998). Variation in Zn efficiency was found among the cultivars of maize. Decrease in shoot dry matter production due to Zn deficiency was most distinct in all cultivars of maize (Table 1 and 2). On average, Zn efficiency ratio was 66.9% for 301 Single Grass (A), 68.3% for 302 Single Grass (B), 61.8 for 307 Single Grass (C) and 78.3% for 400 Single Grass. The cultivated forms of 307 Single Grass (C) possess as well the highest sensitivity to Zn deficiency compared to cultivated rye, triticale, barley, oat and bread wheat cultivars (Cakmak *et al.*, 1998; Ekiz *et al.*, 1998). Under Zn deficiency, Zn uptake was better related to Zn efficiency (Fig. 1 and 2), indicating that Zn-efficient genotypes under Zn deficiency possibly have greater Zn uptake capacity. Enhancements in Zn uptake rate by roots and Zn utilization at the cellular level was shown as important mechanisms affecting expression of high Zn efficiency in wheat (Rengel and Wheal, 1997; Cakmak *et al.*, 1998). Efficient cultivars such as D had high amount of Zn uptake then in-efficient cultivars. The importance of Zn uptake to Zn efficiency agrees with previous studies (Cakmak *et al.*, 1998; Graham and Rengel, 1993). Multiple regression analysis showed that Zn uptake is the most important factor statistically explaining variation in Zn efficiency among the considered rice genotypes (Hajiboland and Salehi, 2006). The higher Zn uptake capacity of Zn efficient genotypes might be attributable to a greater amount of sulfhydryl groups in root-cell plasma membranes, particularly in ion transport-related proteins (Rengel *et al.*, 1995; Rengel and Wheal, 1997).

Zinc applications had positive effect on plant growth leading to increased Chl, LAI and RGR. The apparent mechanism for achieving these improvements was the increase in leaf area index, improved resource generating base for the crop, Chlorophyll content and Relative Growth Rate. Other workers have also reported that zinc application improved Chl, LAI and RGR (Judrth *et al.*, 1977; Khan *et al.*, 2008; Zhoori *et al.*, 2009). It is becoming clear that micronutrient deficiencies, including Zn deficiency, are a significant global problem, affecting more than 2 billion people in the world, with serious implications for human health (Graham and Welch, 1996; Welch *et al.*, 1997).

High consumption of cereal-based foods, of low micronutrient content, has been suggested as a major reason for the widespread occurrence of micronutrient deficiency in humans, particularly in developing countries. Our study confirms that tolerance to Zn deficiency is a complex trait in which many plant characteristics are involved. We also confirmed that a large set of genotypes needs to be considered in order to get a complete view on crop tolerance to Zn deficiency.

CONCLUSION

The cultivars exhibited variation in Zn efficiency (62.3-75.5%) after 40 days and (58.5-81.2%) after 80 days. According to Zn efficiency ratio (%), the cultivars followed the order: D>B>C>A. In efficient cultivar (D), high Shoot dry weight as obtained with two Zn rates (0, 10 mg/kg) were 4.40-7.40 (in stage 1) and 13.90-23.13 gr/pot (in stage 2), respectively. Under Zn-deficient conditions, ranging from 100.80- 231.91 (in stage 1) and 458.01-858.83 µg/pot (in stage 2). Increasing in Zn efficiency caused enhance in Zn uptake. Under Zn-deficient condition efficient cultivars such as D had maximum chlorophyll content, Leaf Area Index and Relative Growth Rate in two stages.

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