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Zinc deficiency as a practical problem in plant and human nutrition in Turkey: A NATO-science for stability project

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Abstract

Zinc (Zn) deficiency is a critical nutritional problem for plants and humans in Turkey. About 14 Mha of cropped land in Turkey are known to be Zn deficient, particularly cereal growing areas of Anatolia. In 1993, a joint research project was started in Turkey with the financial support of the NATO-Science for Stability Programme to select and characterize cereal genotypes with high yield and/or high Zn accumulation in grain under deficient supply of Zn.

Field, greenhouse and growth chamber experiments were carried to study morphological, physiological and genetic factors determining the bases of genotypical differences in Zn efficiency among cereal species and within cultivars of wheat. Among the cereals, rye had particularly high Zn efficiency (high yield under Zn deficiency). There were large genotypical differences among wheat lines. High Zn efficiency was closely associated with enhanced capacity of some lines to take up Zn from soils, but not with increased Zn accumulation per unit dry weight of shoot or grain. Measurement of Zn-containing superoxide dismutase activity in leaves revealed that an efficient utilization of Zn at the tissue or cellular level is an additional major factor involved in Zn efficiency of cereals.

Zinc present in grains from Anatolia seems to be not bioavailable. Phytate : Zn molar ratios in grains, a widely accepted predictor of Zn bioavailability, were extremely high and ranged between 95 and 216 for crops grown severely on Zn-deficient soils of Central Anatolia. In the studies concerning determination of Zn nutritional status of school children in Southeastern Anatolia, most children were found to be of shorter stature and had very low levels of Zn (<100 mg kg^{-1}) in hair.

Keywords: Genotypes; Human nutrition; Wheat; Zinc deficiency; Zinc efficiency

1. Introduction

Zinc (Zn) deficiency in soils has been reported worldwide, particularly in calcareous soils of arid and semiarid regions. In a global soil survey study, Sillanpää (1990) found that ca. 50% of the soil sam-

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ples collected in 25 countries were Zn deficient. Zinc deficiency is a particularly widespread micronutrient deficiency in wheat, leading to severe depressions in wheat production and nutritional quality of grains (Graham et al., 1992; Cakmak et al., 1996d; Graham and Welch, 1996). As in soils and plants, Zn deficiency is also a common nutritional problem in humans, predominantly in developing countries where diets are rich in cereal-based foods and poor in animal protein (Prasad, 1984; Welch, 1993). Foods derived from cereals are not only low in Zn, but also rich in compounds depressing bioavailability (utilization) of Zn to humans, such as phytic acid and fibre.

In Turkey, Zn deficiency is the most widespread micronutrient deficiency in soils and plants. Based on analysis of 1511 soil samples collected from all provinces of Turkey, Eyüpoglu et al. (1994) showed that 50% of the cultivated soils in Turkey are Zn deficient. These Zn-deficient areas are equivalent to 14 Mha of cultivated land in Turkey. Deficiency of Zn in soils on such a large scale and thus in plant foods, has been suggested to be one of the major causes of the widespread occurrence of Zn deficiency in humans in Turkey (Cavdar et al., 1983; Prasad, 1984; Cakmak et al., 1996d).

In 1992, a joint research project with the aim of improving the Zn nutritional status of plants and humans in Turkey by agricultural strategies was submitted to the NATO-Science for Stability Programme, and accepted in March 1993. This paper presents overview information on this NATO-funded project: historical aspects of Zn deficiency; strategies, current results; potential impacts; and future plans.

2. History and initiation of the NATO-funded project

The importance of Zn deficiency for crop production in Turkey was recognised only during the past 10 years although the importance of Zn deficiency in human nutrition was recognised much earlier. Prasad et al., 1961, 1963 were the first to show severe growth retardation in male subjects in the Middle East as a consequence of Zn deficiency. Similar observations were made on workers in Turkey much earlier by Tayanc, 1942; cited in Cavdar et al., 1983 without knowing the underlying cause. The importance and widespread occurrence of Zn deficiency in humans in Turkey were shown through extensive field survey and laboratory studies by Cavdar and her co-workers (Cavdar and Arcasoy, 1972; Cavdar et al., 1980, 1983).

The effects of Zn deficiency on cereal production in Turkey have received attention only since the early 1990s. In 1982 after a soil survey study in different countries, however, Sillanpää (1982) stated that concentrations of Zn in Turkish soils were among the very lowest recorded. Until the 1990/1991 cropping season, there was no scientific evidence that symptoms occurring in wheat, such as necrotic patches on leaves, were definitely caused by Zn deficiency. To identify the underlying cause for the leaf symptoms and low levels of wheat production in some locations in Central Anatolia, two field experiments were conducted in the research stations in Central Anatolia (Eskişeher-Transitional Zone Agricultural Research Station and Konya-International Winter Cereals Research Center) with the participation of CIMMYT representative in Ankara. One experiment was carried out in Eskişeher by M. Kalayci in the 1991–1992 cropping season to test the effect of soil applied Zn and other micronutrients (Fe, Mn, Cu and B) on growth and grain yield of three wheat and one barley cultivars.

The results given in Table 1 revealed that grain yield was significantly depressed only in the absence of Zn fertilization. In the other experiment, a set of bread and durum wheat cultivars differing in sensitivity to micronutrient deficiencies and toxicities were sown in the 1991–1992 cropping season at five locations in Konya and Eskişeher provinces of Central Anatolia (Adelaide University-CIMMYT; R.D. Graham, K.D. Sayre and H.J. Braun, pers. comm., 1992). Among the cultivars in the set, Halberd, a Zn-efficient bread wheat from Australia, showed only slight symptoms of Zn deficiency, while Durati, a Zn-inefficient durum wheat from Australia displayed severe Zn deficiency symptoms. Based on these results, it has been suggested that Zn deficiency is of critical importance for the wheat production in Central Anatolia.

These observations and findings on Zn deficiency in cereals raised questions about the extent and importance of Zn deficiency in soils, plants, foods and humans in Turkey in general and in Central Anatolia in particular. Consequently, a joint NATO project was prepared in 1992 by Cukurova University-Adana, the

Table 1
Effect of soil applications of different micronutrients on grain yield t ha^{-1} of the bread wheat cultivars Bezostaja-1 and Greek-79, durum wheat cultivar Kunduru-1149 and barley cultivar Tokak. Micronutrients were applied at the rate of 5 kg ha^{-1}. (M. Kalayci, unpublished results)

Application	Bezostaja-1	Gerek-79	Kunduru-1149	Tokak	Mean
Control (all micronutrients)	4.64	4.76	2.65	4.73	4.19
–Fe	4.36	4.37	2.38	4.53	3.91
–Mn	4.54	4.71	1.93	5.38	4.14
–Cu	3.93	4.30	2.17	4.91	3.62
–B	4.20	4.37	2.63	4.81	4.00
–Zn	2.96	3.00	1.58	3.07	2.65
$LSD_{0.05}$ (fertilizer×genotype)					0.45

Transitional Zone Agricultural Research Institute-Eskişehir, B.D. International Winter Cereals Research Center-Konya and CIMMYT-Ankara. The project, approved in 1993, has following objectives:

(a) to demonstrate the importance of Zn fertilization for increasing grain yield to farmers and research institutions in Turkey;
(b) to select cereal lines with high efficiency in uptake and/or utilization of Zn when grown on Zn deficient soils;
(c) to characterize physiological, genetic, and morphological mechanisms involved in Zn efficiency; and
(d) to increase Zn concentration in grains by Zn fertilization and/or by selecting lines with high genetic capacity to allocate more Zn into grains and, thus, contribute to higher bioavailability of Zn in diets.

3. Current results

3.1. Zinc deficiency in soils

Zinc deficiency is now known to be particularly widespread in soils of Central Anatolia, where nearly half (4.5 Mha) of the wheat production area of Turkey is located. This region is the driest part of Turkey, and the soils in Central Anatolia are rich in $CaCO_3$ and have high pH values ranging between 7.5 and 8.1. The combination of high pH, $CaCO_3$ and low organic matter (<2%), together with low annual precipitation (ranging from 280 to 400 mm), have been considered the major factors responsible for Zn deficiency in Central Anatolia (Cakmak et al., 1996d). These factors are well known to be involved in immobilization of Zn in soils (Marschner, 1993). Accordingly, in adsorption and desorption studies with soils of Central Anatolia it has been found that 99% of the Zn added to soil at the rate of 520 mg kg^{-1} was fixed and only 6 mg Zn kg^{-1} soil of the adsorbed Zn could be desorbed by 0.5 M $CaCl_2$ (B. Erenoglu, unpublished results). These results indicate that Zn added or present in soils of Central Anatolia is adsorbed and held very strongly by soil constituents.

The concentration of plant available Zn (DTPA-extractable Zn) is extremely low, although the total concentration of Zn in soils is fairly high, ranging from 40 to 80 mg kg^{-1} soil. A soil survey study in Central Anatolia revealed that concentration of DTPA-extractable Zn in 76 soil samples ranged from 0.11 to 0.64 with a mean value of 0.29 mg kg^{-1} soil (Cakmak et al., 1996d). In >90% of the soil samples collected, concentration of plant available Zn was lower than widely accepted critical level (<0.5 mg kg^{-1}). Also, in wheat plants grown in Central Anatolia Zn concentrations in leaves were very low, and based on 136 samples ranged between 4 and 25 mg kg^{-1} dry weight with a mean value of 8.5 mg kg^{-1} dry weight (Cakmak et al., 1996d).

3.2. Response of wheat to zinc fertilization

Wheat plants grown in Central Anatolia were highly responsive to Zn application. In field experiments at six locations in Central Anatolia, improvement of plant growth by Zn application was closely related to the level of DTPA-extractable Zn in soils (Table 2).

Table 2
Effect of soil Zn application (+Zn: 23 kg Zn ha^{-1}) on grain yield of wheat (cv. Gerek-79) grown in various locations in Central Anatolia with different levels of DTPA-extractable Zn in soils (from Cakmak et al., 1996d)

Location	DTPA-extractable Zn (mg Zn kg^{-1} soil)	Grain yield (t ha^{-1})		Increase in yield by Zn application (%)
		−Zn	+Zn	
Konya (Centrum)	0.13	2.8±0.7 [a]	5.9±0.5 [a]	109
Konya (Comakli)	0.11	0.2±0.1	1.4±0.5	554
Eskisehir	0.15	2.5±0.9	3.3±0.2	31
Sarayönü (Cesmelisebil)	0.25	1.1±0.5	2.3±0.5	16
Sarayönü (Gözlü)	0.38	1.1±0.1	1.5±0.3	27
Cumra	0.64	5.4±0.1	5.6±1.1	5
Mean	0.28	2.3	3.3	43

[a] Mean±SD.

Highest increases in grain yield (>100%) were found at locations where DTPA-extractable Zn concentrations were ca. 0.12 mg kg^{-1} soil (Table 2). There was still a large increase in grain yield at the DTPA-extractable Zn level of 0.38 mg kg^{-1} soil, but not at 0.64 mg kg^{-1} soil. This indicates that wheat grown in calcareous soils containing <0.4 mg kg^{-1} DTPA-extractable Zn, as Central Anatolia, will significantly respond to Zn fertilization. Critical DTPA-Zn levels were reported 0.6 mg kg^{-1} for wheat (Singh et al., 1987) and 0.4 mg kg^{-1} for corn and sorghum (Martens and Westermann, 1991).

Further field experiments were carried out with 14 bread and two durum wheat cultivars at Eskişeher and Konya in 1993–1994 and 1994–1995. Application of Zn at the rate of 23 kg Zn ha^{-1} enhanced grain yield at all locations. For example, on average of 16 cultivars, in 1994–1995 cropping season, Zn application enhanced grain yield from 2610 to 3930 kg ha^{-1} in Eskişeher location (51% increase) and from 1860 to 2930 kg ha^{-1} in Konya location (58% increase). Highest increases in grain yield after Zn application (>100%) were found for durum wheat cultivars Kunduru-1149 and Kýzýltan-91 and in bread wheat cultivars BDME-10, Kýrkpýnar-79, Partizanka Niska and Partizanka. Grain yield of bread wheat cultivars Kýrac-66, Kýrgýz-95 and Gerek-79 increased after Zn application by ca. 30% The spectacular increases in grain yield with Zn fertilization evoked a growing interest in project results by farmers and fertilizer companies. Presently, ca. 140 000 t of a new Zn-containing NPK fertilizer are produced per year and used by farmers.

3.3. Effectiveness of different zinc application methods

Selection and/or breeding of Zn-efficient cereal cultivars (those having high yield and/or Zn accumulation in grains in low Zn soils) is the most logical approach to solve Zn deficiency problems in plants and humans. At present, the level of Zn efficiency in wheat is not sufficient, being less than rye and triticale, which are the most Zn-efficient species. Although the genetic variation among wheat genotypes is quite large (Graham and Rengel, 1993; Rengel and Graham, 1995a; Cakmak et al., 1996c, d), even the most Zn-efficient genotypes/cultivars show a decrease in dry matter and or grain yield when grown under Zn-deficient conditions (Graham et al., 1992; Cakmak et al., 1997a). Especially for durum wheats, Zn fertilization is crucial to obtain a reasonable yield under Zn-deficient conditions, because of their low genetic tolerance to Zn deficiency.

Therefore, as a short term solution to Zn deficiency, Zn fertilization seems necessary to overcome yield reductions. In field experiments carried out at a Konya location (DTPA-Zn: 0.10 mg kg^{-1} soil) different Zn application methods were tested for their effectiveness to increase grain yield and grain Zn concentration (Yilmaz et al., 1997). Zinc application methods were: (1) control (no Zn application); (2) soil application (23 kg Zn ha^{-1} as $ZnSO_4{\cdot}7H_20$); (3) seed application (1.0 l of 30% $ZnSO_4{\cdot}7H_20$ for 10 kg of seed), (4) foliar application (2×220 g Zn ha^{-1} as $ZnSO_4{\cdot}7H_20$ in 450 l during tillering and stem elongation stages); (5) soil plus leaf applications (combination of methods 2 and

Table 3
Effects of different Zn application methods on grain yield of bread wheat cultivars Gerek-79 and Bezostaja-1 grown on a Zn-deficient calcareous soil in Central Anatolia (from Yilmaz et al., 1997)

Zinc application methods	Gerek-79 (t ha^{-1})	Bezostaja-1	Mean	Increase by Zn application (%)
Control	0.74	0.81	0.77	—
Soil	2.70	2.34	2.52	226
Seed	2.05	1.96	2.00	160
Leaf	1.47	1.55	1.51	96
Soil+Leaf	2.71	2.33	2.52	227
Seed+Leaf	2.77	2.38	2.57	233
LSD (5%)	0.45	0.74	—	—

4); and (6) seed plus leaf applications (combination of methods 3 and 4). Irrespective of the method, application of Zn significantly increased grain yield (Table 3). Best yield increases resulted from soil, soil plus leaf and seed plus leaf applications, while seed only and, particularly, leaf only applications were less effective in overcoming yield reductions due to Zn deficiency. Seed application of Zn greatly increased grain yield, but remained less effective in increasing Zn concentrations in shoots or grains (Table 4). This can be attributed to dilution of Zn in plant tissues because of enhanced shoot dry matter production, confirming the results of Rengel and Graham (1995b). The results from seed application of Zn also indicate the importance of Zn in seed during germination and early seedling growth, and indicate that sowing seeds with higher Zn content can be considered as a practical solution to alleviate yield depression due to Zn deficiency in soils.

When residual effect of Zn in soil for several years is considered (Martens and Westermann, 1991), soil application of Zn is the most advisable Zn application method. As those authors reported that soil application of 28 kg Zn ha^{-1} as $ZnSO_4$ was sufficient to correct Zn deficiency in plants for 4 to 7 years. Soil application of Zn enhanced Zn concentrations in shoots and grains by two-fold (Table 4). The methods involving leaf applications of Zn were most effective to increase Zn concentration in shoot and grain, which is important for both seeding and diet (see Section 4 below). Soil plus leaf application of Zn can be considered as the most effective method if a high grain yield and high Zn content in grains are desired.

3.4. Genotypic differences

Various morphological and physiological plant factors have been studied to understand the bases of

Table 4
Effects of different Zn application methods on Zn concentrations (mg Zn kg^{-1} dry weight) in whole shoots sampled at the beginning of stem elongation stage and in mature grains of the bread wheat cultivars Gerek-79, and Bezostaja-1 grown on a Zn-deficient calcareous soil in Central Anatolia (from Yilmaz et al., 1997)

Zinc application methods	Whole shoots		Mean	Grain		Mean
	Gerek-79	Bezostaja-1		Gerek-79	Bezostaja-1	
Control	11	9	10	9	10	10
Soil	21	17	19	17	17	17
Seed	14	10	12	11	8	11
Leaf	68	77	73	30	28	31
Soil+Leaf	82	83	83	34	38	34
Seed+Leaf	93	73	83	34	25	33
LSD (5%)	15	15	—	6	9	—

genotypic differences under Zn-deficient conditions. So far, the factors studied were (a) severity of leaf symptoms of Zn deficiency, (b) root and shoot dry matter production, (c) grain yield, (d) release of Zn mobilizing phytosiderophores from roots, (e) concentration and accumulation of Zn in plants, (f) root uptake and root-to-shoot translocation of Zn, and (g) activity of Zn-containing superoxide dismutase (SOD).

The responses to Zn deficiency and Zn fertilization were markedly different among and within the cereal species. Triticale and particularly rye showed an exceptionally high Zn efficiency (ability to grow and yield well under Zn-deficient conditions), while oat and durum wheats were highly sensitive. Based on the severity of Zn-deficiency symptoms and depression in biomass production and grain yield, sensitivity of cereals to Zn deficiency decreased in the following order: durum wheat>oat≥bread wheat>barley>triticale>rye (Cakmak et al., 1997a, 1998).

3.4.1. Root growth

Enhanced root growth under Zn deficiency is often considered an important trait involved in expression of Zn efficiency. In terms of root dry matter production, however, wheat genotypes differing in Zn efficiency were not distinctly different under Zn deficiency. Even in nutrient solution experiments, greater sensitivity of durum wheats to Zn deficiency was associated with greater root growth (Rengel and Graham, 1995a; Cakmak et al., 1996c). However, Zn efficient and inefficient genotypes may differ in root morphology affecting root absorbing surface area (Dong et al., 1995).

3.4.2. Phytosiderophore release

Genotypical differences in Zn efficiency might also be attributed to differences in release of phytosiderophores from roots. Release of phytosiderophores is a well-known phenomenon occurring not only under Fe deficiency, but also under Zn deficiency (Zhang et al., 1989; Cakmak et al., 1994) and is involved in mobilization of Zn both in the rhizosphere (Treeby et al., 1989) and from the root cell walls (Zhang et al., 1991). Under Fe deficiency, the release rate of phytosiderophores was closely associated with the differences in sensitivity to Fe deficiency among and within cereal species (Takagi et al., 1984; Marschner et al., 1986; Jolley and Brown, 1989). Similarly, it was shown in our studies that differences in Zn efficiency between durum and bread wheat lines were related to the differences in release rate of phytosiderophores, predominantly deoxymugineic acid (Cakmak et al., 1994, 1996b). Under Zn deficiency, roots of Zn-efficient bread wheats released and contained larger amounts of the deoxymugineic acid compared to durum wheats (Table 5). Also adaptation of several wild grasses to severely Zn deficient calcareous soils in Central Anatolia was found to be related with enhanced release of phytosiderophores from roots (Cakmak et al., 1996a).

However, for bread wheat cultivars differing widely in sensitivity to Zn deficiency, the release rate of phytosiderophores from roots did not correspond well with the sensitivity of the cultivars to Zn deficiency. Zinc efficient and inefficient bread wheat cultivars were not different in their capacity to release phytosiderophores from roots (Erenoglu et al., 1996). Even the most Zn-efficient rye cultivar Aslim and triticale

Table 5
Release rate of phytosiderophores from roots and concentration of the phytosiderophore deoxymugineic acid in the roots of 14-day-old Zn-deficient bread and durum wheat cultivars (from Cakmak et al., 1996b)

Species/cultivars	Phytosiderophore release (μmol g^{-1} root dry wt 3 h^{-1})	Concentration of deoxymugineic acid (μmol g^{-1} fresh wt root)
Bread wheats		
Kirac-66	8.7±2.8 [a]	2.76
Gerek-79	6.8±2.7	1.90
Durum wheats		
Kunduru-1149	1.2±0.2	0.68
Kiziltan-91	0.8±0.3	0.65

[a] Mean±SD.

cultivar Presto were not superior to the Zn-inefficient bread wheats in release rate of phytosiderophores (Cakmak et al., 1998). Similarly, under Fe deficiency, differential susceptibility of various lines to Fe deficiency was not always related to their capacity to release phytosiderophores as shown in oat (Hansen and Jolley, 1995) and maize (von Wiren et al., 1994). However, these results do not mean that the release of phytosiderophores is of little importance for Zn efficiency. Recently, von Wiren et al. (1996) reported that release rate of phytosiderophores from roots did not differ among maize, but the lines greatly differed in their capacity to take up Zn-chelated phytosiderophore and translocate it from roots into shoots. Phytosiderophores are also effective in mobilizing Zn from cell walls (Zhang et al., 1991) where a substantial proportion of total Zn in plants is located (Schmid et al., 1965). Possibly, Zn can also be translocated within plants as phytosiderophore complexes to sites having high demand for Zn (Welch, 1995).

3.4.3. Root uptake and shoot translocation of Zn

The ability of genotypes to take up greater amounts of Zn under Zn deficiency is a major plant trait responsible for expression of Zn efficiency. Less sensitivity of bread wheats to Zn deficiency than durum wheats was found to be associated with greater Zn-uptake capacity of bread wheats (Cakmak et al., 1996c; Rengel and Graham, 1996). In a nutrient solution experiment with six bread wheat and four durum wheat cultivars, Zn-deficient bread wheats had, on average, 29% more Zn accumulation per plant (shoot plus root) than Zn-deficient durum wheats (Cakmak et al., 1996b). Interestingly, higher Zn accumulation in bread wheats was pronounced in shoots (42% more Zn per shoot), while roots of durum and bread wheats did not differ in Zn accumulation under Zn deficiency (Cakmak et al., 1996c). These results indicate that higher Zn efficiency of bread wheats compared to durum wheats is related to greater Zn uptake and, particularly, to greater root-to-shoot translocation capacity for Zn.

Additionally, the high Zn efficiency in rye was associated with enhanced Zn-uptake capacity. In short-term experiments with labelled Zn (^{65}Zn), rye had highest rate of uptake and root-to-shoot translocation of ^{65}Zn compared to bread and durum wheats (Table 6). The lowest rates of root uptake and shoot translocation of ^{65}Zn under Zn deficiency were found in Kunduru-1149, the most Zn-inefficient durum wheat cultivar. Also, differences in sensitivity to Zn deficiency between bread wheat cultivars Dagdas-94 and BDME-10 tended to be related to the differences in root uptake and shoot translocation of ^{65}Zn (Table 6). It can be concluded that enhanced Zn uptake and translocation into shoots (apical meristems) is an essential step for the expression of Zn efficiency and, thus, for maintenance of higher growth rates under deficient supply of Zn, a phenomenon, where greatest expression is in rye.

3.4.4. Zinc concentrations and accumulation

In the last three years, in greenhouse and field experiments, >550 wheat cultivars and lines, including

Table 6
Uptake and root-to-shoot translocation rates of ^{65}Zn in rye and bread and durum wheat cultivars grown in nutrient solution without Zn for 12 days. ^{65}Zn was supplied as ZnHEDTA for 10 h in chelate-buffered nutrient solution (from Cakmak et al., 1998)

Species/cultivars	Sensitivity to Zn deficiency in field	Uptake rate	Root-to-shoot translocation rate
		(μmol ^{65}Zn g^{-1} root dry wt 10 h^{-1})	
S. cereale			
Aslim	very slight	1.76±0.09 [a]	1.22±0.09 [a]
T. aestivum			
Dagdas-94	mild	0.51±0.08	0.26±0.05
BDME-10	severe	0.45±0.04	0.21±0.02
T. durum			
Kunduru-1149	very severe	0.33±0.05	0.10±0.01

[a] Mean±SD.

several wheat addition, translocation and substitution lines were screened for Zn efficiency. Genotypical differences in sensitivity to Zn deficiency were not related to the Zn concentrations in shoots. Under deficient supply of Zn in soils, all lines contained similarly low amounts of Zn per unit shoot or leaf dry matter. By contrast, the total amount of Zn per shoot (Zn accumulation) greatly differed among the lines and correlated better with the susceptibility of plants to Zn deficiency.

In a greenhouse experiment, 164 entries of the Facultative and Winter Wheat Observation Nursery (FAWWON), a collection of advanced bread wheat lines selected by scientists of Turkey, CIMMYT and ICARDA were tested for their Zn efficiency [(dry matter yield at −Zn/dry matter yield at+Zn)×100], Zn concentration and Zn content. Among the 164 entries, the most Zn-efficient line (efficiency value: 77%) had a Zn concentration of 6.1 mg kg^{-1} shoot dry matter, while the most Zn-inefficient line (efficiency value: 33%) contained 7.7 mg per kg shoot dry matter. Shoot concentrations of Zn in 164 entries ranged from 5.1 to 9.4 mg kg^{-1} dry weight with a mean of 6.9 mg kg^{-1} dry weight. However, the accumulation of Zn per shoot was higher in the most Zn-efficient line (3.1 μg Zn plant^{-1}) than the most inefficient line (1.6 μg Zn plant^{-1}). The relationship between Zn efficiency and Zn concentration in shoots or grains was very poor. A representative example is given in Table 7 where rye cultivar Aslim, the most Zn-efficient cereal cultivar among all the cereal cultivars studied so far by our group, was compared with different bread and durum wheat cultivars for their Zn concentration and Zn content under Zn-deficiency conditions in field. Despite great differences in Zn efficiency, the cereal cultivars were not different in their Zn concentrations. Even in grains, Zn concentrations of efficient cultivars were lower than those of inefficient cultivars. However, Zn-efficient cultivars accumulated markedly more Zn per shoot than did inefficient cultivars (Table 7). The results obtained under field and greenhouse conditions indicate that expression of high Zn efficiency is associated with enhanced Zn uptake capacity of roots, but not necessarily with high accumulation of Zn per unit weight of shoots, leaves or grains. Under Zn deficiency, enhanced uptake of Zn by efficient genotypes is used for additional increases in dry matter production, which results in a corresponding dilution of Zn in tissues to similar concentrations as in inefficient genotypes rather than in an increase in Zn per unit weight of plant. (Marschner, 1995).

3.4.5. *Internal zinc utilization*

Genotypical differences in Zn efficiency may also be related to differences in utilization of Zn at the tissue or cellular level. In most instances, as noted in Tables 7 and 8, under Zn deficiency, Zn-efficient and

Table 7
Zinc-deficiency symptoms (necrotic patches on leaves) and concentration and content of Zn in shoot at the heading state and Zn concentration in grain at maturity in different rye and bread wheat and durum wheat cultivars grown in field on a Zn-deficient calcareous soil (from Cakmak et al., 1996a)

Species/cultivars	Zn deficiency symptoms	Heading stage		Maturity
		Zn concentration (mg kg^{-1} shoot DW)	Zn content (μg shoot^{-1})	Zn concentration in grain (mg kg^{-1} DW)
S. cereale				
Aslim	none	6.8±0.6 [a]	12.1±1.3 [a]	7.0±0.2 [a]
T. aestivum				
Bezostaja	slight/mild	6.1±0.7	6.5±1.2	9.3±1.3
Atay	mild	6.4±0.4	6.3±1.0	11.7±1.7
Turgidum cv. group *durum*				
Kunduru-1149	very severe	7.2±0.8	3.0±0.8	10.5±0.7
C-1252	very severe	7.0±0.6	1.8±0.6	12.0±0.0

[a] Mean±SD.

Table 8
Leaf symptoms of Zn deficiency (necrotic patches on leaf blades), total Zn concentration and activity of Cu- and Zn-containing superoxide dismutase (Cu/Zn-SOD) in leaves of rye and different wheat cultivars grown for 21 days without Zn supply on a Zn deficient soil (Cakmak et al., 1997b)

Species/cultivars	Leaf symptoms	Zn concentration in leaves (mg kg^{-1} dry wt.)	Cu/Zn-SOD in leaves (Units g^{-1} dry wt.)
Rye			
Aslim	absent	7.1±0.9 [a]	781±51 [a]
Bread wheats			
Bezostaja-1	mild	6.4±0.5	695±150
BDME-10	severe	6.2±1.1	515±40
Durum wheats			
Kiziltan-91	very severe	7.3±0.4	527±47
Kunduru-1149	very severe	6.1±0.9	430±29

[a] Mean±SD.

Zn-inefficient lines were not different in amounts of Zn per unit dry weight of leaves and shoots (Graham et al., 1992; Cakmak et al., 1996c). Apparently, Zn-efficient lines may contain a larger proportion of Zn that is mobile within the plants and physiologically active in metabolic reactions.

Thus, differences in severity of Zn deficiency symptoms despite same or similar Zn concentrations in leaves can be explained with corresponding differences in amounts of the physiologically active Zn pool. A typical example is given in Table 8. Under Zn deficiency, the Zn-efficient rye cultivar and Zn-inefficient durum wheat cultivar were not different in concentration of total Zn in leaves despite large differences in severity of Zn-deficiency symptoms. However, activities of Zn-containing superoxide dismutase (Cu/Zn-SOD) were closely related to the severity of Zn deficiency symptoms on leaves (Table 8). In addition, the bread wheat cultivar Bezostaja-1 with less sensitivity to Zn deficiency had greater activity of Cu/Zn-SOD than other bread and durum wheats with more sensitivity to Zn deficiency. These results indicate that efficient utilization of Zn at the tissue or cellular level seems to be an additional critical factor involved in Zn efficiency of cereals. It has to be emphasized that high efficiency in internal utilization of Zn has to be associated with enhanced uptake capacity of roots for Zn in order to ensure higher growth rates at low concentrations of Zn in plant tissues.

3.4.6. Phytic acid and bioavailability of zinc

Bioavailability of Zn is very low in foods containing high amounts of phytic acid (or phytate) and fibre. These compounds have high binding and complexing affinity to Zn and, thus, hamper biological utilization of Zn in human and animal cells (Welch, 1993). Cereal grains and cereal-based foods are rich in phytic acid and fibre. Therefore, consumption of cereal-based foods in large amounts may easily result in Zn deficiency, particularly in children and nursing mothers (Harland et al., 1988).

A total of 54 wheat cultivars, grown in Central Anatolia and South-eastern Anatolia with and without Zn fertilization, were analyzed for phytic acid and Zn concentrations. The results are presented in Table 9. In Central Anatolia, where DTPA-Zn levels are ca. 0.10 mg kg^{-1} soil and Zn deficiency symptoms occur widely, concentration of phytic acid in grain ranged from 7.4 to 14.3 mg g^{-1} with an average of 11.0 mg g^{-1}, and Zn concentrations changed between 7 and 12 mg kg^{-1} with an average value of 8.4 mg kg^{-1}. Application of Zn to plants in Central Anatolia enhanced concentration of Zn in grain from 8 to 13 mg kg^{-1}, while phytic acid levels were reduced from 11 to 8 mg g^{-1}. In South-eastern Anatolia with higher DTPA-Zn (0.36 mg kg^{-1} soil), concentrations of Zn in grain were much higher and averaged 26 mg kg^{-1} for 26 wheats (Table 9). Application of Zn enhanced Zn concentration in grains, but remained without effect on phytic acid levels in South-eastern

Table 9
Effect of Zn fertilization on concentrations of Zn, phytic acid and phytate: Zn molar ratios in grains of 28 wheat cultivars (mostly winter type cultivars) in Central Anatolia and 26 wheat cultivars (mostly summer type cultivars) in South-eastern Anatolia regions differing in DTPA-extractable Zn concentrations in soils (I. Erdal et al., unpublished results)

Region (site) [a]	Zn fertilization (kg Zn ha^{-1})	Zn concentrations (mg kg^{-1})		Phytic acid concentrations (mg g^{-1})		Phytate:Zn molar ratios	
		Mean	Range	Mean	Range	Mean	Range
Central Anatolia	0	8	7–12	11	7.4–14.3	137	95–216
(Konya)	22	13	9–19	8	4.8–11.5	61	41–85
Southeastern Anatolia	0	26	21–33	12	10.0–14.0	49	41–54
(Sanliurfa)	22	31	27–36	12	9.6–13.5	39	35–44

[a] DTPA extractable Zn concentrations in the experimental sites were 0.09 in Konya and 0.36 in Sanliurfa as mg Zn kg^{-1} soil.

Anatolia. These results indicate that phytic acid concentrations in grain can be affected by Zn application, if plants are grown under severe Zn deficiency conditions as in Central Anatolia. Greater concentrations of phytic acid in Zn-deficient wheats (also per grain) from Central Anatolia can be attributed to Zn-deficiency-induced higher root uptake and root-to-shoot transport of P and, thus, P accumulation in grains (Loneragan et al., 1979; Cakmak and Marschner, 1986).

Phytate : Zn molar ratio has often been considered as a suitable predictor for estimation of Zn bioavailability in foods and for risk of occur–rence of Zn deficiency in humans. Usually, ratios above 20 have been proposed to be responsible for inducing Zn deficiency, particularly in children (Solomons, 1982; Harland et al., 1988). The phytate : Zn molar ratios were extremely high in wheats grown in Central Anatolia and ranged between 95 and 216 with an average value of 137 (Table 9). It can be argued that Zn present in grains from Central Anatolia has very low bioavailability. Because Zn application increased Zn levels and reduced phytic acid levels in grains, phytate : Zn molar ratios were markedly decreased by Zn fertilization. As a result of higher Zn concentrations in grains from South-eastern Anatolia, phytate : Zn molar ratios were lower in this region compared to Central Anatolia, but still much higher than proposed critical levels for human nutrition. Extensive consumption of foods made from such cereal grains can potentiate the occurrence of Zn deficiency in humans.

3.5. *Zinc nutritional status of school children*

Zinc deficiency is a widespread micronutrient deficiency in children and women in Turkey (Cavdar et al., 1983), due mainly to a higher proportion of cereal-based foods in Turkish diets. It has been reported that the daily bread consumption of most villagers is quite high, ranging between 500 and 800 g (Braun and Payne, 1995). Such local bread possibly has very little bioavailability of Zn to low levels of Zn and high content of phytic acid (Table 9). In the framework of the NATO project ca. 2000 school children were tested for their Zn nutritional status by measurement of Zn concentration in hair. The results for 581 children (296 male and 285 female) living in South-eastern Anatolia are given in Table 10. The results revealed that most children sampled were in shorter stature. Approximately 80% of children had a height-for-age percentiles below 25% (poor physical development). This result indicated existence of factors limiting linear growth of children. Zinc deficiency may be one of such limiting factors. About 41% of the children had very low concentration of Zn (<100 mg kg^{-1}) in hair (Table 10). The highest concentrations of Zn in hair were found in children having highest height-for-age percentiles (i.e., >90%) (Table 10). Possibly, low socio-economic status of families played an important role in low levels of Zn in children. About 71% of the children sampled were from families with very low income ($US 180 per month). Meat is known as a food that is both high in Zn concentration and in Zn bioavailability. The proportion of meat in the diets of children was very low. The proportion of children

Table 10
Concentration of Zn in hair of school children at different height-for-age percentiles, and cumulative distribution of Zn. Children (*n*=581; male=296, female=285) were sampled in Sanliurfa (South-eastern Anatolia) (Y. Kilinc et al., unpublished results)

Height percentiles (%)	Male (mg kg^{-1})		Female (mg kg^{-1})	
	n	Zn	*n*	Zn
<3	92	166±47 [a]	88	146±43 [a]
10–25	148	161±39	122	140±65
50–75	53	171±48	66	154±42
>90	3	258±76	9	226±35

Hair Zn (mg kg^{-1})	Cumulative distribution of Zn		
	n	Distribution (%)	Cumulative (%)
<50	25	4.4	4.4
50–99	214	36.8	41.2
100–199	255	43.8	85.0
>200	87	15.0	100.0

[a] Mean±SD.

consuming meat only once per week (or more seldom) was 75%. Alternatively, plant-based, particularly cereal-based foods with very low Zn content and bioavailability (see above; Table 9) are predominantly consumed by the children. It can be suggested that due to economical constraints, preference of cereal-based foods and little food diversity in the diet are the main factors responsible for the shorter stature of children and for low levels of Zn in hair.

4. Potential impacts

For various reasons mentioned above, there is an urgent need for improvement of Zn nutritional status of humans and plants in parts of Turkey. Most cereal farmers and many research institutions in Central Anatolia were not aware of the Zn deficiency problem in cereals until the early 1990s. The application of Zn fertilizers to cereals was either never or only rarely carried out in Central Anatolia. The area cropped with cereals (mostly wheat) in Central Anatolia is ca. 4.5 Mha, with average wheat yields near 2250 kg ha^{-1}. If we assume that only 25% of the cereal growing areas in Central Anatolia has Zn deficiency, and Zn application results in only 25% increase in yield, then, an increase of 630 000 t in wheat production by Zn application is possible, which is equivalent to a benefit of ca. $US 140 million. The total expenses for Zn application (ca. $US 40 ha^{-1}) can be estimated as $US 45 million for the corresponding area. Considering the residual effects of applied Zn for >2 to 3 years (see Section 3.3), total net income would be much higher than $US 100 million only for Central Anatolia.

Soil or foliar applications of Zn to correct Zn deficiency and to increase Zn content in edible parts of plants are, however, effective only for a short time, and must be carried out either every year or every 2 to 3 years. Therefore, the activities of the NATO project have been and will be focussed on selection and breeding of cultivars with high efficiency in yield production and Zn loading in edible parts under Zn-deficient conditions. In the last two years, we made excellent progress in selecting and characterizing Zn-efficient lines having high yield capacity under Zn-deficient conditions. Unfortunately, thus far, we were not able to find a cultivar or line, which is effective to allocating more Zn into grain. Apparently, there is a 'genetic block' that restricts both root uptake and grain accumulation of Zn at the amounts exceeding the requirements of plants for their vegetative growth and yield capacity under low supply of Zn in soils.

Improvement of Zn nutritional status of plants is also very beneficial with respect to disease resistance and seedling vigor. Zinc-deficient plants are sensitive to pathogenic fungal root diseases (Graham and Webb, 1991). In Zn-deficient plants, release of various organic compounds from roots, such as sugars, amino acids and phenolics is enhanced (Cakmak and Marschner, 1988), creating a favourable environment for infection. Improvement of Zn nutritional status of plants reduces the exudation of such compounds from roots (Welch et al., 1982; Cakmak and Marschner, 1988) and increases resistance to fungal root diseases (Sparrow and Graham, 1988; Thongbai et al., 1993). In the past, root diseases in Central Anatolia have been largely ignored, but are presently becoming a more important component of research and extension programs in this region of Turkey.

Besides human nutrition, increases in Zn content of grains also have desirable consequences for seedling vigor. Seedlings from seeds with low Zn content are very susceptible to various soil-borne and other pathogens and, thus, to winter-kill (Graham and Webb, 1991; Graham and Rengel, 1993). Seed content of Zn is extremely low in Central Anatolia (Table 9). Obviously, seedlings from such seeds might be sensitive to diseases and 'winter-kill'. This would be one reason why seeding rates (200–300 kg ha^{-1}) in Central Anatolia are three to six times as high as in other countries with similar climate.

5. Future plans

Current experiments are centered on understanding the genetic control of Zn efficiency and localization of genes for Zn efficiency. Experiments with different wild species of wheat revealed that a wide range of genetic variability exists in adaptation to severely Zn-deficient conditions. Studies are in progress with different addition, substitution and translocation series of wheat–alien species, wheat–rye and wheat–wheat to identify chromosome location of Zn efficiency genes. For example, rye chromosomes 1R, 5R and 7R, *Haynaldia* chromosome V2 and *Agropyron* chromosome L1 seem to carry major genes of Zn efficiency (high yield under Zn deficient conditions) (Schlegel et al., 1998). In addition, transfer of a chromosome segment into wheat was highly effective in improving Zn efficiency. Recently, several joint studies have been initiated for introgression of critical alien genes for Zn efficiency into wheat by applying the latest cytogenetic and molecular techniques (RFLPs, RAPDs, microsatellites). All these studies are being realized together with the Institute of Wheat and Sunflower Research-Dobrich (Bulgaria), Cereal Research Department of John Innes Centre, Norwich (UK), Institute of Plant Genetics and Crop Plant Research-Gatersleben (Germany) and CIMMYT (Mexico).

Additionally, using different Zn-efficient and Zn-inefficient wheat cultivars, wild species of wheat and various alien addition and translocation lines, several crossing experiments have been started to produce new cultivars. These new lines will be tested for their Zn efficiency with respect to yield capacity and Zn accumulation in shoot and grain under varied supply of Zn. In the experiments, special attention will be given to measurement of compounds (a) affecting mobility of Zn in the rhizosphere and within plants (i.e., phytosiderophores, nicotianamine), and (b) increasing (i.e., S-containing amino acids) or depressing (i.e., phytic acid) bioavailability of Zn in foods (Welch, 1995; Graham and Welch, 1996).

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