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A review of zinc nutrition and plant breeding

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Abstract

Plants require the proper balance of zinc (Zn) for normal growth and optimum yield. Interest in Zn has risen in the last decade because Zn deficiency stress is extensive in many areas, causing decreases in crop yields. Zn deficiency also decreases the amount of Zn in cereal grain and diminishes its nutritional quality. Hence, increasing the Zn content of the edible portions of crops should be considered in plant breeding. Available data indicate that Zn enrichment traits are present within the genomes of crops that could allow for substantial increases in the Zn concentration of edible parts without negatively impacting yield. Increasing the amount of Zn in food crops can improve the Zn status of people. Furthermore, the use of Zn-dense seeds results in greater seedling vigor and increased crop yields when the seeds are sown in Zn-poor soils. Progress toward developing mineral-dense seed has mainly relied upon conventional plant breeding approaches, a process that is labor-intensive and time-consuming. Hence, the identification of DNA markers that are diagnostic of Zn efficiency can accelerate the development of cultivars that can remain productive even in Zn-deficient soils. Additionally, these markers may be used to begin identifying the specific genes responsible for differences in the response of genotypes to Zn deficiency.

Keywords: Zinc deficiency, genotypic variation, breeding, olecular markers

1. Introduction

Zinc (Zn) deficiency is the most widespread micronutrient deficiency in agricultural lands around the world, causing yield decreases and diminishing the nutritional quality of agricultural plants. Zn is important for plant growth, as plants require a proper balance of all the essential nutrients for normal growth and optimum yield. Plant-based foods are significant sources of Zn for humans (Welch and Graham, 2004).

Zinc is one of the eight trace elements (manganese, copper, boron, iron, zinc, chlorine, molybdenum and nickel) that are essential for normal, healthy growth and reproduction of plants. Zn is required as a structural component of a large number of proteins, such as transcription factors and metalloenzymes (Figueiredo *et al*., 2012). If an insufficient amount of Zn is available, the plants will suffer from physiological stresses due to the failure of metabolic processes in which Zn plays a critical role. Zinc in soils can be separated into fractions based on particle size distribution and/or chemical analysis procedures.

The amount of Zn in each of the chemical pools or forms differs due to the range of Zn concentrations found in the soil parent material and the extent of weathering. Zn^{2+} is released from many primary minerals into the soil solution during rock weathering. The total Zn concentration of soils is related to the composition of the parent rock material and soil mineralogy. In the soil, Zn forms complexes with organic acids, humic substances and other types of dissolved organic carbon. The total Zn concentration is not used for evaluating the availability of soil Zn to the plants because only a small amount of total Zn is exchangeable or soluble. The availability of Zn to plants depends on several soil factors such as the concentration of Zn in solution, ion speciation and the interaction of Zn with other macronutrients and micronutrients (Li *et al*., 2003).

Zinc deficiency may cause large reductions in crop quality and yield. Wheat and barley show significant decreases in growth and grain yield under Zn-deficient conditions in the field (Graham *et al*., 1992; McDonald *et al*., 2001). Zn deficiency in soils also reduces Zn concentration and content in the edible portions of staple food crops and diminishes their nutritional quality (Welch and Graham, 2004). Approximately 40% of world's population suffers from micronutrient deficiencies (the so-called "hidden hunger"), including Zn deficiency. The World Health Organization estimates that Zn deficiency affects one-third of the world's population (approximately two billion people), with prevalence rates ranging from 4 to 73% in various regions (WHO, 2002). A diet consisting of a high proportion of cereal-based foods with low Zn content is considered one of the major reasons for the widespread occurrence of Zn deficiency in humans, especially in developing countries (Biesalski, 2013).

Visible Zn deficiency symptoms in crops usually occur only in cases of relatively severe deficiency. In marginal deficiency, crop quality and yield may be reduced because of hidden Zn deficiency without obvious symptoms (Alloway, 2004). This hidden Zn deficiency may go undetected for several seasons at a high cost to farmers. Most soils with low plant-available Zn can be treated with Zn fertilizers to correct crop Zn deficiency. Several different Zn sources, including $ZnSO_4$, $ZnCO_3$, ZnO, $Zn(NO_3)_2$ and $ZnCl_2$, are currently being used as fertilizers.

When fertilizers are applied to correct Zn deficiency, the added Zn is likely to remain near the surface, even in sandy textured soils. In semi-arid areas, applying liquid forms of nitrogen, phosphate and Zn fertilizers to the subsoil (up to 40 cm deep) can better increase crop Zn uptake and grain yield compared with the application of granular fertilizers to the surface. Moreover, Zn fertilizers may be unavailable or unaffordable in developing countries. Because of the widespread Zn deficiency problems and difficulties in alleviating this deficiency with fertilizers, the development of crops that are efficient Zn accumulators, especially under low soil Zn conditions, is an important approach for improving Zn deficiency tolerance, grain productivity and micronutrient quality. In addition, Zn-efficient genotypes could reduce land degradation by limiting the use of machinery and minimizing fertilizer inputs on agricultural lands. Zn-efficient cultivars of wheat, barley and rice are available (Gregorio *et al*., 2000; Genc and McDonald, 2004) and are grown quite widely in soils with low levels of available Zn.

Plant genotypes vary widely in their tolerance to soils with low plant-available Zn with respect to both Zn uptake and utilization. Tolerance of plant genotypes to Zn deficiency, as a genetic trait, is usually referred to as Zn efficiency and defined as the ability of a cultivar to grow and yield well in soils that are too deficient in Zn to support a standard cultivar. The physiological and molecular mechanisms of Zn deficiency tolerance are just beginning to be understood, and these mechanisms can be exploited in crop breeding programs (Hacisalihoglu and Kochian, 2003) For example, Zn-efficient genotypes with better Zn utilization may contain higher amounts of chelators that bind Zn and increase its physiological availability at the cellular level. A better understanding of the physiological, morphological and genetic bases of Zn efficiency is

needed for the development of fast, simple and reliable screening procedures for identifying and breeding genotypes for Zn efficiency. The first step in breeding for Zn efficiency is the assessment of a large number of segregating populations from crosses of Zn-efficient × Zn-inefficient parents.

Field and pot screening studies have revealed significant genetic variation in Zn efficiency in cereal genotypes, which indicates that selection for improved Zn efficiency is possible. Pot screening is common, being less expensive and faster than fieldwork. In addition, the problem of soil heterogeneity can be eliminated by screening in pots. However, root binding in small pots may be an independent limiting factor, which can obscure a comprehensive view of Zn efficiency. Moreover, the environment is less realistic in glasshouse experiments than in the field.

In the near future, it is anticipated that molecular techniques will dominate screening for Zn efficiency and many other desired traits. In particular, the use of DNA markers for these traits could permit the selection of improved crop cultivars. An increasing number of genetic maps allow traits to be mapped to a particular chromosome, thereby allowing the selection of flanking DNA markers and obviating the need for time-consuming and expensive bioassays.

Over the past two decades, molecular tools have aided the identification, mapping and isolation of genes in a wide range of crop species. Molecular markers have been used for the development of detailed genetic and physical chromosome maps in crops. Another major use of molecular markers in plant systems involves improving the efficiency of conventional plant breeding by indirect selection, which is accomplished using molecular markers linked to qualitative and quantitative trait loci (QTLs). Molecular marker applications have also increased our understanding of the physiological parameters controlling plant responses to biotic and abiotic stress. The main advantages of molecular markers are that they are available for many traits, are not affected by the environment and can be scored at virtually any stage of plant development.

Progress has been made toward identifying Mn- and Zn-efficient parents in wheat and barley and in developing genotypes that are more efficient. However, the variability inherent in field screening has produced some inconsistent results. This issue may be overcome by the use of molecular marker technology, which allows the traits for nutrient efficiency to be selected directly. Molecular markers for micronutrient efficiency (e.g., Mn and Zn) have been identified in barley (Pallotta *et al*., 2000; Lonergan *et al.*, 2001; Sadeghzadeh, 2008; Sadeghzadeh *et al*., 2009), bread wheat (Xu *et al*., 2012), durum wheat (Khabaz-Saberi *et al*., 2002) and maize (Qin *et al*., 2012). This review will first cover Zn deficiency in soils followed by Zn deficiency in plants. Subsequently, breeding for Zn efficiency will be addressed, followed by application of molecular markers in Zn efficiency.

2. Zinc deficiency

When the supply of Zn to plants is inadequate (i.e., there is deficiency of Zn), one or more of many important physiological functions that depend on Zn are impaired, and plant growth is adversely affected. The necessity of Zn for normal growth has only been scientifically recognized for approximately 70 years (Alloway, 2004), and in some areas of the world, the existence of Zn deficiency has only been acknowledged in recent years. Zn deficiency is a severe micronutrient deficiency that threatens world food production (Alloway, 2001; Welch and Graham, 2004). Therefore, understanding and knowledge of Zn deficiency would facilitate the appropriate management of this problem.

Recently, reports of Zn deficiency in various crops have increased because high-yielding plant varieties and intensive cultivation remove large amounts of Zn from the soil at every harvest. Many new breeds and varieties are much more susceptible to Zn deficiency than locally adapted crop genotypes. The increased use of phosphorus (P) fertilizers as well as fertilizers

with less Zn-containing impurities can exacerbate Zn deficiency (Loneragan and Webb, 1993). In Sub-Saharan Africa, the Near East/North Africa and South Asia, the food supply has kept up with population growth. However, simply consuming enough calories has resulted in unforeseen nutritional problems for people, especially those who are poor. Hence, increasing the Zn content of staple crops will enhance the intake of bioavailable Zn and improve the Zn status of deficient populations (Biesalski, 2013).

Briefly, the main causes of Zn deficiency in crops are low Zn availability (high pH, calcareous and sodic soils), low total soil Zn concentration (especially in sandy, sodic and calcareous soils), high levels of nitrogen and phosphate and restricted root exploration due to soil compaction or a high water table, particularly in soils with a marginal Zn status (Alloway, 2004).

3. Zinc in soils

Distribution of zinc deficiency

Zinc deficiency occurs in a wide range of soil types in many parts of the world; however, tropical regions with highly weathered soils, semi-arid areas with calcareous high-pH soils, sandy-textured soils, and acid soils in several different climatic zones tend to be the most seriously affected because of their low Zn content. The results of 190 field trials in 15 countries around the world showed that Zn deficiency was the most frequently occurring micronutrient deficiency. Zn deficiency was recorded in 49% of the trials, with acute forms (with visible symptoms) noted in25%and hidden deficiencies confirmed by yield responses to Zn amendments noted in 24% (Sillanpää, 1990).

It is estimated that approximately 50% of soils used for cereal production in the world have low levels of plant-available Zn (Graham and Welch, 1996). Low levels of plant-available Zn have been reported in the arid and semi-arid regions of India, paddy fields of Pakistan, poorly drained calcareous paddy soils of China and highly alkaline soils with low levels of organic matter in Central Anatolia of Turkey. In Australia, soils with low plant-available Zn are found in southern Australia, Victoria, Queensland, New South Wales and Western Australia. Southwest Australia is considered the largest continuous Zn-deficient area in the world. Such large-scale deficiencies have made Australia a world center of research on micronutrient problems.

Zinc uptake from soil

Zinc enters plants primarily via root absorption of Zn2+ from the soil solution, which is a dynamic and complex process. Uptake depends on ion concentrations at the root surface, plant demand and root absorption capacity. Zn reaches the plant root surface through mass flow, diffusion and root interception mechanisms.

Mass flow is passive nutrient transport from the soil to the roots and is driven by transpiration. When the solution moving through the soil to the root contains a relatively large concentration of Zn, mass flow becomes the dominant mechanism bringing Zn to the root surface.

When the Zn concentration is low, particularly in soils with low plant-available Zn, diffusion plays important role in the transport of Zn and other nutrients, such as P, K, Cu, Fe and Mn, to the root surface because mass flow can only carry a small fraction of the nutrients required by the plants. In contrast to mass flow, diffusion operates only in the immediate volume of soil surrounding a root.

The interception of nutrients by roots is an important uptake mechanism for soil immobile nutrients such as Zn. Thus, root interception (i.e., root growth and root surface area) is also an important factor in determining plant availability of Zn . Poor root interception can limit Zn uptake if granules of $ZnSO_4$ are banded in the soil, particularly at a low rate of $ZnSO_4$ application.

4. Factors affecting the availability of soil zinc to plants

The term “availability” is commonly used to describe the ability of plants to take up nutrients from the soil.

Several authors have extensively reviewed the factors affecting the solubility of Zn in soils and its availability to plants. Zn availability to plants can be affected by factors such as total soil Zn content, soil pH, organic matter, soil temperature and moisture regimes, root distribution and rhizosphere effects.

Soils with a low total Zn concentration are often Zn deficient for crop production. Sandy soils, frequently deficient in available Zn, have an inherently low total Zn concentration. For example, Zn deficiency in plants grown on acid soils is generally associated with a low total soil Zn concentration. These cases of Zn deficiency are related to an absolute Zn deficiency rather than Zn availability.

With the exception of molybdenum, the availability of micronutrients generally decreases as the soil pH increases. Increasing the soil pH stimulates Zn adsorption to the surface of various soil constituents, such as metal oxides and clay minerals; this results in decreases in the solubility and availability of Zn to plants. A high pH decreases the desorption of Zn from soil surfaces, which also reduces the availability of Zn to plants. Zn can precipitate in the form of $Zn(OH)_2$, $ZnCO_3$ and Zn_2SiO_4 at high pH. The Zn concentration in the soil solution is largely dependent on pH; for example, at pH 5.0, the concentration of Zn in the soil solution is approximately 10-4 M, whereas at pH 8.0, this concentration is 10-10 M. Liming of acid soils resulted in an increase in the soil pH from 5.2 to 6.8 and an approximate 10-fold decrease in the Zn concentration in plants (Parker and Walker, 1986).

In acid soils, in contrast, the availability of Zn depends on the amount of soil Zn. In sandy loam, the availability of natural and supplied Zn doubled when the soil pH was reduced from 7 to 5 using ammonium sulfate.

The total soil Zn concentration in calcareous and non-calcareous soils is often similar; however, Zn deficiency is frequently reported for calcareous soils (Singh *et al*., 2005). Calcareous soils (pH>7) with moderate-to-high organic matter content (>15g organic C per kg soil) are likely to be Zn-deficient due to high levels of HCO_3^- in the soil solution (Singh *et al*., 2005) In alkaline soils with a low Zn supply, increasing the Zn application increased the Zn concentration in plants and reduced the deficiency symptoms; however, plant growth was only slightly improved. It was concluded that plant growth on the alkaline soil was more responsive to soil alkalinity than Zn deficiency.

Soil organic matter content is another factor that contributes to Zn deficiency in crops. Zn availability to plants is often reported to be low in soils with high organic matter content due to increased adsorption of Zn by organic ligands and components. An adequate level of organic matter increases the solubility and diffusion rate of Zn in soils. In the United States, Zn deficiency problems frequently occur in areas where the surface soil has been removed by land leveling (Alloway, 2004). The underlying soil has less organic matter than the topsoil, and in many cases, the subsoil also has a higher pH. Several researchers have shown a positive correlation between extractable Zn and organic matter. Both the diethyltriamine pentaacetic acid (DTPA)-extractable Zn and organic matter content decrease with depth in the soil profile (Alloway, 2004). An experiment in wheat showed that the Zn content is positively correlated with the level of soil organic matter.

Other factors that contribute to Zn deficiency include low soil moisture and low temperature (Moraghan and Mascagni Jr, 1991). Soil moisture affects the nutrient supply by impairing diffusion to the root surface. Given that Zn diffusion in soils is highly dependent on soil moisture, plant Zn nutrition may be at risk in semiarid and arid areas where soils are usually water-deficient for long periods during the growing season. Accordingly, in Zn-deficient calcareous soils, wheat yield reductions are more severe under rainfed versus irrigated conditions. However, water-logging alters Zn chemistry in the soil; for example, submerged soils have decreased concentrations of water-soluble Zn compared with well-drained soils. In addition, decreased Zn solubility and low Zn uptake in poorly drained soils is due to the co-

precipitation of Zn with soluble iron and aluminum in the soil. Early in the growing season, Zn deficiency occurs when the soil temperature is still relatively low and subsequently diminishes as the temperature rises. A low soil temperature often increases the incidence and severity of Zn deficiency symptoms (Moraghan and Mascagni Jr, 1991). It was suggested that a colder root zone temperature decreases root colonization with vesicular-arbuscular (VA) mycorrhizae, root growth, Zn uptake and Zn translocation into the shoots (Schwartz *et al.*, 1987; Moraghan and Mascagni Jr, 1991). In barley, shoot Zn uptake was 82% higher in plants grown in solution at 20 °C compared with those grown at 10 °C (Schwartz *et al.*, 1987).

Soil analysis can be used as a tool for predicting nutrient deficiency in existing crops, and it is also more valuable for predicting deficiencies and corrective actions that can be taken to avoid yield losses in subsequent crops. The critical soil levels that lead to Zn deficiency vary between 0.6 and 2.0 mg Zn/kg soil depending on the Zn extraction method (Singh *et al*., 2005). Several soil analytical procedures are available for predicting Zn availability in soils, including extractions with chelating agents, such as DTPA and ethylenediamine tetraacetic acid (EDTA), and salts, such as ammonium acetate. The DTPA method is widely used for predicting plant-available Zn in soils. Based on greenhouse and field experiments, approximately 0.6 mg/kg DTPA-extractable Zn has been suggested as a critical concentration for wheat grown in calcareous soils of arid regions in India. In Australia, the critical DTPA-extractable Zn concentration in acidic soils is 0.25 mg/kg for wheat.

Soils with low plant-available Zn can be treated with Zn fertilizers to provide an adequate supply of Zn to crops. Several different Zn compounds are used as fertilizers; although $ZnSO_4$ is by far the most widely used material. $ZnSO_4$ can also be used as a foliar treatment for crops; however, chelated forms of Zn are usually used for foliar application (Alloway, 2001). The application rates of Zn fertilizers will also vary depending on the crop, the form of Zn applied, the soil conditions and the application method. For soil applications, concentrations can range from 2.5-22 kg Zn/ha for inorganic forms, such as $ZnSO_4$, and 0.3-6 kg Zn/ha for chelated forms (Alloway, 2001).

Finally, the interaction of Zn with other elements decreases Zn availability and influences its adsorption, distribution and utilization in plants. These interactions are mainly due to the influences of other cations on the rate of absorption by plant roots rather than to their effects on the availability of Zn or its forms (Loneragan and Webb, 1993). Zn interactions with P and nitrogen (N) are the most important and widespread in soils with limiting supplies of Zn and P or N. A high level of applied N in the absence of Zn can cause Zn deficiency through a dilution effect, changing the pH of the root environment (Loneragan and Webb, 1993) and increasing the shoot-to-root ratio (Loneragan and Webb, 1993).High levels of P in the soil can also occasionally increase the symptoms of Zn deficiency. Zn and P co-precipitation in the soil may cause the formation of insoluble $ZnO_3(PO_{4)2}$,whichdecreases the Zn concentration of the soil solution and thus lowers Zn availability. Moreover, under limiting or marginal supplies of Zn and P, the application of P causes Zn deficiency due to the dilution of the plant Zn concentration as a result of growth stimulation. Yang *et al*. (2011) reported that with increasing P application, the proportion of Zn and P content in the grain relative to the whole plant decreased. Moreover, P and Zn acted antagonistically in roots; and excess P inhibited Zn uptake in roots.

5. Zinc in plants

The functions of zinc in plants

Zinc is essential for the normal, healthy growth and reproduction of plants. This element is required in small amounts to allow the normal function of several key plant physiological pathways as well as to ensure the structural and functional integrity of membranes. Thus, Zn has important roles in growth regulation, enzyme activation, gene expression

and regulation, phytohormone activity, protein synthesis, photosynthesis, carbohydrate metabolism, fertility, seed production and defense against disease (Marschner, 1995). Zn deficiency will impair these physiological functions and compromise the health and productivity of plants, leading to severe reduction in growth, lower yields (or even crop failure) and poor quality crop products.

Zinc is the only metal that is required in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases). The requirement of Zn for the function of a wide range of enzymes indicates that the metabolism of proteins, carbohydrates and auxin as well as reproductive processes are hampered under Zn deficiency (Römheld and Marschner, 1991). Zn is required for the activity of metalloenzymes that are involved in protein and nucleic acid metabolism.

Zinc is involved in carbohydrate metabolism via its effects on photosynthesis and sugar transformation. Reduced photosynthesis under Zn deficiency can result from a decrease in carbonic anhydrase (CA) activity, the photochemical activity of chloroplasts and chlorophyll content, as well as alterations in chloroplast structure. Low CA may inhibit photosynthetic electron transfer and consequently limit chlorophyll content (Römheld and Marschner, 1991).

Zinc is essential in protein metabolism, and the most important role of Zn in protein synthesis is its involvement in the stability and function of genetic material. Zn is essential in chromatin structure, DNA/RNA metabolism and gene expression. Zn deficiency causes a decrease in protein synthesis (Marschner, 1995) due to RNA degradation (Cakmak *et al.*, 1989), decreased activity of RNA polymerase, ribosomal deformation and a decrease in the number of ribosomes. In Zn-deficient bean leaves, the free amino acid concentration was increased by a factor of 6.5 compared with that of control plants. Upon resupply of Zn, this factor decreased to 5.1 after 24 h, 2.7 after 48 h and 1.4 after 72 h (Cakmak *et al*., 1989).

Zinc has an important physiological role in maintaining the integrity and function of cellular membranes by controlling the generation and detoxification of reactive oxygen species. Reactive oxygen species are potentially damaging to membrane lipids and sulfhydryl groups. When these compounds are damaged due to oxidative stress, there is increased leakage of several organic compounds, such as carbohydrates and amino acids, from Zn-deficient root cells. Due to the increased leakage of carbon-containing compounds into the rhizosphere, Zn-deficient plants may be susceptible to root diseases such as *Fusarium graminearum.*

6. Zinc uptake and movement in plants

Roots take up Zn from the soil solution as a divalent cation (Zn^{2+}), and at high pH, Zn is absorbed as a monovalent ($ZnOH^{+}$) cation (Marschner, 1995). Zn accumulation in plant roots exhibits biphasic kinetics, with initial rapid entry and binding within the root cell wall followed by a slower linear transport phase across the plasma membrane. Zn^{2+} movement from the external solution to the root cell wall free space occurs via diffusion, and Zn is subsequently transported across the plasma membrane via ion transport proteins. The cytoplasm of a plant cell is negatively charged, and there is an electrical gradient that draws cations, including Zn^{2+}, into the cell. Reid *et al*. (1996) reported that it appears unnecessary to invoke an active transport system for Zn transport across the plasma membrane because a Zn influx will be thermodynamically favored. Root Zn uptake is mediated by different transport systems: a high-velocity, low-affinity membrane transporter system (K_m = 2-5 μM) and a low-velocity, high affinity system (K_m = 0.6-2 nM), which is most likely the dominant transport system under low soil Zn conditions (Hacisalihoglu *et al*., 2001). Generally, Zn-inefficient plants have lower rates of uptake and comparable affinity values compared with Zn-efficient plants (Rengel and Wheal, 1997; Hacisalihoglu *et al*., 2001).

From the root surface, nutrients are transported into the root xylem through the epidermis, cortex and endodermis (Marschner, 1995). Zn may pass through

the root to the xylem either through the extracellular spaces between root cells (apoplast) (White *et al.*, 2002) or through the cytoplasmic continuum of root cells linked by plasmodesmata (symplast). The apoplastic cation flux is largely determined by the cation exchange properties of the cell wall, water flows and the existence of Casparian band. Given that the symplastic pathway involves specific transporters in the plasma membrane, particular cations can be selected for transport (Sattelmacher, 2001). In *Thlaspi caerulescens* and *T. arvense*, the entry point for Zn accumulation is across the plasma membrane of root cells, and Zn reaches the xylem via the symplastic pathway in both species (Lasat *et al*., 1996; Lasat and Kochian, 2000). With an increasing external Zn concentration, the apoplastic pathway plays a greater role in Zn uptake and influx to the xylem (White *et al.*, 2002).

Nutrients in the xylem move toward shoots in the transpiration stream. This driving force for transpiration arises from the gradient in water potential between stomatal cells and the atmosphere. An analysis of xylem fluid content showed that Zn moves as a complexed form (e.g., anionic) in the xylem. Zn in the xylem has been measured in a soluble form bound to small proteins and as insoluble complexes, such as Zn-phytate. Metal complexes and ionic activities of micronutrients differ in the xylem and phloem saps (Welch, 1995). The activity of cationic micronutrients, such as Mn, Zn, Fe, Cu and Ni, is low in the phloem sap due to a high phosphate level and high pH. Hence, these micronutrients are likely to form metal complexes to move easily in the phloem stream (Welch, 1995).

7. Zinc deficiency in plants

Many plant species are affected by Zn deficiency in a wide range of soil types in most agricultural regions of the world. The major staple crops (rice, wheat, barley, maize and sorghum) are all affected by Zn deficiency, together with many different fruits, vegetables and other types of crops, including cotton and flax. Low soil concentrations of plant-available Zn may cause not only a decrease in grain yield but also lead to poor nutritional quality of crop products (Graham and Welch, 1996). However, more severe deficiencies (with leaf symptoms) can reduce yield severely and even result in crop failure.

Zinc deficiency can impair many biochemical pathways in crops, which may manifest in visible symptoms such as small and distorted leaves, interveinal chlorosis in young leaves and shortened internodes (Marschner, 1995). In barley, the visual Zn deficiency symptoms include stunted plants and leaves, chlorotic areas on leaves, necrosis, white spots on leaves and mid-leaf collapse (Genc et al., 2003; Lombnaes and Singh, 2003). In wheat, the symptoms of Zn deficiency are usually observed in young seedlings; later, whitish-brown patches and necrotic lesions can be observed on the leaf blades along with mid-leaf collapse (Rengel and Graham, 1995b; Cakmak and Braun, 2001). The appearance of these symptoms can vary with environmental conditions, plant age, deficiency stage and severity as well as the supply of other nutrients.

The Zn efficiency of genotypes can be determined based on phenotypic characteristics such as the severity of visible deficiency symptoms on leaves. The visual symptoms of Zn deficiency vary considerably between barley genotypes and are significantly correlated with Zn efficiency and grain yield (Genc *et al*., 2002b).

Within all crop species, individual varieties (or cultivars) can often vary considerably in their susceptibility to Zn deficiency. It is therefore important to screen crop varieties such that the more tolerant (or Zn-efficient) varieties can be grown on soils with low plant-available Zn. Genotypes can be screened for Zn efficiency based on the expression and severity of visible Zn-deficiency symptoms on leaves. Using a visual score of deficiency symptoms in barley, it was found that a single gene controls increased tolerance to Zn deficiency in a Zn-efficient genotype compared with a Zn-inefficient genotype at the seedling stage. Hence, visual Zn deficiency scores are useful for genetic analysis of tolerance to Zn deficiency (Genc *et al.*, 2003).

The critical (or threshold) concentration (usually defined as 90% of maximum yield) of Zn in tissues varies with plant species, cultivar, plant age, plant part and the environment. Whole shoots, roots, young leaves and grains are used for diagnosing Zn status. Leaves have been considered the most appropriate plant parts to sample for determination of the nutrient status. The critical Zn concentrations in leaves vary between 20 mg Zn/kg in wheat, 15 mg Zn/kg in rice and 22 mg Zn/kg in maize and groundnut. In whole young plants, the reported values are 8 mg Zn/kg for sorghum, 22 mg Zn/kg for rice and 25 mg Zn/kg for wheat and chickpea. However, differences can also occur between different varieties of these crops (Alloway, 2001).

It has been suggested that biochemical analysis can be an excellent indicator of nutrient status, particularly when plant tissues contain a large amount of physiologically inactive nutrients. The activity of carbonic anhydrase was used to diagnose Zn deficiency in wheat (Rengel, 1995), mustard (Chatterjee and Khurana, 2007) and maize. Additionally, the activity of aldolase and ribonuclease appear to be reliable biological indicators of the Zn status of mustard (Chatterjee and Khurana, 2007).

Zinc deficiency in crop production can be ameliorated through agronomy and/or genetic improvement. Substantial crop responses to Zn fertilization were reported in Australia, India and especially in Central Anatolia (Turkey), where wheat grain yields have increased by over 600% since the mid-1990s (Cakmak, 2004). An alternative approach to increasing crop yields in soils with low plant-available Zn involves exploiting genotypic differences in Zn uptake and tissue-use efficiency that exist within crop species (Rengel, 2001; Hacisalihoglu and Kochian, 2003; Alloway, 2004). This approach involves matching the plant to the soil rather than modifying the soil to suit the plant. There are Zn-efficient cultivars of rice, wheat and other crops that are grown widely in soils with low concentrations of plant-available Zn.

8. Mechanisms of Zn efficiency in plants

Various mechanisms have been proposed to explain Zn efficiency at the molecular, physiological, structural and developmental levels in Zn-efficient genotypes; however, the actual mechanisms involved in Zn efficiency are not clear. In general, Zn-efficient genotypes exhibit increased Zn uptake efficiency at the roots and/or more efficient utilization of Zn within the cells. It was suggested that Zn-efficient plant species have the ability to mine Zn from the soil by enhancing availability of Zn in the rhizosphere (Dong *et al*., 1995; Cakmak *et al*., 1996a; Rengel *et al*., 1998). Genc *et al.* (2006) reported that several different mechanisms contribute to Zn efficiency; however, uptake is the major mechanism, and its effect is modified by the physiological efficiency within the barley shoot. Increased Zn uptake might be dependent on root surface area, root colonization by VA mycorrhizae, low pH in the rhizosphere, release of Zn-chelating phytosiderophores (PS) from the roots and induction of polypeptides involved in Zn uptake and transport across the plasma membrane(Rengel and Graham, 1995b; Cakmak and Braun, 2001).

Zinc efficiency can be influenced by root morphology, which varies among plant species (Dong *et al*., 1995). Longer and thinner roots with increased root surface area may influence the availability of Zn and other nutrients, such as Cu, Mn and Fe (Rengel and Graham, 1995b). Furthermore, soil biological activities, such as root colonization by mycorrhizal fungi, cause increases in the uptake of diffusion-limited nutrients, including Zn, P and Cu. Arbuscular mycorrhizae (AM) enable plants to increase Zn uptake by expanding the volume of soil explored by the root system (Kothari *et al*., 1991). The fungal hyphae extend from the roots into the soil and explore greater distances compared with root hairs. In maize, AM increase plant Zn uptake and shoot Zn content in soils with low concentrations of plant-available Zn (Faber *et al*., 1990).

Roots alter the rhizosphere chemistry by changing the rhizosphere pH (Wang *et al*., 2006) and/or releasing PS that can chelate soil Zn and increase its availability

(Cakmak *et al*., 1994). The root-mediated decrease in pH increases Zn availability by solubilizing Zn present in inorganic and organic soil complexes (Hacisalihoglu and Kochian, 2003).

A number of studies have focused on the role of root exudates in Zn efficiency. PS are non-proteinogenic amino acids released from roots of graminaceous species under Fe (Marschner, 1995) and Zn (Zhang *et al*., 1991; Kochian, 1993) deficiency. PS released from roots are highly effective in mobilizing and complexing Zn in calcareous soils. These compounds are involved in mobilizing Zn from the root apoplast of wheat plants (Zhang *et al*., 1991) and may be involved in the translocation and solubility of Zn within plants (Welch, 1995). Under Zn deficiency, release of PS in Zn-inefficient durum wheat is approximately 6-8 times lower than that in Zn-efficient bread wheat (Cakmak *et al*., 1996b; Cakmak *et al.*, 1998). In rice grown in nutrient solution, the efficiency of Zn uptake is correlated with the exudation rates of low molecular weight organic anions (Hoffland *et al.*, 2006).

The results regarding the role of root PS release in Zn efficiency are both contradictory and somewhat controversial. Erenoglu *et al*. (1996) found that PS release did not correlate with differences in the Zn efficiency of efficient or inefficient wheat genotypes subjected to Zn deficiency. In solution culture, zinc deficiency did not significantly induce PS release from barley and wheat cultivars that had been previously reported to exude more PS under Zn deficiency than Zn-inefficient genotypes (Pedler *et al*., 2000). Although root exudation of PS does not appear to play a major role in Zn efficiency, it cannot be ruled out as a factor; thus, further studies are warranted (Rengel, 2001).

Compared with Zn-inefficient genotypes, Zn-efficient genotypes can deliver more Zn from roots to shoots under low Zn conditions but not under Zn-sufficient conditions (Rengel and Graham, 1995b; Cakmak *et al*., 1996a). The high Zn efficiency of rye is mainly related to its capacity to take up and translocate Zn to shoots at much higher rates than other cereals (Cakmak *et al*., 1997a). Khan *et al*. (1998) concluded that efficient Zn uptake coupled with enhanced root-to-shoot transport could be important for Zn efficiency in chickpea genotypes.

Some reports have suggested that Zn transport from the soil to the root and subsequently to the shoot is not the important factor in differential Zn efficiency. There are no significant differences in shoot Zn concentration between inefficient and efficient genotypes grown in soils with low plant-available Zn, even when large differences in visual Zn deficiency symptoms are observed in contrasting genotypes (Cakmak et al., 1999; Hacisalihoglu *et al.*, 2003). These studies appear to indicate that Zn efficiency is a shoot-mediated trait. A shoot-mediated mechanism for Zn efficiency could likely involve (1) changes in subcellular Zn compartmentation and homeostasis such that efficient genotypes accumulate higher levels of Zn in the cytoplasm of leaf cells and (2) more efficient biochemical use of cellular Zn, such that Zn-requiring macromolecules can efficiently incorporate Zn as a cofactor under low-Zn conditions (Hacisalihoglu and Kochian, 2003).

The results of leaf tissue compartmentation studies of wheat cultivars grown under low-Zn conditions showed that both the efficient and inefficient cultivars had similar Zn contents in the cytoplasm (9–11%) and vacuole (83–85%) (Hacisalihoglu *et al*., 2003). These results suggest that subcellular Zn compartmentation may not be involved in Zn efficiency; however, more studies are obviously needed in this area.

Metal-responsive elements (MREs) may play a role in Zn homeostasis by controlling gene expression in relation to changes in plant metal status. The MRE-binding transcription factor-1 (MTF1) mediates the regulation of genes involved in Zn homeostasis, including responses to both Zn deficiency and toxicity. MTF1 appears to be involved in the regulation of the free Zn concentration in the cell by allowing Zn to bind to MREs and initiate metallothionein gene transcription (Andrews, 2001).

There is a positive correlation between biochemical utilization involving Zn-requiring enzymes and Zn efficiency in wheat and bean (Hacisalihoglu *et al*., 2003). Zn-efficient genotypes might contain a higher amount of Zn that readily participates in metabolic reactions and binds to Zn-requiring enzymes. Zn-efficient wheat genotypes exhibit increased activity of Zn-requiring enzymes (carbonic anhydrase and Cu/Zn superoxide dismutase) than Zn-inefficient genotypes under Zn-deficient conditions and at similar Zn concentrations in leaves (Rengel, 1995; Cakmak *et al*., 1997c). It is possible that genotypes with the Zn-efficiency trait are better able to utilize Zn through the action of the Zn-containing enzymes carbonic anhydrase and Cu/Zn superoxide dismutase (Hacisalihoglu and Kochian, 2003).

Internal utilization of Zn is also considered to be an important potential Zn efficiency mechanism when Zn-efficient and -inefficient plants have similar leaf Zn concentrations and only Zn-inefficient plants show severe Zn-deficiency symptoms (Rengel and Graham, 1995b). Genc *et al*. (2002a) indicated that the greater efficiency of barley genotypes may be attributed to more efficient Zn utilization at the cellular level.

9. Breeding for Zn efficiency

The primary objective of plant breeding has been to enhance farm productivity, usually by developing crops with higher yields. In contrast, improving micronutrient efficiencies and increasing nutrient content in plants has rarely been a breeding objective. In fact, crop nutritional problems have mostly been ignored in breeding.

Some nutritional problems cannot be easily resolved by altering soil fertility or chemistry, and the application of modern breeding techniques to breed crops that are adapted to soils with a poor nutritional status is required. In the case of micronutrient deficiencies induced by high pH (i.e., Zn, Fe and Mn deficiencies), agronomic solutions (i.e., fertilizers) are not always successful, and a genetic solution is necessary (Cakmak and Braun, 2001). Furthermore, the correction of Zn deficiency induced by subsoil constraints, topsoil drying and diseases is not effective via fertilization (Graham and Rengel, 1993). Hence, breeding and use of Zn-efficient plant genotypes that can more effectively function under Zn deficiency is an effective and sustainable solution to address Zn deficiency limitations in crop production.

Progress has been made with respect to the first stage of breeding, which consists of screening for genetic variability in the trace mineral concentration in plant tissues. Crops show significant genotypic variation with respect to the concentration of minerals such as Zn. For example, in field-grown barley genotypes, the grain Zn concentrations ranged from 22 to 61 mg/kg (Sadeghzadeh, 2008). Screening of wheat genotypes revealed grain Zn concentrations ranging from 14 to 42 mg/kg, and similar ranges of Zn concentrations were found in rice (Bouis, 1995).

Due to the large genotypic variation in Zn efficiency among crops (Cakmak *et al.*, 1998), there is a need for targeted selection and/or breeding of plants with greater efficiency, both in terms of higher grain yield and grain Zn content. In the past, lack of a suitable procedure for screening a large number of genotypes in a short time hampered breeding for Zn efficiency. However, screening in the field at nutrient-responsive sites and comparing yields under limiting and non-limiting rates of Zn application has been used extensively to assess efficiency (Graham *et al*., 1992). The results of such screening experiments can be variable because the severity of the nutrient deficiency varies between sites and years due to the effects of other growth-limiting factors, such as drought and diseases. Hence, reliable alternative methods are required.

The use of controlled environments for screening is a common practice. Due to funding and time constraints, the screening of large populations for the development of molecular markers requires a pot culture screening system. Soil-based pot assays under controlled conditions allow for the relative efficiency

of genotypes to be assessed (Cakmak *et al.*, 1997b; Genc and McDonald, 2004); however, the efficiency is generally assessed based on seedling growth rather than grain yield. Additionally, a major challenge will be to demonstrate the relevance of these screening methods under field conditions.

Lombnaes and Singh (2003) selected four approaches to characterize tolerance to Zn deficiency in barley and wheat: (1) relative shoot weight at low compared with high Zn supply ("Zn efficiency index"), (2) relative shoot-to-root ratio at low compared with high Zn supply, (3) total shoot uptake of Zn under deficient conditions and (4) shoot dry weight under deficient conditions. Evaluating the severity of Zn deficiency symptoms on leaves together with the Zn efficiency ratio (yield under -Zn / yield under +Zn) appears to be a reasonable approach for reliably and quickly screening large numbers of genotypes for Zn efficiency within a short time (Cakmak and Braun, 2001; Genc *et al*., 2003).

10. Role of high Zn reserves in seeds

Some crop genotypes have a large capacity to take up trace minerals and accumulate them in the grain, even when grown in soils with low plant-available micronutrients (Graham and Welch, 1996); however, the physiological processes controlling micronutrient accumulation in seeds are not well understood (Welch and Graham, 2002). Micronutrient uptake and accumulation traits in plants are heritable and could therefore be improved by selective breeding. Hence, screening for genetic variability in these traits is commonly the first step in plant breeding. There is significant genotypic variation for seed Zn accumulation in several staple crops, including rice, wheat, maize and bean (Gregorio *et al*., 2000; Genc *et al*., 2002b; Mantovi *et al.,* 2003). For example, the seed Zn concentration of wheat cultivars ranged from 25 to 64 mg/kg (Frossard *et al*., 2000), and the seed Zn content of field-grown barley varied from 0.7 to 2.9 µg/seed (Sadeghzadeh, 2008). There is increasing evidence that an elevated grain nutrient concentration may allow good crop establishment when such seeds are sown in soils with low plant-available nutrients. Sowing seeds with high Zn concentration can be a practical solution for increasing yield under Zn deficiency (Cakmak and Braun, 2001). In glasshouse experiments, wheat plants grown from high-Zn seed had improved growth and grain yield compared with plants derived from seed with low Zn content (Rengel and Graham, 1995a). In Zn-deficient fields, wheat plants derived from seed with high and intermediate Zn content had a significantly greater grain yield compared with plants grown from seed with low Zn content (Cakmak and Braun, 2001). In barley, Genc *et al*. (2000) reported that high seed Zn content significantly reduced the visual Zn deficiency symptoms and improved vegetative growth, especially when the Zn supply from the rooting medium was deficient for plant growth.

Breeding for micronutrient-dense seeds can confer resistance to root diseases, resulting in lower dependence on fungicides. Seed nutrient levels that fail to provide enough nutrition to seedlings in deficient soils may greatly increase plant susceptibility to some diseases, especially fungal root diseases of major crops (Streeter *et al*., 2001). From an agronomic point of view, micronutrient-dense seeds may have enough nutrient reserves to last until a large root system is developed to compensate for the low nutrient supply in micronutrient-poor soils.

Enhancing the nutrient content and nutritional quality of crops for the purpose of improving human nutrition is currently a global challenge because it has been mostly ignored by previous breeding programs. Recently, interest in increasing the micronutrient concentration in crop grains has been increasing (Welch and Graham, 2002; Welch and Graham, 2004; Uauy *et al*., 2006). Such increases result in improved human health and better crop production. The micronutrient concentration in grains destined for human consumption is a more important parameter than the micronutrient content. The nutrient concentration in seeds is dependent on the parental plant growth conditions, including soil type, nutrient availability, crop species and genotype.

It is desirable to combine the ability to load Zn into the grain with a high yield potential. Efficient Zn uptake from the soil and efficient Zn loading into the seed are achievable together with high yield through breeding techniques. The grain Zn concentration of a genotype reflects its ability to take up Zn from the soil, mobilize Zn from different parts of the plant and load Zn in the grain (Genc *et al*., 2006). However, to obtain reliable information regarding the level of genetic variation in grain nutrient concentration, any possible dilution and concentration effects associated with differences in yield potential must be taken into account. Farmers can be expected to adopt genotypes with Zn-dense seeds to achieve higher profits. The use of seed with high Zn content could provide a practical approach for alleviating the Zn-deficiency problem, especially when farmers are not aware of Zn deficiency and in areas where Zn fertilization is not practical.

11. Genotypic variation in Zn efficiency

Plant genotypes vary widely with respect to their tolerance to Zn deficiency, both in terms of Zn utilization and uptake. A wide range of wheat, barley, rice, bean, chickpea and maize germplasms has been studied, indicating there is enough genotypic variation to allow breeding for nutritional improvement (Gregorio *et al*., 2000). Nutritional traits are generally stable across environments, despite some reported genotype by environment (G x E) interactions. Thus, it is possible to combine high micronutrient traits with high yield.

Rengel and Wheal (1997) indicated that the differences in Zn efficiency between wheat cultivars are closely related to differences in Zn uptake capacity. Similarly, the higher Zn efficiency of rye versus wheat cultivars is accompanied by an increase in the Zn concentration of shoots; rye has a higher genetic ability to absorb Zn from soils with low plant-available Zn (Cakmak *et al*., 1997a). In field experiments performed on Zn-deficient calcareous soils, Zn efficiency was positively correlated with the total amount (uptake) of Zn in shoots (Graham et al., 1992; Cakmak *et al*., 1997b). Although Zn-efficient genotypes have greater Zn uptake ability, they do not necessarily have a higher Zn concentration (amount of Zn per unit of dry weight) in leaves, shoots or grains (Graham *et al*., 1992). Zn-inefficient wheat genotypes may even have greater Zn concentrations in leaves or grains than Zn-efficient genotypes (Cakmak *et al*., 1997b; Cakmak *et al.*, 1998). Under Zn deficiency, increased Zn uptake by efficient genotypes improves dry matter production and results in corresponding decreases in Zn concentration similar to those present in Zn-inefficient genotypes (i.e., dilution due to growth) (cf. Marschner, 1995).

12. Genetics of Zn efficiency

Most research related to Zn efficiency in crops has concentrated on the physiological aspects of Zn uptake or has compared genotypes with respect to their relative efficiency to grow in soils with low plant-available Zn. Although some physiological mechanisms involved in Zn efficiency have been documented, limited information is available on the genetic control of these mechanisms and the molecular backgrounds or genes responsible for Zn efficiency. An understanding of the genetic variability for Zn efficiency and its simple inheritance pattern could allow progress toward improving Zn efficiency in crops.

A limited number of studies have yielded some preliminary evidence regarding the genetics of Zn efficiency in several crop species. Studies of chromosome addition lines in rye have shown that Cu, Zn and Mn efficiencies were independent characteristics and were located on different chromosomes. The results from a diallel analysis in rice suggested that the genes controlling Zn efficiency are additive and, to a lesser degree, dominant (Majumder *et al.*, 1990). In maize, four additive genes were reported to affect the Zn concentration in the ear leaf (El-Bendary *et al*., 1993). Several loci on chromosomes 1R, 2R and 7R enhance Zn efficiency in rye, with genes on the short arms of 1R and 7R being the most effective (Cakmak *et al*., 1997a). The distribution of F3 populations from across between Zn-efficient and Zn-inefficient

genotypes showed that only a few genes control Zn efficiency in soybean based on measurements of foliar Zn concentrations (Hartwing *et al*., 1991). The results from a diallel experiment comparing seven wheat cultivars differing in Zn efficiency with their F1 derivatives showed that genes controlling Zn efficiency are dominant (Cakmak and Braun, 2001).

As in *Thlaspi* species, the over expression of Zn transporters in cereals may affect plant growth, seed mineral content and Zn transport rates. Lasat *et al*. (1996) found that enhanced expression of genes encoding Zn transporters can increase Zn uptake in *Thlaspi caerulescens* and *T. arvense*. Further study in this system revealed that a Zn transporter gene, ZNT1, was highly expressed in *T. caerulescens*, allowing enhanced Zn uptake (Lasat *et al.*, 2000; Pence *et al*., 2000; Assuncao *et al*., 2001). The ZNT1 gene is a member of the ZIP family of metal transporters (Guerinot, 2000), which are known to transport a variety of divalent cations. Transgenic *Arabidopsis* over expressing the plasma membrane ion transporter gene ZAT exhibited enhanced Zn content in the roots when the plants were grown under high external Zn (Van der Zaal *et al*., 1999). In barley, over expression of an *Arabidopsis* Zn transporter increased short-term Zn uptake and seed cation content (Ramesh *et al*., 2004).

Uauy (2006) reported that reduction in the RNA levels of the *T. aestivum No Apical Meristem (TaNAM)* genes is associated with a decrease in wheat grain Zn and Fe concentrations and an increase in residual N, Zn and Fe in the flag leaf. These results suggest that the reduced grain Zn and Fe concentrations were the result of reduced translocation from leaves rather than the result of a dilution effect caused by larger grains.

Using visual Zn deficiency symptoms, it was reported that a single dominant gene controls tolerance to Zn deficiency in common bean (Singh and Westermann, 2002). Genc *et al*. (2003) determined the inheritance of Zn efficiency in barley using a visual score of deficiency symptoms and reported that tolerance to Zn deficiency at the seedling stage is controlled by a single gene with no dominance.

13. Identifying molecular markers for Zn efficiency

Selection using molecular markers could be an efficient complementary breeding tool, especially when selection is performed under unfavorable conditions (Cakmak and Braun, 2001). Molecular markers for Mn efficiency have been identified in barley (Pallotta *et al*., 2000), and putative markers have been identified in durum wheat (Khabaz-Saberi *et al*., 2002). Screening for higher seed Zn accumulation in double haploid populations arising from a cross between Zn-efficient and Zn-inefficient genotypes could lead to the development of molecular markers for Zn efficiency.

Molecular markers can be broadly classified into the following three groups: hybridization-based markers, such as restriction fragment length polymorphism (RFLPs); DNA chip- and sequencing-based markers, such as single nucleotide polymorphism (SNPs); and PCR-based markers, such as random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), sequence tagged sites (STS), amplified fragment length polymorphisms (AFLPs), microsatellite-anchored fragment length polymorphisms (MFLPs) and others.

With the ever-increasing number of linkage maps being produced by various research groups, it is anticipated that the mapping of nutritional characteristics will become more common. Before developing a genetic linkage map and identifying molecular markers, large mapping populations that segregate for the particular trait must be produced. Wide crosses usually provide segregating populations with a relatively large array of polymorphisms.

The most commonly used plant materials for genetically dissecting and mapping traits are segregating populations descended from two varieties showing divergence for the target trait(s). F2 populations are suitable for mapping; however,

when traits are controlled by multiple genes, more advanced generations are needed to minimize environmental effects on traits. Development of a complete genetic linkage map requires genetically stable selfed populations that have advanced through several recombination cycles via self-pollination. Doubled haploid and recombinant inbred lines (RILs) provide permanent mapping resources. In addition, doubled haploid lines (DH) and RILs are very useful for mapping because phenotypic analysis is carried out using several plants from each family rather than a single F2 plant. Thus, the analysis is more accurate, and the total population size required is smaller than that required with an F2 population. DH lines and RILs are also better suited for the analysis of quantitative traits.

Polymorphic molecular markers are used for genotyping a segregating population during the development of a linkage map. By statistically evaluating segregating marker alleles and linkages among different marker alleles from previous studies, markers can be placed in linkage groups. When marker locations in the genome are known (e.g., RFLPs or SSRs), linkage groups can be assigned to chromosomes. When the genome of a crop species has adequate marker coverage, the number of linkage groups observed should match the number of haploid chromosomes in the genome (i.e., the barley linkage map should have 7 linkage groups). Although constructing a linkage map is necessary for identifying genes controlling quantitative traits, when the target trait is regulated by major genes, a full linkage map is not always required to identify genes and associate them with markers (Ribaut *et al*., 2001).

The next step after constructing a linkage map is to identify the chromosomal location of the genetic locus by identifying a DNA marker that co-segregates with the trait of interest, such as Zn accumulation. Theoretically, tightly linked DNA markers can speed up the breeding process because breeders may then select for the DNA markers rather than attempting more expensive, time-consuming bioassays or relying on field data. Effective mapping studies depend on two types of data: field or glasshouse data on the segregation of the trait(s) and laboratory data on marker segregation. The quality of the phenotypic data is crucial for the success of gene/QTL analysis because laboratory data on marker segregation within a population must be correlated with field or glasshouse data to identify QTLs. Therefore, phenotypic evaluation must be carefully planned and performed with adequate replications to minimize error (Ribaut *et al*., 2001).

QTLs are genetic factors that are responsible for a fraction of the observed phenotypic variation in a quantitative trait. QTL analysis for cereal grain quality traits has been pursued by several research groups (Septiningsih *et al*., 2003; Li *et al.*, 2004). To identify the genetic loci involved in establishing seed Zn content in Arabidopsis, Vreugdenhil *et al.* (2004) identified four QTLs on chromosomes 1, 2, 3 and 5 that are involved in seed Zn content and explain up to 42% of the variation. In an F2 cross of Arabidopsis halleri with A. petraea, three QTLs were identified on chromosomes 4, 6 and 7 that are involved in determining the seed Zn content (Filatov *et al*., 2007). In common bean, one QTL on linkage group IV was associated with seed Zn content, explaining 15% of the phenotypic variance for this trait (Guzman-Maldonado *et al*., 2003). Using RILs from the cross W7984 x Opata 85, Balint *et al*. (2007) identified QTLs controlling shoot Zn and Mn contents on wheat chromosomes 3AL and 3BL, respectively.

In a DH barley population derived from a cross between Clipper and Sahara, Lonergan *et al*. (2001) identified a region on the long arm of chromosome 4H as being associated with both shoot Zn concentration and content. In a related study, one QTL on the short arm of chromosome 2H was also found to be involved with increased seed Zn content in barley (Lonergan, 2001).

In another study on 150 doubled haploids from the Clipper × Sahara cross grown in the field and glasshouse, Sadeghzadeh *et al.* (2008) identified two regions located on chromosomes 2HS and 2HL that were associated with seed Zn concentration and

content of field-grown plants. The first region was flanked by the RFLP markers Xbcd175 and Xpsr108; the second region was flanked by the morphological marker for six/two rowed ear-types, vrs1, and the RFLP marker XksuF15. These two regions accounted for 45% of the total variation in seed Zn concentration and 59% of the total variation in seed Zn content. In a glasshouse experiment, these two regions located on 2HS and 2HL were also associated with seed Zn concentration and content, and they explained 37% and 55% of the total variation in seed Zn concentration and content, respectively. Furthermore, in a glasshouse experiment, two additional QTLs were detected on chromosomes 3HL and 4HS, explaining 13% of the phenotypic variance in seed Zn concentration (Sadeghzadeh *et al.*, 2013).

Moreover, Sadeghzadeh *et al*. (2008) identified one dominant DNA polymorphism using the MFLP technique. Then, the candidate MFLP marker was converted into a simple sequence-specific and PCR-based marker. This converted sequence-specific marker, designated SZnR1 (Seed Zn Regulator-1), is located on the short arm of chromosome 2H and might be useful in marker-assisted selection (MAS) for the improvement of barley productivity and nutritional quality in Zn-deficient environments.

Verification of QTLs is necessary prior to their use in MAS for cultivar improvement. Putative QTLs could not be confirmed in an independent set of genotypes obtained from the same cross used for mapping (Romagosa *et al*., 1999), which may occur when QTLs are not accurately located in the genome and the flanking molecular markers used do not adequately tag the desirable alleles. A vanishing QTL may also be due to other factors, such as inaccurate phenotyping, accepting a QTL with marginal logarithm of odds (LOD) score and a small sample size. Thus, confirmation of the QTL effects in an independent sample of genotypes within the same cross is warranted. Using a different population from the same cross allows different environmental effects and QTL × environment interactions for a putative QTL to be tested (Romagosa *et al.*, 1999).

A significant development that is likely to occur in the next few years will be the use of markers to identify desirable Zn efficiency gene(s). Markers will allow selection for Zn efficiency independently of the environmental variability or the growth stage. If markers are close enough to the gene of interest, they can be directly used for MAS. One of the major drawbacks occurs when the linked marker used for selection is distant from the gene of interest, leading to potential crossovers between the marker and the gene. This produces a high percentage of false positives or negatives during the screening process (Mohan *et al.*, 1997). Hence, marker density and the accuracy of the current unsaturated genetic maps should be increased, and additional markers are needed to facilitate MAS for complex traits such as Zn efficiency and identification of relevant gene(s). PCR-based markers, such as AFLPs, SSRs and MFLPs, can be used to increase the number of loci on the genetic map and thus increase map saturation (Karakousis *et al*., 2003). With a more saturated map, the genomic position of loci controlling high Zn accumulation can be determined more accurately than with current genetic maps.

Following QTL identification, candidate loci or genes can be determined through fine mapping and map-based cloning, and this information could be used for MAS breeding. Importantly, knowledge regarding the genes and/or chromosomal loci controlling seed or shoot Zn in one plant species can be used in different target crops by exploiting gene homology and/or genome collinearity (Ghandilyan *et al.*, 2006).

14. Conclusions

Low plant availability of soil Zn is a critical problem for cereal production, causing severe reduction in yield and nutritional quality of the grains. Combining improved tolerance to Zn deficiency in soils and increased Zn concentration and content in seeds is a high priority research topic, especially in Zn-deficient areas. High Zn efficiency in crops appears to be

related to various morphological and physiological traits, such as root surface area, Zn-mobilizing root exudates and better utilization of Zn at the cellular level. However, finding a solution to Zn deficiency requires a comprehensive exploration of potential genetic resources and an in-depth understanding of their role in micronutrient accumulation mechanisms.

Field and growth room screening revealed significant genetic variation for Zn efficiency in cereals, suggesting that selection for improved Zn efficiency is possible. Evaluating the severity of Zn deficiency symptoms on leaves as well as monitoring shoot and seed Zn concentration and content appear to be useful approaches for screening large populations for Zn efficiency. However, the variability inherent in field and growth room screening has produced some inconsistent results. In view of the difficulty in developing fast and reliable screening methods for Zn efficiency (such as seedling selection in pots) that accurately reflect field screening, molecular markers for Zn efficiency are considered important for success in cereal breeding programs. A lack of suitable markers for Zn efficiency has long hampered the application of this idea in practice.

Markers are now applied routinely to replace time-consuming or expensive tests. As technology continues to evolve, the availability of markers at low costs will become a reality for many crops. This opens up a range of new options through which information obtained from linkage between markers and genes or QTLs can be utilized. It is essential that plant breeders make use of all tools at hand to develop improved varieties. The recent identification of DNA markers that are diagnostic of Zn efficiency will accelerate the development of cultivars that can remain productive on soils with low plant-available Zn. Additionally, these markers may be used to begin identifying the specific genes responsible for differences in the response of crops to Zn deficiency.

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