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Zinc Application Affects Tissue Zinc Concentration and Seed Yield of Pea (*Pisum sativum* L.)

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ABSTRACT

A 2-year field experiment was conducted to assess the effect of applied zinc (Zn) on the seed yield of pea (*Pisum sativum* L.) and to determine the internal Zn requirement of pea with emphasis on the seed and leaves as index tissues. The experiment was carried out at two different locations (Talagang, Chakwal district and National Agricultural Research Centre (NARC), Islamabad) in the Potohar Plateau, Pakistan by growing three pea cultivars (Green feast, Climax, and Meteor). The soils were fertilized with 0, 2, 4, 8, and 16 kg Zn ha^{-1} along with recommended basal fertilization of nitrogen (N), phosphorus (P), potassium (K), and boron (B). Zinc application increased seed yield significantly for all the three cultivars. Maximum increase in the pea seed yield (2-year mean) was 21% and 15% for Green feast, 28% and 21% for Climax, and 34% and 26% for Meteor at Talagang and NARC, respectively. In all cultivars, Zn concentrations in leaves and seed increased to varying extents as a result of Zn application. Fertiliser Zn requirement for near-maximum seed yield varied from 3.2 to 5.3 kg ha^{-1} for different cultivars. Zinc concentrations of leaves and seeds appeared to be a good indicator of soil Zn availability. The critical Zn concentration range sufficient for 95% maximum yield (internal Zn requirement) was 42–53 mg kg^{-1} in the pea leaves and 45–60 mg kg^{-1} in the seeds of the three pea cultivars studied.

Key Words: calcareous soils, diagnostic criteria, vegetable crops, zinc fertiliser, zinc uptake

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Zinc (Zn) deficiency is a widespread and frequent micronutrient disorder in crops, predominantly in calcareous soils of arid and semi-arid regions worldwide (Takkar and Walker, 1993; Welch and Graham, 2002) including Pakistan (Anonymous 1998, Rafique *et al.*, 2006) because of its low solubility and high fixation under such soil conditions (Lindsay, 1979). Nearly half of the agricultural soils contain low levels of plant-available Zn (Graham and Welch, 1996), thus reducing crop yield and nutritional quality.

The soils across much of the cultivated areas in Pakistan are developed from calcareous alluvium and loess, and low in organic matter as well as many essential plant nutrients (Rashid and Ahmad, 1994). Multiple factors like free carbonates, low organic matter, high pH, and continuous nutrient removal with intensive cultivation coupled with inadequate and imbalanced fertiliser use are associated with deficiencies of nutrient in crops including Zn (Rafique *et al.*, 2006).

Pea (*Pisum sativum* L.), a cool season vegetable crop belonging to family *Leguminosae*, is one of the leading and popular vegetables in Pakistan. It is a valuable supplement to cereals and other starchy food in the human diet due to high contents of lysine and tryptophan. It is categorized as less sensitive to Zn deficiency (Alloway, 2008). However, Zn deficiency does occur in peas as Zn has many important roles in plant growth and a lack of Zn was linked to reduced seed formation (Bell and Dell, 2008). Zinc deficiency in human also appears to be a critical nutritional and health problem in the world. Challenge is being faced to increase seed/grain Zn concentration in crops to overcome widespread malnutrition especially in developing countries (Bouis and Welch, 2010). Thus, increasing Zn levels in seed could deliver more Zn to people who rely directly or indirectly on pea-derived food. Zinc application was also an effective strategy of biofortification to increase grain Zn concentration in wheat and rice (Cakmak, 2008; Hossain *et al.*, 2008; Shivay *et al.*, 2008), but information specific to pea is limited.

Foliar analysis at a particular crop growth stage is widely used as a diagnostic guide for fertilization. Whole shoots or recently matured leaves at early flowering are the tissues usually recommended for analysis

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(Jones *et al.*, 1991). According to Jones *et al.* (1991), seed usually is not a better index tissue for estimating the nutrient status of plants. Nevertheless, seed analyses have also been used for determining Zn supply to young plants (Rashid and Fox, 1992). Zinc concentration of seeds reflects differences among soils in their ability to supply Zn and the ability of plants to accumulate Zn (Rashid and Fox, 1992; Rafique *et al.*, 2011).

Much of the information regarding sensitivity of pea species to Zn deficiency is based on field observations, whereas corresponding experimental work is rarely reported in literature. Moreover, information concerning Zn requirement and critical concentration in plant parts of the crop is limited. Further, plant analysis diagnosing Zn concentration values published in the literature may not be appropriate for various crop genotypes grown in different agro ecological zones. Even the cultivars of the same plant species demonstrate a variable response to a specific nutrient supply/deficiency (Rengel and Romheld, 2000; Hacisalihoglu *et al.*, 2004). The objective of this study was to assess the effect of Zn application on the yield and internal Zn requirement of pea with emphasis on the seed and leaves as index tissues for determining the Zn status of crop under field conditions.

MATERIALS AND METHODS

Sites description

A 2-year (2010–2012) field experiment was conducted at two locations, *i.e.*, Talagang, Chakwal district (32°56′ N, 72°25′ E), a sandy loam Balkassar soil (coarse loamy mixed, hyper thermic Udic Haplustalf) and National Agricultural Research Centre (NARC), Islamabad (33°43′ N, 73°5′ E), a loam Nabipur soil (fine loamy mixed, hyper thermic Udic Ustochrept) in the Potohar Plateau, Pakistan. The Balkassar soil is relatively coarse-textured with lesser soil organic matter (OM), compared with Nabipur soil (Table I).

Nutrient treatments, experimental design and crop management

The experiment consisted of 45 plots, each comprised of three raised beds of 1.25-m width and 9.0-m length, arranged in split-plot design with three replications. Three pea cultivars, *i.e.*, Green feast, Climax, and Meteor were in main-plots and Zn doses, *i.e.*, 0, 2, 4, 8, and 16 kg Zn ha^{-1} as $ZnSO_4 \cdot 7H_2O$ were applied in sub-plots. Additionally, 60 kg N ha^{-1} as urea, 100 kg P_2O_5 ha^{-1} as single super phosphate, 60 kg K_2O ha^{-1} as sulphate of potash, and 1 kg B ha^{-1} as boric acid were applied as basal dose to produce normal mature plants, plus extra nutrient to provide a margin of safety in the soil until harvest. Full doses of P, K, B, Zn and one-third of N were applied at the time of sowing. Remaining amount of N was applied in two splits, *i.e.*, at apparent flowering and pod formation stages. The pea seeds were sown on both sides of beds 5 cm apart in mid October. Irrigation, weeding, and other cultural practices were done according to regular recommenda-

TABLE I

Selected initial physico-chemical characteristics of soils at two field experimental sites in the Potohar Plateau of Pakistan

Soil characteristic	Experimental site	
	Talagang	NARC[a)]
Soil series	Balkassar	Nabipur
Soil family	Coarse-loamy mixed hyper thermic Udic Haplustalf	Fine-loamy mixed hyper thermic Udic Ustochrept
Clay (%)	10	15
Silt (%)	22	45
Texture	Sandy loam	Loam
pH (1:1)	8.1	8.3
EC[b)] (1:1) (dS m^{-1})	0.40	0.54
Organic matter (g kg^{-1})	3.4	4.9
$CaCO_3$ (g kg^{-1})	24	31
NO_3^--N (mg kg^{-1})[c)]	2.2	3.0
P (mg kg^{-1})[c)]	1.3	1.9
K (mg kg^{-1})[c)]	90	120
Zn (mg kg^{-1})[c)]	0.28	0.42
B (mg kg^{-1})[d)]	0.17	0.25

[a)]National Agricultural Research Centre.
[b)]Electric conductivity.
[c)]Ammonium bicarbonate-DTPA-extractable.
[d)]Hot water-extractable.

tions for the region. Nutrient treatments were same at both locations for 2 years.

Composite diagnostic plant tissue, *i.e.*, recently matured leaves at flower initiations, were collected (Jones *et al.*, 1991). Picking was started when pods were well filled with young and tender seeds, and three pickings were taken. Seed and stalk yield was recorded and representative samples were kept for Zn analysis.

Zinc analysis

Diagnostic plant parts, *i.e.*, leaf, stalks, and seeds, were digested in a double acid mixture (2:1), *i.e.*, nitric acid and perchloric acid. Zinc in the digests was determined by atomic absorption spectrophotometry (AAnalyst 800, Perkin Elmer, USA) (Wright and Stuczynski, 1996). Internal Zn requirement, the concentration of Zn in specific tissue sufficient for 95% maximum yield (Rashid and Fox, 1992), was determined from relative yield *versus* Zn concentration using a boundary line technique (Webb, 1972).

Statistical analysis

Analysis of variance (ANOVA) of the measured parameters was performed using MSTAT-C and the Zn doses and cultivars means were compared using Duncan's multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Seed yield

There were no significant differences in pea seed yield and change pattern across the years at both locations, so data of 2 years were pooled in this study. Analysis of variance showed significant effects of Zn and cultivar on pea seed yield at both locations (Table II, $P < 0.05$). However, interaction effect (Zn rates $\times$ cultivars) was non-significant. Zinc application increased seed yield of all pea cultivars. Increasing Zn application rate to 8 kg Zn ha^{-1} increased seed yield of Green feast at both locations. However, increased seed yields of Meteor was observed at 16 kg Zn ha^{-1} application. The yield differences between 0 and 8 kg Zn ha^{-1} were significant, while no significant difference was found between 8 and 16 kg ha^{-1}. Among cultivars, increase in seed yield with Zn fertilization over control, was highest for Meteor and lowest for Green feast. Maximum increase in pea seed yield (2-year mean) at Talagang and NARC was 21% and 15% for Green feast, 28% and 21% for Climax, and 34% and 26% for Meteor. Better impact of applied Zn over control was more in coarse-textured Balkassar soil at Talagang compared with that in Nabipur soil at NARC. It was probably related to lesser native OM content in the former soil than in Nabipur soil at NARC (Table I). In this study, seed yield increased up to 8 kg Zn ha^{-1} application. No toxicity symptom was observed at this rate. In a pot study conducted by Shukla and Raj (1980) on a calcareous Zn-deficient soil using pigeon pea as test crop, 93% increase in seed yield was observed with 5 mg Zn kg^{-1} soil application. However, 50 mg Zn kg^{-1} soil did not increase seed yield significantly over 5 mg Zn kg^{-1} soil application. As Zn has many important roles in plant growth, *i.e.*, photosynthesis, enzyme activity such as carbonic anhydrase (Rengel, 1995), chlorophyll concentration, and stomatal conductance (Hu and Sparks, 1991), a continuous supply of Zn is necessary for optimum plant growth and yield.

TABLE II

Effect of Zn fertilization on the seed yields of three pea cultivars at two field experimental sites in the Potohar Plateau of Pakistan

Zn applied	Seed yield							
	Talagang				NARC[a]			
	Green feast	Climax	Meteor	Mean	Green feast	Climax	Meteor	Mean
kg ha^{-1}	t ha^{-1}							
0	1.676	1.498	1.375	1.516D[b]	1.945	1.670	1.538	1.718D
2	1.868	1.758	1.635	1.754C	2.121	1.861	1.713	1.898C
4	1.935	1.848	1.732	1.838B	2.169	1.979	1.798	1.982B
8	2.027	1.915	1.821	1.921A	2.237	2.021	1.907	2.055A
16	2.002	1.909	1.841	1.917A	2.228	2.029	1.938	2.065A
Mean	1.901a[c]	1.785b	1.681c		2.140a	1.912b	1.779c	

[a] National Agricultural Research Centre.
[b] For each Zn application rate, means in a column followed by the same uppercase letter are not significantly different at $P \leq 0.05$. Least significant difference (LSD, $P < 0.05$) was 0.06 and 0.04 for Talagang and NARC, respectively.
[c] For each pea cultivar, means in a row followed by the same lowercase letter are not significantly different at $P \leq 0.05$. Least significant difference (LSD, $P < 0.05$) was 0.05 and 0.03 for Talagang and NARC, respectively.

Genotypic variation

In the present study, the term "Zn efficiency" is employed as the ability of a plant to grow and yield well in a Zn-deficient soil (Graham, 1984). Based on the reduction in seed yield, Zn efficiency of the cultivars, *i.e.*, ratio of seed produced under Zn deficiency to Zn fertilization was: 86%–89% for Green feast, 81%–85% for Climax, and 78%–84% for Meteor (Table III). Thus, under given conditions, efficiency of the grown pea cultivars declined in the order of Green feast > Climax > Meteor. It is most likely that the Zn taken up by more efficient cultivars is used for dry matter production under Zn deficient condition and thus diluted to similar concentrations as in the inefficient cultivars, and not accumulated in seeds. The reason for higher sensitivity of Meteor to Zn deficiency as compared to Green feast is not known. These differences among the cultivars might be related to the higher Zn accumulating capacity of Meteor. The observed variation in Zn accumulation among the genotypes supported earlier results of Rafique *et al.* (2011) who reported that the Zn accumulation was related to the genotypic variation in onion. Differential susceptibility to Zn deficiency among onion and wheat genotypes is attributed to the differential capacity of genotypes in acquisition of Zn from soils (Graham *et al.*, 1992; Cakmak *et al.*, 1996a; Rafique *et al.*, 2011). Cakmak *et al.* (1994, 1996b) also reported that differences in Zn efficiency among wheat genotypes might be related to their efficiency to release Zn mobilizing phytosiderophores (phytometallophores) from their roots to the rhizosphere and absorption and translocation of Zn from roots to shoot meristem. Phytosiderophores are known to be effective in mobilizing Zn by chelating sparingly soluble Zn compounds in calcareous soils (Treeby *et al.*, 1989).

Fertiliser Zn requirement

The observed fertiliser Zn requirement for 95% maximum yield of pea cultivars was 3.2 kg Zn ha^{-1} for Green feast, 3.4 kg Zn ha^{-1} for Climax, and 5.3 kg Zn ha^{-1} for Meteor (Fig. 1). The field study revealed that despite low sensitivity of peas to Zn deficiency, this nutrition disorder caused a considerable yield loss. Anonymous (1998) reported that a dose of about 4 kg Zn ha^{-1} can help ameliorate the deficiency not only in the current crop but also in subsequent crop grown in the same field. Thus, a nominal investment on Zn fertiliser, in soil-deficient situation, can improve crop productivity, increase plant Zn concentration, enhance growers' income, and help sustain the soil resource base.

Leaf Zn composition

Pea leaf Zn concentration increased significantly

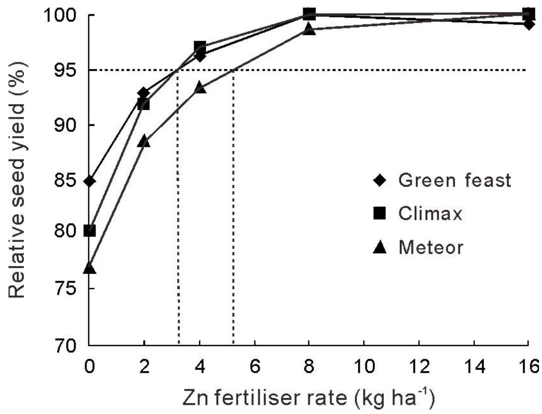

Fig. 1 Relationships between Zn fertiliser rates and relative seed yields of three pea cultivars (Green feast, Climax, and Meteor). Maximum yields of the pea cultivars were 2.132 t ha^{-1} for Green feast, 1.972 t ha^{-1} for Climax, and 1.900 t ha^{-1} for Meteor.

TABLE III

Effect of Zn application on seed yield and Zn efficiency of three pea cultivars grown in two Zn-deficient soils at two field experimental sites in the Potohar Plateau of Pakistan

Cultivar	Talagang				NARC[a)]			
	Seed yield		Increase over control	Zn efficiency[d)]	Seed yield		Increase over control	Zn efficiency
	Control[b)]	Zn applied treatments[c)]			Control	Zn applied treatments		
	t ha^{-1}		%		t ha^{-1}		%	
Green feast	1.676	1.958	17	86	1.945	2.179	12	89
Climax	1.498	1.857	24	81	1.670	1.960	17	85
Meteor	1.375	1.757	28	78	1.538	1.827	19	84
Mean	1.516	1.857	22	82	1.718	1.989	16	86

a) National Agricultural Research Centre.
b) 0 kg Zn ha^{-1}.
c) Zn applied at 2, 4, 8, and 16 kg ha^{-1}.
d) Zinc efficiency was calculated as the ratio of dry seed yield at 0 kg Zn ha^{-1} to the mean dry seed yield at 2, 4, 8, and 16 kg Zn ha^{-1}.

with Zn fertiliser rates at both locations (Table IV). However, the magnitude of increase varied among cultivars. Leaf Zn concentration increase was relatively higher at Talagang than NARC for all the cultivars. Extent of increase was 65% in Green feast, 74% in Climax and 76% in Meteor with applied Zn at Talagang. Similar trend was observed at NARC but the Zn concentration was relatively low at NARC, *i.e.*, 44% in Green feast, 58% in Climax and 63% in Meteor. The largest range of Zn concentration among Zn rates occurred in Meteor and the smallest in Green Feast, that might be attributed partially to a dilution effect as the result of greater seed production of Green feast (Table II). The data of 2 years at both locations were pooled in this study for determining internal Zn requirement of leaves (plotting leaf Zn concentration *versus* relative seed yield of peas) and estimated internal Zn requirement was 42 mg kg^{-1} for Green feast, 46 mg kg^{-1} for Climax and 53 mg kg^{-1} for Meteor (Fig. 2).

Total Zn uptake

Zinc application increased total Zn uptake (seeds + stalks) at both locations (Table IV). Total Zn uptake differed significantly among the Zn rates and cultivars, while interaction effect (Zn rate × cultivar) was non-significant. Increase in total Zn uptake over control was high at Talagang, *i.e.*, 94% in Green feast, 119% in Climax, and 147% in Meteor. While this increase was less at NARC probably due to greater yield potential; *i.e.*, 75% in Green feast, 84% in Climax, and 111% in Meteor. Obviously higher uptake by Meteor at both sites was due to its larger internal Zn requirement of leaves compared with the other cultivars. The total Zn uptake was positively related with seed yields and the correlation coefficient (r) values were 0.95, 0.96, and 0.96 for Green feast, Climax, and Meteor, respectively. Even strong correlation ($r = 0.998$) between total uptake and leaf Zn concentration was observed.

Seed Zn composition

Zinc concentration in seeds differed widely among cultivars and influenced by Zn application to the soils in which the plants grew (Table IV). The Zn concentrations in seeds, attributable to Zn fertilization, were

TABLE IV

Zinc concentration in tissues and total Zn uptake by three pea cultivars (Green feast, Climax and Meteor) (mean of 2 years) as affected by Zn fertilization at two field experimental sites in the Potohar Plateau of Pakistan

Zn applied	Talagang				NARC[a]			
	Green feast	Climax	Meteor	Mean	Green feast	Climax	Meteor	Mean
kg ha^{-1}								
	Zn concentration in leaves (mg kg^{-1})							
0	32	35	37	35E[b]	34	36	36	35E
2	37	44	45	42D	39	42	43	41D
4	44	50	54	49C	44	47	50	47C
8	49	56	59	55B	47	51	54	51B
16	53	61	65	60A	49	57	59	55A
Mean	43c[c]	49b	52a		42a	47b	49a	
	Zn concentration in seeds (mg kg^{-1})							
0	34	39	42	38E	35	41	44	40E
2	42	49	52	48D	42	46	51	46E
4	48	57	59	55C	45	51	58	51E
8	52	62	65	60B	48	58	65	57B
16	57	66	72	65A	52	61	70	61A
Mean	47c	54b	58a		44c	51b	57a	
	Total Zn uptake (mg kg^{-1})							
0	113	108	102	108E	130	126	124	127E
2	153	158	151	154D	166	157	165	163D
4	179	190	187	186C	191	188	194	191C
8	201	218	220	213B	211	213	230	218B
16	219	237	252	236A	228	233	262	241A
Mean	173b	182a	183a		185b	183b	195a	

[a] National Agricultural Research Centre.
[b] For each Zn application rate, means in a column followed by the same uppercase letter are not significantly different at $P \leq 0.05$. Least significant difference (LSD, $P < 0.05$) was 1.28 and 1.00 for Zn concentration in leaves, 1.34 and 1.35 for Zn concentration in seeds, and 5.78 and 3.96 for total Zn uptake for Talagang and NARC, respectively.
[c] For each pea cultivar, means in a row followed by the same lowercase letter are not significantly different at $P \leq 0.05$. LSD ($P < 0.05$) was 1.26 and 0.58 for Zn concentration in leaves, 0.67 and 1.11 for Zn concentration in seeds, and 2.85 and 4.91 for total Zn uptake for Talagang and NARC, respectively.

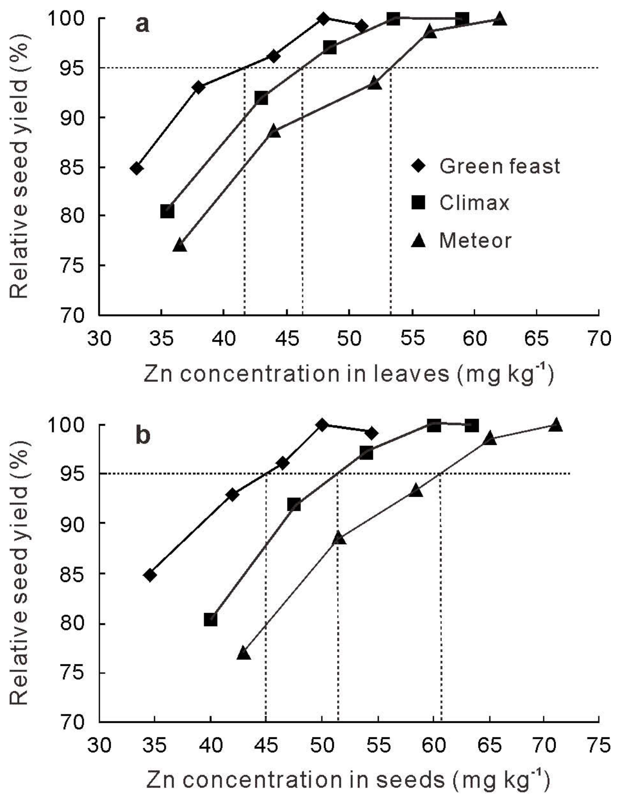

Fig. 2 Relationships between the Zn concentrations of leaves (a) and seeds (b) and the relative seed yields of three pea cultivars (Green feast, Climax and Meteor).

higher in Meteor compared with the other two cultivars, *i.e.*, Green feast and Climax. Seed Zn concentrations ranged from 34 to 57 mg kg^{-1} in Green feast, from 39 to 66 mg kg^{-1} in Climax, and from 42 to 72 mg kg^{-1} in Meteor at Talagang. The extent of variation in Zn concentration was relatively lower at NARC as it ranged from 35 to 52 mg kg^{-1} in Green feast, from 41 to 61 mg kg^{-1} in Climax, and from 44 to 70 mg kg^{-1} in Meteor. Seeds of pea may be taken as a diagnostic tissue like other crops because of a range of Zn concentration and better analytical precision (Rashid and Fox, 1992). Internal Zn requirement of seed was estimated by plotting seed Zn concentration *versus* relative seed yield (Fig. 2). Data of 2 years at both locations were pooled in the present study. Estimated internal Zn requirement associated with the 95% of maximum seed yield was 45 mg kg^{-1} for Green feast, 51 mg kg^{-1} for Climax, and 60 mg kg^{-1} for Meteor, respectively.

The use of seed as diagnostic tissue has several advantages like easy collection, cleaning, and processing. Moreover, date of seed sampling is not critical, analyzing mature seeds may minimize differences in Zn concentration due to stage of development, and seed use may increase analytical precision because seeds contain little silica and are well suited to dry ashing. A probable disadvantage for seed use is that while leaf analysis might be useful for current crop, seed analysis can only be used to diagnose former problems and apply for future strategy.

Critical level of Zn in diagnostic plant parts especially in seed is not well documented in literature. However, Huett *et al.* (1997) reported 61 mg Zn kg^{-1} as the adequate concentration in pea seed and 53 mg Zn kg^{-1} in leaflets at early flowering stage. A relatively wide range, *i.e.*, 25–100 mg Zn kg^{-1}, has been reported by Jones *et al.* (1991) as the sufficient concentration in recent fully developed leaflets at first bloom. The result of this study indicates that critical concentration varies with cultivars and was not the same in all cultivars even grown under the same conditions. Variation in critical levels of nutrients can occur most probably due to differences in crop genotypes and plant age (Jones *et al.*, 1991). It is obvious that no single critical Zn concentration can be used to predict Zn deficiency. Further, plant's internal requirement of a nutrient may also vary because of plant growth interaction with the supply of other nutrients and with environmental factors such as temperature, CO_2 concentration, diseases and pests, and errors involved in derivations (Munson and Nelson, 1990; Smith and Loneragan, 1997). Critical values can also vary with environmental factors affecting Zn uptake, such as water, soil texture, and soil pH (Sims and Johnson, 1991). It is not only the external factors but also the plant species differing in their abilities to absorb Zn from the same soil that affect the plant in accumulating Zn (Cakmak *et al.*, 1997).

CONCLUSIONS

Despite the general perception regarding pea's less sensitivity to Zn deficiency, Zn use enhanced pea's productivity in alkaline calcareous soils. Zinc centrations of pea leaves as well as mature seeds appeared to be a good indicator of soil Zn availability status. Extensive research work has established the utility of Zn application in agronomic crops. However, application of micronutrients such as Zn, B, and Fe is not commonly being practiced for vegetable crops. The results of this study clearly demonstrated the beneficial effect of Zn application on vegetable yields and plant tissue Zn concentrations.

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