Improving zinc efficiency of cereals under zinc deficiency

Bhupinder Singh[1,*], Senthil Kumar A. Natesan[2], B. K. Singh[1] and K. Usha[3]

[1]Nuclear Research Laboratory, [2]Division of Plant Physiology, [3]Division of Fruits and Horticulture Technology, Indian Agricultural Research Institute, New Delhi 110 012, India

One of the widest ranging abiotic stresses in world agriculture arises from low zinc (Zn) availability in calcareous soils, particularly in cereals. Cereal species greatly differ in their zinc efficiency (ZE), defined in this article as the ability of a plant to grow and yield well under Zn deficiency. ZE has been attributed mainly to the efficiency of acquisition of Zn under conditions of low soil Zn availability rather than to its utilization or (re)-translocation within a plant. A higher Zn acquisition efficiency, further, may be due to either or all of the following: an efficient ionic Zn uptake system, better root architecture, i.e. long and fine roots with architecture favouring exploitation of Zn from larger soil volume, higher synthesis and release of Zn-mobilizing phytosiderophore by the roots and uptake of Zn-phytosiderophore complex. Seed Zn content has also been suggested to affect ZE. This article attempts to examine critically the scanty and scattered reports available on the status of Zn deficiency globally; morphological, biochemical and physiological basis of regulation of ZE in cereals and approaches to improve ZE in terms of grain productivity and grain Zn *vis-à-vis* its bioavailability under conditions of poor Zn availability.

A causal relationship between important Zn-containing enzymes, viz. carbonic anhydrase (CA), Cu/Zn-superoxide dismutase (SOD) activities and ZE is reported in wheat and other cereal species. Enhanced production and release of Fe-mobilizing phytometallophores known as phytosiderophores (PS), is another mechanism relevant for cereal species in adaptation to zinc deficiency.

Zinc deficiency: A global concern

LOW availability of Zn in calcareous soils is one of the widest ranging abiotic stresses in world agriculture, particularly in Turkey, Australia, China and India. Global studies initiated by FAO record Zn deficiency in 50% of the soil samples collected from 25 countries[1]. It is one of the most widespread nutritional constraints in crop plants, especially in cereals[2–5]. Among cereals, wheat and rice in particular, suffer from Zn deficiency. Grain-yield reduction of up to 80% along with reduced grain Zn level have been observed under Zn deficiency[6]. This has serious implication for human health in countries where consumption of cereal-based diets predominate[7]. Further, plants grown on zinc-deficient soils tend to accumulate heavy metals, which again is a potential human health hazard[8,9].

Zinc in soil

Zinc deficiency is common on neutral and calcareous soils, intensively cropped soils, paddy soils and poorly drained soils, sodic and saline soils, peat soils, soils with high available phosphorus and silicon, sandy soils, highly weathered acid and coarse-textured soils. Factors such as topsoil drying, subsoil, disease interactions and high cost of fertilizer also contribute to zinc deficiency[2]. The critical soil levels for occurrence of zinc deficiency are between 0.6 ppm and 2.0 mg zinc kg^{-1} depending on the method of extraction used. Calcareous soils (pH > 7) with moderate to high organic matter content (>1.5% organic C) are likely to be Zn-deficient due to high HCO_3^- in the soil solution. A ratio of more than 1 for exchangeable Mg: Ca in soil may also indicate Zn deficiency.

In the Indian context, more than 50% of the agricultural soils is zinc-deficient. The causes for occurrence of Zn deficiencies of this magnitude are related to the introduction of high-yielding varieties, neglect of application of bulky organic manures, imbalanced use of fertilizer and low Zn uptake and accumulation of Zn which depends upon the pH, soil organic matter, temperature, light intensity, crop species, etc. Zn deficiency is quite widespread in the Indo-Gangetic plain and other important cereal-growing states like Punjab, Uttar Pradesh, etc. which account for almost three-fourths of the country food grain production. The total area under Zn deficiency is about 10 Mha in India and approximately 85% of rice–wheat system cropping takes place in the Indo-Gangetic plain which has calcareous soils with high pH and thus low Zn availablility. Improving production from this cereal belt is therefore vital for sustaining grain production in the country. Zinc occurs in soil as sphalerite, olivine, hornblende, augite and biotite; however, availability of Zn from these sources is guided by several factors mentioned above.

Correction of Zn deficiency through addition of Zn fertilizers (Table 1) is a common practice. The application of 62.5 kg $ZnSO_4$ to the first crop of the cereal-based cropping system such as cotton–wheat, bajra–wheat or rice–wheat, is sufficient to meet the Zn requirement for three

*For correspondence. (e-mail: bhupindersinghiari@yahoo.com)

years or six crops. This practice is widely followed in several states such as Punjab and Haryana. However, this approach is neither economical nor environment-friendly in the long run, as only 20% of the applied Zn is available for plant uptake, while the remainder gets adsorbed on soil colloids and is, therefore, rendered immobile. As only a small fraction of the applied Zn is utilized by the crop to which it is applied, Zn accumulation in agricultural soils is on the increase, which is an environmental concern. With regard to human Zn-nutrition, fortification of Zn in food is practised, but is expensive and difficult to implement in developing countries like India, Bangladesh, Nepal, etc. Development of crop plants that are efficient Zn accumulators, especially under Zn-deficiency is, therefore, a potentially important endeavour for improving zinc deficiency tolerance of cereal species *vis-à-vis*, grain productivity and micronutrient quality. There is a need for selection and/or breeding of plant genotypes with higher resistance to Zn deficiency both in terms of a higher grain yield and a higher grain Zn content[10]. Realization of this approach is plausible in view of the large genotypic differences in Zn sensitivity among crop plants, particularly when its availability to the roots is limited[6,11,12].

Zinc in human nutrition

In biological systems, Zn is involved in the activity of more than 300 enzymes. In these enzymes, Zn plays either catalytic, co-catalytic or structural roles. Zinc also plays a critical role in the synthesis of proteins and metabolism of DNA and RNA. There is also increasing evidence that several zinc-containing proteins exist, which affect gene expression directly. The recommended dietary allowances for Zn are 5 mg/day for infants, 10 mg/day for children less than 10 yrs, 15 mg/day for males more than 10 yrs, 12 mg/day for females more than 10 yrs and 15 mg/day for women during pregnancy; however, these intake limits are seldom met. Consequently, Zn deficiency in humans results in a multitude of health problems such as impairment in linear growth, sexual immaturation, impairment of learning ability and immune functions and malformations in central nervous system[7].

Table 1. Commonly used Zn fertilizers

Compound	Formula	Zn content (%)
Zinc sulphate monohydrate	$ZnSO_4.H_2O$	36
Zinc sulphate heptahydrate	$ZnSO_4.7H_2O$	22
Zinc oxysulphate	$ZnSO_4.xZnO$	20–50
Basic zinc sulphate	$ZnSO_4.4Zn(OH)_2$	55
Zinc oxide	ZnO	50–80
Zinc carbonate	$ZnCO_3$	50–56
Zinc chloride	$ZnCl_2$	50
Zinc nitrate	$Zn(NO_3)_2.3H_2O$	23
Zinc frits	–	10–30
Disodium zinc EDTA	$Na_2ZnEDTA$	8–14
Sodium zinc HEDTA	NaZnHEDTA	6–10
Sodium zinc EDTA	NaZnEDTA	9–13

Zinc in plant nutrition

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[3]. Depending on the zinc level, zinc deficiency status of plants can be classified as follows: less than $10\,mg\,kg^{-1}$ – definite zinc deficiency; between 10 and $15\,mg\,kg^{-1}$ – likely to be zinc-deficient; between 15 and $20\,mg\,kg^{-1}$ – likely to be zinc-deficient; more than $20\,mg\,kg^{-1}$ – Zn-sufficient. The ratios of P: Zn and Fe: Zn in the shoot at tillering to pod initiation stage are good indicators of zinc deficiency, while leaf Zn concentration is a less reliable indicator of zinc deficiency, except in extreme cases. Leaf Zn concentration below $15\,mg\,kg^{-1}$ is regarded as Zn-deficient. Critical concentrations[3] of zinc in different plant tissues of cereals are presented in Table 2.

Zinc efficiency

Genotypic variation for zinc efficiency

Zinc efficiency, defined herein as the ability of a plant to grow and yield well under zinc-deficient conditions, varies among cereal species[13,14]. Genotypic differences for zinc use efficiency have been reported for several crops species[10,11,15,16]. Physiological mechanism(s) conferring Zn efficiency and their relative significance on low Zn soil/solution culture have been investigated by several workers[14,17–20]. Genotypic differences in Zn efficiency have been related to various mechanisms operative in the rhizosphere and within a plant system. Considerable progress has been made over the past few years to identify mechanisms that the plant species and genotypes possess for efficient acqui-

Table 2. Critical concentration of Zn in different plant tissues of cereals[3]

Crop	Tissue	Critical concentration (mg Zn/kg dry matter)
Rice	Seedling	22
Rice	Whole plant	15
Rice	Pre-flowering plant top	17.4
Maize	Upper 3rd leaf	16
Maize	Whole plant	22
Wheat	Shoot	24.5
Wheat	Pre-flowering plant top	14.5
Wheat	Whole plant	20–25
Wheat	Grain	12
Sorghum	Whole plant	8
Sorghum	Blade 1	10
Sorghum	Blade 5	25

sition of Zn from soils low in Zn availability[17,21]. These include, higher uptake of zinc (Zn^{2+}) by roots, protection against superoxide free radicles, i.e. efficient antioxidative defence mechanism, efficient utilization and (re)-translocation of Zn[9,17]. Cakmak *et al.*[4,5] showed that Zn efficiency of cereals is mainly related to difference in acquisition of Zn by the roots (Table 3). However, physiological and biochemical processes that control Zn efficiency, in general, and Zn acquisition by the roots, in particular, are among the less thoroughly studied aspects of plant Zn-nutrition. Graham and Rengel[13] 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[22].

Crop response to zinc deficiency

Symptoms of zinc deficiency

Zinc-deficient plants, in general, show a marked reduction in plant height and develop whitish-brown patches which turn necrotic with increasing severity of deficiency. Wheat plants show dusty brown spots on upper leaves of stunted plants, shoot growth is more inhibited than root growth, tillering decreases, spikelet sterility increases, midrib becomes chlorotic particularly near the leaf base of younger leaves, leaves lose turgor and turn brown as brown blotches and streaks appear on lower leaves. A white line sometimes appears along the leaf midrib and size of the leaf blade is reduced[6]. Symptoms may be more pronounced during early growth stages due to Zn immobilization. Based on field evaluation, Zn deficiency response of genotypes can be termed as Zn-efficient (showing no or relatively mild symptoms of Zn deficiency) and Zn-sensitive, (showing severe leaf symptoms, Table 2)[6]. In maize, Zn deficiency appears as a yellow striping of the leaves. Areas of the leaf near the stalk may develop a general white to yellow discolouration, i.e. white bud. In case of severe deficiency, the plants are stunted due to shortened internodes and the lower leaves show a reddish or yellowish streak about one-third of the distance from the margin. During Zn-deficient condition, barley leaves show uniform chlorosis and drying, and tip growth decreases. Deficiency symptoms in sorghum grains, are similar to those in maize, but less pronounced. In oat, the leaves become pale green; older leaves show collapsed areas at the margins and tips are greyish in colour. Necrosis extends down the leaf and remainder of the leaf is grey to bronze-green[6,18–20].

Table 3. Effect of Zn supply on amount of Zn in shoots of different cereals grown for six weeks in Zn-deficient soil

Cereal	Amount of Zn[6] (µg/shoot) –Zn	(µg/shoot) +Zn	(µg/g dw) –Zn	(µg/g dw) +Zn	Symptoms of Zn deficiency in leaf (necrotic patches on leaf blades)
Secale cereale	6.7	40	9.0	46	Very slight or absent
Hordeum vulgare	5.7	69	8.4	62	Mild
Triticum aestivum	2.9	36	6.5	46	Mild
Triticum durum	1.8	39	6.4	47	Very severe
Avena sativa	2.3	42	6.3	38	Very severe

Plant growth under zinc deficiency

For a genotype to be zinc-efficient, it should not only be able to absorb more zinc from deficient soils, but should also produce more dry matter and grain yield. It, however, may not necessarily have the highest zinc concentration in tissue or grain[10]. It is evident from the available literature that the crop response to zinc deficiency in terms of dry mass production is diverse and there is no unanimity in using root and shoot dry mass production or shoot : root ratio as an indicator for zinc efficiency of cereals under low Zn condition. Although root and shoot growth is distinctly reduced under zinc deficiency[10,23], shoot dry weight is depressed to a greater extent than root dry weight[17,24,25]. Among wheat species, durum wheat is more sensitive to zinc deficiency than bread wheat[4], as evident from the fact that the decline in shoot growth of Zn-sensitive durum wheat (durati) under zinc deficiency was much more than that of Zn-deficient tolerant Warigal, a bread wheat genotype[24]. In some cereal genotypes, root growth was enhanced under Zn deficiency[3–5]. Higher sensitivity of durum wheat to Zn deficiency was associated with higher root growth at the expense of shoot growth[12]. In nutrient solution experiments, decrease in shoot dry matter production induced by Zn deficiency was more pronounced in durum wheat than in bread wheat[3,19,26]. Root and shoot weight significantly increased with application of Zn and there was an increase in root density with an increase in root volume[27]. Zn-efficiency based on shoot dry weight and shoot growth showed marked differences among chickpea genotypes for which the shoot dry weight was lower under Zn deficiency compared with the Zn-sufficient condition[28]. The root : shoot ratio in general, increases[29] as an initial response to Zn deficiency. Cakmak *et al.*[30] observed a decrease in shoot dry matter production of about 16% in rye, 36% in bread and 47% in durum wheat as a result of zinc deficiency.

It is observed that Zn content (accumulation) per shoot and not Zn concentration is better correlated with the sensitivity of genotypes to Zn deficiency[3–5,19,26]. In wheat genotypes grown under controlled environmental condition in nutrient solution for 25 days, Zn content in the dry matter was much lower in plants grown without Zn compared to those supplied with Zn[15]. Concentration of Zn was significantly higher in plants supplied with Zn than those without Zn supply. Root Zn concentrations were greater than shoot Zn concentrations under Zn-deficient conditions, since

under deficient Zn supply the transport of Zn from root to shoot[12] is inhibited. Zn-efficient bread wheat genotypes, in general, contained more Zn in shoots than Zn-inefficient durum wheat genotypes in field[10], greenhouse[3] and nutrient solution experiments[12,19]. Zn-efficient chickpea was reported to have higher zinc content per plant and higher zinc uptake per gram of root dry weight than those of inefficient-genotypes[28].

Factors regulating zinc efficiency of cereals

Root characteristics

Root is the main mineral nutrient uptake organ of plants, and its growth undoubtedly affects nutrient uptake and transport. The micronutrient uptake depends largely on root activities, which affect the root characteristics that control the uptake rate[31]. A number of mathematical models of nutrient uptake by plants were developed based on soil chemistry, kinetics of nutrient uptake and root architecture and morphology[32]. Of these, root morphology and architecture are functionally important in efficient acquisition of soil resources and in plant adaptation to sub-optimal condition of both water and nutrients[33–35]. Dong *et al.*[23] suggested that the difference in root morphology among genotypes is more likely to be a property of the genotype. Zinc uptake by higher plants appears to be mostly controlled by the transport of zinc across the plasma membrane, which is largely metabolism-dependent and genetically controlled. Zn-efficient genotypes may be able to maintain structural and functional stability of their root-cell plasma membranes better than Zn-inefficient genotypes under Zn deficiency[36]. Different traits associated with root morphology are: root length, diameter, density and volume. Plant species or cultivars that produce finer roots with diameter <0.3 mm can explore a large volume of soil and hence, can more efficiently scavenge off the small amounts of immobile zinc ion, than plants that produce thicker roots. Excalibur, a zinc-efficient wheat genotype, develops smaller roots than the cv. Gatcher, a zinc-inefficient genotype[2]. In addition, plant species with longer root system are expected to be more zinc-efficient, as a deeper rooting zone can explore zinc more efficiently in the subsoil[37]. Growing longer and thinner roots and having a greater proportion of thinner roots in the total root biomass early in the growth period are the two characters associated with Zn-efficient genotypes[23]. Zn^{2+} ions have to travel less distance to root absorption sites in case of plants having higher root absorbing surface area[38]. Rengel and Wheal[22] showed that Zn uptake is reduced in wheat genotypes having a lower proportion of finer roots (diameter < 0.2 mm) in the total root mass. A vigorous root system may be beneficial in extracting more of the slowly diffusible Zn from a given soil volume[37]. It is suggested that the ability of Zn-efficient genotypes to produce greater proportion of fine roots (< 0.2 mm in diameter) with higher surface area to volume ratio may be related to greater Zn efficiency of the genotypes[23]. It can be amply deduced from studies made so far that breeding cereals with root system capable of greater mobilization of Zn from soils of low Zn availability is a promising and environmental-friendly strategy that not only reduces Zn-fertilizer use, but also increases resistance of cereals to soil-borne fungal disease[13].

The term root architecture is used to represent the shape of root system, i.e. the spatial configuration of the root system. Since soil resources are distributed unevenly, spatial coverage or distribution of the root system will determine the ability of plants to exploit the unevenly distributed soil resources effectively. Hence, it is highly desirable to breed cereal species with root systems (finer and longer roots) that are capable of mobilizing Zn on low Zn soils[10].

Phytosiderophore production and release

In recent years, considerable progress has been made towards identification of adaptive mechanisms that enable plant species in efficient uptake of nutrients from soils low in nutritional quality. One such mechanism that has been found in graminaceous species under Fe deficiency is the release of phytosiderophores of the mugineic acid (MA) family (phytometallophores), which are highly effective in mobilizing, by chelation, the sparingly soluble inorganic Fe compounds such as Fe III hydroxides and oxides in the rhizosphere. The phytometallophore–Fe (III) complex is actively transported across the plasma membrane without Fe (III) reduction. In graminaceous plants, tolerance to Fe deficiency is suggested to depend on the amount of MA secreted from the roots[39–41]. Studies have been conducted to elucidate the genetic regulation of phytosiderophore biosynthesis in graminaceous species[42–45]. Activities of nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT), the chief enzymes of phytosiderophore biosynthesis, were found to be correlated with the release of phytosiderophore in barley and rice under Fe deficiency and therefore for Fe deficiency tolerance[42,46].

Plant roots can absorb Zn not only as a divalent cation but also in a chelated form, namely as Zn-phytosiderophores. In monocotyledon species, Zn deficiency increased root exudation of amino acids, sugars and phenolics and Zn-deficient plants showed increased mobilization of both Zn and Fe from various sources. In contrast, the root exudates of Zn-deficient dicotyledonous species did not enhance Zn mobilization from a synthetic resin. These differences in capability of mobilization of Zn and Fe between plant species are due to an enhanced release of phtosiderophores under Zn deficiency in the graminaceous species[47]. This enhanced release of phytosiderophores was inversely related to the Zn nutritional status of the plants. Root exudates may also be of importance in enhancing the mobilization and uptake of micronutrients from the apoplasmic pool. These micronutrients may be bound in exchangeable form or precipitated in the apoplast or at the root surface[48]. Root

exudates mobilized increasing amounts of various micronutrients in the following order: Cu < Fe < Zn < Mn[49]. Merckx *et al.*[50] has demonstrated complexation of Zn and Mn by organic compounds released from maize and wheat roots and determined that the carbon they contained originated from the plant.

Treeby *et al.*[49] and Crowley *et al.*[51] suggested that phytosiderophore production is a general response of plants to micronutrient deficiency. Marschner *et al.*[52] reported enhanced phytosiderophore production under nutrient-deficient conditions. Further, the role of phytosiderophore in acquisition of iron and other micronutrients (Zn, Cu, Mn) along with genotypic differences in the release of phytosiderophore and uptake of metal chelates was highlighted by Romheld and Marschner[39]. Phytosiderophores possess greater ability to complex Zn and enhance its mobility in the rhizosphere[49] and root apoplast[47]. Differences in Zn uptake capacity between bread and durum wheat cultivars were attributed to differential release of phytosiderophores (Table 4) from roots[3–5,24]. Zn-deficient roots of two wheat cultivars, i.e. Durati and Warigal, released respectively, five and three times more phytosiderophore than the respective Zn-sufficient Durati and Warigal roots[24]. The resupply of Zn to Zn-deficient plants completely repressed the release of phytosiderophores within 72 h[53]. A 100-fold excess of phytosiderophores over Zn availability considerably represses the uptake of free Zn, whereas under the same conditions uptake rate of phytosiderophore-chelated Zn may be 5 to 10 times higher than that of free Zn[54]. It is, therefore, suggested that the phytosiderophore release under zinc deficiency stress may be causally involved in determining zinc efficiency of graminaceous species. The stability of Fe (III)–PS complex, however, is much higher than that of Zn–PS[55] and unlike Fe, there is no direct evidence on record of a Zn–PS translocator. It is suggested that both Zn–(PS) and Fe–(PS) complexes are taken up by plant roots through the same transport system[54,56]. Zhang *et al.*[47] observed that the peak in Zn uptake coincided with the maximum rate of phytosiderophore release in Fe-deficient plants, while in Fe-sufficient plants the release of phytosiderophores was low and no such peak in Zn uptake rate could be observed. A much higher uptake rate of Zn in Fe-deficient barley plants supplied with inorganic Zn in nutrient solutions[49] indicated an enhanced mobilization of apoplasmic Zn by the phytosiderophores released under Fe deficiency. The relationship between Fe transport to shoots and differential exudation of phytosiderophores by wheat genotypes has been proposed as a physiological mechanism behind differential genotypic tolerance to Zn deficiency[11,57]. Zn-deficient plants were unable to achieve phytosiderophore exudation as high as those observed in Fe-deficient plants, but were capable of sustaining phytosiderophore exudation for a longer time[17]. The mechanism behind greater tolerance to Zn deficiency in the wheat germplasm is lower transport of Fe from roots to shoots, with shoots responding to physiological deficiency of Fe by sending signals to the roots to increase exudation of phytosiderophores[57]. However, this reasoning has been argued by Singh *et al.*[19], who observed a higher and almost similar Fe-uptake by Zn-efficient and inefficient wheat genotypes under Zn deficiency.

Furthermore, it has been shown that in Fe[58] and Zn-deficient plants[15], the release of phytosiderophores follows the same diurnal rhythm. Similar type of phytosiderophores are believed to be released under both Zn and Fe deficiencies[19,53]. Zn-phytosiderophores have similar structural confirmations as Fe-phytosiderophores[59] and a similar regulatory mechanism for the biosynthesis and/or release of phytosiderophores under both Zn and Fe deficiencies has been suggested[17]. Since methionine is a precursor for the biosynthesis of various phytosiderophores in graminaceous species[60], inhibition of protein synthesis, both under Fe and Zn deficiencies, may cause an accumulation of free amino acids, which may consequently result in enhanced biosynthesis of phytosiderophores in Fe-deficient and Zn-deficient roots[53, 61].

Release of phytosiderophores from the root is also affected by root zone temperature. Decrease in root zone temperature from 30 to 5°C decreased the rate of release of phytosiderophores[62]. Role of light in the release of phytosiderophores under Fe and Zn deficiency has also been shown[6]. Plants release phytosiderophores at higher amounts about a few hours after the onset of the light period. Under continuous darkness or continuous light, the rate of release of phytosiderophores is lower[6,62]. The diurnal pattern of phytosiderophore release and its influence on zinc uptake have been investigated by several workers[6,19,47]. They observed a sharp rise in phytosiderophore production 3h after onset of the light period, which gradually declined thereafter. In Fe-deficient plants, release of phytosiderophores from the roots followed a distinct diurnal rhythm with a steep peak about 4 h after the onset of the light period[47]. A similar pattern of diurnal release of phytosiderophores was reported in Zn-deficient graminaceous species[19,53]. Since phytosiderophores can mobilize Zn not only in the rhizosphere, but also from the root apoplast, the apoplasmic pool of Zn has to be taken into account as potential source for both uptake and diurnal variation in uptake rates of Zn[47]. HPLC analysis has revealed that DMA (2′-deoxy mugineic acid) is the predominant phytosiderophore released from the roots of zinc-efficient wheat cultivars under Zn deficiency[15,19], while in rice it is mugenic acid[46].

Table 4. Relative release of phytosiderophores from roots of bread and durum wheat in nutrient solution under Fe and Zn deficiency[19]

Wheat species	Relative release of phytosiderophore	
	Fe deficiency	Zn deficiency
T. aestivum (bread wheat)	High (++++)	Moderate (+++)
T. durum (durum wheat)	High (++++)	Low (+)

Furthermore, under zinc deficiency the organic and inorganic compounds released from the root can stimulate microbial activity in the rhizosphere, since the rhizosphere microorganisms derive energy from root exudates and secretions, sloughed-off cells and other root debris[33]. In addition, zinc deficiency increased the number of fluorescent pseudomonas in the rhizosphere of all wheat genotypes tested, but the effect was particularly obvious for genotypes tolerant to zinc deficiency[33]. Enhanced production and release of Zn-mobilizing phytosiderophores, therefore, is a mechanism relevant for cereal species in adaptation on zinc-deficient soils[6,14,15,19,53,63,64]. On the contrary, Erenoglu *et al.*[65] and Pedlar *et al.*[66] did not find any relationship between the release of phytosiderophores and zinc efficiency. Therefore, despite reports favouring a relationship between phytosiderophore release and Zn efficiency of cereal species under zinc deficiency, at the present juncture, their significance in Zn nutrition is still an open question. There is a need to investigate the genetic and molecular regulation of phytosiderophore biosynthesis under zinc deficiency in order to underline the contribution of phytosiderophores in zinc efficiency of cereals[64].

Zinc uptake kinetics

Genotypic variation in uptake efficiency may not only be due to differences in morphology and architecture, but also due to differences in the affinity of the uptake system (K_m), maximum uptake rate (I_{max}) and threshold concentration (C_{min}) – minimum concentration at which the root can deplete nutrient in the external solution. Epstein[67], based on depletion technique which permits establishment of uptake–substrate relation in low concentration range for intact plants, has shown that the net uptake rate of a nutrient can be related to the external concentration of that nutrient. Kinetic parameters of zinc uptake determined for wheat plants pre-grown at deficient or sufficient zinc supply, showed a saturation response for net zinc uptake with increasing solution zinc concentration. Zinc deficiency caused an increase in I_{max} in zinc-efficient genotypes, but not in zinc-inefficient genotypes. Zinc-efficient genotypes possess a better absorption and root-to-shoot transport, probably due to a more efficient transport system such as ion channel or ion pump, compared with the zinc-inefficient genotypes[28,37].

Rengel and Wheal[22] studied kinetic parameters of Zn uptake in bread wheat cultivars differing in Zn efficiency and showed that under Zn deficiency, Zn-efficient cultivars showed greater I_{max} value (maximum net uptake rate) than Zn-inefficient cultivars. They also found that Zn efficient bread wheat cultivar had a 30% higher rate of net Zn uptake than Zn-inefficient bread wheat. Zinc deficiency over a bnger period (24 days) increased I_{max} and K_m in zinc-efficient genotypes, but zinc-inefficient genotypes did not show any increase in I_{max} following a period of zinc deficiency[68]. With an increase in severity of Zn-deficiency stress between 14 and 18 days of growth at 0 Zn, uptake of ^{65}Zn increased by 170% in Zn-deficiency tolerant wheat cultivar Warigal, but remained unchanged in Zn-deficiency-sensitive Durati. Zn-deficient Warigal plants transported larger amounts of ^{65}Zn to the shoot compared with Zn-sensitive Durati[24]. On the other hand, no clear difference was found between Zn-efficient and Zn-inefficient bread wheat cultivars in either uptake or root-to-shoot translocation rates[69,70] of ^{65}Zn.

Radiotracer techniques were employed to characterize Zn^{2+} influx into the root symplasm and translocation to the shoot in *Thalaspi caerulescens* (zinc hyper accumulator) and *Thalaspi arvense* (zinc non-accumulator). Concentration-dependent Zn^{2+} influx in both the species yielded non-saturating kinetic curves that could be resolved into linear and saturable components. These saturable components followed Michaelis–Menton kinetics. ^{65}Zn content and uptake in root, sheath and blade of maize and barley plants increased significantly with increased levels of zinc application. The labelled Zn rapidly accumulated in the roots of wheat plants upon immersion into the isotope solution[71]. Root uptake and root-to-shoot transport of Zn and particularly internal utilization of Zn may be an equally important mechanism involved in the expression of Zn efficiency in cereal genotypes[65].

Zinc (re)-translocation

Little is known about transport of Zn from roots to leaves and from leaves to other plant organs. Enhanced translocation of Zn from root to shoot meristems and its (re)translocation from senescing to growing organs under deficient Zn supply might also contribute towards Zn efficiency in cereals. The enhanced capacity of genotypes for Zn translocation from root to shoot and its utilization under deficient Zn supply was shown to contribute to Zn efficiency in wheat genotypes[4]. Haslett *et al.*[72] showed that Zn is highly mobile within the plant system and foliar applied ^{65}Zn is translocated to leaves both above and below the treated leaf as well as to the root tips. Hajiboland *et al*[73] found that Zn deficiency tolerance of a Zn-efficient rice genotype is related to its ability to re-translocate zinc from older to growing/emerging leaves. Erenoglu *et al.*[70,74], however, were unable to confirm these findings in wheat. Among dicots, Zn-efficient chickpea genotypes, CTS-11308 and T-1587, transported more than 70% of the total absorbed zinc to the shoot compared with genotpes, Tyson and Dooen[28].

Zinc utilization

One of the main roles of micronutrients in plants is derived from their presence in the active centres of many enzyme molecules[75]. As a result, metalloenzyme activities of several micronutrients have been used as specific parameters for the appraisal of biologically active metals involved in plant metabolic processes[76]. Utilization efficiency in terms

of dry matter production per unit of zinc present in the dry matter may be linked to differences in the ability of a genotype to maintain an optimal activity of the important zinc-regulated enzymes, viz. super oxide dismutase (SOD) and carbonic anhydrase (CA). There are also a large number of enzymes in which zinc is an integral component of the enzyme structure (zinc enzymes). Activity of these enzymes has been correlated with zinc availability to the plants. Differences in internal utilization or mobility of Zn have been shown to be involved in expression of Zn efficiency[20].

Carbonic anhydrase can occur as a dimer, tetrameter, hexamer or octamer, with a zinc atom in every subunit and a molecular mass ranging from 42 to 250 kDa[77]. CA is present in leaves of higher plants in abundant quantities (1–2% of total soluble leaf protein) and thus represents a significant storage pool of Zn in leaf cells. Generally, CA is present in excess of what may be required for photosynthesis, particularly in C_3 plants. CA activity is much lower in wheat compared to a number of other species[78].

Activity of CA decreases in a number of plant species as a consequence of Zn deficiency[79]. CA activity is closely related to zinc content in C_3 plants. Under extreme zinc deficiency, CA activity is almost absent. High CA activity is required in the mesophyll chloroplast of C_4 plants and removal of zinc from the CA molecule *in vitro* results in an irreversible loss of catalytic activity[80]. There is a quantitative difference between total and physiologically active Zn in leaves. Activity of CA was suggested to be a suitable indicator for the levels of physiologically active Zn in the leaf tissue[79].

Deficiency of Zn is known to decrease CA activity drastically in several plant species[81]. Under zinc deficiency, there was twofold higher CA activity in zinc-efficient than zinc-inefficient genotypes of wheat, indicating a higher level of physiologically active Zn pool in leaves of Zn-efficient genotypes. Upon resupply of zinc to zinc-deficient plants, zinc-inefficient wheat genotype lost the ability to increase CA activity, while zinc-efficient genotype Warigal showed a saturating, curvilinear increase in the CA activity, indicating a positive relationship between CA activity and Zn efficiency of the model wheat genotypes[36]. Ability of Zn-efficient wheat genotype to maintain greater CA activity under Zn-deficient conditions may be beneficial in maintaining the photosynthetic rate and dry matter production at a higher level; a characteristic that may be especially important for wheat as a species with inherently lower CA activity compared to other species[36]. Zn appears to have a regulatory influence on stomatal opening, possibly as a constituent of CA[82].

In the metalloenyme SOD, zinc is associated with copper (Cu–Zn–SOD) and represents the structural component of SOD. Under zinc deficiency, SOD activity is much lower but can be restored *in vitro* by resupply of zinc to the assay medium. This indicates that zinc atom is essential for the normal functioning of Cu–Zn–SOD[3,83]. The activities of Cu–Zn–SOD and in part, total SOD, but not Mn-SOD are closely related with the sensitivity of wheat and rye cultivars to Zn deficiencies[6]. Recently, Kochian's group[20] has shown that zinc efficiency (ZE) in wheat is correlated with enhanced expression and activity of zinc-requiring enzymes and thus, biochemical Zn utilization is an important component of ZE in wheat. They found no correlation between ZE and Zn translocation to the shoot. Further, total and water-soluble concentrations of leaf Zn were not associated with ZE, and no differences in sub-cellular Zn compartmentation were found between Zn-efficient and Zn-inefficient genotypes. The group provides Northern analysis evidence to suggest that Cu–Zn–SOD gene expression was upregulated in Zn-efficient genotype, but not in inefficient type. An efficient utilization of Zn, therefore, at the cellular level appears to be a major factor determining the expression of Zn efficiency in cereals growing under deficient supply of zinc.

Conclusion

It can be amply surmised from the available literature that zinc efficiency of cereals under zinc deficiency is regulated by several factors, most importantly, the presence of an efficient Zn^{2+} and Zn–phytosiderophore complex uptake system. Manipulation of phytosideriophore biosynthesis and release is a promising strategy to improve Zn efficiency in cereal crops[19]. Higuchi *et al.*[84] and Takahashi *et al.*[44] have already shown that manipulation of phytosiderophore biosynthesis for higher phytosiderophore production and release can effectively improve Fe deficiency tolerance of iron-inefficient rice on low Fe-alkaline soils. It may be quite plausible that zinc deficiency tolerance of graminaceous species can also be achieved through manipulation of key enzymes of phytosiderophore biosynthesis, i.e. NAS and NAAT[19]. This will help in reducing and may be even totally eliminating the application of fertilizer zinc to the soil. Further, the future Zn-efficient plant types should be so modelled to mobilize Zn from the unavailable or distant Zn-pools through improved root architecture in terms of thinner and longer roots having a larger surface area of absorption or utilization, and maintaining higher activities of Cu–Zn–SOD and CA or translocation in terms of high root to shoot transport of Zn and its retranslocation from older to younger and growing plant tissues. Improved Zn efficient types are likely to give a higher grain yield and grain Zn on Zn-deficient soil. Further, this will also help in alleviating the Zn deficiency problems in human population.

1. Graham, R. D., Breeding wheats for tolerance to micronutrient deficient soils: present status and priorities. In *Wheat for the non-Traditional Warm Areas CIMMYT* (ed. Saunders, D. A.), Mexico D.F., 1991, pp. 315–332.
2. Sillanpaa, M., Micronutrients and the nutrient status of soils. A global study. *FAO Soil Bulletin* No. 48, FAO. Rome, Italy, 1982.
3. Marschner, H., *Mineral Nutrition of Higher Plants*, Academic Press, New York, 1995.

4. Cakmak, I., Yilmaz, A., Kalayci, M., Ekiz, H., Torun, B., Erenoglu, B. and Brown, H. J., Zinc deficiency as a critical problem in wheat production in central Anatolia. *Plant Soil*, 1996, **180**, 165–172.
5. Cakmak, I., Sari, N., Marschner, H., Kalayci, M., Yilmaz, A., Eker, S. and Gulut, K. Y., Dry matter production and distribution of zinc in bread and durum wheat genotypes differing in zinc efficiency. *Plant Soil*, 1996, **180**, 173–181.
6. Cakmak, I. *et al.*, Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica*, 1998, **100**, 349–357.
7. Welch, R. M., Impact of mineral nutrients in plants on human nutrition on a worldwide scale. In *Plant Nutrition – Food Security and Sustainability of Agro-ecosystems* (eds Horst, W. J. *et al.*), Kluwer, Dordrecht, The Netherlands, 2001, pp. 284–285.
8. Wolink, K. A., Fricke, F. L., Capar, S. G., Braude, G. L., Meyer, M. W., Satzger, R. D. and Bonnin, E., Elements in major raw agricultural crops in the United States. 1. Cadmium and lead in lettuce peanuts, potatoes, soybeans, sweet corn and wheat. *J. Agric. Food Chem.*, 1983, **31**, 1240–1244.
9. Hart, J. J., Norvell, W. A., Welch, R. M., Sullivan, L. A. and Kochian L. V., Characterization of zinc uptake, binding, and translocation of bread and durum wheat cultivars. *Plant. Physiol.*, 1998, **118**, 219–226.
10. Graham, R. D., Ascher, J. S. and Hynes, S. C., Selecting zinc-efficient cereal genotypes for soil of low zinc status. *Plant Soil*, 1992, **146**, 241–250.
11. Rengel, Z. and Graham, R. D., Wheat genotypes differ in Zn efficiency when grown in chelated buffer: I. *Growth Plant Soil*, 1995, **176**, 307–316.
12. Rengel, Z. and Graham, R. D., Wheat genotypes differ in Zn efficiency when grown in chelate-buffered nutrient solution: II. Nutrient uptake. *Plant Soil*, 1995, **176**, 317–324.
13. Graham, R. D. and Rengel, Z., Genotypic variation in Zn uptake and utilization by plants. In *Zinc in Soils and Plants* (ed. Robson, A. D.), Kluwer, Dordrecht, The Netherlands, 1993, pp. 107–114.
14. Erenoglu, B., Eker, S., Cakmak, I., Derici, R. and Romheld, V., Effect of iron and zinc deficiency on release of phytosiderophores by barley cultivars differing in zinc efficiency. *J. Plant Nutr.*, 2000, **23**, 1645–1656.
15. Cakmak, I., Gulut, K. Y., Marschner, H. and Graham, R. D., Effect of zinc and iron deficiency on phytosiderophore release in wheat genotype differing in zinc efficiency. *J. Plant Nutr.*, 1994, **17**, 1–17.
16. Wiren, N. V., Mori, S., Marschner, H. and Romheld, V., Iron inefficiency in maize mutant YS1 (*Zea mays* L. cv yellow-stripe) is caused by a defect in uptake of iron phytosiderophores. *Plant Physiol.*, 1994, **106**, 71–77.
17. Rengel, Z. and Romheld, V., Root exudation and Fe uptake and transport in wheat genotypes differing in tolerance to Zn deficiency. *Plant Soil*, 2000, **222**, 25–34.
18. Hacisalihoglu, G., Hart, J. J. and Kochian, L. V., High- and low-affinity zinc transport systems and their possible role in zinc efficiency in bread wheat. *Plant Physiol.*, 2001, **125**, 456–463.
19. Singh, Bhupinder, Erenoglu, B., Neumann, G., Römheld, V. and von Wiren, N., Role of phytosiderophores in zinc efficiency of wheat. In *Eco-Physiology of Rhizosphere* (ed. Merbach, W.), 2002, pp. 52–60.
20. Gökhan, H., Hart, J. J., Wang, Yi-Hong, Cakmak, I. and Kochian, L. V., Zinc efficiency is correlated with enhanced expression and activity of zinc-requiring enzymes in wheat. *Plant Physiol.*, 2003, **131**, 595–602.
21. Marschner, H., Romheld, V. and Kissel, M., Different strategies in higher plants in mobilization and uptake of iron. *J. Plant Nutr.*, 1986, **9**, 695–713.
22. Rengel, Z. and Wheal, M. S., Basic parameters of Zn uptake by wheat are affected by herbicides chlorsulfuran. *J. Exp. Bot.*, 1997, **48**, 935–941.
23. Dong, B., Rengel, Z. and Graham, R. D., Root morphology of wheat genotypes differing in zinc efficiency. *J. Plant Nutr.*, 1995, **18**, 2761–2773.
24. Rengel, Z., Romheld, V. and Marschner, H., Uptake of zinc and iron by wheat genotypes differing in tolerance to zinc deficiency. *J. Plant Physiol.*, 1998, **152**, 433–438.
25. Natesan, A. and Senthil Kumar, Studies on zinc use efficiency in wheat genotypes. Division of Plant Physiology, IARI, 2001, New Delhi.
26. Singh, Bhupinder, Role of phytosiderophores in zinc mobilisation and exploitation of the trait for improving zinc use efficiency in wheat. DST Young Scientist Project Completion Report. DST, 2001.
27. Malewar, G. U., Indulkar, B. S. and Takankhar, V. G., Root characteristics and yield attributes as influenced by Zn levels and groundnut varieties. *Ann. Agric. Res.*, 1993, **14**, 478–481.
28. Khan, H. R., McDonald, G. K. and Rengel, Z., Chickpea genotypes differ in their sensitivity to Zn deficiency. *Plant Soil*, 1998, **198**, 11–18.
29. Loneragan, J. F., Krik, G. J. and Webb, M. J., Translocation and function of Zn in roots. *J. Plant Nutr.*, 1987, **10**, 1247–1254.
30. Cakmak, I., Ozturk, L., Eker, S., Torun, B., Kalfa, H. I. and Yilmaz, A., Concentration of zinc and activity of copper/zinc-superoxide dismutase in leaves of rye and wheat cultivars differing in sensitivity to zinc deficiency. *J. Plant Physiol.*, 1997, **151**, 91–95.
31. Barber, S. A., Nutrient bioavailability: A mechanistic approach, Wiley-Interscience, New York, 1984.
32. Rengel, Z., Mechanistic simulation models of nutrient uptake: A review. *Plant Soil*, 1993, **152**, 161–173.
33. Lynch, J. M. and Whipps, J. M., Substrate flow in the rhizosphere. *Plant Soil*, 1990, **129**, 1–10.
34. Sattelmacher, B., Horst, W. J. and Becker, H. C., Factors that contribute to genetic variation for nutrient efficiency of crop plants. *Z. Pflanzenernaehr. Bodenkd.*, 1994, **157**, 215–224.
35. Barber, S. A. and Silberbush, M., Plant root morphology and nutrient uptake. In *Roots, Nutrient and Water Influx and Plant Growth* (eds Barber, S. A. and Bouldin, D. R.), Soil Science Society of America, Madison, WI, 1984, pp. 65–87.
36. Rengel, Z., Carbonic anhydrase activity in leaves of wheat genotypes differing in Zn efficiency. *J. Plant Physiol.*, 1995, **147**, 251–256.
37. Grewal, H. S., Stangoulis, J. C. R., Potter, T. D. and Graham, R. D., Zinc efficiency of oilseed rape (*Brassica napus* and *B. juncea*) genotypes. *Plant Soil.*, 1997, **191**, 123–132.
38. Marschner, H., Zinc uptake from soils. In *Zinc in Soils and Plants* (ed. Robson, A. D.), Kluwer, Dordrecht, The Netherlands, 1993, pp. 59–77.
39. Romheld, V. and Marschner, H., Genotypical differences among graminaceious species in release of phytosiderophores and uptake of iron phytosiderophores. *Plant Soil*, 1990, **123**, 147–153.
40. Singh, K., Chino, M., Nishizawa, N. K., Ohata, T. and Mori, S., Genotypic variation among Indian graminaceous species with respect to phytosiderophore secretion. In *Genetic Aspects of Plant Mineral Nutrition* (ed. Randall, P. J.), Kluwer, Dordrecht, The Netherlands, 1993, pp. 335–339.
41. Marschner, H. and Romheld, V., Strategies of plants for acquisition of iron. *Plant Soil*, 1994, **165**, 261–274.
42. Kanazawa, K., Higuchi, K., Nishizawa, N. K., Fushiya, S., Chino M. and Mori, S., Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretions under Fe-deficient conditions in Gramineae. *J. Exp. Bot.*, 1994, **45**, 1903–1906.
43. Higuchi, K., Kanazawa, K., Nishizawa, N. K. and Mori, S., The role of nicotianamine synthase in response to Fe nutrition status in gramineae. *Plant Soil*, 1996, **178**, 171–177.
44. Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N. K. and Mori, S., Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nature Biotechnol.*, 2001, **19**, 466–469.
45. Itai, R., Suzuki, K., Yamaguchi, H., Nakanishi, H., Nishizawa, N. K., Yoshimura, E. and Mori, S., Induced activity of adenine phosphoribosyltransferase (APRT) in iron deficient barley roots: a possible

role for phytosiderophore production. *J. Exp. Bot.*, 2002, **51**, 1179–1188.
46. Higuchi, K., Suzuki, K., Nakanishi, H., Yamaguchi, H., Nishizawa, N. K. and Mori, S., Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores. *Plant Physiol.*, 1999, **119**, 471–479.
47. Zhang, F. S., Romheld, V. and Marschner, H., Release of zinc mobilising root exudates in different plant species as affected by zinc nutritional status. *J. Plant Nutr.*, 1991, **14**, 675–686.
48. Clarkson, D. T. and Sanderson, J., Sites of absorption and translocation of iron in barley roots. *Plant Physiol.*, 1978, **61**, 731–736.
49. Treeby, M., Marschner, H. and Romheld, V., Mobilization of iron and other micronutrient cations from a calcareous soil by plant-borne, microbial, and synthetic metal chelators. *Plant Soil*, 1989, **114**, 217–226.
50. Merckx, R., van Ginkel, J. H., Sinnaeve, J. and Cremes, A., Plant-induced changes in the rhizosphere of maize and wheat. *Plant Soil*, 1986, **96**, 95–107.
51. Crowley, D. E., Reid, C. P. P. and Szanislo, P. J., Microbial siderophores as iron sources for plants. In *Iron Transport in Microbes, Plants and Animals* (eds Winkelmann, G., vander Helm, D. and Neilands, J. B.), VCH, Weinheim, 1987, pp. 375–386.
52. Marschner, H., Treeby, M. and Romheld, V., Role of root-induced changes in the rhizosphere for iron acquisition in higher plants. *Z. Pflanzenernaehr. Bodenkd.*, 1989, **152**, 197–204.
53. Zhang, F., Romheld, V. and Marschner, H., Effect of zinc deficiency in wheat on the release of zinc and iron mobilising root exudates. *Z. Pflanzenernaehr Bodenkd.*, 1989, **152**, 205–210.
54. Wiren, N. V., Marschner, H. and Romheld, V., Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. *Plant Physiol.*, 1996, **111**, 1119–1125.
55. Murakami, T., Ise, K., Hayakawa, M., Kamei, S. and Broekmann, R. A., Stabilities of metal complexes of mugineic acids and their specific affinities for iron(III). *Chem. Lett.*, 1989, 2137–2140.
56. Kochian, L. V., Zinc absorption from hydroponic solution by plant roots. In *Zinc in Soils and Plants* (ed. Robson, A. D.), Kluwer, Dordrecht, The Netherland, 1993, pp. 45–57.
57. Walter, A., Romheld, V. and Mori, S., Is the release of phytosiderophores in zinc-deficient wheat plants a response to impaired iron utilization? *Physiol. Plant.*, 1994, **92**, 493–500.
58. Takagi, S., Naturally occurring iron-chelating compounds in oat and rice-root washings. *Soil Sci. Plant Nutr.*, **22**, 423–433.
59. Iwashita, T., Mino, Y., Naoki, H., Sugiura, Y. and Nomoto, K., High-resolution proton nuclear magnetic resonance analysis of solution structures and conformational properties of mugineic acid and its metal complexes. *Biochemistry*, 1983, **22**, 4842–4845.
60. Mori, S., Nishizawa, N., Kawai, S., Sato, Y. and Takagi, S., Dynamic state of mugineic acid analogous phytosiderophores in Fe deficient barley. *J. Plant Nutr.*, 1987, **10**, 1003–1011.
61. Takahashi, M., Yamaguchi, H., Nakanishi, H., Shirori, T., Nishizawa, N. K. and Mori, S., Cloning two genes for nicotianamine amino transferase, a critical enzyme in iron acquisition (strategy II) in graminaceous plants. *Plant Physiol.*, 1999, **121**, 947–956.
62. Kissel, M., Eisenmangel-induzierte Abgabe von phytosiderophoren aus Gerstenwurzeln als effizienter Mechannismus zur Eisenmobilisierung. PhD thesis, University of Hohenheim, Stuttgart, Germany, 1987.
63. Hopkins, B. G., Whitney, D. A., Lamond, R. E. and Jolley, V. D., Phytosiderophore release by sorghum, wheat and corn under zinc deficiency. *J. Plant Nutr.*, 1998, **21**, 2623–2637.
64. Singh, Bhupinder, Singh, B. K., Kumar, J., Yadav, S. S. and Garg, N., Effects of salt stress on growth, nodulation, nitrogen and carbon fixation of ten genetically diverse lines of chick-pea. *Indian J. Plant Physiol.*, 2003 (in press).
65. Erenoglu, B. *et al.*, Phytosiderophore release does not correlate well with zinc efficiency in different bread wheat genotypes. *J. Plant Nutr.*, 1996, **19**, 1569–1580.
66. Pedlar, J. F., Parker, D. R. and David, E. C., Zinc deficiency-induced phytosiderophore release by the triticaceae is not consistently expressed in solution culture. *Planta*, 2000, **211**, 120–126.
67. Epstein, E., *Mineral Nutrition of Plants: Principles and Perspectives*, Wiley, New York, 1972.
68. Rengel, Z., Root exudation and microflora populations in phase of crop genotypes differing in tolerance to micronutrient deficiency. *Plant Soil*, 1997, **196**, 255–260.
69. Erenoglu, B., Cakmak, I., Romheld, V., Derici, R., Rengel, Z., Uptake of zinc by rye, bread wheat and durum wheat cultivars differing in zinc efficiency. *Plant Soil*, 1999, **209**, 245–252.
70. Erenoglu, B., Nikolic, M., Romheld, V. and Cakmak, I., Uptake and transport of foliar applied zinc (^{65}Zn) in bread and durum wheat cultivars differing in zinc efficiency. *Plant Soil*, 2002, **241**, 251–257.
71. Pearson, J. N. and Regnel, Z., Uptake and distribution of ^{65}Zn and ^{54}Mn in wheat grown at sufficient and deficient levels of Zn and Mn during vegetative growth. *J. Exp. Bot.*, 1995, **46**, 833–839.
72. Haslett, B. S., Reid, R. J. and Rengel, Z., Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. *Ann. Bot.*, 2001, **87**, 379–386.
73. Hajiboland, R., Singh, B. and Romheld, V., Retranslocation of Zn from leaves as important contributing factor for zinc efficiency of rice genotypes. In *Plant Nutrition – Food Security and Sustainability of Agro-ecosystems* (eds Horst, W. J. *et al.*), Kluwer, Dordrecht, The Netherlands, 2001, pp. 226–227.
74. Erenoglu, B., Romheld, V. and Cakmak, I., Retranslocation of Zn from older leaves to younger leaves and roots in wheat cultivars differing in Zn efficiency. In *Plant Nutrition – Food Security and Sustainability of Agro-ecosystems* (eds Horst, W. J. *et al.*), Kluwer, Dordrecht, The Netherlands, 2001, pp. 224–225.
75. Vallee, B. L. and Wacker, W. E. C., Metalloproteins. In *The Proteins. Composition, Structure and Function* (ed. Neurath, H.), Academic Press, New York, 1970, vol. V, 2nd edn, pp. 25–60.
76. Bar-Akiva, A., Functional aspects of mineral nutrients in use for the evaluation of plant nutrient requirement. *Recent Advances in Plant Nutrition* (ed. Samish, R. M.), Gordon and Breach, New York, 1971, vol. 1, pp. 115–139.
77. Graham, R. D., Reed, M. L., Patterson, B. D., Hockley, D. G. and Dwyer, M. R., Chemical properties, distribution and physiology of plant and algal carbonic anhydrases. *Ann. N.Y. Acad. Sci.*, 1984, **429**, 222–237.
78. Makino, A., Sahashita, H., Hidema, J., Mae, T. Ojima, K. and Osmond, B., Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to the CO_2-transfer resistance. *Plant Physiol.*, 1992, **100**, 1737–1743.
79. Gibson, T. S. and Leece, D. R., Estimation of physiologically active zinc in maize by biochemical assay. *Plant Soil*, 1981, **146**, 241–250.
80. Guliev, N. M., Bairamov, S. H. M. and Aliev, D. A., Functional organization of carbonic anhydrase in higher plants. *Sov. Plant Physiol.*, 1992, **39**, 537–544.
81. Reed, M. L. and Graham, D., Carbonic anhydrase in plants: distribution, properties, and possible physiological functions. In *Progress in Phytochemistry* (eds Reinhold, L., Harborne, J. B. and Swain, T.), Pergamon Press, Oxford, 1980, vol. 7, pp. 47–94.
82. Sharma, P. N., Tripathi, A. and Bisht, S. S., Zinc requirement for stomatal opening in cauliflower. *Plant Physiol.*, 1995, **107**, 751–756.
83. Cakmak, I., Ozturk, L., Karanlik, S., Marschner, H. and Ekiz, H., Zinc-efficient wild grasses enhance release of phytosiderophore under zinc deficiency. *J. Plant Nutr.*, 1996, **9**, 551–563.
84. Higuchi, K., Watanabe, S., Takahashi, M., Kawasaki, S., Nakanishi, H., Nishizawa, N. K. and Mori, S., Nicotianamine synthase gene expression differs in barley and rice under Fe-deficient conditions. *Plant J. Cell Mol. Biol.*, 2001, **25**, 159–167.

Received 15 December 2003; revised accepted 6 September 2004