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Micronutrient Deficiency: A Global Challenge and Physiological Approach to Improve Grain Productivity under Low Zinc Availability

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ABSTRACT

Micronutrient deficiency in soils is a fast emerging phenomenon and a challenging abiotic stress in world agriculture. Most important micronutrients that the developing and developed world is concerned from point of view of sustaining grain productivity and malnutrition in human beings are iron and zinc. Biofortification of staple food crops with micronutrients by either breeding for higher uptake efficiency or fertilization can be an effective strategy to address widespread dietary deficiency in human populations. Cereal species greatly differ in their micronutrient efficiency (MiE), defined in this paper as the ability of a plant to grow and yield well under micronutrient deficiency. MiE generally has been attributed to the efficiency of acquisition of nutrients under conditions of their low soil availability rather than to its utilisation or (re)-translocation within a plant. A higher zinc and iron acquisition efficiency of genotypes could be attributed to either or all of the following; an efficient ionic metal uptake system, better root architecture i.e., long and fine roots with architecture favouring exploitation of micronutrients from larger soil volume, higher synthesis and release of metal mobilising phytosiderophore by the roots and uptake of Fe- and Zn-phytosiderophore complex. Seed Zn content has also been suggested to affect the respective MiE. Root morphology and characteristics and interaction between micronutrients and other ionic radicals have been implicated as determinants of MiE. This review attempts to examine critically the scanty and scattered reports available on status of micronutrient deficiency with special reference to Zn, globally; morphological, biochemical and physiological basis of regulation of MiE in cereals and approaches to improve MiE in terms of grain productivity and grain Fe and Zn *vis-à-vis* its bioavailability under conditions of poor micronutrient availability.

Keywords: iron, micronutrient deficiency, metal uptake and transport, phytosiderophore, seed micronutrient

Abbreviations: CA, carbonic anhydrase; **Fe**, iron; **MiE**, micronutrient efficiency; **PS**, phytosiderophore; **SOD**, superoxide dismutase; **Zn**, zinc

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INTRODUCTION: MICRONUTRIENTS

Agricultural crops in general, require 16 essential elements for their growth and development. The elements include carbon (C), hydrogen (H) and oxygen (O), which are derived from air and water and comprise 94.0 to 99.5% of the fresh plant tissue. The remaining nutrients used by plants come from soil in the form of inorganic salts. Depending on their requirement for plant metabolism the nutrients are classified as macro and micronutrients. The macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S) are taken up by plants either from native minerals or from fertilizers applied to soil. Legumes are an exception because they can also fix nitrogen from the air (Marschner 1995). There are some other essential elements that are needed by plants in relatively small amount "micronutrients" i.e., iron (Fe), zinc (Zn), boron (B), chlorine (Cl), copper (Cu), manganese (Mn) and molybdenum (Mo). Crops, in general, vary considerably in their response to various micronutrients. Brassicas and legumes are highly responsive to Mo and B, whereas corn and other cereals are more responsive to Zn and Cu. The availability and uptake of micronutrients by plants also depends on the growing micro environment and soil characteristics. Micronutrient deficiencies are more common in humid temperate regions, as well as in humid tropical regions, because of intense leaching associated with high precipitation. Soil pH is one of the most important factors affecting the availability of micronutrients to plants. The availability of micronutrients, in general, is

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Invited Review

Table 1 Micronutrient removed by plant tissues for obtaining good yields of various crops.*

Crops harvested and portion used for analysis		**Micronutients removed (kg/ha)**						
		Yield level (t/ha)	**Chlorine (Cl)**	**Boron (B)**	**Copper (Cu)**	**Iron (Fe)**	**Manganese (Mn)**	**Zinc (Zn)**
Alfalfa (*Medicago sativa*)	Hay	1.3	6	0.10	< 0.1	0.20	0.70	0.70
Barley (*Hordeum vulgare*)	Grain	4.0	8	0.10	< 0.1	0.30	0.10	0.10
	Straw	-	1	0.02	< 0.1	0.01	0.70	0.10
Corn (*Zea mays*)	Grain	9.5	2	0.70	< 0.1	0.20	0.10	0.20
	Straw	-	1	0.06	< 0.1	1.0	1.70	0.30
Cotton (*Gossypium* spp.)	Seed	2.5	-	0.012	0.011	0.140	0.019	0.480
Mung bean (*Vigna radiata*)	Seed	-	-	0.07	0.01	0.78	0.18	0.07
Flax (*Linum usitassimun*)	Seed	-	-	0.16	0.05	0.29	0.09	0.17
Oats (*Avena sativa*)	Grain	4.0	1	-	< 0.1	1.00	0.20	0.10
	Straw	-	1	-	< 0.1	0.20	0.20	0.40
Peas (*Pisum sativum*)	Vines, pods	-	-	0.07	< 0.1	0.70	0.50	0.10
Peanut (*Arachis hypogia*)	Nuts	2.0	-	0.025	0.060	0.480	0.040	0.050
Potato (*Solanum tuberosum*)	Tuber	-	-	0.28	0.14	3.10	0.18	0.70
Rapeseed (*Brassica* spp.)	Seed	3.0	-	0.025	0.017	0.150	0.090	0.050
Rice (*Oryza sativa*)	Grain	50	-	0.060	0.020	0.810	0.060	0.215
Soybean (*Glycine max*)	Seed	-	-	0.1125	0.1125	1.93	0.068	0.1125
Sunflower (*Helianthus annus*)	Seed	-	-	0.28	0.07	0.53	0.19	0.12
Wheat (*Triticum aestivum*)	Grain	4.0	6	0.06	< 0.1	0.50	0.20	0.20
	Straw	-	2	0.02	< 0.1	0.20	0.30	0.10

* Based on Epstein 1972; Khan and Nortcliffe 1982; Marschner 1995; Broadley *et al.* 2001, 2003, 2004; Chadha 2001; Grusak 2002; Sharma 2006; Gupta *et al.* 2008; Anonymous 2009; Yang *et al.* 2009

reduced at high pH, with the exception of Mo whose availability increases as soil pH increases. Micronutrient removal by different tissues in some important crops is given in **Table 1** (Epstein 1972; Khan and Nortcliffe 1982; Marschner 1995; Broadley *et al.* 2001, 2003; Grusak 2002; Broadley *et al.* 2004; Sharma 2006; Gupta *et al.* 2008; Khoshgoftarmanesh *et al.* 2010). Essentiality of Zn in plants was first shown in maize by Maze (1915) and subsequently in barley and sunflower (Sommer and Lipman 1926). Based on the concentration, the plant could be classified as deficient or sufficient or toxic for a mineral (Kramer 2005). Like, in most crops the optimal leaf Zn concentration required to maintain adequate growth works out to be 15-20 mg Zn Kg^{-1}on dry mater basis (Alloway 2007). However, to maintain an optimum mineral nutrient concentration a plant has to overcome negative influences and other influential factors like genotype, soil nutrient availability, rhizospheric modifications, abiotic stress, mineral uptake and utilization efficiency, etc. Even after achieving a mineral nutrient rich produce, the task of improving their bioavailability poses a greater challenge (Lyons *et al.* 2008). Micronutrient bioavailability in human and animal populations is poor and depends chiefly on inter-nutrient interaction, phytate level, binding proteins besides several others (Andreini *et al.* 2006). No doubt that micronutrient deficiency is a global phenomenon affecting the lives of more than two billion people. These deficiencies increase the risk of severe disease in approximately 40% of the world's population (Welch and Graham 2004; Merchant 2010). Further, Fe, Zn, and vitamins A, B, and C have immunomodulating functions and thus influence the susceptibility of a host to infectious diseases and their courses and outcomes (Failla 2003).

Micronutrient in soils

Soils vary widely in their micronutrient content and in their ability to supply micronutrients in quantities sufficient for optimal crop growth. Micronutrient deficient soils are alarmingly widespread across the globe, and the problem is aggravated by high sensitivity of most of the modern cultivars of major crops to low micronutrient levels. A crude estimate suggests that more than 50% of the soils globally are deficient of one or the other nutrient (Sillanpaa 1982; Salwa *et al.* 2010). However, recent advances including the global positioning system (GPS), geographic information systems (GIS), inductively coupled plasma (ICP) spectrometry, geostatistics, and precision agriculture can be used to construct an accurate soil micronutrient mapping and provide quantitative support for decision and policy makers to devise and improve agricultural approaches for a balanced micro-nutrition of crops (White and Zasoski 1999).

Table 2 Micronutrient levels in soils.*

Micronutrient	**Deficient**	**Medium**	**Adequate**
Boron (hot water extractable, ppm)	0.0 - 0.4	0.5 - 1.2	> 1.2
Chlorine (water Extractable, ppm)	0.0 - 8.0	-	-
Copper (DTPA extractable, ppm)	0.0 - 0.2	0.3 - 1.0	> 1.0
	0.0 - 0.5	0.6 - 1.0	> 1.0
	0.0 - 2.5	-	> 2.5
Iron (DTPA extractable, ppm)	0.0 - 2.0	2.0 - 4.5	> 4.5
Manganese (DTPA extractable, ppm)	0.0 - 1.0	-	> 1.0
Molybdenum (ppm)	0.0 - 0.4	0.4 - 0.50	>0.5
Zinc (DTPA extractable, ppm)	0.0 - 0.5	0.5 - 1.0	> 1.0

* Based on Sillanpaa 1990; Prasad *et al.* 2005; Wei *et al.* 2006; Nadeem *et al.* 2009; Akporhonor *et al.* 2009; Pirzadeh *et al.* 2010

The micronutrient content of the soil depends greatly on the parent material from which the soil developed (**Table 2**) and on the soil forming processes (Sillanpaa 1990; Prasad *et al.* 2005; Wei *et al.* 2006). Two sources of readily available micronutrients exist in soil such as nutrients that are adsorbed onto soil colloids (very small soil particles) and nutrients that are in the form of salts dissolved in the soil solution. Their total level is rarely indicative of plant availability which depends on soil pH, organic matter content, adsorptive surfaces, and other physical, chemical, and biological conditions in the rhizosphere (Grusak 2002; Pati and Mukhopadhyay 2010). Organic matter is also an important secondary source of some micronutrients (Dabkowska-Naskret 2003; Hoffland *et al.* 2006). Most micronutrients are held tightly in complex organic compounds and may not be readily available to plants. However, they can be an important source of micronutrients when they are slowly released into a plant available form as the organic matter decomposes (Rico *et al.* 2009).

Assessment of micronutrient status of plants

Diagnosing a micronutrient deficiency can be at times difficult and tricky as symptoms induced by insect infestation and those in response to certain chemicals could cause misinterpretation of visual deficiency symptom assessment. Before concluding ones assessments, one must ensure that

Table 3 Response* of crops to micronutrient fertilizers.**

Crop	Boron	Copper	Manganese	Molybdenum	Zinc	Iron
Alfalfa (*Medicago sativa*)	High	Medium	Low	Medium	Low	—
Asparagus (*Asparagus officinalis*)	High	Medium	Low	Medium	Low	Medium
Mustard (*Brassica juncea*)	High	High	Medium	High	Medium	—
Broccoli (*Brassica oleracea*)	High	Medium	Medium	High	—	High
Backwheat (*Fagopyrum tataricum*)	Medium	Medium	Medium	Medium/High	Low	—
Barley (*Hordeum vulgare*)	Medium	Low	Low	Low	Low	—
Cabbage (*Brassica oleracea* var. *capitata*)	Low	Medium	Medium	—	—	Medium
Cauliflower (*Brassica oleracea* var. *botrytis*)	Medium	Medium	Medium	Low	Low	High
Celery (*Apicum graveolens*)	High	Medium	Medium	High	—	—
Corn (*Zea mays*)	High	Medium	Medium	Low	—	—
Cucumber (*Cucumis sativa*)	Low	Medium	Medium	Low	High	—
Carrot (*Daucus carota*)	High	Medium	Medium	Medium	Medium	—
Canola (*Emblica officinalis*)	Medium	Medium	Medium	Medium	Low	—
Flax (*Linum usitassimun*)	Low	Medium	Medium	Low	Medium	—
Grasses (*Graminae* spp.)	High	Medium	Low	Medium	Low	—
Kidney bean (*Phaseolus vulgaris*)	Low	Medium	Medium	Low	Medium	—
Oat (*Avena sativa*)	Low	Low	Medium	Low	Low	Medium
Onion (*Allium cepa*)	Low	Medium	High	Low	Low	—
Lettuce (*Lactuca sativa*)	Medium	High	High	Medium	High	—
Lupin (*Lupinus* spp.)	Medium	High	High	High	Medium	—
Melon (*Cucumis melo*)	Low	Low	Low	Medium	Medium	—
Pasture	Low	Low	Medium	Low	Low	—
Pea (*Pisum sativum*)	High	Medium	Low	High	Low	—
Pepper (*Piper nigrum*)	Low	Low	Medium	Medium	Low	—
Potato (*Solanum tuberosum*)	Low	Low	High	Medium	Low	—
Pumpkin (*Curcurbita moshata*)	Low	Low	Medium	Low	Medium	—
Red clover (*Trifolium pratense*)	Low	Low	Medium	Low	Low	—
Rye (*Secale cereale*)	Medium	Medium	Low	Medium	Low	Low
Sorghum (*Sorghum bicolor*)	Low	Medium	High	Low	High	—
Soybean (*Glycine max*)	Low	Medium	High	Low	Medium	—
Spinach (*Spinacea oleracea*)	Low	Low	High	Medium	Medium	High
Sugar beet (*Beta vulgaris*)	Medium	Medium	High	Medium	Medium	High
Sunflower (*Helianthus annus*)	Medium	High	High	High	High	—
Sweet corn (*Zea mays* cv. Rugosa)	Low	Medium	Medium	Low	High	Medium
Tobacco (*Nicotiana tabacum*)	Medium	Low	Medium	—	Medium	—
Tomato (*Lycopersicon esculantum*)	High	High	Medium	Medium	Medium	High
Triticale (*Triticum secale*)	High	High	Medium	Medium	Medium	—
Wheat (*Triticum aestivum*)	Medium	Medium	—	—	—	—

* Certain crops and even varieties may vary in sensitivity to micronutrient deficiencies. Highly responsive crops often respond to micronutrient fertilizer if the micronutrient concentration in the soil is low. Medium responsive crops are less likely to respond and low responsive crops do not usually respond even at the lowest micronutrient levels.
** Based on Singh and Balasubramanian 1983; El-Fouly 1987; Ishag 1992; Abosetugn 1993; Soomro *et a*l. 2000; Chadha 2001; Gupta 2005; Lisuma *et al.* 2006; FAO databases (various); Alloway 2008; Anonymous 2009; Cakmak 2010

Table 4 Optimal level of micronutrient in different crops at whole plant level prior to flowering (in ppm).*

Crop	Boron	Copper	Iron	Manganese	Molybdenum	Zinc
Alfalfa (*Medicago sativa*)	31-80	11-30	31-250	31-100	1.0 -5.0	21-70
Corn (*Zea mays*)	4-25	6-20	21-250	2.0-150	1.0-2.0	20.70
Potato (*Solanum tuberosum*)	15-40	7-30	30-300	30-200	0.5-4.0	30-100
Broad leaf vegetable (spinach)	30-60	8-20	50-250	30-200	0.5-5.0	30-100
Soybean (*Glycine max*)	21-55	10-30	51-350	21-100	1.0-5.0	21-50
Sugar beet (*Beta vulgaris*)	26-80	11-40	51-200	21-150	0.15-5.0	19-60
Cucurbits (*Cucurbitacae* sp.)	30-60	8-20	50-250	30-200	0.5-5.0	30-100
Wheat (*Triticum aestivum*)	6-40	6-50	11-300	16-200	0.03-5.0	21-70

* Based on Chadha 2001; Sharma 2006; Alloway 2007; Anonymous 2009

poor crop growth is not the result of a macronutrient deficiency, drought, salinity, disease or insect problem, herbicide injury or some other physiological disorder and only then one should determine mineral nutrient deficiency based on visual symptoms (Grusak 2002). In addition to the visual deficiency symptoms, an analysis of the soil and plant samples for their micronutrient profile could complement and support visual assessment (Augusto *et al.* 2009). Crop response to a micronutrient deficiency and its correction can be achieved by applying micronutrient to a specific, clearly marked out affected area of land or go in for foliar sprays to correct deficiency wherein dose of application will vary from crop to crop and on growth stage (Alloway 2007). Additionally the response to different crops to micronutrient application varies and greatly depends upon its requirement for growth that differs with crop and stage of growth. Data in **Table 3** summarizes the response of crops to different micronutrient fertilizers and based on which the crops were classified as insensitive ('low') to 'high' responsive for different micronutrients (Singh and Balasubramanian 1983; El-Fouly 1987; Ishag 1992; Abosetugn 1993; Soomro *et al.* 2000; Gupta 2005; Lisuma *et al.* 2006; FAO Databases (various); Alloway 2008). Optimal micronutrient requirements in crop species, prior to flowering stage, are summarized in **Table 4** (Sharma 2006; Alloway 2007, 2008).

Micronutrient deficiency symptoms

Certain micronutrients have characteristic deficiency symptoms. However, symptoms can be easily confused with other nutrient deficiencies, salinity, disease, drought, herbicide injury or other physiological problems. Visual symptoms alone are not a reliable method of determining a

Table 5 Visual micronutrient deficiency symptoms*, ** in different crops***.

Elements	Crops	Deficiency symptoms
Boron	Barley (*Hordeum vulgare*)	Stems appear swollen, leaves develop necrotic margins and break; growing points die and young growths fail to expand. Heads distorted and dwarfed.
	Broadbean (*Vicia faba*)	Stems somewhat stiff; growing points die and lateral growths develop from bases of shoots; leaves slight intervenal chlorosis.
	Cabbage (*Brassica oleracea*)	Longitudinal section showing necrosis and splitting of pith.
	Carrot (*Daucus carota*)	Growth of young leaves restricted giving a rosette effect, older leaves orange tints; growing point may die.
	Cauliflower (*Brassica oleracea* var. *botrytis*)	Browning of curd and lesions in pith.
	Clover (*Trifolium alexandrinum*)	Stem thickened and stiff; growing point killed, and young growths distorted; older leaves, marginal aeas high purple and red tints.
	Flax (*Linum usitassimun*)	Thickened and stiff; tip leaves yellow and fail to expand; growing points die.
	Pea (*Pisum sativum*)	Stems thickened and stiff, growth squat and bushy habit; foliage chlorotic, young leaflets small and tips brown; growing points die.
	Potato (*Solanum tuberosum*)	Growth stunted; growing point killed; leaves dull grayish green, changing to yellow before dying off.
	Sugarbeet (*Beta vulgaris*)	Early stage of boron deficiency. Young leaves distorted and fail to expand.
	Tomato (*Lycopersicon esculentum*)	Stems stiff; terminal buds die and growths die back; lateral shoots developed, giving plant flat top; leaves highly tinted purple, brown and yellow. Fruits pitted and corky areas in skin; ripening uneven.
	Wheat (*Triticum aestivum*)	Terminal shoots die; leaves die back from tips and young leaves remain rolled. (Similar to calcium deficiency)
Copper	Wheat (*Triticum aestivum*)	Head may fail to emerge; where formed, grain production restricted. Tips of heads chlorotic and distorted. Leaves subtending heads slightly chlorotic and deformed and in most severe specimens from spirals.
Iron	Apple (*Malus malus*)	Tip leaves chlorotic; small veins show as fine network in early stages; margins develop brown patches.
	Broccoli (*Brassica oleracea*)	Chlorosis of leaves beginning as a chlorotic mottling.
	Cabbage (*Brassica oleracea* var. *capitata*)	Severe chlorosis of leaves beginning as a chlorotic mottling.
	Cauliflower (*Brassica oleracea* var. *botrytis*)	Restricted lateral growth of leaf laminae and curling and brown spotting of margins.
	Cherry (*Prunus cerasus*)	Severe chlorosis; veins, including small sublaterals prominent green; occasional brown marginal patches.
	Clover (*Trifolium alexandrinum*)	Younger leaves strongly chlorotic.
	Flax (*Linum usitassimun*)	Chlorosis, especially near tips.
	Gooseberry (*Rubus raspberry*)	Terminal leaves strongly chlorotic.
	Oat (*Avena sativa*)	Young leaves severe chlorosis; chlorosis begins as intervenal stripes. Young leaves severe chlorosis; chlorosis begins as intervenal stripes.
	Pear (*Pyrus communis*)	Leaves severely chlorotic and brown marginal areas; fruits ground color, pale and highly flushed
	Plum (*Prunus domestica*)	Leaves severely chlorotic, with fine sublateral veins showing green in early stages. Tip foliage strongly chlorotic
	Potato (*Solanum tuberosum*)	Young leaver strongly chlorotic; veins may remain green; margins and tips brown patches
	Raspberry (*Rubus disambiguation*)	Terminal leaves severe chlorosis followed by marginal and intervenal necrosis.
	Strawberry (*Fragaria ananosa*)	Leaves severe chlorosis; green network of fine veins distinct in early stages.
	Sugarbeet (*Beta vulgaris*)	Younger leaves chlorotic mottling.
	Tomato (*Lycopersicon esculantum*)	Tip leaves, especially basal areas of leaflets, intense chlorotic mottling; stem near tip also yellow.
	Wheat (*Triticum aestivum*)	Severe chlorosis of leaves, most severe on younger growths; die-back of chlorotic leaves.
Manganese	Apple (*Malus malus*)	Leaves severe chlorosis over most of tree; young leaves of terminal shoots not as severely affected as older leaves.
	Barley (*Hordeum vulgare*)	Leaves slightly chlorotic especially near tips, and show intervenal brown spots.
	Broadbean (*Vicia faba*)	Brown lesions in centers of cotyledons.
	Cabbage (*Brassica oleracea* var. *capitata*)	Leaves severe intervenal chlorotic marbling and necrosis.
	Cherry (*Prunus cerasus*)	Smooth intervenal chlorosis beginning at margins and progressing towards midrib; fine sublateral veins obliterated.
	Clover (*Trifolium alexandrinum*)	Young leaflets, margins chlorotic followed by fine brown spotting and scorching.
	Flax (*Linum usitassimun*)	Yellowing of tip leaves.
	Gooseberry (*Rubus raspberry*)	Somewhat faint intervenal chlorosis as well defined pattern.
	Oat (*Avena sativa*)	Irregular, grayish-brown lesions, which coalesce and bring about collapse of leaf (gray speck symptoms.
	Pea (*Pisum sativum*)	Intervenal chlorosis beginning at margins. Brown lesions in centers of cotyledons (Marsh Spot).
	Pear (*Pyrus communus*)	Somewhat faint intervenal chlorosis beginning near margins.
	Clover (*Trifolium alexandrinum*)	Intervenal chlorotic yellowing beginning near margins and progressing towards midrib as well defined smooth pattern
	Potato (*Solanum tuberosum*)	Leaves from near tips of shoots still green, but showing characteristic brown spotting, mainly along veins.
	Raspberry (*Rubus disambiguation*)	Leaves intervenal chlorosis beginning near margins and progressing towards midribs as well defined pattern; terminal leaves green or less chlorotic than older leaves, in contrast to iron deficiency.
	Rye (*Secale cereale*)	Leaves intervenal chlorotic strips and intervenal white streaky lesions.
	Strawberry (*Fragaria ananosa*)	Somewhat faint intervenal chlorosis beginning at margins and progressing towards midrib; where severe, chlorotic areas yellowish appearance.

Table 5 (Cont.)

Elements	Crops	Deficiency symptoms
Manganese	Spinach (*Spinacea oleracea*)	Leaves severe chlorosis.
	Sugarbeet (*Beta vulgaris*)	Leaves severe "Speckled Yellows" and leaf margins curled forward.
	Tomato (*Lycopersicon esculentum*)	Intervenal chlorotic mottling distributed over entire surfaces of leaflets; mottled areas become necrotic.
	Wheat (*Triticum aestivum*)	Intermittent intervenal chlorotic streaks.
Molybdenum	Cabbage (*Brassica oleracea* var. *capitata*)	Leaves cupped and show chlorotic mottling, especially around margins; tips and margins develop dead patches; plants fail to heart
	Cauliflower (*Brassica oleracea* var. *botrytis*)	Leaves slightly cupped and show chlorotic mottling; tips develop dead patches; cotyledons remain dark green. (Similar to manganese toxicity).
	Tomato (*Lycopersicon esculentum*)	Leaflets somewhat chlorotic, strongly incurled and die back from tips.
Zinc	Apple (*Malus malus*)	Buds along shoots fail to develop, leaves small and narrow ("Little Leaf" condition) and tend to form rosettes at tips of shoots.
	Wheat (*Triticum aestivum*)	Whitish lesions.

*Appearance of deficiency symptoms depended on tolerance and susceptibility of a crop to low availability of the micronutrient

** Chlorine deficiency-symptoms are rarely observed

***Based on Arnon and Stout 1939; Bowen 1979; Marschner 1995; Loneragan 1997; Grusak 2002; Chadha 2001; Berry 2006; Sharma 2006; Singh *et al*. 2007; Alloway 2007; Anonymous 2009

micronutrient, problem, but they are useful indicators when used with other diagnostic tools. Among edible crops, wheat, tomato and cabbage are sensitive to most of the micronutrient deficiencies. A guide to the typical micronutrient deficiency symptoms observed in different crops is presented in **Table 5** (Arnon and Stout 1939; Bowen 1979; Marschner 1995; Loneragan 1997; Grusak 2002; Berry 2006; Sharma 2006; Singh *et al*. 2007; Alloway 2007). While a list of typical features that correspond to the deficiency of respective micronutrients is given below.

Iron

Chlorosis of the younger leaves characterizes an iron deficiency. The tissue between the veins gradually turns yellow, while the veins tend to stay green. The tips and margins of some leaves may turn brown and become dry and brittle (Diaz *et al.* 2009).

Zinc

Zn is partly mobile in cereals, wherein the symptoms of Zn deficiency vary from pale yellow chlorotic patches, on middle leaves, to grey or dark brown necrotic areas on the whole leaf. Zn deficient plants are stunted. Younger leaves will have a more normal healthy green color but are smaller. Old leaves remain dark green and appear healthy. A Zn deficiency prevents the elongation of internodes and leaves, which results crowding of leaves together at the top in a fan-shaped ‘rosette’ appearance (Wissuwa *et al.* 2006; Singh *et al.* 2007).

Molybdenum

Molybdenum deficiency symptoms are similar to those of nitrogen. Most conspicuous symptom is whiptail in leaves of *Brassica* species wherein the leaf lamina fails to grow and the petiole gives a whip like appearance (Weir 2004).

Manganese

Manganese is partly mobile in cereals. White to grey flecks or specks first appear and become more severe on mature leaves about halfway up the shoot. If a deficiency persists, symptoms spread to old leaves then to the youngest leaves. In wheat and barley, affected young leaves frequently turn pale green and have a limp or wilted appearance as manganese is not readily transferred from old to young leaves. Small white to grey spots, specks or strips appear a short distance from the end of the leaf tip on young leaves (Staff 2009).

Boron

Dark brown, irregular lesions appear, followed by pale yellow chlorosis of young leaves in response to boron deficiency. Stems are short and growing points may die. In canola, the symptoms of a boron deficiency can be confused with a sulphur deficiency. In alfalfa, boron deficiency symptoms include death of the terminal bud, rosetting, yellowtop and poor flowering. When a boron deficiency is moderate, seed yield is often reduced without any evidence of severe deficiency symptoms during vegetative growth (Staff 2009).

Chlorine

This deficiency is very rare; therefore, symptoms are seldom observed. Symptoms may include stubby roots, some chlorosis of new leaves and plant wilting (Berry 2006).

Copper

In wheat copper deficiency causes yellowing of younger leaves, limpness, wilting, curling of the upper leaves and necrosis of the leaf tips. Excessive tillering, aborted heads, delayed maturity and poor grain filling resulting in a high straw to grain ratio are also observed under deficiency. Since copper is not readily transferred from old to young leaves, older leaves remain darker and relatively healthy while younger leaves show symptoms (Berry 2006).

ZINC DEFICIENCY: A GLOBAL CONCERN

Low availability of Zn in calcareous soils is one of the widest ranging a biotic stresses in the 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 (Sillanpaa 1982; Cakmak 2004, 2010). It is one of the most widespread nutritional constraint in crop plants especially in cereals (Marschner 1995; Cakmak *et al.* 1996a, 1996b, 1996c; Welch and Graham 2004; Martin *et al*. 2007, Malakouti 2007; Cakmak 2010). Among cereals, wheat, in particular, suffers from Zn deficiency in large areas of the world particularly in Turkey, Australia, China, and India. In India, presently, up to 50% of the agricultural land has been reported to be Zn deficient (Singh *et al* 2005; Alloway 2007). Use of high yielding varieties and high input approach, in the last few decades, has resulted in wide spread nutrient imbalance in the cereal growing tracts of India. Correction of soil Zn deficiency through addition of Zn fertilisers is neither economical nor environmental friendly as only 20% of the applied Zn is available for plant uptake while the remainder gets adsorbed on soil minerals and is, therefore, rendered immobile. However, a recent report by Cakmak (2010) shows that biofortification of cereals with Fe and Zn is possible through fertilization strategy. He advocates that by improving the N availability micronutrient accumulation in cereals can bge enhanced. Further, grain yield reductions, up to 80%, along with reduced grain Zn concentrations have been observed under Zn deficiency (Welch 2001; Alloway 2004). This has serious implication for human health in countries where cereal-based diets predominate. Development of crop plants that are efficient Zn accumulators is, therefore, a potentially important endeavour. Additionally, plants grown on Zn-deficient soils tend to accumulate heavy metals which again are a potential human health hazard (Hart *et al.* 1998; Korkmaz *et al.* 2010). There is a need for selection and /or breeding of plant genotypes with higher resistance to Zn deficiency in terms of both a higher grain yield and higher grain Zn content (Welch and Graham 2004; Ghandilyan *et al.* 2006; Ahmadi 2010). Realisation of this approach is plausible in view of the large genotypic differences in Zn acquisition among crop plants particularly when its availability to the roots is limited (Rengel and Graham 1995a, 1995b). Differences in susceptibility to Zn deficiency among cereal genotypes are known and attributed chiefly to the differential capacity of genotypes in acquisition of Zn from low Zn soils (Genc *et al.* 2006). Zn efficiency, defined, herein, as the ability of a plant to grow and yield well in Zn deficient conditions, varies among cereal species (Singh *et al.* 2002, 2006; Sudhalakshmi *et al.* 2007; Ghasemi-Fasaei and Ronaghi 2008; Chen *et al.* 2009). Considerable progress has been made in identification of adaptive mechanisms which plant species and genotypes have developed for efficient Zn acquisition from soils low in Zn availability (Rengel and Romheld 2000; Genc *et al.* 2006; Bashir *et al.* 2006; Song *et al.* 2006; Martin *et al.* 2007; Suzuki *et al.* 2008). Genotypic differences in Zn efficiency have been attributed to various mechanisms operative in the rhizosphere (Pati and Mukhopadhyay 2010) and within the plant, which include higher uptake of Zn by roots, protection against superoxide free radicles, efficient utilisation and (re)-translocation of Zn from older to younger growing parts (Hart *et al.* 1998; Rengel and Romheld 2000; Hajiboland *et al.* 2001). Cakmak *et al.* (1994) showed that Zn efficiency of cereals was mainly related to difference in acquisition of Zn by roots. Thus, there is a need to understand the physiological and biochemical processes that control Zn acquisition by roots under Zn deficiency. Zn acquisition by plants is one of the less thoroughly studied aspects of plant nutrition, and there are scattered literatures on the biochemical, morphological, and physiological effects of Zn deficiency on plants. This review provides an overview of the physiological and biochemical adaptations of cereals, to acquire and utilise Zn under conditions of limited Zn availability (Graham and Rengel 1993) have mooted that more than one mechanism could be responsible for establishing Zn efficiency in a genotype. It is likely that different genotypes subjected to Zn deficiency under different environmental conditions will respond by, one or more, different efficiency mechanisms (Hacisalihoglu *et al.* 2001; Singh 2001; Genc *et al.* 2006; Ahmadi 2010; Cakmak 2010). There are several key attributes in a plant that can play a significant role in determining zinc efficiency or zinc deficiency tolerance of crops. These involve root characteristics like root volume, root mass, root surface area and root length, which can influence bioavailability of Zn from soil, Zn uptake by root (Dong *et al.* 1995; Rengel *et al.* 1998) and its translocation from root to shoot (Kochian 1993); altered sub cellular compartmentation of Zn in shoot cells; and more efficient utilization of Zn in shoots at biochemical level. Zn uptake by roots could also be influenced by phytosiderophore release in cereal species (Rengel 2001). It also appears that root cationic Zn uptake is mediated by two different transport systems i.e., a high velocity, low affinity system (Km = 2-5 μM) and a low velocity, high affinity system (Km = 0.6-2 nM). Later is the probable dominant transport system on low available Zn soils (Hacisalihoglu *et al.* 2001). All organisms have homeostatic mechanisms to maintain the required concentration of essential nutrients for optimal biochemical and physiological functioning. Cellular Zn homeostasis is complex and is regulated by overlapping influence of transport, chelation, trafficking and sequestration (Clemens 2001). The contention is supported by the observation of Cakmak *et al.* (1999) and Torun *et al.* (2000), who found no difference in shoot Zn concentration but found clear contrasting Zn deficiency symptoms between efficient and inefficient genotypes when grown on Zn deficient soil or solution. This could happen due to difference in efficiency for internal utilization of Zn. Radiotracer studies, using ^{65}Zn on subcellular compartmentation of Zn have revealed that about 8% root Zn is in the cytoplasm, 76% in the vacuole while the remaining is in the cell wall. Further, roots of Zn-efficient and wheat cultivars were found to have a similar level of Zn in cytoplasm and vacuole thus suggesting that subcellular Zn compartmentation may have a limited role in zinc efficiency (Maria and Cogliatti 1988; Hacisalihoglu and Kochian 2003). It may however, be a genotype-dependent characteristic. One or more of the above mechanisms may regulate zinc efficiency difference between crop species. Role of mycorrhiza and the functional significance of this symbiosis is worth investigating (Chen *et al.* 2003). The above regulatory and adaptive approaches to zinc deficiency tolerance have been discussed at length later in this review.

Zn in soil

Zn deficiency is common on neutral and calcareous soils, intensively cropped soils, paddy soils and very poorly drained soils, sodic and saline soils, peat soils, soils with high available phosphorous and silicon, sandy soils, highly weathered acid and coarse-textured soils (Alloway 2009; Pati and Mukhopadhyay 2010). Factors such as topsoil drying, subsoil, disease interactions and high cost of fertilizer also contribute to Zn deficiency (Sillanpaa 1982). The critical soil levels for occurrence of Zn deficiency are between 0.6 mg Zn kg^{-1} to $^{2.0}$ mg Zn 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 HCO3^{-} in the soil solution. A ratio of more than 1 for exchangeable Mg, Ca in soil may also indicate Zn deficiency (Wei *et al.* 2006).

Most of the cultivable soils in India are Zn deficient (Cakmak 2004; Alloway 2007). 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 is depend upon the pH, soil organic matter, temperature, light intensity, crop species, etc. Zn deficiency is quite widespread in the Indo-Gangetic plains and other important cereal growing states like Punjab, Uttar Pradesh, etc which account for almost three fourth of countries food grain production. The total area under Zn deficiency is about 10M ha in India and approximately 85% of rice wheat system cropping takes place in Indo-Gangetic plain which has calcareous soils with high pH and thus low Zn availability. Improving production from this cereal belt is therefore, vital for sustaining grain production in the country (Sharma 2006). Zn occurs in soil as sphalerite, olivine, hornblende, augite and biotite however, availability of Zn from these sources is guided by several factors described above. On deficient soils, correction of Zn deficiency through addition of Zn fertilisers (**Table 6**) is a common practice (Bowen 1979; Martens and Westermann 1991; Mortvedt and Gilkes 1993; Srivastava and Gupta 1996; White and Zasoski 1999; Alloway 2007; Ahmadi 2010; Anonymous 2010). Assessing the transformation of Zn in maize cultivated neutral soils, Alvarej and Gonzalez (2006) found that highest amounts of Zn were taken up by the plants when Zn was applied as Zn−EDTA (20 mg kg^{-1}, 7.44 mg of Zn pot^{-1}; 10 mg kg^{-1} Zn rate, 3.93 mg of Zn pot^{-1}) or as Zn−EDDHA fertilizer (20 mg kg^{-1} Zn rate, 4.66 mg Zn pot^{-1}) and even after the maize crop was harvested, sufficient quantities of available Zn still re-

Table 6 Commonly used Zn fertilizers.*

Compound	Formula	Zn content (%)
Basic zinc sulphate	$ZnSO_4{\cdot}4Zn(OH)_2$	55
Chelated Zn (EDTA)	Zn (EDTA)	12
Disodium Zinc EDTA	$Na_2ZnEDTA$	8-14
Sodium Zinc HEDTA	NaZnHEDTA	6-10
Sodium Zinc EDTA	NaZnEDTA	9-13
Zinc carbonate	$ZnCO_3$	50-56
Zinc frits	-	10-30
Zn lignosulphonate		5-8
Zinc nitrate	$Zn(NO_3)_2{\cdot}3H_2O$	23
Zinc oxide	ZnO	50-80
Zinc oxysulphate	$xZnSO_4\,xZnO$	20-50
Zinc phosphate	$Zn_3(PO_4)_2{\cdot}4H_2O$	19.5
Zinc polyflavonoids	Organically bound Zn	10
Zinc sulphate heptahydrate	$ZnSO_4{\cdot}7H_2O$	22
Zinc sulphate monohydrate	$ZnSO_4{\cdot}H_2O$	36
Zincated urea	Zn+N	2 (Zn); 43 (N)

*Based on Bowen 1979; Martens and Westermann 1991; Mortvedt and Gilkes 1993; Srivastava and Gupta 1996; White and Zasoski 1999; Alloway 2007; Staff 2009; Anonymous 2010

mained in the soil (DTPA- or Mehlich-3-extractable Zn) to meet Zn requirement for another harvest. To exemplify it further, an application of 62.5 kg $ZnSO_4$ to first crop of the cereal based cropping system such as cotton-wheat, bajra-wheat and rice-wheat is sufficient to meet Zn requirement for three years or six crops. This practice is widely followed in several states of India. However, this approach is neither economical nor environmental 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 (Cappuyns *et al.* 2006). As only a small fraction of the applied Zn is utilised by the crop to which it is applied, Zn accumulation in agricultural soils is on the increase, which is an environmental concern. Regarding human Zn-nutrition, fortification of Zn in food is practiced but is expensive and difficult to implement in developing countries like India, Bangladesh, Nepal, etc. (Genc and McDonald 2004c). Development of crop plants that are efficient Zn accumulators especially under Zn deficiency is, therefore, a potentially important endeavour for improving Zn 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 (Graham *et al.* 1992; Genc *et al.* 2004b; Palmgren *et al.* 2008). Realisation 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 (Cakmak *et al.* 1998; Singh *et al.* 2005; Welch and Graham 2004; Alloway 2007).

Zn in human nutrition

Zinc is an essential micronutrient for human growth, development, and immune function. Zinc deficiency impairs overall immune function and resistance to infection (Fischer and Black 2004; Mafra and Cozzolino 2004). In biological systems, Zn is involved in activity of more than 300 enzymes. In these enzymes, Zn plays either catalytic, co-catalytic and structural roles. Zn also plays a critical role in synthesis of proteins and metabolism of DNA and RNA. There is also increasing evidence that several Zn containing proteins exist which affect gene expression directly. Symptoms resulting from zinc deficiency in human beings are as diverse as the enzymes with which the metal is associated. If chronic, severe, and untreated, zinc deficiency can be fatal. Less drastic symptoms include infections, hypogonadism, weight loss, emotional disturbance, dermatitis, alopecia, impaired taste acuity, night blindness, poor appetite, delayed wound healing, and elevated blood ammonia levels (Evans 1986; Bhowmik *et al.* 2010). The chief reason for realization of Zn deficiency symptoms is the poor diet consumption, but often the most severe symptoms result from other factors including excessive alcohol use, liver diseases, renal disease, and sickle cell disease. Hambidge (2000) emphasized the need to understand and develop a clinical spectrum of severe zinc deficiency, identify ideal biomarkers for defining milder zinc deficiency states and better understanding of human zinc metabolism and homeostasis (including its limitations) at a molecular, cellular, organ-system and whole body level and of factors that affect zinc bioavailability and potential strategies for the prevention and management of human zinc deficiency. Hunt (2003) reviewed the nutritional significance of vegetarian diets for human health and concluded that with elimination of meat and increased intake of phytate-containing legumes and whole grains, the absorption of both iron and zinc is lower with vegetarian than with nonvegetarian, diets. 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 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 immaturities, learning ability and immune functions and malformations in central nervous system (Welch 2001; Jeejeebhoy 2007; Cetin *et al.* 2009; Bhowmik *et al.* 2010). Low Zn availability also risks heart ailments (Lopez *et al.* 2008). These reports make it clear that while ingesting grams of Zn metal can be toxic; however, human beings cannot live without a few µg in their diet.

Zn in plant nutrition

Zn is an important micronutrient. Plant response to Zn deficiency occurs in terms of decrease in membrane integrity, susceptible to heat stress, decreased synthesis of carbohydrates, decreased cytochrome and nucleotide synthesis, decreased auxin synthesis, and decreased chlorophyll synthesis. Further, Zn-containing enzymes are also inhibited which includes Alcohol dehydrogenase, carbonic anhydrase, Cu-Zn-Superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase, and RNA Polymerase (Marschner 1995). Zn is also regarded as a novel intracellular second messenger (Yamasaki *et al.* 2007). Depending on Zn level, Zn deficiency status of plants can be classified as follows, < 10 mg kg^{-1}- definite Zn deficiency; between 10-15 mg kg^{-1}- very likely to be Zn deficient; between 15-20 mg kg^{-1} - likely Zn deficient; > 20 mg kg^{-1}- Zn-sufficient. The ratios of P, Zn and Fe, Zn in the shoot at tillering to the pod initiation stage are good indicators of Zn deficiency while, leaf Zn concentration is a less reliable indicator of Zn deficiency, except in extreme cases Leaf Zn concentration below 15 mg kg^{-1} is regarded as Zn-deficient. Based on available literature (Brennan *et al.* 1993; Marschner 1995; Srivastava and Gupta 1996; Brennan *et al.* 2002; Taiz and Zeiger 2006; Sharma 2006; Alloway 2007; Merchant 2010) critical concentrations of Zn in different crop plant tissues are compiled and depicted in **Table 7**.

ZN EFFICIENCY

Genotypic differences in Zn efficiency

Zn efficiency, defined herein, as the ability of a plant to grow and yield well under Zn deficient conditions, varies among cereal species (Graham and Rengel 1993; Erenoglu *et al.* 2000; Singh *et al.* 2006). Genotypic differences for Zn use efficiency have been reported for several crops species (Graham *et al.* 1992; Cakmak *et al.* 1994; von Wiren *et al.* 1994; Taylor and Macnair 2006; Sudhalakshmi *et al.* 2007). Physiological mechanism(s) conferring Zn efficiency and their relative significance on low Zn soils/solution culture has been investigated by several workers (Erenoglu *et al.* 2000; Rengel and Romheld 2000; Hacisalihoglu *et al.* 2001; Singh *et al.* 2002; Hacisalihoglu *et al.* 2003). Genotypic dif-

Table 7 Critical concentration of Zn in different plant tissues of cereals and other crops.*

Crop	Tissue	Critical concentration (mg Zn/Kg dry matter)
Alfalfa (*Medicago sativa*)	Top 15 cm at 1/10 bloom	20
Canola (*Brassica campestris*)	YFEL	20
Cassava (*Manihot utilissima*)	YEB 63 days after seeding	30
Corn (*Zea mays*)	Whole plant	22
	Upper 3rd leaf	16
Cotton (*Gossypium* spp.)	37 days after seeding YEB	11
Peanut (*Arachis hypogea*)	YFEL/early pegging	8-10
	Whole plant	15-22
Rice (*Oryza sativa*)	Whole plant	15
	Pre-flowering plant top	17.4
Sorghum (*Sorghum bicolor*)	Whole plant	8
	Blade 1	10
	Blade 5	25
Small grains (minor millets)	Head emergence	15
Soybean (*Glycine max*)	Most recently mature trifoliate at early bloom	20
Sugar beet (*Beta vulgaris*)	Leaves	20
Wheat (*Triticum aestivum*)	Shoot	24.5
	Pre-flowering plant top	14.5
	Whole plant	20-25
	Grain	12

* Based on Brennan *et al.* 1993; Marschner 1995; Srivastava and Gupta 1996; Brennan *et al.* 2002; Chadha 2001; Taiz and Zeiger 2006; Sharma 2006; Alloway 2007; Anonymous 2009

Table 8 Effect of Zn supply on the amount of Zn in shoots of different cereals grown for six weeks in a Zn-deficient soil.*

Cereals	Amount of Zn				Leaf symptoms of Zn deficiency (necrotic patches on leaf blades)
	(µg/shoot)		(µg/g dw)		
	-Zn	+Zn	-Zn	+Zn	
Barley (*Hordeum vulgare*)	5.7	69	8.4	62	Mild
Bread wheat (*Triticum aestivum*)	2.9	36	6.5	46	Mild
Durum wheat (*Triticum durum)*	1.8	39	6.4	47	Very severe
Einkorn wheat (*Triticum monococcum*)	-	-	7.9	47	Severe
Oats (*Avena sativa*)	2.3	42	6.3	38	Very severe
Rice (*Oryza sativa*)	22.0	119.8	1.9	6.5	Severe bronzing
Rye (*Secale cereale*)	6.7	40	9.0	46	Very slight or absent
Tausch's goatgrass (*Aegilops tauscii*)	-	-	7.0	41	Mild

* Based on Cakmak *et al.* 1997; Tolay *et al.* 2001; Singh 2001; Chadha 2001; Genc *et al.* 2006; Wissuwa *et al.* 2006; Anonymous 2009

ferences 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 acquisition of Zn from soils low in Zn availability (Marschner *et al.* 1986; Rengel and Romheld 2000; Hajoboland *et al.* 2001; Genc *et al.* 2006). These include, higher uptake of Zn (Zn^{2+}) by roots, protection against superoxide free radicals i.e., efficient antioxidative defense mechanism, efficient utilization and (re)-translocation of Zn (Cakmak *et al.* 1996c; Hart *et al.* 1998; Rengel and Romheld 2000; Singh *et al.* 2005). Research has shown (Cakmak *et al.* 1997; Singh 2001; Tolay *et al.* 2001; Genc *et al.* 2006; Wissuwa *et al.* 2006; Ahmadi 2010) that Zn efficiency of cereals is mainly related to difference in acquisition of Zn by root (Table 8). However, a higher Zn efficiency could also be achieved through greater distribution to younger parts and lower internal requirement (Genc *et al.* 2004a). Scrutiny of the available literature shows that physiological and biochemical processes that control Zn efficiency, in general, and Zn acquisition by roots, in particular, are one of the less thoroughly studied aspects of plant Zn-nutrition (Callahan *et al.* 2006). Graham and Rengel (1993) suggested that more than one mechanism could be responsible for establishing Zn efficiency in a genotype and it is likely that different genotypes subjected to Zn deficiency under different environmental conditions will respond by, one or more, different efficiency mechanisms (Rengel and Wheal 1997). While there is considerable genetic variation in tolerance to Zn deficiency (also known as Zn efficiency), phenotypic selection is difficult and would benefit from the development of molecular markers. Endeavors are being made to identify QTL linked to growth under low Zn and to those associated with the concentrations of Zn and Fe in leaf tissue and in the grain (Assunção *et al.* 2006; Genc *et al.* 2009).

CROP RESPONSE TO ZN DEFICIENCY

Symptoms of Zn deficiency

Zn-deficient plants, in general, show a marked reduction in plant height and develop whitish-brown patches which turn necrotic with increasing severity of deficiency. Zn deficient wheat plants show dusty brown spots on upper leaves of stunted plants, shoot growth more inhibited than root growth, uneven plant growth decreased tillering, increased spikelet sterility, chlorotic midribs particularly near the leaf base of younger leaves, leaves lose turgor and turn brown as brown blotches and streaks appear on lower leaves, enlarge, and coalesce. White line sometimes appears along the leaf midrib and leaf blade size is reduced (Cakmak *et al.* 1998). 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 8**) (Cakmak *et al.* 1998). In maize, Zn deficiency appears as a yellow striping of the leaves. Areas of leaf near the stalk may develop genera 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 way from the margin. In Zn deficient condition, the barley leaves show uniform chlorosis and drying and tip growth decreases. Deficiency symptoms in grain sorghum 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 color. Necrosis extends down the leaf and the remainder of the leaf is grey to bronze-green (Cakmak *et al.* 1998; Hacisalihoglu *et al.* 2003; Singh *et al.* 2005; Alloway 2007).

Plant growth under Zn deficiency

For a genotype to be Zn efficient, it should not only be able to absorb more Zn from deficient soils but should also produce more dry matter and grain yield. It, however, may not necessarily have the highest Zn concentrations in tissue or grain (Graham *et al.* 1992). It is evident from the available literature that the crop response to Zn deficiency in terms of dry mass production is very diverse and there is no unanimity in using root and shoot dry mass production or shoot/root ratio as an indicator for Zn efficiency of cereals under low Zn condition. Although both root and shoot growth are distinctly reduced under Zn deficiency (Graham *et al.* 1992; Dong *et al.* 1995), shoot dry weight is depressed to a greater extent than root dry weight (Rengel and Romheld 2000; Rengel *et al.* 1998; Natesan 2001; Sudhalakhsmi 2007; Ahmadi 2010; Widodo *et al.* 2010). Among wheat species, durum wheat is more sensitive to Zn deficiency than the bread wheat (Singh *et al.* 2006) as evident from the fact that the decline in shoot growth of Zn-sensitive durum wheat (kunduru) under Zn deficiency was much more than that of Zn-deficient tolerant Bezostaja, a bread wheat genotype (Singh *et al.* 2002). In some other cereal genotypes; however, root growth was enhanced under Zn deficiency (Cakmak *et al.* 1996a). Higher sensitivity of durum wheat to Zn deficiency was associated with higher root growth at the expense of shoot growth (Rengel and Graham 1995b). In nutrient solution experiments, decrease in shoot dry matter production induced by Zn deficiency was more pronounced in durum wheat than in bread wheat (Marschner 1995; Singh 2001; Singh *et al.* 2002, 2006). Root and shoot weight significantly increased with application of Zn and there was an increase in root density with an increase in root volume (Malewar *et al.* 1993). Zn-efficiency based on shoot dry weight and shoot growth showed marked differences among chick pea genotypes for which the shoot dry weight was lower under Zn deficiency than compared with the Zn sufficienct condition (Khan *et al.* 1998). The root: shoot ratio in general increases (Loneragan *et al.* 1987) as an initial response to Zn deficiency. Cakmak *et al.* (1998) 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 Zn 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 (Cakmak *et al.* 1996b; Singh 2001; Natesan 2001; Genc *et al.* 2006). In wheat genotypes grown under controlled environmental conditions in nutrient solution for 25 days, Zn contents in the dry matter were much lower in plants grown without Zn compared to the plants supplied with Zn (Cakmak *et al.* 1994). Concentrations of Zn were significantly higher in plants supplied with Zn than the plants without Zn supply. Root Zn concentrations were greater than the shoot Zn concentrations under Zn deficient conditions, since under deficient Zn supply transport of Zn from roots to shoots (Rengel and Graham 1995b) is inhabited. Zn-efficient bread wheat genotypes, in general, contained more Zn in shoots than Zn-inefficient durum wheat genotypes in field (Graham *et al.* 1992), greenhouse (Marschner 1995) and nutrient solution experiments (Rengel and Graham 1995b; Singh *et al.* 2002, 2006). Zn efficient chickpea were reported to a have higher Zn content per plant and higher Zn uptake per gram of root dry weight than those of inefficient-genotypes (Loneragan 1997; Ahmadi 2010).

PLANT FACTORS REGULATING ZN 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 their root characteristics that control the uptake rate (Barber 1984). A number of mathematical models of nutrient uptake by plants were developed based on the soil chemistry, kinetics of nutrient uptake and root architecture and morphology (Rengel 1993; Qian *et al.* 2005; Lehto *et al.* 2006). 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 (Barber and Silberbush 1984; Lynch and Whipps 1990; Sattelmacher *et al.* 1994; Dong *et al.* 1995; Briat *et al.* 2006; Chen *et al.* 2009). 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, the spatial coverage or distribution of root system will determine the ability of the plants to exploit the unevenly distributed soil resources effectively (Graham *et al.* 1992).

Available literature on this aspect clearly suggests that the difference in root morphology among genotypes is more likely to be the property of the genotype. Zn uptake by higher plants appears to be mostly controlled by the transport of Zn 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 (Rengel 1997). 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 Zn ion, than plants that produce thicker roots. Excalibur, a Zn efficient wheat genotype, develops smaller roots than the cv. 'Gatcher', a Zn-inefficient genotype (Dong *et al.* 1995). In addition, plant species with longer root system are expected to be more Zn efficient, as a deeper rooting zone can explore Zn more efficiently in subsoil's (Chen *et al.* 2009). 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 the Zn-efficient genotypes (Dong *et al.* 1995). Zn^{2+} ions have to travel less distance to root absorption sites in case of plants having higher root absorbing surface area (Marschner 1993). Rengel and Wheal (1997) 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 (Grewal *et al.* 1997). It is suggested that ability of Zn-efficient genotypes to produce a 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 (Dong *et al.* 1995; Chen *et al.* 2009). It can be amply deduced from the studies made so far that breeding cereals with root system capable of greater mobilisation of Zn from soils of low Zn availability is promising, and environmentally-friendly strategy that can not only reduce Zn-fertiliser use but can will also increase resistance of cereals to soil-borne fungal disease (Graham and Rengel 1993). Studies to elucidate the physiological mechanisms of Zn efficiency in rice (*Oryza sativa* L.) involved comparison of root morphology, ultrastructure, and oxidative enzyme activities between Zn-efficient (IR8192) and Zn-inefficient rice genotype (Erjiufeng). Under moderate Zn deficient conditions, many swollen mitochondria were observed in the root tip cells of 'Erjiufeng', whereas most root cells in 'IR8192' remained intact. Disturbances in the ultrastructure of these organelles were accompanied with elevated oxidative stress in both genotypes and the increases were less in 'IR8192' than in 'Erjiufeng'. The study indicates that ability of the Zn efficient genotype to produce more fine roots and maintain a relatively efficient antioxidative system and intact root tip cell and functions were the contributing factors responsible for its Zn deficiency tolerance characteristics (Chen *et al.* 2009). 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. There are indications that association of mycorrhiza could benefit the plants in terms of total tissue Zn accumulation on soils rich or poor in micro mineral resource (Vogel-Mikus *et al.* 2005). Very recent report of Widodo *et al.* (2010) in Zn-efficient and Zn-inefficient rice takes credit from Zn uptake transporters in favor of root characteristics and root exudation as the determinants of Zinc deficiency tolerance and improved Zn efficiency.

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 mugineic acid (MA) family (phytometallophores), which are highly effective in mobilising, by chelation, the sparingly soluble inorganic Fe compounds such as Fe III hydroxides and oxides in the rhizosphere. Very recently using most advanced available physical tools Xuan and co-workers provided unquestionable evidence for phytosiderophore metal complexation (Xuan *et al.* 2006, 2007; Dell'mour *et al.* 2010). The phytometallophore-Fe (III) or Zn complexes are not only stable but are also actively transported across the plasma membrane without Fe (III) reduction (Schaaf *et al.* 2005). In graminaceous plants, tolerance to Fe deficiency is suggested to depend on the amounts of MAs secreted from their roots (Romheld and Marschner 1990; Singh *et al.* 1993; Marschner and Romheld 1994; Weber *et al.* 2006; Suzuki *et al.* 2008). Studies have been conducted to elucidate the genetic regulation of phytosiderophore biosynthesis in graminaceous species (Kanazawa *et. al* 1994; Higuchi *et al.* 1996, 1999, 2001; Takahashi *et al.* 2001; Itai *et al.* 2002; Song *et al.* 2006). Activities of nicotianamine synthase (NAS) and nicotianamine amino transferase (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 (Schaaf *et al.* 2005; Murata *et al.* 2006; Bashir *et al.* 2006; Estelle *et al.* 2006; Ishimaru *et al.* 2006; Harada *et al.* 2007).

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 the plant species are due to an enhanced release of phtosiderophores under Zn deficiency in the graminaceous species (Zhang *et al.* 1991). 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 (Clarkson and Sanderson 1978). Root exudates mobilized increasing amounts of the various micronutrients in the following order, Cu < Fe < Zn < Mn (Treeby *et al.* 1989). Merckx *et al.* (1986) 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.* (1986) and Crowley *et al.* (1987) suggested that phytosiderophore production is a general response of plants to micronutrient deficiency. Singh *et al.* (2006) reported an 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 (1990) and Xuan *et al.* (2007). Complexation of metals by phytosiderophores was unequivocally revealed by CE-ESI-MS and CE-ICP-MS (Dell'mour *et al.* 2010). Literature shows that phytosideropores possess a high ability to complex Zn and enhance its mobility in the rhizosphere (Singh 2001; Singh *et al.* 2002, 2005; Xuan *et al.* 2006) and root apoplast (Zhang *et al.* 1991). Differences in Zn uptake capacity between bread and durum wheat cultivars were attributed to differential release of phytosiderophores from roots (Cakmak *et al.* 1996b; Rengel *et al.* 1998; Singh *et al.* 2002, 2006). 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 (Rengel *et al.* 1998). The resupply of Zn to Zn-deficient plants completely repressed the release of phytosiderophores within 72 h (Zhang *et al.* 1989). 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 (von Wiren *et al.* 1996). It is, therefore, suggested that the phytosiderophore release under Zn deficiency stress may be causally involved in determining Zn efficiency of graminaceious species. Data in **Table 9** gives information on the release of phytosiderophore by different cereal species, raised on Fe and Zn deficient nutrient solution (Cakmak *et al.* 1998; Shenker *et al.* 2001; Tolay *et al.* 2001; Hansen *et al.* 2006; Singh *et al.* 2006; Buxton *et al.* 2009). The stability of Fe (III)-PS complex, however, is much higher than that of Zn-PS (Murakami *et al.* 1989) 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 same transport system (Kochian 1993; von Wiren *et al.* 1996; Harada *et al.* 2007). Zhang *et al.* (1991) observed that the peak in Zn uptake coincided with the maximum rate of phytosiderophore release in Fe-deficient plants while, in the Fe-sufficient plants the release of phytosiderophores was very 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 (Treeby *et al.* 1989) 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 (Walter *et al.* 1984; Rengel and Graham 1995a). 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 (Rengel and Romheld 2000). The mechanism behind the 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 roots to increase exudation of phytosiderophores (Walter *et al.* 1984). The above findings, however, have been argued by Singh *et al.* (2001, 2002, 2006) who observed a higher and almost similar Fe-uptake by Zn-efficient and inefficient wheat genotypes under Zn deficiency.

Further, it has been shown that in Fe (Takagi 1976) and Zn deficient plants (Cakmak *et al.* 1994; Singh *et al.* 2006) the release of phytosiderophores follows the same diurnal rhythm. Similar types of phytosiderophores were found to be released under both Zn and Fe deficiencies (Singh *et al.*

Table 9 Phytosiderophore release from roots of different cereal species between 10-14 days after transfer to Fe- and Zn-deficient nutrient solution.*

Cereal species	Phytosiderophore release (μmol g^{-1} root DW (3 h)$^{-1}$)	
	Fe deficiency	**Zn deficiency**
Barley (*Hordeum vulgare*)	18.9	11
Bread wheat (*Triticum aestivum)*	22.5	8.4
Corn (*Zea mays*)	6.0	-
Oats (*Avena sativa*)	3.1*	-
Rice (*Oryza sativa*)	3.1	-
Rye (*Secale cereale*)	8.1	-
Sorghum (*Sorghum bicolor*)	3.6	-
Sugarcane (*Saccharum officinarum)*	Nd	-
Tausch's goatgrass (*Aegilops tauschii*)	22	3.1

* Based on Cakmak *et al.* 1998; Shenker *et al.* 2001; Tolay *et al.* 2001; Chadha 2001; Singh *et al.* 2006; Hansen *et al.* 2006; Anonymous 2009; Buxton *et al.* 2009
nd. Not detected; -. Not done; Fe and Zn sufficient cereal and dicot species in general do not release any phytosiderophore

2002; Kobayashi *et al.* 2005). Zn-phytosiderophores have similar structural confirmations as Fe-phytosiderophores (Iwashita 1983; Xuan *et al.* 2006, 2007) and a similar regulatory mechanism for the biosynthesis and/or release of phytosiderophores under both Zn and Fe deficiencies have been suggested (Rengel and Romheld 2000). Since methionine is precursor for the biosynthesis of the various phytosiderophores in Graminaceous species (Mori *et al.* 1987), the 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 (Zhang *et al.* 1989; Takahashi *et al.* 1999). Release of phytosiderophores from 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 (Kissel 1987). Role of light in the release of phytosiderophores under Fe and Zn deficiency is also shown (Wolink *et al.* 1983). Plants release phytosiderophores at higher amounts about a few hours after the onset of light period, while under continuous darkness or continuous light the rate of release of phytosiderophores is lower (Singh 2001; Schaaf *et al.* 2005). The diurnal pattern of phytosiderophore release and its influence on Zn uptake has been investigated by several workers (Wolink *et al.* 1983; Zhang *et al.* 1991; Hacisalihoglu *et al.* 2003; Singh *et al.* 2006). They observed a sharp rise in phytosiderophore production 3 h after onset of the light period, which gradually declined thereafter. In the Fe-deficient plants, the 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 (Zhang *et al.* 1991). A similar pattern of diurnal release of phytosiderophores was reported in Zn-deficient Graminaceous species (Zhang *et al.* 1989; Singh *et al.* 2006). 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 (Zhang *et al.* 1991). In wheat, DMA (2'-deoxy mugineic acid) is the predominant phytosiderophore released from the roots of Zn efficient wheat cultivars under Zn deficiency (von Wiren *et al.* 1994; Singh *et al.* 2002), while in rice mugenic acid dominates (Higuchi *et al.* 1999).

Furthermore, under Zn deficiency the organic and inorganic compounds released from 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 (Lynch and Whipps 1990). In addition, in their study, Zn deficiency increased numbers of fluorescent pseudomonas in the rhizosphere of all wheat genotypes tested but the effect was particularly obvious for genotypes tolerant to Zn deficiency. Enhanced production and release of Zn mobilising phytosiderophore, therefore, is a mechanism relevant for cereal species in adaptation on Zn deficient soils (Wolink *et al.* 1983; Cakmak *et al.* 1994; von Wiren *et al.* 1994; Hopkins *et al.* 1998; Hacisalihoglu *et al.* 2003; Singh *et al.* 2005; Ishimaru *et al.* 2006). However, on the contrary, Erenoglu *et al.* (1996) and Pedler *et al.* (2000) did not find a relationship between the release of phytosiderophores and the Zn efficiency. Therefore, despite reports favoring a relationship between phytosiderophore release and Zn efficiency of cereal species under Zn deficiency, at the present juncture, their significance in Zn nutrition is still a research area with more questions than answers.

Zn 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 (1972), 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 Zn uptake determined for wheat plants pre-grown at deficient or sufficient Zn supply, showed a saturation response for net Zn uptake with increasing solution Zn concentration. Zn deficiency caused an increase in I_{max} in the Zn efficient genotype but not in Zn-inefficient genotype. Zn-efficient genotype possess a better absorption and root to shoot transport, probably due to a more efficient transport system such as ion channel or ion pump when compared with the Zn-inefficient genotypes (Khan *et al.* 1998; Grewal *et al.* 1997). Rengel and Wheal (1997) studied kinetic parameters of Zn uptake in bread wheat cultivars differing in Zn efficiency and showed that under Zn deficiency, Zn efficient cultivar showed a greater I_{max} value (maximum net uptake rate) than Zn-inefficient cultivar. They also found that the Zn efficient bread wheat cultivar had a 30% higher rate of net Zn uptake than Zn-inefficient bread wheat. Zn deficiency over a longer period (24 days) increased I_{max} and K_m in Zn-efficient genotypes, but Zn-inefficient genotypes did not show an increase in I_{max} following a period of Zn deficiency (Rengel 1997; Singh 2001). With an increase in severity of the Zn-deficiency stress between 14 and 18 days of growth at 0 Zn, uptake of Zn increased by 170% in the Zn-deficiency tolerant wheat cultivar Warigal but remained unchanged in Zn-deficiency sensitive Durati. Zn-deficient Warigal plants transported larger amounts of Zn (Erenoglu *et al.* 2001) to the shoot than compared with Zn sensitive (Rengel *et al.* 1998). 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 of Zn (Erenoglu *et al.* 1996, 1999, 2002). Radiotracer techniques were employed to characterise Zn^{2+} influx into the root symplasm and translocation to the shoot in the *Thalaspi caerulescens* (Zn hyper accumulator) and *Thalaspi arvense* (Zn non-accumulator) (Bernard *et al.* 2004; Broadhurst *et al.* 2004; Papoyan *et al.* 2004). 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 (Erenoglu *et al.* 1996; Singh 2001; Gumaelius *et al.* 2004; Ma *et al.* 2004; Zha *et al.* 2004). Zn content and uptake in root, sheath and blade of maize and barley plants increased significantly with increased levels of Zn application. The labelled Zn rapidly accumulated in the roots of wheat plants upon immersion into the isotope solution (Pearson and Regnel 1995). A vacuolar membrane Zn transporter AtMTIP has been shown to govern leaf accumulation of Zn (Drager *et al.* 2004; Desbrosses *et al.* 2005). It so appears that the root uptake, root-to-shoot transport of Zn and its internal utilization are crucial for the expression of

Zn efficiency in cereal genotypes (Erenoglu *et al.* 1996; Richaud 2004; Genc *et al.* 2006). However, on the contrary importance of Zn transporter in determining Zn efficiency of rice could not be supported (Widodo *et al.* 2010).

Zn (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 roots to shoot and its utilization under deficient Zn supply was shown to contribute to Zn efficiency in wheat genotypes (Cakmak *et al.* 1996a). Haslett *et al.* (2001) showed that Zn is highly mobile within plant system and foliar applied Zn is translocated to leaves both above and below the treated leaf as well as to the root tips (Erenoglu *et al.* 1996). Hajiboland *et al.* (2001) found that Zn deficiency tolerance of a Zn efficient rice genotype is related to its ability to re-translocate Zn from older to growing/ emerging leaves. Erenoglu *et al.* (2001, 2002), Verret *et al.* (2004), Poynton *et al.* (2004) and Mendoza-Cozatl *et al.* (2008), however, were unable to confirm these findings. Compared to cereals, among dicots, Zn-efficient chickpea transported more Zn to the shoot when compared with inefficient genotypes (Khan *et al.* 1998).

Activities of Zn-containing enzymes

One of the main roles of micronutrients in plants is derived from their presence as the active centers of many enzyme molecules (Salt 2004). As a result of this function, metalloenzyme activities of several micronutrients have been used as specific parameters for the appraisal of biologically active metals involved in plant metabolic processes (Singh *et al.* 2005). Utilization efficiency in terms of dry matter production per unit of Zn present in the dry matter may be linked to the differences in the ability of a genotype to maintain an optimal activity of the important Zn regulated enzymes viz., super oxide dismutase (SOD) and carbonic anhydrase (CA). There are also a large number of enzymes in which Zn is an integral component of enzyme structure (Zn enzymes). Activity of these enzymes has been correlated with Zn availability to the plants. Differences in internal utilization or mobility of Zn have been shown to be involved in expression of Zn efficiency (Marschner *et al.* 1986; Genc *et al.* 2006). Carbonic anhydrase can occur as a dimer, tetrameter, hexamer or octamer, with a Zn atom in every subunit and a molecular mass ranging from 42 to 250 Kda (Graham *et al.* 1984). 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 when compared to a number of other species (Makino *et al.* 1992). Activity of carbonic anhydrase decreases in a number of plant species as a consequence of Zn deficiency (Hacisalihoglu *et al.* 2003). CA activity is closely related to the Zn content in C_3 plants. Under extreme Zn deficiency CA activity is almost absent. High CA activity is required in the mesophyll chloroplast of C_4 plants and removal of Zn from the CA molecule *in vitro* results in an irreversible loss of catalytic activity (Singh *et al.* 2005). There is a quantitative difference between the 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 (Tiwari *et al.* 2005). Deficiency of Zn is known to decrease CA activity drastically in several plant species (Guliev *et al.* 1992). Under Zn deficiency, there was two-fold higher CA activity in the Zn-efficient than the Zn-inefficient genotypes of wheat indicating a higher level of physiologically active Zn pool in leaves of Zn efficient genotypes. Upon resupply of Zn to the Zn deficient plants, Zn-inefficient wheat genotype lost the ability to increase CA activity, while Zn-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 (Rengel 1995). 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 (Rengel 1995). Zn appears to have a regulatory influence on stomatal opening, possibly as a constituent of CA (Sharma *et al.* 1995). In the metalloenyme SOD, Zn is associated with copper (Cu-Zn-SOD) and represents the structural component of SOD. Under Zn deficiency, SOD activity is much lower but can be restored in vitro by resupply of Zn to the assay medium. This indicates that the Zn atom is essential for the normal functioning of Cu-Zn-SOD (Marschner 1995; Cakmak *et al.* 1997). The activities of Cu-Zn-SOD and in part, total SOD, but not Mn-SOD is very closely related with the sensitivity of wheat and rye cultivars to Zn deficiencies (Wolink *et al.* 1983). Of late Zn efficiency (ZE) of crops has been correlated with enhanced expression and activity of Zn-requiring enzymes. No correlation was observed between ZE and Zn translocation to the shoot (Wojcik *et al.* 2006). 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 -inefficient genotypes. Northern Blot analysis provides evidence to suggest that Cu-Zn-SOD gene expression was up regulated in the Zn-efficient genotype but not in inefficient type (Hacisalihoglu *et al.* 2003). 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 Zn.

ZN INTERACTIONS *IN VIVO*

Significance of Zn in determining the rates and fate of certain critical metabolic processes through either direct or indirect hormonal influence in plants and human beings is known. Blindauer and Schmid (2010) attempted to understand the mechanisms that govern metal ion trafficking in plants particularly the mechanisms for intracellular zinc trafficking. Research efforts are required to pinpoint the determinants for zinc specificity in particular in the context of predicting protein function, concerning discrimination between metal ions in a biological system functions. Ahmadi (2010) and Cakmak (2010) emphasized on Zn and N interaction and advocated that a better N nutrition leads to a better Zn nutrition for a crop. Deeper understanding of beneficial interactions between the nutrients could lead to their exploitation to improve the efficiency and effectiveness of supplementation, fortification, and biofortification programs. There is now evidence for important interaction of Se with Zn and Fe. Zn can increase Se concentration in various organs, including brain, spleen, kidney, liver, lung, and heart, and Se can increase Zn concentration in liver, small intestine, blood, kidney, spleen, brain, and lung. Se compounds regulate Zn delivery from metallothionein to Zn enzyme, copper, Zn–superoxide dismutase. Thus, Se, Zn, and Cu are linked in cytosolic defense against reactive oxygen and nitrogen species (Lyon *et al.* 2008). The relationship among accumulation of selenium, auxin, and some nutrient elements viz., Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} in roots, mesocotyls, and leaves of maize was studied by Kocot *et al.* (2008). Using much reliable inductively coupled plasma optical emission spectroscopy (ICP-OES) technique they showed that the selenite and IAA, present in the external medium of growing plants, changed the uptake and accumulation of some cations in the plant tissues. The change of transport conditions of these nutrient elements is probably one of the first observed symptoms of selenium effects on plants. He and coworkers investigated the antagonistic effects of Zn and Se soil supplementation against lead and cadmium and provided evidence for the ability of Zn and Se to restrain the accumulation of Pb and Cd and improve the absorption of some other nutritional elements, like Fe, Mn, Cu, Ca and Mg. Zn application along with Se, according to them could be effectively used to improve trace elements nutrition in the vegetables (He *et al.* 2004). Mohammad and Moheman (2010) conducted through a nutrient culture experiment in tomato reported a significant inhibitory effects of Zn on Cd accumulation in all plant organs at toxic levels (100 and 1000 mg kg^{-1} soil). A reduction in Cd concentration did not improve shoot and root biomass and suggested that Zn concentration is a dominant factor affecting plant growth. An examination of Cd-Zn interactions and their transfer to soil-plant system indicates a synergistic effect wherein an increase in Cd and Zn concentrations in soil resulted in an increase in the accumulation of Cd or Zn in the plant. Zn-protein interaction is another area of research receiving attention these days (Blindauer and Sadler 2005; Maret 2005). AtMTP3, a metal tolerance protein from *Arabidopsis thaliana* has been shown to maintain metal homeostasis by mediating Zn exclusion from the shoot under excess availability of Zn (Arrivault *et al.* 2006). Interaction of phosphate with Zn is known for long. Phosphorus is normally taken up through high-affinity P transporter proteins embedded in the plasma membrane of certain cell types in plant roots. Expression of the responsible genes responds to P availability and their transcription is tightly controlled. However, this tight control of P uptake is lost under Zn deficiency, leading to very high accumulation of P in plants Huang *et al.* (2000) examined the effect of plant Zn status on the expression of the genes encoding the HVPT1 and HVPT2 high-affinity P transporters in barley (*Hordeum vulgare* L. cv. 'Weeah') roots and showed that the above genes encoding the P transporters were expressed under Zn deficiency in plants grown in either P-sufficient or -deficient conditions. Moreover, the role of Zn in the regulation of these genes is specific in that it cannot be replaced by manganese (a divalent cation similar to Zn). In another study, Fe application was found to increase the plant Fe uptake but decreased the uptake of manganese, Zn and copper. Uptake of Zn, however, was not affected by Mn application. An antagonistic effect of Fe with Mn, Zn, and Cu was attributed as the main reason for no observed increase in dry matter yield of wheat on application of iron (Ghasemi-Fasaei and Ronaghi 2008). Field experiment conducted to study the effects of boron (B), molybdenum (Mo), Zn (Zn) and their interactions on seed yield and yield formation of rapeseed show that a combined application of B with Mo or Zn resulted in higher seed yield than the application of B, Mo or Zn alone, and the seed yield of the B+Mo+Zn treatment was the highest in all treatments, 68.1% above the control (Yang *et al.* 2009).

ZN BIOFORTIFICATION

The main objective of biofortification is to develop plants that have are better in terms of an increased content of bioavailable nutrients in their edible parts. Cereals serve as the main staple food for a large proportion of the world population but have the shortcoming, from a nutrition perspective, of being low in Zn and other essential nutrients. Major bottlenecks in plant biofortification appear to be the root–shoot barrier and – in cereals – the process of grain filling (Pfeiffer and McClafferty 2010). Not only the availability of minerals but their bioavailability has to be ensured (Prasad 2010). Grusak and Cakmak (2005) and Cakmak (2010) describe various available and possible approaches to improve crop delivery of minerals to humans and livestock.

New findings demonstrate that the root–shoot distribution of Zn is controlled mainly by heavy metal transporting P_{1B}-ATPases and the metal tolerance protein (MTP) family (Palmgren *et al.* 2008). A greater understanding of Zn transport seems important to improve crop quality and also to help alleviate accumulation of any toxic metals in plant tissues. Grain Zn concentration can be increased by Zn fertilization. However, since fertilization with Zn could create more problem than solution, it is important to explore the native genetic variation in grain Zn concentration and wild relatives of barley, here, may offer potential for crop improvement for this trait (Genc *et al.* 2004b). Synthetic hexaploid wheats (*Triticum aestivum* L) obtained through cross between durum (*Triticum turgidum*) and diploid wheat (*Aegilops tauschii*) have could be an effective means of transferring desirable nutritional characteristics of *Aegilops tauschii* in addition to traits of disease resistance and abiotic stress tolerance into modern bread wheat genotypes. Genc and Mcdonald (2004c) found considerable genetic variation in expression of Zn deficiency symptoms (slight to severe), Zn efficiency (70-100%), shoot Zn concentration (5.8-10.5 and 33-53 mg/kg DW under deficient and sufficient Zn, respectively), shoot Zn content (3.8-10.6 and 34.0-64.6 μg/plant, under deficient and sufficient Zn, respectively) and Zn utilization (0.096-0.172 and 0.019-0.033 g DW/μg Zn under deficient and sufficient Zn, respectively) within synthetic accessions of wheat. They show that synthetic hexaploids can be used to improve current levels of Zn efficiency in modern wheat genotypes and that the synthetics may also be a good source of high grain Zn concentration (28-66 mg Zn/kg seed DW) (Genc and Mcdonald 2004c).

Research efforts to identify QTL for Zn and also iron accumulation are gaining momentum (Assunção *et al.* 2006). Screening and characterization of doubled haploid population derived from a cross between the Zn inefficient genotype RAC875-2 and the moderately efficient genotype Cascades was done by Genc *et al.* (2009) to identify QTL linked to growth under low Zn and with the concentrations of Zn and iron (Fe) in leaf tissue and in the grain. The QTLs were identified using an improved method of analysis, whole genome average interval mapping. Depending on the traits and the site, the QTL accounted for 12–81% of the genetic variation. Most of the QTL linked to seedling growth under Zn deficiency and to Zn and Fe concentrations were associated with height genes with greater seedling biomass associated with lower Zn and Fe concentrations. Their work led to the identification of a QTL for kernel weight on chromosome 4A, four QTL for grain Zn concentration on chromosomes 3D, 4B, 6B and 7A and one QTL for grain Fe concentration on chromosome 3D (Genc *et al.* 2009).

Apart from molecular or physiological routes to biofortification, Zuo and Zhang (2009) describe agronomic approach for Zn and Fe biofortification i.e., intercropping between dicots and Gramineous species. Intercropping is common in developing countries such as China, India, Southeast Asia, Latin America and Africa. Inter specific interaction will facilitate the iron and Zn nutrition of intercropping systems such as peanut/maize or wheat/chickpea. Intercropping can also increase iron and Zn content in the seeds. In intercropping of chickpea and wheat, the Fe contents in wheat and chickpea seed were increased 1.26 and 1.21 times, respectively, and Zn concentration in chickpea seed was 2.82 times higher than that in monocropping (Zuo and Zhang 2009). These micronutrients are also important food components. At molecular level, Le and An (2009) generated transgenic rice plants over-expressing OsIRT1 gene and showed that this iron transporter gene could also help in improving uptake of Zn. The transgenic rice showed elevated level of both Fe and Zn in the shoots, roots and mature seeds. This demonstrates that molecular manipulation of genes involved in even iron acquisition could enhance micronutrient levels in grains of cereal crops.

CONCLUSION

A critical analysis of the available literature on varied aspects of zinc research in crop plants suggests that while there are a number of different mechanisms contributing to Zn efficiency, uptake is the major mechanism and the effect is modified further by the physiological efficiency within the shoot. Root:shoot partitioning was not strongly associated with Zn efficiency. Visual symptoms of the severity of Zn deficiency was a good predictor of Zn efficiency and was correlated with Zn uptake. Genetic variation in grain Zn concentration should be explored and wild relatives of barley may offer potential for crop improvement for this trait. It can be amply surmised from the available literature that Zn efficiency of cereals under Zn 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. Researchers 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 Zn deficiency tolerance of Graminaceous species can also be achieved through manipulation of the key enzymes of phytosiderophore biosynthesis i.e., nicotianamine synthase and nicotianamine aminotransferase. This will help in reducing and may be even totally eliminating the application of fertilizer Zn to the soil for sufficing plant Zn requirements. 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 in terms of maintaining a higher activities of Cu-Zn-SOD and CA or translocation in terms of high root to shoot transport of Zn and its re translocation from older to younger and growing plant tissues. It is now clear that the root–shoot distribution of Zn is controlled mainly by heavy metal transporting P_{1B}-ATPases and the metal tolerance protein (MTP) family. A greater understanding of Zn transport is important to improve crop quality and also to help alleviate accumulation of any toxic metals. The biofortification for Zn should use complementation of physiological, agronomic and molecular approach to develop micronutrient efficient plant types and achieve traversing of root, shoot, grain barrier in Zn transport.

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