Research review

Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine

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Summary

The diets of over two-thirds of the world's population lack one or more essential mineral elements. This can be remedied through dietary diversification, mineral supplementation, food fortification, or increasing the concentrations and/or bioavailability of mineral elements in produce (biofortification). This article reviews aspects of soil science, plant physiology and genetics underpinning crop biofortification strategies, as well as agronomic and genetic approaches currently taken to biofortify food crops with the mineral elements most commonly lacking in human diets: iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), iodine (I) and selenium (Se). Two complementary approaches have been successfully adopted to increase the concentrations of bioavailable mineral elements in food crops. First, agronomic approaches optimizing the application of mineral fertilizers and/or improving the solubilization and mobilization of mineral elements in the soil have been implemented. Secondly, crops have been developed with: increased abilities to acquire mineral elements and accumulate them in edible tissues; increased concentrations of 'promoter' substances, such as ascorbate, β-carotene and cysteine-rich polypeptides which stimulate the absorption of essential mineral elements by the gut; and reduced concentrations of 'antinutrients', such as oxalate, polyphenolics or phytate, which interfere with their absorption. These approaches are addressing mineral malnutrition in humans globally.

Abbreviations: ABC, ATP-binding cassette; CAX, Ca^{2+}/H^{+} antiporter; CCC, cation chloride co-transporter; CDF, cation diffusion facilitator; CLC, chloride channel; CNGC, cyclic nucleotide gated channel; CTR, copper transporter; FRO, ferric reductase oxidase; GLR, glutamate receptor; HAST, high-affinity sulphate transporter; HMA, heavy metal P_{1B}-ATPase; lpa, low phytic acid; IRT, iron-regulated transporter; MATE, multidrug and toxin efflux; MRS2, mitochondrial RNA splicing 2; MscS, mechanosensitive channel of small conductance; MSL, MscS-like; MTP, metal tolerance protein; NA, nicotianamine; NAS, nicotianamine synthase; NRAMP, natural

resistance-associated macrophage protein; *O*PHS, *O*-phosphohomoserine; OPT, oligopeptide transporter; PIC1, permease in chloroplasts 1; QTL, quantitative trait locus; SAM, S-adenosyl methionine; SeCys, selenocysteine; SeMet, selenomethionine; VIT1, vacuolar iron transporter 1; VSP, vegetative storage protein; YSL, yellow stripe like; ZIP, ZRT-, IRT-like protein; ZRT, zinc-regulated transporter.

Mineral elements required by humans

Humans require at least 22 mineral elements for their wellbeing (Welch & Graham, 2004; White & Broadley, 2005a; Graham *et al.*, 2007). These can be supplied by an appropriate diet. However, it is estimated that over 60% of the world's 6 billion people are iron (Fe) deficient, over 30% are zinc (Zn) deficient, 30% are iodine (I) deficient and *c.* 15% are selenium (Se) deficient (see Supporting Information References S1). In addition, calcium (Ca), magnesium (Mg) and copper (Cu) deficiencies are common in many developed and developing countries (Frossard *et al.*, 2000; Welch & Graham, 2002, 2005; Rude & Gruber, 2004; Grusak & Cakmak, 2005; Thacher *et al.*, 2006). This situation is attributed to crop production in areas with low mineral phytoavailability and/or consumption of (staple) crops with inherently low tissue mineral concentrations, compounded by a lack of fish or animal products in the diet (Welch & Graham, 2002, 2005; Poletti *et al.*, 2004; White & Broadley, 2005a; Gibson, 2006; Graham *et al.*, 2007). Currently, mineral malnutrition is considered to be among the most serious global challenges to humankind and is avoidable (Copenhagen Consensus 2004; http://www.copenhagenconsensus.com). Mineral malnutrition can be addressed through dietary diversification, mineral supplementation, food fortification and/or increasing mineral concentrations in edible crops (biofortification). However, strategies to increase dietary diversification, mineral supplementation and food fortification have not always been successful (see References S2). For this reason, biofortification of crops through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements, is advocated as an immediate strategy not only to increase mineral concentrations in edible crops but also to improve yields on infertile soils (Graham *et al.*, 2001, 2007; Bouis *et al.*, 2003; Genc *et al.*, 2005; White & Broadley, 2005a; Pfeiffer & McClafferty, 2007). In addition, as mineral elements in edible portions of biofortified crops must be bioavailable to humans, parallel attempts are advocated (1) to increase the concentrations of 'promoter' substances, such as ascorbate (vitamin C), β-carotene, cysteine-rich polypeptides and certain organic and amino acids, which stimulate the absorption of essential mineral elements by the gut, and (2) to reduce the concentrations of 'antinutrients', such as oxalate, polyphenolics (tannins) or phytate (IP_6), which interfere with their absorption (References S3). This review will focus on strategies to increase the concentrations and bioavailability of the seven mineral elements most often lacking in human diets: Fe, Zn, Cu, Ca, Mg, Se and I.

Phytoavailability of mineral elements

Plants can only acquire mineral elements supplied to them in specific chemical forms. For a biofortification strategy to prove successful, it is necessary to be aware of the forms of mineral elements acquired by plant roots, and the limitations to the supply and phytoavailability of mineral elements in the rhizosphere solution. The supply and phytoavailability of mineral elements in the rhizosphere solution ultimately limit the accumulation of mineral elements by crops, unless foliar fertilizers are applied.

Roots of all plant species can take up Fe, Zn, Cu, Ca and Mg in their cationic forms and graminaceous species can also take up Fe, Zn and Cu as metal-chelates (see section 'Uptake, distribution and accumulation of mineral elements by plants'; Marschner, 1995; White, 2003). Selenium can be taken up by plant roots as selenate, selenite or organoselenium compounds (White *et al.*, 2004, 2007b; Li *et al.*, 2008) and iodine can be taken up as either iodide or iodate (Umaly & Poel, 1971; Mackowiak & Grossl, 1999). The occurrence of these chemical forms in the rhizosphere solution is a function of the soil's physicochemical and biological properties, which will ultimately determine the phytoavailability of these elements in the soil.

Mineral elements can be present in the soil as free ions, or ions adsorbed onto mineral or organic surfaces, as dissolved compounds or precipitates, as part of lattice structures or contained within the soil biota. The most important soil properties governing mineral availability are soil pH, redox conditions, cation exchange capacity, activity of microbes, soil structure, organic matter and water content (Shuman, 1998; Frossard *et al.*, 2000). Indeed, although high concentrations of Fe, Zn and Cu occur in many soils, the phytoavailability of these mineral elements is often restricted by soil properties (see References S4), which predetermine both genetic and agricultural strategies for their effective utilization.

Concentrations of cationic Fe, Zn and Cu in the rhizosphere solution are determined by soil-specific precipitation, complexation and adsorption reactions, and high pH is often the major factor limiting the phytoavailability of these elements. Iron deficiency in plants often occurs on well-aerated, calcareous or alkaline soils (Shuman, 1998; Schmidt, 1999; Frossard *et al.*, 2000). These soils cover 25 to 30% of the land

surface and are distributed throughout the world (Frossard *et al.*, 2000; Cakmak, 2002, 2008; Alloway, 2004). In such soils, soluble Fe species rarely exceed 10^{-10} M (Frossard *et al.*, 2000). Both Zn and Cu deficiencies also occur in plants growing on calcareous or alkaline soils, especially in arid and semi-arid environments (see References S5). It is estimated that about half the agricultural soils in India and Turkey, a third of agricultural soils in China, and most soils in Western Australia lack sufficient phytoavailable Zn (Frossard *et al.*, 2000; Cakmak, 2004; Broadley *et al.*, 2007; Ismail *et al.*, 2007). In nonpolluted areas, typical Zn^{2+} concentrations in the soil solution range from 10^{-8} to 10^{-6} M and Cu^{2+} concentrations range from 10^{-9} to 10^{-6} M (Barber, 1995; Welch, 1995; Frossard *et al.*, 2000; Broadley *et al.*, 2007). Because of their low concentrations in the soil solution and small diffusion coefficients, Zn^{2+} and Cu^{2+} have limited mobility in the soil (Gupta, 1979; Barber, 1995; Shuman, 1998; Whiting *et al.*, 2003; Broadley *et al.*, 2007; Cakmak, 2008) and plant roots must forage through the soil to acquire sufficient Zn and Cu for plant nutrition (Rengel, 2001; Hacisalihoglu & Kochian, 2003). Processes that increase Fe, Zn and Cu phytoavailability in the rhizosphere, such as the exudation of protons, phytosiderophores and organic acids by roots, generally increase the concentrations of these elements in crops (Welch, 1995; Rengel, 2001; Abadía *et al.*, 2002; Hoffland *et al.*, 2006; Wissuwa *et al.*, 2006; Ismail *et al.*, 2007; Degryse *et al.*, 2008).

Plants rarely lack a Ca supply from the soil solution sufficient for growth, and Ca^{2+} concentrations in the rhizosphere solution generally lie in the millimolar range (White & Broadley, 2003). However, Ca deficiency can occur in plants growing on highly weathered tropical soils, because of their low total Ca content (Richey *et al.*, 1982), on strongly acidic soils, where Al^{3+} may inhibit Ca^{2+} uptake, and on sodic or saline soils, where excessive sodium (Na^{+}) inhibits Ca^{2+} uptake (Epstein, 1972; Marschner, 1995). Sodic or saline soils occur worldwide, but mostly in the arid subtropics (Frossard *et al.*, 2000). In addition, several costly Ca-deficiency disorders occur in horticulture, which arise when sufficient Ca is temporarily unavailable to developing tissues (Shear, 1975; Ho & White, 2005). The supply of Ca^{2+} to field crops is determined by various aspects of soil chemistry including cation exchange capacity, representation of Ca in the base cation pool, the rate at which mineralization of soil organic matter releases Ca^{2+}, and the pH of the soil solution (McLaughlin & Wimmer, 1999).

Magnesium is present as a divalent cation in the soil solution, which, because it binds less avidly to soil particles than other cations, is prone to leaching. This is considered to be an important factor influencing Mg phytoavailability in shallow or coarse-textured soils (Wilkinson *et al.*, 1990). Magnesium deficiency in plants occurs worldwide, especially on strongly acidic soils (Metson, 1974; Wilkinson *et al.*, 1990; Hailes *et al.*, 1997; Aitken *et al.*, 1999), and is aggravated by high concentrations of competing cations, particularly Al^{3+} and Mn^{2+}, in the soil solution. On alkaline soils, carbonate formation and excess Ca, potassium (K) and Na reduce Mg phytoavailability. It is also possible that the incidence of Mg deficiency in crop plants is increasing as a result of intensive crop production without concomitant Mg fertilization. The Mg concentrations in soil solutions extracted at field capacity generally lie between 125 μM and 8.5 mM, which is sufficient for mass flow to supply Mg to plant roots (Wilkinson *et al.*, 1990; Barber, 1995; Hailes *et al.*, 1997).

The concentration and forms of Se in soils are determined primarily by their geochemistry (Gissel-Nielsen, 1998; Combs, 2001; Gupta & Gupta, 2002; Lyons *et al.*, 2003; Broadley *et al.*, 2006b; White *et al.*, 2007b; Rayman, 2008). Although seleniferous soils can have Se concentrations in excess of 1200 mg Se kg^{-1}, the Se concentrations in most soils lie between 0.01 and 2.0 mg Se kg^{-1}. Selenate is the major Se species in alkaline and oxidized soils (pe + pH > 15), whereas selenite predominates in well-drained mineral soils with a neutral to acidic pH (pe + pH = 7.5 to 15). Selenide species are stable only under low redox conditions (pe + pH < 7.5). Selenate is relatively mobile in the soil solution, but selenite is strongly absorbed by Fe and aluminium (Al) oxides/hydroxides and, to a lesser extent, by clays and organic matter. In most cultivated soils, selenate is the form of Se available to plants (Broadley *et al.*, 2006b; White *et al.*, 2007b).

Iodine is present in soils as iodide, iodate and organic I compounds. Little I is present in the soil solution and most soil I is associated with organic matter, clays and oxides of Fe and Al (Fuge & Johnson, 1986). The prevalent form of I in the soil solution is often iodide, but iodate may also occur depending upon pH and redox conditions (Fuge & Johnson, 1986; Yuita, 1992; Mackowiak & Grossl, 1999; Kodama *et al.*, 2006). As atmospheric I deposition is an important source of soil I, mid-continental soils often lack I (Fuge & Johnson, 1986), but soils with low I concentrations or phytoavailability occur worldwide (Lyons *et al.*, 2004).

Many strategies for the biofortification of crops with essential mineral elements rely on increasing the acquisition of these elements from the soil. However, it is obvious that if the soil contains insufficient amounts of these elements then they must be added to the agricultural system as fertilizers. If sufficient amounts of these elements are present in the soil, then the focus turns to increasing the supply and phytoavailability of these elements in the rhizosphere, and their uptake by plant roots and redistribution to edible portions, such that biofortification is effective.

Uptake, distribution and accumulation of mineral elements by plants

To increase mineral concentrations in edible tissues, without loss of yield, there must be increased uptake by roots (of minerals present in the soil solution) or leaves (for foliar applied minerals), effective redistribution within the plant to the edible

portion, and accumulation in edible tissues in a nontoxic form (Welch & Graham, 2005). This section provides a brief overview of the molecular and physiological processes involved in the uptake, distribution and accumulation of mineral elements in plants.

Iron

Plants have two strategies for acquiring Fe from the soil (Marschner, 1995; Welch, 1995; Schmidt, 1999; Gross *et al.*, 2003; Grotz & Guerinot, 2006; Puig *et al.*, 2007a). In strategy I, which is employed by nongraminaceous species, roots acidify the rhizosphere and release organic acids and phenolic compounds to increase Fe^{3+} concentrations in the soil solution. These compounds chelate Fe^{3+}, which is subsequently reduced to Fe^{2+} by ferric reductases in the plasma membrane of root epidermal cells, which are encoded by members of the ferric reductase oxidase (*FRO*) gene family (Robinson *et al.*, 1999; Wu *et al.*, 2005; Mukherjee *et al.*, 2006). Members of the zinc-regulated transporter (ZRT)-, iron-regulated transporter (IRT)-like protein (ZIP) family, such as AtIRT1 in Arabidopsis, then mediate Fe^{2+} influx to root cells (Vert *et al.*, 2002; Ishimaru *et al.*, 2006). In strategy II, which is employed by cereals and grasses, phytosiderophores (structural derivatives of mugineic acid) are released into the rhizosphere to chelate Fe^{3+} and the Fe^{3+}-phytosiderophore complex is taken up by root cells (von Wirén *et al.*, 1995; Ishimaru *et al.*, 2006). The chemistry of phytosiderophores is species-specific and determines the contrasting abilities of different grasses and cereals to acquire Fe (Marschner, 1995; Bashir *et al.*, 2006). It is thought that homologues of the maize (*Zea mays*) yellow stripe 1 (YS1) protein belonging to the oligopeptide transporter (OPT) family are responsible for Fe^{3+}-phytosiderophore uptake by strategy II plants (von Wirén *et al.*, 1995; Curie *et al.*, 2001; Roberts *et al.*, 2004; Ishimaru *et al.*, 2006; Haydon & Cobbett, 2007a; Puig *et al.*, 2007a). The YS1 protein is a proton-coupled metal-complex symporter (Schaaf *et al.*, 2004). Intriguingly, the genomes of both Arabidopsis and rice (*Oryza sativa*) appear to contain the genes necessary for both strategy I and strategy II Fe acquisition, but there are quantitative differences in their number and qualitative differences in their structure and activity (Gross *et al.*, 2003; Puig *et al.*, 2007a).

Proteins that load Fe into the xylem have not been identified yet, but they are believed to transport Fe^{2+}. Within the xylem, Fe is transported as Fe^{3+}-citrate (Brown & Chaney, 1971; Welch, 1995; von Wirén *et al.*, 1999; Abadía *et al.*, 2002; Mukherjee *et al.*, 2006). FRD3, a member of the multidrug and toxin efflux (MATE) family present in the root pericycle, is important for Fe transport from root to shoot and appears to be involved in loading citrate into the xylem in Arabidopsis (Durrett *et al.*, 2007; Haydon & Cobbett, 2007a; Puig *et al.*, 2007a). Presumably, members of the ZIP family are responsible for Fe^{2+} uptake by shoot cells. Members of the natural resistance-associated macrophage protein (NRAMP) family are not thought to be responsible for Fe uptake from the soil, but have been implicated in Fe homeostasis within plant cells. In particular, NRAMP3 and NRAMP4 are thought to facilitate Fe^{2+} release from the vacuole (Thomine *et al.*, 2003; Gross *et al.*, 2003; Hall & Williams, 2003; Lanquar *et al.*, 2005; Grotz & Guerinot, 2006; Puig *et al.*, 2007a), opposing the activity of the vacuolar iron transporter 1 (VIT1) protein which catalyses Fe influx to the vacuole (Kim SA *et al.*, 2006b). In leaves of plants overloaded with Fe, and in some seeds, Fe can accumulate as Fe-chelates in the vacuole (Pich *et al.*, 2001; Lanquar *et al.*, 2005; Kim SA *et al.*, 2006b). However, under most environmental conditions, the majority of cellular Fe is located in the plastid, where it is associated with the Fe-storage protein ferritin (Briat *et al.*, 1999; Petit *et al.*, 2001). The permease in chloroplasts 1 (PIC1) protein is thought to transport Fe from the cytoplasm into the plastid (Duy *et al.*, 2007). It is thought that yellow stripe like (YSL) proteins, and related OPTs, load and unload Fe^{2+}-nicotianamine (Fe^{2+}-NA) complexes into and out of the phloem for Fe redistribution within the plant (see References S6).

The expression of genes encoding many of the proteins responsible for Fe uptake and redistribution within the plant are up-regulated during Fe deficiency. These include genes encoding FROs (Robinson *et al.*, 1999; Wintz *et al.*, 2003; Li *et al.*, 2004; Ishimaru *et al.*, 2006; Mukherjee *et al.*, 2006), ZIPs (Curie *et al.*, 2000; Bereczky *et al.*, 2003; Wintz *et al.*, 2003; Li *et al.*, 2004; Ishimaru *et al.*, 2005; Grotz & Guerinot, 2006), NRAMPs (Curie *et al.*, 2000; Bereczky *et al.*, 2003; Lanquar *et al.*, 2005; Grotz & Guerinot, 2006; Krämer *et al.*, 2007), YS1 (Curie *et al.*, 2001), YSLs (Wintz *et al.*, 2003; Koike *et al.*, 2004; Roberts *et al.*, 2004; Suzuki *et al.*, 2006; Ogo *et al.*, 2007; Stacey *et al.*, 2008) and enzymes involved in the biosynthesis of NA, such as nicotianamine synthase (NAS), and phytosiderophores (Wintz *et al.*, 2003; Bashir *et al.*, 2006; Suzuki *et al.*, 2006; Ogo *et al.*, 2007). The expression of strategy I responses is regulated by the basic helix-loop-helix (bHLH) transcription factor LeFER in tomato (*Solanum lycopersicum*) (Ling *et al.*, 2002; Bereczky *et al.*, 2003; Li *et al.*, 2004) or its orthologue AtFIT1 in Arabidopsis (Colangelo & Guerinot, 2004; Jakoby *et al.*, 2004; Yuan *et al.*, 2005). However, overexpression of neither *LeFER* nor *AtFIT1* leads to constitutive Fe uptake (Colangelo & Guerinot, 2004; Yuan *et al.*, 2005), suggesting the existence of additional regulatory cascades. It is possible that one of these is initiated by a shoot-derived signal (Enomoto *et al.*, 2007). In rice, the ABI3/VP1 transcription factor OsIDEF1 appears to regulate the expression of strategy II responses through the bHLH transcription factor OsIRO2 (Kobayashi *et al.*, 2007; Ogo *et al.*, 2007).

Zinc

It is commonly assumed that most Zn is transported symplastically across the root to the xylem, although a substantial fraction may traverse the root and reach the xylem

via the apoplast (White *et al.*, 2002b; Broadley *et al.*, 2007). Zinc can be taken up across the plasma membrane of root cells as Zn^{2+} or as a Zn-phytosiderophore complex (von Wirén *et al.*, 1996; Grotz & Guerinot, 2006; Suzuki *et al.*, 2006; Broadley *et al.*, 2007; Ismail *et al.*, 2007). Although some plasma membrane Ca^{2+} channels are permeable to Zn^{2+} (Demidchik *et al.*, 2002; White *et al.*, 2002a), it is thought that most Zn^{2+} influx to the cytoplasm is mediated by ZIPs (ZIP1, ZIP3 and ZIP4; Pence *et al.*, 2000; Assunção *et al.*, 2001; López-Millán *et al.*, 2004; Colangelo & Guerinot, 2006; Broadley *et al.*, 2007; Palmgren *et al.*, 2008), and that YSL proteins catalyse the uptake of Zn-phytosiderophore complexes in strategy II plants (von Wirén *et al.*, 1996; Suzuki *et al.*, 2006; Haydon & Cobbett, 2007a). As the cytoplasm of plant cells contains an abundance of proteins that bind Zn^{2+}, cytoplasmic Zn^{2+} concentrations are likely to be vanishingly small (Broadley *et al.*, 2007).

Members of the cation diffusion facilitator (CDF) family, such as the metal tolerance proteins MTP1 and MTP3 (see References S7), the Mg^{2+}/H^{+} antiporter, MHX (Shaul *et al.*, 1999; Elbaz *et al.*, 2006), and the zinc-induced facilitator 1 (ZIF1) transporter (Haydon & Cobbett, 2007b) appear to transport Zn into the vacuole via a Zn^{2+}/H^{+} antiport mechanism, whilst NRAMPs have been implicated in Zn mobilization from the vacuole (Thomine *et al.*, 2003). Members of the heavy metal P_{1B}-ATPase family (HMA1, HMA2, HMA3 and HMA4) appear to have Zn-transport functions throughout the cell, as well as in loading Zn into the xylem (see References S8). It is thought that Zn is sequestered in the vacuole as an organic acid complex (Broadley *et al.*, 2007). Within the xylem, Zn may be transported as Zn^{2+} or complexed with organic acids, histidine or nicotianamine (Welch, 1995; von Wirén *et al.*, 1999; White *et al.*, 2002b; Broadley *et al.*, 2007; Palmgren *et al.*, 2008). Members of the ZIP family are thought to mediate Zn^{2+} influx to leaf cells and to the phloem (Ishimaru *et al.*, 2005). In addition, YSL proteins may load Zn into the phloem, where Zn is transported as a Zn-NA complex, or as a complex with small proteins, to sink tissues (Krüger *et al.*, 2002; Gross *et al.*, 2003; Haydon & Cobbett, 2007a; Puig *et al.*, 2007a; Waters & Grusak, 2008a). Although Zn mobility in the phloem is generally considered to be low, this may not always be the case (Welch, 1995; Haslett *et al.*, 2001).

The genes encoding many of the proteins responsible for Zn uptake, sequestration and redistribution within the plant show up-regulated expression during Zn deficiency. These include *ZIP*s (Pence *et al.*, 2000; Wintz *et al.*, 2003; Ishimaru *et al.*, 2005; Filatov *et al.*, 2006; Hammond *et al.*, 2006; Talke *et al.*, 2006; van de Mortel *et al.*, 2006; Krämer *et al.*, 2007; Milner & Kochian, 2008), *HMA*s (Wintz *et al.*, 2003; Papoyan & Kochian, 2004; van de Mortel *et al.*, 2006), *YSL*s (Wintz *et al.*, 2003; Suzuki *et al.*, 2006; van de Mortel *et al.*, 2006), *MTP*s (Arrivault *et al.*, 2006; van de Mortel *et al.*, 2006), *ZIF1* (Haydon & Cobbett, 2007b), *FRD3* (van de Mortel *et al.*, 2006), *NAS*s and genes encoding enzymes involved in the biosynthesis of phytosiderophores (Wintz *et al.*, 2003; Suzuki *et al.*, 2006; Talke *et al.*, 2006; van de Mortel *et al.*, 2006). Interestingly, genes encoding ZIPs, MTPs, NRAMPs, HMAs, FRD3 and YSL proteins and NAS exhibit constitutively high expression in plants that hyper-accumulate Zn (see References S9).

Copper

Copper is taken up as Cu^{+} by high-affinity transporters belonging to the copper transporter (CTR) family (COPT1, COPT2, COPT3 and COPT4; Sancenón *et al.*, 2003, 2004; Grotz & Guerinot, 2006; Puig *et al.*, 2007a) and/or as Cu^{2+} by ZIPs (ZIP2 and ZIP4) whose expression in roots is up-regulated by Cu deficiency (Grotz *et al.*, 1998; Wintz *et al.*, 2003). Once inside plant cells, Cu^{+} is bound by metallotheioneins (Guo *et al.*, 2003, 2008) and Cu chaperone proteins, such as the Arabidopsis AtCCH, AtCCS, AtATX1 and AtCOX17 metallochaperones, which deliver it to specific apoproteins to form biologically active Cu-proteins (Hall & Williams, 2003; Grotz & Guerinot, 2006; Krämer *et al.*, 2007; Puig *et al.*, 2007a,b). The expression of genes encoding metallotheioneins is strongly induced by Cu in the environment and has been shown to correlate with Cu tolerance among Arabidopsis ecotypes and populations of *Silene vulgaris* and *Silene paradoxa* (Murphy & Taiz, 1995; van Hoof *et al.*, 2001; Mengoni *et al.*, 2003). Members of the heavy metal P_{1B}-ATPase family remove Cu from the cytoplasm. In Arabidopsis, AtHMA1, AtHMA2, AtHMA3 and AtHMA4 transport divalent Cu^{2+}, whereas AtHMA5, AtHMA6, AtHMA7 and AtHMA8 transport monovalent Cu^{+} (Baxter *et al.*, 2003; Hall & Williams, 2003; Grotz & Guerinot, 2006; Puig *et al.*, 2007a). These transporters appear to function in Cu detoxification (AtHMA5) or in delivering Cu for the formation of Cu-proteins in the secretory pathway or plastids (AtHMA1; AtHMA6 = AtPAA1; AtHMA7 = AtRAN1; AtHMA8 = AtPAA2; Shikanai *et al.*, 2003; Abdel-Ghany *et al.*, 2005; Andrés-Colás *et al.*, 2006; Seigneurin-Berny *et al.*, 2006; Puig *et al.*, 2007b). Similarly, the rice ATPase OsHMA9 has been implicated in Cu efflux from the cytoplasm (Lee *et al.*, 2007). The NRAMPs have been implicated in Cu^{2+} transport to the vacuole (Hall & Williams, 2003) and Cu-binding vegetative storage proteins (VSPs) appear to have a role in Cu homeostasis and Cu detoxification (Mira *et al.*, 2002; Kung *et al.*, 2006). It is not known how Cu is loaded into the xylem, but it is transported in a complexed form, probably as Cu^{2+}-NA (von Wirén *et al.*, 1999). Estimates of Cu mobility in the phloem vary widely (Welch, 1995). Copper is probably loaded into the phloem by a YSL protein and transported complexed with small proteins or as Cu-NA (Mira *et al.*, 2001; Guo *et al.*, 2003; DiDonato *et al.*, 2004; Puig *et al.*, 2007a; Waters & Grusak, 2008a). Interestingly, the YSL proteins may transport both Cu-NA complexes and the free Cu^{2+} and Fe^{2+} cations (Wintz *et al.*, 2003).

Calcium

It is thought that Ca can reach the xylem solely via the root apoplast in regions where Casparian bands are absent or via the cytoplasm of unsuberized endodermal cells where Casparian bands are present (White, 2001; Moore *et al.*, 2002). However, the relative contributions of apoplastic and symplastic pathways to the delivery of Ca to the xylem are unknown. Calcium influx to root cells is mediated by a variety of Ca^{2+}-permeable cation channels (White, 2000; White *et al.*, 2002a; White & Broadley, 2003; Demidchik & Maathuis, 2007; Haswell, 2007; Roux & Steinebrunner, 2007; Miedema *et al.*, 2008; Wheeler & Brownlee, 2008). These include hyperpolarization-activated Ca^{2+} channels, thought to be formed by plant annexins, voltage-independent cation channels, thought to be formed by members of the cyclic nucleotide gated channel (CNGC) and/or glutamate receptor (GLR) protein families, and depolarization-activated Ca^{2+} channels, one of which may be encoded by homologues of the *AtTPC1* gene (see References S10). The plasma membrane may also contain mechanosensitive ion channels permeable to Ca^{2+} encoded by members of the mechanosensitive channel of small conductance (MscS)-like (*MSL*) gene family (Haswell, 2007). The activity of all these ion channels is exquisitely regulated, because explicit perturbations in cytosolic Ca^{2+} concentrations co-ordinate specific responses to many developmental and environmental stimuli (White & Broadley, 2003). Homologues of the low-affinity cation transporter of wheat (TaLCT1) can also facilitate Ca^{2+} influx to root cells (Clemens *et al.*, 1998). Cytosolic Ca^{2+} is maintained at submicromolar concentrations by Ca^{2+}-ATPases, encoded by members of the P_{2B}-ATPase (*ECA*/*ACA*) gene family, and Ca^{2+}/H^+ antiporters, such as those encoded by the Ca^{2+}/H^+ antiporter (*CAX*) genes, which export Ca^{2+} to the apoplast, endoplasmic reticulum, plastids or vacuoles (Hirschi, 2001; Baxter *et al.*, 2003; White & Broadley, 2003; Shigaki & Hirschi, 2006; George *et al.*, 2008). CAX-mediated Ca^{2+} influx to vacuoles is energized by the H^+ gradient generated by vacuolar ATPases and/or PPiases (Shigaki & Hirschi, 2006). Within the cytosol Ca^{2+} is complexed to diverse proteins including calmodulin, calmodulin-related proteins, calcineurin-B-like proteins, Ca^{2+}-dependent protein kinases and annexins (White & Broadley, 2003). The Ca^{2+}-binding proteins calreticulin, calsequestrin, calnexin and the lumenal binding protein (BiP) are present in the endoplasmic reticulum (White & Broadley, 2003) and vacuoles may also contain Ca^{2+}-binding proteins, such as the radish (*Raphanus sativus*) vacuolar calcium-binding (RVCaB) protein (Yuasa & Maeshima, 2000). Calcium is released from the vacuole through Ca^{2+}-permeable cation channels (White, 2000), which again may include homologues of AtTPC1 and/or annexins in some plant species (Peiter *et al.*, 2005; Pottosin & Schönknecht, 2007; Mortimer *et al.*, 2008; Wheeler & Brownlee, 2008). No gene encoding any ligand-gated, vacuolar Ca^{2+} channel has been identified to date (Nagata *et al.*, 2004; Krinke *et al.*, 2007; Wheeler & Brownlee, 2008).

Calcium transport to the shoot occurs largely from the root apex and/or regions of lateral root initiation (White, 2001) and, within the xylem, Ca appears to be transported either as Ca^{2+} or complexed with organic acids (Welch, 1995). When grown in the same environment, differences between taxa in their shoot Ca concentrations correlate well with cell wall chemistry and cation-binding capacity (White & Broadley, 2003; White, 2005), but the absolute shoot Ca concentration found in leaves depends greatly upon the phytoavailability of Ca in the rhizosphere and the transpirational water flux (White, 2001; White & Broadley, 2003). As Ca is almost immobile in the phloem, fruits, seeds and tubers rely on its delivery via the xylem and, consequently, contain low Ca concentrations (Welch, 1999; White & Broadley, 2003; Ho & White, 2005; White *et al.*, in press). In vacuoles, Ca is present as Ca^{2+}, as soluble complexes with proteins and/or organic acids, and in insoluble forms, such as Ca-oxalate and Ca-phytate, depending upon the plant species and the phytoavailability of Ca in the environment (Kinzel, 1982; Kinzel & Lechner, 1992; White & Broadley, 2003; Franceschi & Nakata, 2005; White, 2005). Some plants accumulate soluble Ca in specific cell types, such as leaf trichomes (White & Broadley, 2003), and the formation Ca-oxalate crystals also occurs in specific cell types, according to genetic predisposition (Franceschi & Nakata, 2005).

Magnesium

It is thought that Mg enters root cells through Mg^{2+}-permeable cation channels (White, 2000; White & Broadley, 2003) and/or that members of the mitochondrial RNA splicing 2 (MRS2) family of transport proteins (AtMGT1 and AtMGT10) catalyse Mg^{2+} influx across the plasma membrane (Schock *et al.*, 2000; Li *et al.*, 2001b; Shaul, 2002; Gardner, 2003). Most cellular Mg is associated with proteins, and cytosolic Mg^{2+} concentrations approximate 0.4 mM (White *et al.*, 1990). The vacuole is the main storage compartment for Mg in plants and the MHX transporter, which is encoded by a single gene in Arabidopsis, is thought to dominate Mg^{2+} transport into the vacuole (Shaul *et al.*, 1999; David-Assael *et al.*, 2006). Interestingly, AtMHX co-localizes with a major chromosomal quantitative trait locus (QTL) affecting seed Mg concentration in Arabidopsis (Vreugdenhil *et al.*, 2004). Magnesium is released from the vacuole through Mg^{2+}-permeable cation channels, including the slow vacuolar (SV) channel (White, 2000; Pottosin & Schönknecht, 2007). It is possible that ATPases catalyse Mg^{2+} efflux from root cells into the xylem, where it is transported either as Mg^{2+} or complexed with organic acids (Welch, 1995).

Shoot Mg concentrations in plants supplied with adequate Mg generally approximate 1 to 10 mg g^{-1} dry matter (DM) (Wilkinson *et al.*, 1990; Broadley *et al.*, 2004). Approximately 75% of leaf Mg appears to be associated with protein synthesis via its roles in ribosomal structure and function

(Wilkinson *et al.*, 1990). In leaves, the MRS2-11 transporter is thought to facilitate Mg entry to the chloroplast (Drummond *et al.*, 2006), where between 15 and 20% of the Mg in leaves is associated with chlorophyll (Wilkinson *et al.*, 1990). Because Mg is a phloem-mobile element, it is readily translocated to fruit, seed and tubers (Wilkinson *et al.*, 1990; White *et al.*, in press).

Selenium

Plant roots can take up Se as selenate, selenite or organoselenium compounds, such as selenocysteine (SeCys) and selenomethionine (SeMet), but cannot take up colloidal elemental Se or metal selenides (White *et al.*, 2004, 2007b). Selenate is transported across the plasma membrane of root cells by high-affinity sulphate transporters (HASTs; Terry *et al.*, 2000; White *et al.*, 2004, 2007b; Sors *et al.*, 2005b; Broadley *et al.*, 2006b; Hawkesford & Zhao, 2007), whilst selenite is thought to be transported by phosphate transporters (Li *et al.*, 2008). Selenite is rapidly converted to organoselenium compounds in the root, whereas selenate is delivered to the xylem and transported to the shoot, where it is assimilated into organoselenium compounds and redistributed within the plant in a manner analogous to S (Terry *et al.*, 2000; Broadley *et al.*, 2006b; Sors *et al.*, 2005b; White *et al.*, 2007b; Hawkesford & Zhao, 2007; Li *et al.*, 2008).

The enzymes of the Se/S assimilation pathway are generally encoded by extensive gene families whose products are directed to different intracellular compartments (Hawkesford & De Kok, 2006). Selenate is thought to be activated by adenosine triphosphate sulphurylase to form adenosine 5′-phosphoselenate (APSe), which then is reduced to selenite by adenosine 5′-phosphosulphate (APS) reductase. The activity of APS reductase appears to exert a major effect on the flux through the Se/S assimilation pathway (Vauclare *et al.*, 2002). Selenite is then reduced to selenide by a sulphite reductase located in the chloroplast. SeCys is synthesized from serine and selenide by cysteine synthase, an enzyme complex containing both serine acetyl transferase and *O*-acetylserine (thiol) lyase subunits. The activity of cysteine synthase is regulated dynamically by the concentration of *O*-acetylserine. Selenomethionine is synthesized from SeCys and *O*-phosphohomoserine (*O*PHS) through the sequential actions of cystathionine γ-synthase, which produces selenocystathionine, cystathionine β-lyase, which produces homoselenocysteine, and methionine synthase. It is noteworthy that *O*PHS is also the precursor of threonine and an increase in the concentration of S-adenosyl methionine (SAM), which is synthesized from methionine by SAM synthase, accelerates the conversion of *O*PHS to threonine, rather than methionine, by allosteric activation of threonine synthase (Curien *et al.*, 1998). The number of cystathionine γ-synthase transcripts is also reduced by increasing SAM or methionine concentrations (Chiba *et al.*, 1999; Hesse & Hoefgen, 2003). Both SeCys and SeMet can either be incorporated into proteins or methylated. For example, Se-methylselenocysteine (SeMSeCys), γ-glutamyl-SeMSeCys and Se-methylselenomethionine are characteristic Se assimilation products of species in the genera *Allium* and *Brassica* (Broadley *et al.*, 2006b; White *et al.*, 2007b). Unfortunately, the nonspecific replacement of cysteine by SeCys and/or methionine by SeMet in proteins can alter their stability and activity. The methylation of SeCys and SeMet is thought to reduce the incorporation of these amino acids into proteins and may account for the ability of some plants to accumulate high tissue Se concentrations (Brown & Shrift, 1982). Shoot Se concentrations tend to increase to a maximum during seedling growth, then decline before, or upon, flowering (Rosenfeld & Beath, 1964; Xue *et al.*, 2001; Turakainen *et al.*, 2004; White *et al.*, 2007b), which is consistent with transcriptional analyses suggesting that Se/S assimilation occurs predominantly in the first leaves that a plant produces (White *et al.*, 2007b). Selenium is thought to be redistributed within the plant as selenate and/or organoselenium compounds via the phloem (White *et al.*, 2007b).

Iodine

It is thought that plant root cells take up I as the iodide anion (Umaly & Poel, 1971; Mackowiak & Grossl, 1999; Zhu *et al.*, 2003; Blasco *et al.*, 2008) and that I^- follows the chloride (Cl^-) transport pathway with H^+/anion symporters catalysing I^- uptake and anion channels releasing I^- into the xylem (White & Broadley, 2001; Roberts, 2006). However, the molecular identities of transporters catalysing these fluxes are not firmly established. Putative H^+/halide transporters belong to the chloride channel (CLC) transporter family (De Angeli *et al.*, 2006; Marmagne *et al.*, 2007) and a subset of the ATP-binding cassette (ABC) protein superfamily (Verrier *et al.*, 2008), whilst Na:K/Cl symporters belong to the cation chloride co-transporter (CCC) gene family (Colmenero-Flores *et al.*, 2007). Homologues of the band 3 protein are also suspected to transport I^- (Frommer & von Wirén, 2002; Bruce *et al.*, 2004). Plant Cl^- channels are readily permeable to I^- (Barbier-Brygoo *et al.*, 2000; White & Broadley, 2001; Roberts, 2006). These are thought to be encoded by *CLC* genes (Barbier-Brygoo *et al.*, 2000; White & Broadley, 2001; Nakamura *et al.*, 2006), although other members of this family are I^--permeable H^+/anion antiporters (De Angeli *et al.*, 2006). Halide fluxes may also be facilitated by organic acid transporters (White & Broadley, 2001). Iodide-permeable H^+/anion antiporters and anion channels in the tonoplast are likely to mediate I^- fluxes into and out of the vacuole (White & Broadley, 2001; De Angeli *et al.*, 2006; Nakamura *et al.*, 2006). Although fertilizer I is readily accumulated in roots and leaves (Mackowiak & Grossl, 1999; Zhu *et al.*, 2003; Dai *et al.*, 2004, 2006; Kashparov *et al.*, 2005; Mackowiak *et al.*, 2005; Blasco *et al.*, 2008), little I is redistributed via the phloem to fruits or seeds (Muramatsu *et al.*, 1993, 1995).

Variation in tissue concentrations of mineral elements among plant species

Tissue concentrations of mineral elements can differ markedly between plant species growing in the same environment. Systematic variation in shoot concentrations of Fe, Zn, Cu, Ca, Mg and Se has been documented (Thompson *et al.*, 1997; Broadley *et al.*, 2001, 2003, 2004, 2007; White *et al.*, 2004, 2007a; Watanabe *et al.*, 2007). There is also variation in leaf I concentration among angiosperm species, although this has not been quantified explicitly (Fuge & Johnson, 1986; Dai *et al.*, 2004).

Much of the genetic variation in shoot Ca and Mg concentrations occurs at the ordinal level or above (Thompson *et al.*, 1997; Broadley *et al.*, 2003, 2004; Watanabe *et al.*, 2007). This implies that concentrations of these elements in shoot tissues are constrained by an ancient evolutionary heritage. Commelinoid monocots, such as cereals and grasses, have lower shoot Ca concentrations than eudicot species (Sleper *et al.*, 1989; Thompson *et al.*, 1997; Broadley *et al.*, 2003, 2004). This has been attributed to differences in their cell wall chemistry and cation exchange capacity (White & Broadley, 2003; White, 2005). Among the eudicots, members of orders within the rosid (Brassicales, Cucurbitales, Malvales and Rosales) and asterid (Apiales, Asterales, Lamiales and Solanales) clades typically have higher shoot Ca concentrations (Broadley *et al.*, 2003, 2004). Within the magnoliids, members of noncommelinoid orders, such as the Asparagales, have higher shoot Ca concentrations than members of the commelinoid orders (Broadley *et al.*, 2003, 2004). There is a strong correlation between the ability of a plant to accumulate Ca and its ability to accumulate Mg (White, 2001, 2005; Broadley *et al.*, 2004). Thus, phylogenetic variation in shoot Mg concentrations generally resembles that for shoot Ca concentrations. However, species from families within the Caryophyllales, including the Amaranthaceae and Caryophyllaceae, have a tendency towards uncommonly high leaf Mg concentrations and therefore lower leaf Ca/Mg quotients than most other angiosperms (Broadley *et al.*, 2004, 2008; Watanabe *et al.*, 2007). For example, Ca/Mg quotients for 322 spinach (*Spinacia oleracea*, Amaranthaceae) accessions grown hydroponically were typically 5-fold lower than the Ca/Mg quotients for over 400 accessions of *Brassica oleracea* (Brassicaceae) grown under a variety of conditions (Broadley *et al.*, 2008). At the subordinal level, species and ecotypes adapted to Ca-rich environments generally have lower tissue Ca concentrations than those adapted to low-Ca soils when they are grown together (Zohlen & Tyler, 2004) and nonCaryophyllales species and ecotypes adapted to Mg-rich, serpentine environments generally have lower shoot Mg concentrations than congenerics or conspecifics adapted to other soils, when grown in the same environment (Bradshaw, 2005; O'Dell *et al.*, 2006).

In contrast to Ca and Mg, relatively little variation in shoot Fe, Zn, Cu and Se concentrations occurs at the ordinal level or above (Broadley *et al.*, 2001, 2007; White *et al.*, 2004, 2007a; Watanabe *et al.*, 2007). This implies that closely related species will often differ substantially in their tissue Fe, Zn, Cu and/or Se concentrations. Indeed, some plant species 'hyperaccumulate' Zn, Cu and Se, and can contain leaf concentrations of these elements several orders of magnitude greater than those in closely-related species growing on the same substrate (Rosenfeld & Beath, 1964; Reeves & Baker, 2000; Broadley *et al.*, 2001, 2007; White *et al.*, 2007a). Traits determining the accumulation of extraordinarily high concentrations of these elements appear to have evolved by convergent evolution of appropriate transport and metabolic pathways in several distinct angiosperm clades (Brown & Shrift, 1982; Reeves & Baker, 2000; Broadley *et al.*, 2001, 2007; White *et al.*, 2004, 2007a,b). Members of the Brassicaceae (e.g. *Arabidopsis halleri* and *Noccaea* spp.), Caryophyllaceae (e.g. *Minuartia verna*), Polygonaceae (e.g. *Rumex acetosa*) and Dichapetalaceae (e.g. *Dichapetalum gelonioides*) have been observed to hyperaccumulate Zn (Broadley *et al.*, 2007). Members of several angiosperm orders, including the Fabales, Malvales, Asterales, Solanales, Lamiales, Caryophyllales and Poales, appear to hyperaccumulate Cu (Reeves & Baker, 2000). Members of the Fabaceae (e.g. *Astragalus bisulcatus* and *Astragalus racemosus*), Asteraceae (e.g. *Aster occidentalis* and *Machaeranthera ramosa*) and Brassicaceae (e.g. *Stanleya pinnata*) hyperaccumulate Se in their leaves (Rosenfeld & Beath, 1964; White *et al.*, 2004, 2007a), whilst some members of the Lecythidaceae family accumulate large Se concentrations in their fruits and seeds (Broadley *et al.*, 2006b; White *et al.*, 2007b).

In addition to the phenomenon of hyperaccumulation, there are general differences among angiosperm orders in their shoot Zn, Cu and Se concentrations (Broadley *et al.*, 2001, 2007; White *et al.*, 2004, 2007a). Shoot Cu concentrations appear to be lower in members of the Brassicales and Poales, and higher in members of the Malvales and Malphigiales (Broadley *et al.*, 2001). Shoot Zn concentrations are lower in the Ericales and commelinoid monocotyledons, and higher in the Caryophyllales and noncommelinoid monocotyledons. In a meta-analysis of 1108 studies comparing shoot Zn concentrations among 365 species from 48 plant families and 12 key angiosperm clades, Broadley *et al.* (2007) reported that the lowest shoot Zn concentrations occurred in the Linaceae, Poaceae and Solanaceae, and the highest shoot Zn concentrations occurred in the Amaranthaceae and Salicaceae. Similarly, differences in Se metabolism occur among angiosperm orders that affect tissue Se concentrations and bioavailability to animals. For example, brassicas and alliums accumulate unique organo-Se compounds in their tissues and, consequently, have higher tissue Se concentrations than many other plants grown under the same conditions (Broadley *et al.*, 2006b; White *et al.*, 2007a,b).

One consequence of the strong phylogenetic control of tissue Ca and Mg concentrations, and above all the lower tissue Ca

and Mg concentrations in commelinoid monocotyledon species than in species from other angiosperm families, is an increased risk of Ca- and Mg-related deficiency disorders in populations changing from bean-rich to cereal-rich diets (Graham *et al.*, 2001; Welch & Graham, 2004; White & Broadley, 2005a). Similarly, there has been an increase in Fe-deficiency anaemia and Zn-deficiency disorders as cereals have replaced traditional, more mineral-rich dicotyledonous crops such as pulses, vegetables and fruits (Welch & Graham, 2004). The seeds of cereals generally have far lower concentrations of Fe and Zn than seeds of legumes (Table 1; Rengel *et al.*, 1999; White & Broadley, 2005a). This highlights the importance of the choice of plant species in strategies designed to increase the delivery of mineral elements to vulnerable populations.

In addition to the phylogenetic heritage of different plant species affecting their ability to accumulate essential mineral elements, the concentrations of mineral elements in edible tissues are also influenced by their mobility within the plant. For example, although Se and Mg are transported readily in the phloem, Fe, Zn, Cu and I are not, and Ca has little phloem mobility (Epstein, 1972; Mackowiak & Grossl, 1999; Welch, 1999; White & Broadley, 2003). Thus, phloem-fed tissues such as fruits, seeds and tubers are often poor sources of Fe, Zn, Cu, I and Ca, whilst leafy vegetables are rich sources of these elements (Table 1; Welch, 1999; White & Broadley, 2005a). The bioavailability of Ca also depends on whether tissues also contain oxalate, as Ca oxalate is not readily absorbed in the human gut. For example, edible portions from species within the Oxalidales (e.g. carambola (*Averrhoa carambola*) and oca (*Oxalis tuberose*)), Caryophyllales (e.g. beet/chard (*Beta vulgaris*), amaranth (*Amaranthus* spp.), rhubarb (*Rheum rhabarbarum*) and spinach (*S. oleracea*)) and Malpighiales (e.g. castor bean (*Ricinus communis*) and linseed (*Linum usitatissimum*)) often contain high Ca concentrations, but the Ca bioavailability is low because of high oxalate concentrations (White & Broadley, 2003; White, 2005; Kim *et al.*, 2007; Titchenal & Dobbs, 2007).

Historical trends in the concentrations of mineral elements in edible tissues

Analyses of historical data have suggested that the concentrations of certain mineral elements in edible produce from developed countries have declined over the last half century (Davis, in press). It appears that the mean concentrations of Cu, and possibly also Mg, in the dry matter of vegetables available in the UK declined significantly between the 1930s and the 1980s (White & Broadley, 2005b; Broadley *et al.*, 2006a; Davis, 2006), that the mean concentrations of Fe, Cu and Ca in the dry matter of horticultural produce available in the USA has declined significantly since the mid twentieth century (Davis *et al.*, 2004, White & Broadley, 2005b; Davis, 2006), and that the Zn and Cu concentrations in the dry matter of cereal products, vegetables and fruits from Finland have declined over the last 25 yr (Ekholm *et al.*, 2007). Holden *et al.* (in press) have cautioned that historical data for the mineral composition of foods can be compromised by differences in crop genotype, crop husbandry, environmental factors, geographical sampling strategy, portion analysed and analytical methods, and by inter-laboratory variability. However, because similar changes in the concentrations of mineral elements in produce have occurred in different countries, which share similar historical farming practices, it has been suggested that this phenomenon might be a consequence of the adoption of modern varieties and/or agronomic practices (White & Broadley, 2005b). The best evidence of this phenomenon, to date, shows that a decline in the concentrations of Fe, Zn, Cu and Mg in wheat (*Triticum aestivum*) grain coincided with the introduction of semi-dwarf high-yielding cultivars in the Broadbalk Wheat Experiment at Rothamsted, UK (Fan *et al.*, 2008).

Recent research has focused on the effects of increased yield, whether achieved by agronomic or genetic improvement, on the concentrations of mineral elements in produce. It has long been appreciated that environmental factors accelerating plant growth rates, such as higher temperatures, light intensity, CO_2 concentrations and irrigation, often result in reduced concentrations of mineral elements in plant tissues (Jarrell & Beverly, 1981; Loladze, 2002), and a number of recent studies have shown that the concentrations of various mineral elements are lower in higher yielding genotypes. For example, weak negative relationships have been found between grain Fe and Cu concentrations and grain yield among triticale cultivars (Feil & Fossati, 1995), and between seed Fe or Zn concentrations and seed yield among sorghum (*Sorghum bicolor*) genotypes (Reddy *et al.*, 2005). Similarly, negative relationships between Fe, Zn, Mg, Se and phosphorus (P) concentrations in grain and grain yield have been observed among cultivars of bread (*T. aestivum*) and durum (*Triticum durum*) wheat (Bänziger & Long, 2000; Monasterio & Graham, 2000, Garvin *et al.*, 2006; Oury *et al.*, 2006; Distelfeld *et al.*, 2007; Ortiz-Monasterio *et al.*, 2007; McDonald *et al.*, 2008), although the strength of these relationship is influenced greatly by the environment. In leafy vegetables, Farnham *et al.* (2000) found a strong negative relationship between Ca and Mg concentrations and head weight among 27 broccoli (*Brassica oleracea* var. *italica*) genotypes, and Broadley *et al.* (2008) observed weak negative relationships between biomass yield and shoot Ca concentrations among genotypes of the *gongylodes* (kohlrabi) and *sabauda* (Savoy cabbage) subtaxa of *B. oleracea* and between biomass yield and shoot Mg concentrations among genotypes of the *gongylodes* and *acephala* (kale) subtaxa (Broadley *et al.*, 2008). However, negative relationships between the concentrations of mineral elements and the yield of edible produce are not always observed in crop genotypes (White *et al.*, in press). For example, some studies report no significant relationships between the concentrations of particular mineral elements in grain and the yield of cereals (Feil & Fossati, 1995; Graham

Table 1 Examples of variation in the concentrations of essential mineral elements in edible tissues among genotypes of common crops grown under the same conditions

Crop	Genotypes, trial site and reference	[Fe] (mg kg^{-1} DW)	[Zn] (mg kg^{-1} DW)	[Ca] (g kg^{-1} DW)	[Mg] (g kg^{-1} DW)	[Cu] (mg kg^{-1} DW)
Rice (*Oryza sativa*); brown grain	Core collection	6–24	14–58	–	–	–
	Field trial	Q = 3.87	Q = 4.34			
	Gregorio *et al.* (2000)	(n = 1138)	(n = 1138)			
Rice (*Oryza sativa*); polished grain	Core collection	4–30	8–95	–	–	2.5–143.5
	Field trial	Q = 7.38	Q = 11.6			Q = 57.4
	Yang *et al.* (1998)	(n = 285)	(n = 285)			(n = 285)
Wheat (*Triticum* species); grain	Selected genotypes	25–73	25–92	–	–	–
	Field trial (Obregon)	Q = 2.92	Q = 3.68			
	Monasterio & Graham (2000)	(n = 324)	(n = 324)			
Wheat (*Triticum aestivum*); grain	Bread wheat genotypes	29–57	25–53	0.25–0.73	0.92–1.43	–
	Field trial (El Batan)	Q = 1.96	Q = 2.12	Q = 2.92	Q = 1.56	
	Graham *et al.* (1999)	(n = 132)	(n = 132)	(n = 132)	(n = 132)	
Wheat (*Triticum aestivum*); grain	Commercial varieties	24–43	16–26	–	–	1.7–2.9
	Field trial (Hutchinson)	Q = 1.75	Q = 1.64			Q = 1.62
	Garvin *et al.* (2006)	(n = 14)	(n = 14)			(n = 14)
Wheat (*Triticum aestivum*); grain	Elite genotypes	26–45	14–20	–	0.80–1.39	–
	Field trial	Q = 1.71	Q = 1.38		Q = 1.74	
	Oury *et al.* (2006)	(n = 51)	(n = 51)		(n = 51)	
Triticale (*Triticosecale*); grain	Selected cultivars	28–36	21–31	0.35–0.50	1.20–1.46	4.7–7.5
	Six field trials	Q = 1.27	Q = 1.50	Q = 1.42	Q = 1.22	Q = 1.60
	Feil & Fossati (1995)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Maize (*Zea mays*); grain	Core collection	16–63	13–58	–	–	–
	Field trial (Harare)	Q = 3.85	Q = 4.46			
	Bänziger & Long (2000)	(n = 1417)	(n = 1417)			
Maize (*Zea mays*); kernel	Elite varieties	17–24	16–25	–	–	–
	Field trials (mean 3 sites)	Q = 1.45	Q = 1.49			
	Oikeh *et al.* (2003a)	(n = 49)	(n = 49)			
Pearl millet (*Pennisetum glaucum*); grain	Diverse germplasm	30–76	25–65	–	–	–
	Field trial (2 seasons)	Q = 2.51	Q = 2.64			
	Velu *et al.* (2007)	(n = 120)	(n = 120)			
Pearl millet (*Pennisetum glaucum*); grain	Commercial germplasm	70–180	53–70	0.10–0.80	1.80–2.70	10.0–18.0
	Field trial	Q = 2.57	Q = 1.32	Q = 8.00	Q = 1.50	Q = 1.80
	Abdalla *et al.* (1998)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Barley (*Hordeum vulgare*); grain	Core collection	21–83	–	–	–	–
	Field trail	Q = 3.95				
	Ma *et al.* (2004)	(n = 409)				
Barley (*Hordeum vulgare*); grain	Commercial cultivars	104–189	30–38	0.82–1.18	2.09–2.35	–
	Field trial	Q = 1.83	Q = 1.26	Q = 1.44	Q = 1.12	
	P. J. White & I. J. Bingham, unpublished	(n = 16)	(n = 16)	(n = 16)	(n = 16)	
Sorghum (*Sorghum bicolor*); grain	Diverse germplasm	20–37	13–31	–	–	–
	Field trial	Q = 1.84	Q = 2.31			
	Reddy *et al.* (2005)	(n = 84)	(n = 84)			
Bean (*Phaseolus vulgaris*); seed	Core collection	35–92	21–59	0.5–3.1	–	–
	Field trial	Q = 2.65	Q = 2.86	Q = 6.20		
	Islam *et al.* (2002)	(n = 1072)	(n = 1072)	(n = 1072)		
Bean (*Phaseolus vulgaris*); seed	Selected genotypes	48–74	17–28	1.39–2.04	1.47–1.96	5.0–10.0
	Field trials	Q = 1.54	Q = 1.65	Q = 1.46	Q = 1.33	Q = 2.00
	Ariza-Nieto *et al.* (2007)	(n = 8)	(n = 8)	(n = 8)	(n = 8)	(n = 8)
Pea (*Pisum sativum*); seed	Core collection	23–105	16–107	0.28–2.56	1.06–2.47	1.4–13.8
	Glasshouse trial	Q = 4.5	Q = 6.6	Q = 9.1	Q = 2.3	Q = 10.1
	Grusak & Cakmak (2005)	(n = 481)	(n = 481)	(n = 481)	(n = 481)	(n = 481)

Table 1 *continued*

Crop	Genotypes, trial site and reference	[Fe] (mg kg^{-1} DW)	[Zn] (mg kg^{-1} DW)	[Ca] (g kg^{-1} DW)	[Mg] (g kg^{-1} DW)	[Cu] (mg kg^{-1} DW)
Soybean (*Glycine max*); seed	Commercial lines	–	38–67	1.8–3.4	2.2–3.4	–
	Field trial		*Q* = 1.76	*Q* = 1.89	*Q* = 1.55	
	Raboy *et al.* (1984)		(*n* = 38)	(*n* = 38)	(*n* = 38)	
Soybean (*Glycine soja*); seed	Commercial lines	–	59–83	2.3–4.8	2.2–3.1	–
	Field trial		*Q* = 1.41	*Q* = 2.09	*Q* = 1.41	
	Raboy *et al.* (1984)		(*n* = 20)	(*n* = 20)	(*n* = 20)	
Peanut (*Arachis hypogaea*); seed	Diverse germplasm	24–41	25–41	0.4–0.7	1.7–2.3	8.0–17.0
	Field trial	*Q* = 1.70	*Q* = 1.64	*Q* = 2.00	*Q* = 1.35	*Q* = 2.13
	Branch & Gaines (1983)	(*n* = 26)	(*n* = 27)	(*n* = 26)	(*n* = 26)	(*n* = 26)
Chickpea (*Cicer arietinum*); seed	Core collection	42–133	45–123	0.81–3.02	1.09–2.59	0.9–9.3
	Trial	*Q* = 3.17	*Q* = 2.72	*Q* = 3.73	*Q* = 2.38	*Q* = 10.6
	Grusak (2006)**	(*n* = 239)	(*n* = 239)	(*n* = 239)	(*n* = 239)	(*n* = 239)
Chickpea (*Cicer arietinum*); seed	Commercial cultivars	24–41	35–60	1.9–2.2	0.04–0.05	107–122
	Field trial	*Q* = 1.71	*Q* = 1.71	*Q* = 1.18	*Q* = 1.16	*Q* = 1.14
	Zia-Ul-Haq *et al.* (2007)	(*n* = 4)	(*n* = 4)	(*n* = 4)	(*n* = 4)	(*n* = 4)
Chickpea (*Cicer arietinum*); edible leaf	Diverse accessions	79–120	56–137	14.4–22.6	3.2–4.6	1.3–3.0
	Glasshouse trial	*Q* = 1.51	*Q* = 2.46	*Q* = 1.57	*Q* = 1.44	*Q* = 2.31
	Ibrikci *et al.* (2003)	(*n* = 19)	(*n* = 19)	(*n* = 19)	(*n* = 19)	(*n* = 19)
***Brassica oleracea*; leaves**	Core collection	23–1045	9–221	14.3–39.2	4.0–9.6	1.4–9.3
	Glasshouse trial (P4)	*Q* = 45.2	*Q* = 25.9	*Q* = 2.75	*Q* = 2.43	*Q* = 6.76
	Broadley *et al.* (2008)	(*n* = 345)	(*n* = 339)	(*n* = 348)	(*n* = 348)	(*n* = 346)
***Brassica oleracea*; leaves**	Commercial varieties	71–338	34–122	19.1–35.0	4.2–8.3	2.9–6.4
	Glasshouse trial (P4)	*Q* = 4.79	*Q* = 3.56	*Q* = 1.83	*Q* = 1.98	*Q* = 2.24
	Broadley *et al.* (2008)	(*n* = 74)	(*n* = 74)	(*n* = 74)	(*n* = 74)	(*n* = 74)
Kale and collards (*B. oleracea* var. *acephala*); leaves	Commercial varieties	69–109	33–60	14.6–26.1	2.9–4.8	–
	Glasshouse trial	*Q* = 1.57	*Q* = 1.78	*Q* = 1.79	*Q* = 1.68	
	Kopsell *et al.* (2004b)	(*n* = 22)	(*n* = 22)	(*n* = 22)	(*n* = 22)	
Broccoli (*B. oleracea* var. *italica*); inflorescences	Commercial cultivars	–	–	3.40–5.13	1.34–1.68	–
	Field trial (SS-P)			*Q* = 1.51	*Q* = 1.25	
	Rosa *et al.* (2002)			(*n* = 11)	(*n* = 11)	
***Brassica rapa*; leaves**	Core collection	60–350	23–156	–	–	–
	Hydroponic trial	*Q* = 5.80	*Q* = 6.72			
	Wu *et al.* (2007)	(*n* = 111)	(*n* = 111)			
Spinach (*Spinacia oleracea*); leaves	Core collection	50–139	31–387	3.0–11.5	5.2–14.7	2.2–11.0
	Growth chamber trial	*Q* = 2.7	*Q* = 12.3	*Q* = 3.8	*Q* = 2.9	*Q* = 4.9
	Grusak & Cakmak (2005)	(*n* = 327)	(*n* = 327)	(*n* = 327)	(*n* = 327)	(*n* = 327)
Carrot (*Daucus carota*); roots	Commercial cultivars	32–198	18–39	2.7–4.5	0.8–2.3	–
	Field trial	*Q* = 6.19	*Q* = 2.17	*Q* = 1.65	*Q* = 2.89	
	Nicolle *et al.* (2004b)	(*n* = 20)	(*n* = 20)	(*n* = 20)	(*n* = 20)	
Cassava (*Manihot esculenta*); roots	Selected genotypes	6–230	3–38	0.31–2.50	0.52–2.40	0.8–40.3
	Field trial	*Q* = 38.3	*Q* = 14.3	*Q* = 8.1	*Q* = 4.6	*Q* = 51.0
	Chávez *et al.* (2005)	(*n* = 600)	(*n* = 600)	(*n* = 600)	(*n* = 600)	(*n* = 599)
Potato (*Solanum tuberosum*); tubers	Commercial varieties	32–374	7–17	0.27–0.67	0.87–1.23	2.3–4.9
	Field trial	*Q* = 11.6	*Q* = 2.40	*Q* = 2.48	*Q* = 1.41	*Q* = 2.12
	White *et al.* (in press)	(*n* = 26)	(*n* = 26)	(*n* = 26)	(*n* = 26)	(*n* = 26)
Yam (*Dioscorea alata*); tubers	Core collection	9–176	8–25	0.14–0.49	0.20–0.36	5.0–13.0
	Field trial	*Q* = 19.6	*Q* = 3.13	*Q* = 3.5	*Q* = 1.82	*Q* = 2.6
	Agbor-Egbe & Trèche (1995)	(*n* = 23)	(*n* = 23)	(*n* = 23)	(*n* = 23)	(*n* = 23)

Data show minimum-to-maximum concentrations (Fe, Zn and Cu, mg kg^{-1} dry matter; Mg and Ca, g kg^{-1} dry matter), the maximum/minimum quotient (Q) and the number of genotypes surveyed (*n*). *For carrot roots, a fresh weight (FW):dry matter (DM) ratio of 10 was assumed. **Data for chickpea accessions obtained from the Germplasm Resources Information Network (GRIN) website (http://www.ars-grin.gov/cgi-bin/npgs/html/eval.pl?492937). DW, dry weight.

et al., 1999; Ortiz-Monasterio *et al.*, 2007), between seed Fe and Zn concentrations and yield in common bean (*Phaseolus vulgaris*; Graham *et al.*, 2001), between shoot mineral concentrations and biomass production within most subtaxa of *B. oleracea* (Broadley *et al.*, 2008), or between the concentrations of mineral elements in tubers and tuber yield among potato (*Solanum tuberosum*) varieties (White *et al.*, in press). These observations suggest that the biofortification of crops with mineral elements can be achieved without compromising yield.

Agronomic biofortification strategies

Agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvement of the solubilization and mobilization of mineral elements in the soil. When crops are grown where mineral elements become immediately unavailable in the soil, targeted application of soluble inorganic fertilizers to roots or to leaves is practised. In situations where mineral elements are not readily translocated to edible tissues, foliar applications of soluble inorganic fertilizers are made. It has been observed that the human population of the world has exceeded the carrying capacity of low-input agriculture, and modern inorganic fertilizers are necessary to obtain the crop yields required to prevent starvation (Graham *et al.*, 2007). It is argued, therefore, that the use of inorganic fertilizers must be included in any future strategy for food security. If the widespread use of inorganic fertilizers is facilitated, it might be possible to incorporate mineral elements essential for human nutrition before their distribution, as is practised for Se in Finland and Zn in Turkey.

Inorganic fertilizers

Soils often contain large amounts of Fe, but little of this is phytoavailable. The application of inorganic Fe fertilizers to such soils is usually ineffective as it rapidly becomes unavailable to plant roots through adsorption, precipitation and oxidation reactions. For this reason, Fe-chelates are often used as soil Fe fertilizers (Shuman, 1998; Rengel *et al.*, 1999). In addition, the availability of Fe in the rhizosphere can be increased by soil acidification with elemental S (Shuman, 1998). This has the added benefit of crop S fertilization. Foliar applications of Fe fertilizers are often made to crops growing in Fe-deficient soils, but, because Fe is not readily translocated within plants, these must be repeated throughout the growing season (Loneragan, 1997; Cakmak, 2002). Nevertheless, by appropriate Fe fertilization, Fe concentrations in edible portions of cereals, vegetables and fruits can be increased (Shuman, 1998; Rengel *et al.*, 1999).

Zinc is commonly applied to crops as $ZnSO_4$ or as synthetic chelates (Shuman, 1998; Broadley *et al.*, 2007; Cakmak, 2008). The application of Zn fertilizers to the soil is effective in increasing grain Zn concentrations in cereals growing on most, but not all, soils and foliar applications of either $ZnSO_4$ or Zn-chelates can increase grain Zn concentrations in plants with adequate Zn mobility in the phloem (Rengel *et al.*, 1999; Cakmak, 2002, 2004, 2008; Genc *et al.*, 2005; Oury *et al.*, 2006; Harris *et al.*, 2007; Fang *et al.*, 2008). Similarly, soil and/or foliar applications of Zn fertilizers can increase leaf, tuber and fruit Zn concentrations (Shuman, 1998; Rengel *et al.*, 1999; Broadley *et al.*, 2007). In some soils, the residual effects of a single application of Zn fertilizer can be appreciated over several years.

The phytoavailability of Cu in many agricultural soils is low, and Cu applied to the soil often becomes rapidly unavailable to plants (Gupta, 1979). Nevertheless, Cu concentrations in cereals, vegetables and fruits can be increased by Cu fertilization (Gupta, 1979; Sterrett *et al.*, 1983; Shuman, 1998; Rengel *et al.*, 1999; Bunzl *et al.*, 2001; Tamoutsidis *et al.*, 2002). Crops are generally supplied with Cu as a soil application of $CuSO_4$ or as sewage sludges and manures (Gupta, 1979; Shuman, 1998). These amendments improve plant growth on soils with low Cu phytoavailability and increase Cu concentrations in edible tissues. However, the combination of crop variety and Cu fertilization must be managed appropriately to ensure that Cu fertilization is adequate but not excessive, as too much Cu can be toxic to both plants and humans (Gupta, 1979; Shuman, 1998; White & Broadley, 2005a; Puig *et al.*, 2007a). Foliar applications of Cu fertilizers are occasionally recommended under specific circumstances (Gupta, 1979).

Common Ca fertilizers include lime (CaO and $CaCO_3$), gypsum ($CaSO_4$), calcium phosphate and calcium nitrate. The application of Ca fertilizers to the soil generally increases Ca concentrations in tubers and leaves and sometimes, but not always, in fruits and seeds (Shear, 1975; McLaughlin & Wimmer, 1999; Welch, 1999; White, 2001; White & Broadley, 2003; Ho & White, 2005; White *et al.*, in press). It is thought that the application of Ca fertilizers to soils increases fruit and seed Ca concentrations markedly only when these can be supplied with Ca via the xylem, as Ca transport in the phloem is severely restricted. Foliar applications of soluble Ca fertilizers are commonly made to horticultural crops to prevent Ca-deficiency disorders (Shear, 1975; Ho & White, 2005). The liming of soil increases the pH of the soil solution and provides a Ca source in the topsoil, whilst water-soluble gypsum provides Ca throughout the soil profile.

Magnesium is generally supplied to crops as its sulphate (Epsom salts or kieserite), carbonate or, most commonly, oxide (Metson, 1974; Draycott & Allison, 1998). In addition, the use of magnesium ammonium phosphate (struvite) has recently received attention, as it has potential as a sustainable P source for agriculture (Parsons & Smith, 2008). Magnesium fertilizers are frequently applied to the soil surface or, when less soluble, incorporated into the subsoil (Metson, 1974; Draycott & Allison, 1998). Magnesium sulphate provides readily available Mg^{2+}, whereas MgO behaves as a slow-release fertilizer (Metson,

1974; Draycott & Allison, 1998). Foliar applications of $MgSO_4$ are also common on some crops (Metson, 1974; Draycott & Allison, 1998). The application of Mg fertilizers increases Mg concentrations in plant tissues and there is a strong positive relationship between Mg^{2+} in the soil solution and Mg concentrations in produce (Metson, 1974; Wilkinson *et al.*, 1990; Draycott & Allison, 1998; Oury *et al.*, 2006).

Tissue Se concentrations in plants can be increased by soil or foliar applications of Se fertilizers and this has been shown to have beneficial effects on animal health and deliver Se to the human diet (Gissel-Nielsen, 1998; Combs, 2001; Gupta & Gupta, 2002; Lyons *et al.*, 2003, 2005; Hartikainen, 2005; Broadley *et al.*, 2006b; Hawkesford & Zhao, 2007; Rayman, 2008). The use of inorganic Se fertilizers to increase crop Se concentrations has been particularly successful in both Finland and New Zealand (Eurola *et al.*, 1989, 1991; Lyons *et al.*, 2003; Hartikainen, 2005). For example, since the incorporation of Se into all multielement fertilizers used in Finnish agriculture became mandatory in July 1984, Se concentrations in many indigenous food items have increased over 10-fold (Eurola *et al.*, 1989, 1991, 2004; Ekholm *et al.*, 2007). Both Na_2SeO_4 and K_2SeO_4 provide phytoavailable Se for immediate uptake by crops, but the application of selenite or less soluble forms of selenate, such as $BaSeO_4$, provides longer lasting effects (Gissel-Nielsen, 1998; Gupta & Gupta, 2002; Broadley *et al.*, 2006b). Soil applications are generally recommended, especially for crops subject to late-season moisture and heat stress (Lyons *et al.*, 2005), but foliar applications have also been deployed (Fang *et al.*, 2008). One intriguing proposal is to use the Se-rich straw of plants grown on naturally seleniferous soils as a 'green manure' in areas with inadequate soil Se concentrations (Terry *et al.*, 2000).

In most soils, I is present in solution as iodide, although iodate can also be present under strongly oxidizing conditions (Fuge & Johnson, 1986). Fertilization with soluble iodide and/or iodate salts has been practised in agriculture, and the iodinization of irrigation water has successfully increased the delivery of I to humans through edible crops (Jiang *et al.*, 1997; Lyons *et al.*, 2004). The I concentrations in root crops and leafy vegetables can be increased greatly by the application of I fertilizers, and, although I is not readily mobile in the phloem, I concentrations in tubers, fruits and seeds can also be increased by I fertilization to nutritionally significant concentrations (Jiang *et al.*, 1997; Rengel *et al.*, 1999; Dai *et al.*, 2004). It has been suggested that, because human dietary I requirements are quite low, I fertilizers might be added to large areas of agricultural production from aeroplanes (Graham *et al.*, 2007).

From the foregoing discussion, it is clear that the application of inorganic fertilizers can undoubtedly increase the concentrations of mineral elements commonly lacking in human diets in edible produce. However, these fertilizers must be applied regularly and can be costly to manufacture, distribute and apply. Furthermore, the manufacture and use of inorganic fertilizers can incur environmental costs, such as those caused by the production of greenhouse gasses and mineral enrichment of the environment. The supply of certain mineral elements may also become limiting in the future. For example, reserves of Zn and Cu are estimated to be 480 and 940 million tonnes (US Geological Survey, 2007), respectively, and it has been estimated that, at their current rate of consumption, the supply of both these elements may be exhausted within 60 yr (Cohen, 2007; Kesler, 2007). Similarly, the world's Se reserves amount to only 170 000 tonnes (US Geological Survey, 2007). If all 210 Mha of the world's wheat was fertilized at 20 g Se ha^{-1} (Broadley *et al.*, 2006b), this would consume 4322 t Se yr^{-1}, and the Se supply would be exhausted in less than 40 yr. By contrast, the supplies of Fe, Ca, Mg and I are considered to be secure for over 100 yr at their current rate of consumption (Kesler, 2007). The amounts of mineral elements removed by crops, and, therefore, the minimum required in fertilizer applications to maintain soil mineral concentrations can be estimated from their concentrations in harvested portions (USDA-ARS, 2007; http://www.ars.usda.gov/ba/bhnrc/ndl) and global production statistics (FAO, 2006; http://faostat.fao.org/site/291/default.aspx). These data suggest that, in contrast to Se, the amounts of Fe, Zn, Cu, Ca, Mg and I required for the biofortification of edible crops are negligible compared with their global reserves. Hence, the supply of inorganic fertilizers for agriculture could be secure for many centuries, if it were prioritized.

Increasing the acquisition of mineral elements from unfertilized soils

The total mineral concentrations of Fe, Zn and Cu in most soils would be sufficient to support mineral-dense crops, if these elements were phytoavailable (Loneragan, 1997; Shuman, 1998; Schmidt, 1999; Graham *et al.*, 1999; Frossard *et al.*, 2000; Rengel, 2001). Hence, there is considerable interest in developing management systems that exploit soil and fertilizer sources of mineral elements more effectively and in breeding mineral-efficient crops that produce high yields and accumulate minerals from previously infertile soils. In developing countries, breeding for increased yields on infertile soils is a major objective (Lynch, 2007). This work aims to improve both the acquisition of mineral elements and their physiological utilization in the plant for improved yields (Lynch, 2007).

Crop yields in developing countries are restricted principally by drought, low phytoavailability of P and/or nitrogen (N), and soil acidity, which is often associated with Al toxicity and low phytoavailability of Ca, Mg and K (Lynch, 2007; Kirkby & Johnston, 2008). In addition, the phytoavailability of mineral elements, such as Fe, Zn and Cu, limits crop yields on many calcareous soils of the world (see section 'Phytoavailability of mineral elements'). The acquisition of mineral elements with restricted mobility in the soil, such as P, K, Fe, Zn and Cu, can be improved by investing more biomass in

the root system, by producing a greater number and more even spread of roots, by developing a more extensive root system, with longer, thinner roots with more root hairs, and by proliferating lateral roots in mineral-rich patches (White *et al.*, 2005; Lynch, 2007; Kirkby & Johnston, 2008; White & Hammond, 2008). In addition, the efflux of organic acids, which displace cations from their binding sites in the soil, and the secretion of enzymes capable of degrading organic compounds, such as phytate, that chelate cations can also improve the acquisition of Fe, Zn and Cu (Morgan *et al.*, 2005; Lynch, 2007). There is considerable intraspecific genetic variation in root architecture and root exudation that might improve the acquisition of all these elements from unfertilized soils (White *et al.*, 2005; Lynch, 2007; White & Hammond, 2008). Similarly, rotations and intercropping of plants that are better able to access and mobilize mineral elements with low solubility and/or movement in the soil solution can be utilized to increase their tissue concentrations and crop yield (Rengel *et al.*, 1999; Jolley *et al.*, 2004; Graham *et al.*, 2007; Inal *et al.*, 2007). It has been suggested that such plants might include micronutrient-rich, indigenous food crops that acquire mineral elements more effectively from unfertilized soils (Welch & Graham, 2005). The diversification of rotations to include species with greater concentrations of essential mineral elements for human nutrition in their edible tissue also has the potential to increase the delivery of these elements to the human diet independently (Graham *et al.*, 2007).

Soil micro-organisms can also be exploited to increase the volume of soil explored by crop plants and the phytoavailability of mineral elements (Rengel *et al.*, 1999; Barea *et al.*, 2005; Morgan *et al.*, 2005; Lynch, 2007; Kirkby & Johnston, 2008). Many crops are associated with mycorrhizal fungi, which have the potential to increase the volume of soil exploited for the acquisition of immobile mineral elements, and release organic acids, siderophores and enzymes capable of degrading organic compounds (Rengel *et al.*, 1999; Barea *et al.*, 2005; Morgan *et al.*, 2005; Smith & Read, 2007). Recently, He & Nara (2007) suggested that the agricultural management of mycorrhizal fungi could be used to increase mineral concentrations in edible produce, and several studies have found that mycorrhizal associations increase Se, Fe, Zn and Cu concentrations in crop plants (Kothari *et al.*, 1991; Caris *et al.*, 1998; Rengel *et al.*, 1999; Harrier & Watson, 2003; Larsen *et al.*, 2006; Cavagnaro, 2008). However, because the symbiotic relationship between plants and mycorrhizal fungi is fuelled by photosynthate from plants, such associations can reduce yields in well-fertilized soils (Morgan *et al.*, 2005; Lynch, 2007). Relationships with N_2-fixing bacteria, whether symbiotic or associative, are especially important in N-limited environments (Rengel *et al.*, 1999; Hardarson & Broughton, 2003). Thus, the deployment of legumes in N-limited environments is essential, but is often, although not always (Houlton *et al.*, 2008), compromised by their high demand for P and other mineral elements for growth (White & Hammond, 2008). Again, this might be addressed by including plants that are better able to access and mobilize mineral elements in rotations and intercropping schemes (Kirkby & Johnston, 2008). Finally, exudates from plant roots and mycorrhizal fungi can provide carbon for other soil microbes that affect the phytoavailability of mineral elements. Hence, inoculants of growth-promoting bacteria can increase the acquisition of Fe, Zn and Cu by plant roots, tissue mineral concentrations, plant growth and yield (Rengel, 2001; Whiting *et al.*, 2001; Barea *et al.*, 2005).

Genetic biofortification strategies

Genetic variation in the concentrations of mineral elements in edible portions of crop plants

Increasing the concentrations of essential mineral elements in produce through the application of mineral fertilizers can be complemented by breeding crops with an increased ability to acquire and accumulate these minerals in their edible portions. However, it must be recognized that genotypic enhancement can only improve the acquisition, utilization or accumulation of mineral elements available to the crop. Considerable genetic variation appears to exist in the concentrations of the mineral elements most frequently lacking in human diets in the edible portions of most crop species.

Data are being amassed on within-species genetic variation in Fe, Zn, Cu, Ca, Mg and Se concentrations in the edible tissues of crop plants (Table 1). However, published data on genetic variation in I concentrations in plant tissues are scarce. Nevertheless, within-species variation in leaf I concentration has been reported for both perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and shown to be heritable (Butler & Glenday, 1961; Alderman & Jones, 1967). These studies provide strong evidence that leaf I concentration is under genetic control, which provides the rationale to establish the extent of within-species genetic variation in the I concentration of edible portions of crop plants (Lyons *et al.*, 2004).

Most surveys of genetic variation in mineral concentrations have been performed on the carbohydrate-rich portions of staple crops. These include rice (*O. sativa*), wheat (*Triticum* spp.) and maize (*Z. mays*) grains, common bean (*P. vulgaris*) seeds, sweet potato (*Ipomoea batatas*) tubers, and cassava (*Manihot esculenta*) and yam (*Dioscorea alata*) roots (Grusak & Cakmak, 2005; White & Broadley, 2005a; Pfeiffer & McClafferty, 2007). In addition, genetic variation in the mineral concentrations of the edible portions of other commonly consumed cereals (such as barley (*Hordeum vulgare*), millet (*Panicum miliaceum*) and sorghum), legumes (such as pea (*Pisum sativum*), soybean (*Glycine max* and *Glycine soja*), lentil (*Lens culinaris*), pigeonpea (*Cajanus* spp.), cowpea (*Vigna unguiculata*), groundnut (*Vigna subterranea*), chickpea (*Cicer arietinum*) and peanut (*Arachis hypogaea*)), vegetables (such as brassicas (*Brassica* spp.), spinach, carrot (*Daucus carota*) and potato (*S. tuberosum* and

Solanum phureja)) and fruits (such as tomato (*S. lycopersicum*) and bananas/plantains (*Musa* spp.)) that contribute significantly to human diets has also been surveyed (Grusak & Cakmak, 2005; White & Broadley, 2005a; Johns & Eyzaguirre, 2007; Pfeiffer & McClafferty, 2007). These data suggest that there is sufficient genetic potential within cultivated species to manipulate the tissue concentrations of all the mineral elements lacking in human diets through breeding programmes.

There is genetic variation in the concentrations of mineral elements in the grains of most cereal species. Concentrations of Fe and Zn in cereal grain vary 1.5- to 4-fold among genotypes depending on the genetic diversity of the material tested (Table 1; see References S11). Some of this variation can be attributed to differences in grain yield (see section 'Historical trends in the concentrations of mineral elements in edible tissues'). Although the cultivated germplasm of some cereals, such as wheat, may have a limited genetic variation in grain Fe and Zn concentrations, wild relatives often possess considerable variation (Monasterio & Graham, 2000; Cakmak *et al.*, 2004; Welch *et al.*, 2005; Chhuneja *et al.*, 2006; Pfeiffer & McClafferty, 2007; Cakmak, 2008; Tiwari *et al.*, 2008) and accessions with grain Fe and Zn concentrations at least 2-fold higher than the most widely grown varieties are available for many cereal species (White & Broadley, 2005a). Differences in grain Zn and Fe concentrations between species of wild and cultivated wheat have been attributed, in part, to allelic variation at a chromosomal locus that promotes early senescence and remobilization of protein, Fe, Zn and Mn from senescing leaves to seeds, and the introgression of the high grain protein content (Gpc-B1) locus from a wild tetraploid wheat (*Triticum turgidum* ssp. *dicoccoides*) to a cultivated wheat (*Triticum durum*) resulted in higher concentrations of Fe and Zn in its grain (Distelfeld *et al.*, 2007). In addition, highly significant positive correlations between grain Fe and Zn concentrations have been observed in maize (Maziya-Dixon *et al.*, 2000; Long *et al.*, 2004), wheat (Graham *et al.*, 1999; Garvin *et al.*, 2006), sorghum (Reddy *et al.*, 2005) and pearl millet (*Pennisetum glaucum*; Velu *et al.*, 2007), which increase the possibilities of breeding for increased concentrations of both Fe and Zn simultaneously. Interestingly, studies of related *Triticum* species showed strong positive correlations between grain protein and Zn concentrations (Ozturk *et al.*, 2006; Distelfeld *et al.*, 2007). Chromosomal loci affecting grain Fe and Zn have been mapped in rice (Gregorio *et al.*, 2000) and wheat (cited by Ortiz-Monasterio *et al.*, 2007). Grain Ca has been found to vary almost 3-fold and grain Mg *c.* 1.5-fold among wheat accessions (Peterson *et al.*, 1986; Graham *et al.*, 1999; Bálint *et al.*, 2001; Oury *et al.*, 2006) and genetic variation in grain Cu concentrations has been reported for wheat (McGrath, 1985; Peterson *et al.*, 1986; Bálint *et al.*, 2001; Garvin *et al.*, 2006) and pearl millet (Abdalla *et al.*, 1998). Genetic variation in grain Se has also been reported for wheat (Lyons *et al.*, 2005; Garvin *et al.*, 2006) and oat (*Avena sativa*; Eurola *et al.*, 2004), although the expression of this trait is strongly dependent upon weather conditions, crop husbandry and selenium fertilization (Eurola *et al.*, 2004; Lyons *et al.*, 2005; Garvin *et al.*, 2006). Although it is often suggested that little variation exists in grain Se concentrations amongst modern bread and durum wheats, the wild wheats (*Triticum dicoccum* and *Triticum spelta*) and their relatives (*Aegilops tauschii*) have significantly higher Se concentrations than cultivated wheat and can be integrated into breeding programmes (Graham *et al.*, 2001; Lyons *et al.*, 2003, 2005; White & Broadley, 2005a; White *et al.*, 2007b).

Because the seeds of many cereals are often consumed after milling or polishing, it is pertinent to consider whether genetic variation in the distribution of mineral elements within the seed can be utilized in biofortification strategies. Mineral elements are nonhomogenously distributed within the seed and the concentrations of many mineral elements are highest in the husk and/or aleurone layers (see References S12). Milling or polishing cereal seeds can, therefore, remove large quantities of mineral elements from the diet and the extent of these losses is genotype dependent (Gregorio *et al.*, 2000; Vasconcelos *et al.*, 2003; Ma *et al.*, 2004; Lyons *et al.*, 2005; Prom-u-thai *et al.*, 2007). The partitioning of mineral elements such as Fe and Zn within cereal grains is affected by aspects of grain morphology, such as grain size, embryo size, and the number and thickness of the tissue layers (Welch *et al.*, 1993; Oikeh *et al.*, 2003a; Cakmak *et al.*, 2004). For example, grain Fe, Zn, Ca, Mg, and Cu concentrations in maize, rice and barley are related to the number of aleurone cell layers, which is cultivar dependent (Welch *et al.*, 1993; Prom-u-thai *et al.*, 2007).

Seeds of legumes, such as common bean, pea, lentil, soybean, mungbean (*Vigna radiata*), chickpea, peanut and groundnut, generally have higher concentrations of Fe, Zn, Ca and Mg than cereal grains, and significant within-species genetic variation in the concentrations of all these elements has been observed (Table 1; White & Broadley, 2005a; Pfeiffer & McClafferty, 2007). Seed Fe and Zn concentrations have been found to vary from 1.4- to 6.6-fold among genotypes of legume species grown together in the same environment (Table 1). Some of this variation is associated with seed morphology, as the tissue distribution of Zn and Fe in legume seeds is under genetic control (Moraghan *et al.*, 2002; Ariza-Nieto *et al.*, 2007). Domestication does not appear to have affected the mean concentration or range of Fe or Zn in bean seeds (Beebe *et al.*, 2000). Seed Ca and Mg concentrations also vary considerably among genotypes of legume species grown in the same environment. For example, seed Ca concentrations varied 9-fold among 120 segregating $F_{2,3}$s from a wide cross between a wild and a cultivated *P. vulgaris* genotype (Guzmán-Maldonado *et al.*, 2003), and seed Ca and Mg concentrations ranged from 0.03 to 0.26% dry weight (DW) and from 0.11 to 0.25% DW, respectively, among 481 *P. sativum* accessions (Grusak & Cakmak, 2005). Seed Ca concentrations varied up to 2.6-fold among 10 chickpea genotypes (Abbo *et al.*, 2000) and 2-fold among

soybean cultivars (Raboy *et al.*, 1984; Horner *et al.*, 2005). Similarly, seed Cu concentrations show appreciable within-species genetic variation (Deosthale, 1981; Branch & Gaines, 1983; Grusak & Cakmak, 2005; Ariza-Nieto *et al.*, 2007; Zia-Ul-Haq *et al.*, 2007). Little information is available on within-species variation in seed Se or I concentrations in legumes, although variation in Se accumulation among soybean genotypes has been documented (Yang *et al.*, 2003; Zhang Y *et al.*, 2003b). Seed Fe, Zn and Ca concentrations all behave as quantitative traits in legume species and genetic loci influencing them can be mapped using QTL analysis (Beebe *et al.*, 2000; Guzmán-Maldonado *et al.*, 2003; Cichy *et al.*, 2005; Gelin *et al.*, 2007) and introgressed into commercial germplasm (Islam *et al.*, 2004). It is thought that most genetic variation in Zn concentration in common bean is controlled by a single locus (Guzmán-Maldonado *et al.*, 2003; Cichy *et al.*, 2005; Gelin *et al.*, 2007). The positive correlations among the concentrations of Zn and Fe, and Ca and Mg in beans (Beebe *et al.*, 2000; House *et al.*, 2002; Guzmán-Maldonado *et al.*, 2003; Gelin *et al.*, 2007) suggest the possibility of breeding for increased concentrations of these elements simultaneously.

The concentrations of some mineral elements, such as Ca, Mg, Fe and Zn, are often greater in leafy vegetables than in grain, seed, fruit or tuber crops (Table 1). In a core collection of *B. oleracea* thought to incorporate approx. 99% of the common allelic polymorphisms in this species, leaf Fe varied 45-fold and leaf Zn varied 26-fold between accessions in one glasshouse trial (M. R. Broadley, J. P. Hammond and P. J. White, unpublished observations). In the same glasshouse trial, leaf Fe varied 4.8-fold and leaf Zn varied 3.6-fold among 74 commercial varieties representing all subtaxa of *B. oleracea*. Among 22 accessions of *B. oleracea* var. *acephala* (kale/collards), leaf Fe varied 1.6-fold and leaf Zn varied 1.8-fold, and the rankings of cultivars were consistent between years despite substantial year-to-year variation in leaf Fe and Zn concentrations (Kopsell *et al.*, 2004b). Among 327 accessions of spinach, leaf Fe varied 2.7-fold, and leaf Zn varied 12-fold (Grusak & Cakmak, 2005). Genetic variation in shoot Fe and Zn concentrations has also been observed in *Brassica rapa* (Wu *et al.*, 2007, 2008) and onion (*Allium cepa*; Alvarez *et al.*, 2003). Shoot Ca and Mg concentrations varied up to 3-fold among accessions of *B. oleracea* (Farnham *et al.*, 2000; Rosa *et al.*, 2002; Kopsell *et al.*, 2004b; Broadley *et al.*, 2008), *B. rapa* (Wu *et al.*, 2008), spinach (Grusak & Cakmak, 2005), onion (Alvarez *et al.*, 2003) and chickpea (Ibrikci *et al.*, 2003), and the concentrations of these elements in shoot tissues appear to be correlated (White & Broadley, 2005a; Broadley *et al.*, 2008; Wu *et al.*, 2008). Several QTLs affecting shoot Ca and Mg concentrations have been found in a cross between a rapid-cycling *B. oleracea* var. *alboglabra* and an F_1 *B. oleracea* var. *italica* (Broadley *et al.*, 2008), and a QTL affecting shoot Mg concentration has been found in *B. rapa* (Wu *et al.*, 2008), but no candidate genes have been identified yet. Within-species genetic variation in shoot Mg concentrations has also been reported for forage of Jerusalem artichoke (*Helianthus tuberosus*; Seiler & Campbell, 2006) and herbage of forage grasses, such as crested wheatgrass (*Agropyron cristatum*), Italian ryegrass (*Lolium multiflorum*), orchard grass, perennial ryegrass (*Lolium perenne*), reed canary grass (*Phalaris arundinacea*), Russian wildrye (*Psathyrostachys juncea*) and tall fescue (*Festuca arundinacea*), which have been studied because of the negative economic consequences of grass tetany in ruminant animals when their diets contain insufficient Mg (Hides & Thomas, 1981; Sleper *et al.*, 1989; Smith *et al.*, 1999; Jefferson *et al.*, 2001). Leaf Cu concentrations vary many fold among genotypes of *B. oleracea* (M. R. Broadley, P. J. White and J. P. Hammond, unpublished observations), spinach (Grusak & Cakmak, 2005), chickpea (Ibrikci *et al.*, 2003) and onion (Alvarez *et al.*, 2003). Genetic variation in shoot Se concentrations has been observed in *B. oleracea* (Kopsell & Randle, 2001; Farnham *et al.*, 2007) and onions (Kopsell & Randle, 1997).

The concentrations of some elements, such as Fe, Ca and Mg, are also generally higher in root crops than in cereal grains, legume seeds, fruits or tubers (Table 1). There is considerable within-species genetic variation in the concentrations of these elements in root crops. For example, there is 51-fold variation in root Cu, 38-fold variation in root Fe, 14-fold variation in root Zn, 8-fold variation in root Ca, and 4.6-fold variation in root Mg among 600 cassava genotypes (Chávez *et al.*, 2005). Considerable genetic variation in root Fe and Zn concentrations has also been observed in sweet potato (Pfeiffer & McClafferty, 2007; Courtney *et al.*, 2008) and carrot (Nicolle *et al.*, 2004b), and in root Ca and Mg concentrations in carrot (Nicolle *et al.*, 2004b) and sugar beet (*Beta vulgaris*) (Baierová & Baier, 1993).

Fruits generally have low concentrations of mineral elements that are less mobile in the phloem, and the concentrations of mineral elements essential to human nutrition do not appear to vary greatly among cultivars. For example, fruit Fe, Zn, Ca and Mg concentrations differed less than 2-fold between six strawberry (*Fragaria* spp.) cultivars (Hakala *et al.*, 2003), as did Fe and Zn concentrations in fruits of three apple (*Malus domestica*) varieties (Iwane, 1991) and Fe, Zn, Mg and Cu concentrations in representative fingers of three plantain varieties (Davey *et al.*, 2007). However, mean fruit Ca concentrations differed 2.5-fold among these plantain varieties (Davey *et al.*, 2007) and fruit Cu concentrations varied 2.5-fold among the apple varieties (Iwane, 1991). Similarly, there was 2.3-fold variation in Ca concentrations and 8-fold variation in Fe concentrations among 11 plum (*Prunus domestica*) varieties (Nergiz & Yildiz, 1997). Some genetic variation in the Se concentrations in tomato has also been reported (Shennan *et al.*, 1990, Pezzarossa *et al.*, 1999).

Limited within-species genetic variation in the concentrations of mineral elements has also been observed in tubers (Table 1; Pfeiffer & McClafferty, 2007; White *et al.*, in press). In

general, tuber concentrations of mineral elements differ 2- to 3-fold among genotypes, although greater variation in tuber Fe concentrations is occasionally observed (Agbor-Egbe & Trèche, 1995; White *et al.*, in press). Genetic variation in tuber Fe, Zn, Ca, Mg and Cu concentrations has been observed in yams (Agbor-Egbe & Trèche, 1995; Pfeiffer & McClafferty, 2007), oca (Sangketkit *et al.*, 2001) and cultivated potato species (Pfeiffer & McClafferty, 2007; White *et al.*, in press and references therein). As tubers acquire most of their mineral elements via the phloem (Westermann, 2005), the concentrations of Ca, Mg and Zn in tubers are often lower than in other edible tissues (Table 1).

Genetic variation in the concentrations of promoters and antinutrients in the edible portions of crop plants

The bioavailability of essential mineral elements depends greatly on the presence in a meal of substances that promote or inhibit their absorption by the gut (Frossard *et al.*, 2000; Reddy, 2002; Hotz & Brown, 2004; Welch & Graham, 2004; White & Broadley, 2005a; Slingerland *et al.*, 2006; Bohn *et al.*, 2008). The main substances known to inhibit the absorption of Fe, Zn, Ca and Mg are phytate from cereal grains and legume seeds and polyphenolics from beverages such as tea and coffee, beans and sorghum. The absorption of Ca is inhibited additionally by oxalate present in certain fruit and vegetables (Franceschi & Nakata, 2005; Titchenal & Dobbs, 2007). It is estimated that only *c.* 5% of the Fe and 25% of the Zn present in legume and cereal seeds is bioavailable (Pfeiffer & McClafferty, 2007). The bioavailability of Fe is reduced at dietary phytate/Fe molar quotients greater than 1 and the bioavailability of Zn is reduced when the phytate/Zn molar quotient exceeds about 6 (Lönnerdal, 2002; Hurrell, 2003). Substances enhancing the absorption of Fe and Zn include ascorbic acid and β-carotene from fruits and vegetables, whilst cysteine-rich polypeptides from plant and animal sources promote the absorption of Fe, Zn and Cu. Inulin, a polysaccharide of fructose often with a terminal glucose unit that is present in significant amounts in a wide range of edible crops, promotes the absorption of both Ca and Mg (Roberfroid, 2005, 2007).

Phytate concentrations vary considerably among cereal, legume and vegetable crops (Holland *et al.*, 1991, 1992; Frossard *et al.*, 2000; Reddy, 2002; Hotz & Brown, 2004; Vreugdenhil *et al.*, 2005). Significant within-species genetic variation has been found for grain phytate concentration in rice (Welch *et al.*, 2000, Glahn *et al.*, 2002), wheat (Lolas *et al.*, 1976; Raboy *et al.*, 1991; Erdal *et al.*, 2002; Welch *et al.*, 2005), barley (Dai *et al.*, 2007), pearl millet (Abdalla *et al.*, 1998), oat (Lolas *et al.*, 1976), triticale (Feil & Fossati, 1997) and sorghum (Reddy *et al.*, 2005; Slingerland *et al.*, 2006). Similarly, seed phytate concentrations vary among genotypes of common bean (Lolas & Markakis, 1975; Coelho *et al.*, 2002; House *et al.*, 2002; Cichy *et al.*, 2005; Ariza-Nieto *et al.*, 2007) and soybean (Lolas *et al.*, 1976; Raboy *et al.*, 1984; Horner *et al.*, 2005) and among accessions of *B. rapa* (Zhao *et al.*, 2007, 2008). In addition, natural and induced mutants with similar total P concentrations to conventional varieties, but reduced seed phytate concentrations, named low phytic acid (*lpa*) mutants, have been described in wheat, barley, maize, rice and soybean (reviewed by Raboy, 2003, 2007; Bohn *et al.*, 2008). Although many of these mutants have reduced rates of germination and agronomic yield, this is not always the case (Ertl *et al.*, 1998, Pilu *et al.*, 2003; Hulke *et al.*, 2004; Oltmans *et al.*, 2005; Bregitzer & Raboy, 2006; Guttieri *et al.*, 2006a; Raboy, 2007). Two phenotypes of *lpa* mutants are commonly observed. Seeds of one phenotype do not accumulate greater concentrations of inositol phosphates (IPs), whereas seeds of the other phenotype accumulate IP_3, IP_4 and IP_5 (Raboy, 2003, 2007). The *lpa* mutations do not appear to have much effect on the concentrations or distributions of mineral elements within the seed (Hatzack *et al.*, 2000; Guttieri *et al.*, 2004, 2006b; Liu *et al.*, 2004a, 2007; Ockenden *et al.*, 2004; Bryant *et al.*, 2005; Joyce *et al.*, 2005; Lin *et al.*, 2005), but replacing conventional varieties with *lpa* mutants in diets improves the mineral nutrition of monogastric animals, including humans (Adams *et al.*, 2002; Mendoza *et al.*, 1998, 2001; Hambidge *et al.*, 2004, 2005; Mazariegos *et al.*, 2006; see reviews by Mendoza, 2002; Raboy, 2007). In humans, this beneficial effect is most apparent when the dietary consumption of minerals is low. Several QTLs affecting the phytate concentration in seeds of rice (Stangoulis *et al.*, 2007) and common bean (Cichy *et al.*, 2005) and in seeds and leaves of *B. rapa* (Zhao *et al.*, 2007, 2008) have been identified for use as molecular markers in breeding programmes. Weak correlations between phytate and Fe, Zn, Ca or Mg concentrations in sorghum grain (Reddy *et al.*, 2005), soybean seeds (Raboy *et al.*, 1984) and common bean seeds (Cichy *et al.*, 2005), and a lack of coincidence of QTLs affecting seed phytate and those affecting Fe or Zn concentrations (Vreugdenhil *et al.*, 2004; Cichy *et al.*, 2005; Stangoulis *et al.*, 2007; Waters & Grusak, 2008b) suggest that these traits are controlled by different genes and that it would be possible to breed for Fe-, Zn-, Ca- and/or Mg-dense edible portions with low phytate concentrations.

It has long been known that P and phytate concentrations in cereal grains and legume seeds are increased greatly by the application of P fertilizers and reduced by the application of Zn fertilizers (Reddy *et al.*, 1989; Ryan *et al.*, 2008). Thus, judicious applications of inorganic fertilizers could complement any genetic approaches to reduce dietary phytate intake and increase dietary Zn/phytate quotients.

Genetic variation in the concentrations of polyphenolics in seeds of sorghum (Dicko *et al.*, 2002) and common bean (Guzmán-Maldonado *et al.*, 2000, 2003; House *et al.*, 2002) has also been reported. However, because polyphenolic compounds differ in their abilities to bind Fe (Brune *et al.*, 1989; Hurrell *et al.*, 1999), and many polyphenols have been

shown to be beneficial to human health (Scalbert *et al.*, 2005), it will be necessary to reduce the concentrations of only those polyphenols that bind Fe most avidly to increase Fe bioavailability.

Cereal grains, vegetables and fruits vary widely in their oxalate concentrations (USDA, 1984; Libert & Franceschi, 1987; Holland *et al.*, 1992; Kim *et al.*, 2007; Massey, 2007). Most angiosperms deposit Ca-oxalate crystals in cell vacuoles or, occasionally, the cell wall, although members of some commelinoid and noncommelinoid monocot families, including the Poaceae, Liliaceae and Zingiberaceae, appear to lack Ca-oxalate crystals (Prychid & Rudall, 1999; Franceschi & Nakata, 2005). The shapes and tissue distributions of oxalate crystals differ among plant species. Plants that can accumulate large quantities of oxalate (> 5% DM) in their edible tissues include members of the Caryophyllales (e.g. amaranth, beet/chard, purslane (*Portulaca oleracea*), spinach, tetragona (*Tetragonia tetragonioides*) and rhubarb), Araceae (e.g. taro (*Colocasia esculenta*)), and Oxalidaceae (e.g. carambola and oca). Seeds of some legumes, and roots of carrot and cassava can also accumulate high concentrations of oxalate on occasion (USDA, 1984; Massey, 2007). In general, oxalate concentrations are far greater in leaves than in roots or fruits (Libert & Franceschi, 1987). In some species, such as amaranth, beet/chard and spinach, much of the oxalate is insoluble, whereas in other species, such as oca, sweet pepper (*Capsicum annuum*), eggplant (*Solanum melongena*) and carrot, the oxalate is soluble (Libert & Franceschi, 1987; Albihn & Savage, 2001; White & Broadley, 2003; Franceschi & Nakata, 2005; White, 2005; Kim *et al.*, 2007). In plants that precipitate Ca-oxalate, the accumulation of oxalate is directly proportional to tissue Ca concentration and is, therefore, strongly dependent upon Ca phytoavailability and plant growth rate (Libert & Franceschi, 1987; Kinzel & Lechner, 1992; Franceschi & Nakata, 2005). Some within-species genetic variation in oxalate concentrations has been observed in beet (Libert & Franceschi, 1987), spinach (Kitchen *et al.*, 1964; Libert & Franceschi, 1987; Kawazu *et al.*, 2003; Mou, 2008), rhubarb (Libert & Creed, 1985; Libert, 1987; Libert & Franceschi, 1987), carambola (Wilson *et al.*, 1982), oca (Ross *et al.*, 1999; Albihn & Savage, 2001; Sangketkit *et al.*, 2001), taro (Tanaka *et al.*, 2003) and soybean (Massey *et al.*, 2001; Horner *et al.*, 2005), and several mutants have been identified in the forage legume *Medicago truncatula* with reduced or altered accumulation of Ca-oxalate in their leaves (Nakata & McConn, 2000; McConn & Nakata, 2002). For two of the *M. truncatula calcium oxalate deficient* mutants (*cod5* and *cod6*), reduced accumulation of Ca-oxalate has been correlated with increased Ca bioavailability in herbage (Nakata & McConn, 2006, 2007; Morris *et al.*, 2007).

The concentrations of β-carotene and ascorbic acid vary among plant tissues and among plant species (Bhaskarachary *et al.*, 1995, 2007; Frossard *et al.*, 2000; FSA, 2002; USDA-ARS, 2007). Fruits and vegetables are important dietary sources of ascorbic acid and β-carotene. Significant within-species genetic variation in β-carotene concentration has been found in edible portions of a number of crop species, including cassava (Chávez *et al.*, 2000, 2005; Maziya-Dixon *et al.*, 2000; Thakkar *et al.*, 2007), carrot (Nicolle *et al.*, 2004b; Baranska *et al.*, 2006), lettuce (*Lactuca sativa*; Nicolle *et al.*, 2004a; Mou, 2005), *B. oleracea* (Schonhof & Krumbeln, 1996; Kurilich *et al.*, 1999; Kopsell *et al.*, 2004a), sweet potato (Nestel *et al.*, 2006), potato (Nesterenko & Sink, 2003; Morris *et al.*, 2004), sweet pepper (Simonne *et al.*, 1997; Howard *et al.*, 2000), tomato (Fraser & Bramley, 2004), chickpea (Abbo *et al.*, 2005), immature soybean (Simonne *et al.*, 2000), common bean, rice, wheat (Welch & Graham, 2005; Howitt & Pogson, 2006), sorghum (Kapoor & Naik, 1970; Reddy *et al.*, 2005), pearl millet (Kapoor & Naik, 1970), maize (Kurilich & Juvik, 1999; Maziya-Dixon *et al.*, 2000; Hulshof *et al.*, 2007; Ortiz-Monasterio *et al.*, 2007; Harjes *et al.*, 2008) and plantains (Davey *et al.*, 2007). In addition, natural mutations in either *LeDET1* or *LeDDB1* have been found to lead to increased concentrations of β-carotene and ascorbic acid in tomato fruit (Liu *et al.*, 2004b; Bino *et al.*, 2005), a spontaneous, semidominant mutation of the *orange* (*Or*) gene encoding a plastid-associated DnaJ-related protein (Lu *et al.*, 2006) has been found to lead to high concentrations of β-carotene in cauliflower (*Brassica oleracea* var. *botrytis*) curd (Li *et al.*, 2001a), and natural mutations in the promoter of the *yellow1* (*Y1*) gene encoding a phytoene synthase have been found to be associated with greater concentrations of β-carotene in the endosperm of maize (Palaisa *et al.*, 2003). Both cauliflower *Or* hybrids and maize with yellow endosperm are available commercially.

There is also significant genetic variation in the concentration of ascorbic acid in edible tissues of many crop species including carrot (Nicolle *et al.*, 2004b), cassava (Chávez *et al.*, 2000), lettuce (Nicolle *et al.*, 2004a), *B. oleracea* (Kurilich *et al.*, 1999), sweet pepper (Simonne *et al.*, 1997; Howard *et al.*, 2000), strawberry (Hakala *et al.*, 2003) and banana (Wall, 2006). In addition to genotypic effects, ascorbate concentrations in plant tissues are affected by other factors such as developmental stage, time of day, and growth environment (Smirnoff *et al.*, 2001; Ishikawa *et al.*, 2006; Smith *et al.*, 2007). Ascorbate concentrations increase in response to diverse environmental stresses and, in particular, high light intensities. This is thought to be related to the role of ascorbate as a key antioxidant in plants (Smirnoff *et al.*, 2001; Noctor, 2006).

Transgenic approaches to biofortification

Transgenic approaches to biofortification rely on improving the phytoavailability of mineral elements in the soil, their uptake from the rhizosphere, translocation to the shoot and accumulation in edible tissues (White & Broadley, 2005a; Davies, 2007; Puig *et al.*, 2007a; Zhu *et al.*, 2007). In addition, transgenic approaches may be used to reduce the concentrations of antinutrients and increase the concentrations of promoter substances.

Increasing concentrations of mineral elements

Iron acquisition from the soil can be improved in strategy I plants by overexpressing genes encoding Fe(III) reductases (Samuelsen *et al.*, 1998; Grusak, 2000; Rogers & Guerinot, 2002; Vasconcelos *et al.*, 2006) and Fe^{2+} transporters of the root plasma membrane (Ramesh *et al.*, 2004; Grotz & Guerinot, 2006), and in strategy II plants by increasing the synthesis and exudation of phytosiderophores (Takahashi *et al.*, 2001; Douchkov *et al.*, 2005), together with increased expression of genes encoding YSL proteins. However, it has been observed that, although the overexpression of *FRO* genes is sufficient to increase Fe accumulation in leaves, increased biosynthesis of Fe-chelates in the shoot is often required to increase Fe concentrations in seeds, fruits and tubers, which rely on Fe supplied via the phloem (Grusak, 2000; Rogers & Guerinot, 2002; White & Broadley, 2005a). Nevertheless, increasing the capacity of edible tissues to sequester Fe can promote their accumulation of Fe, possibly through feedback mechanisms impacting on plant Fe homeostasis. Thus, altering the activity of vacuolar transporters, such as NRAMPs and VIT1, in seeds can increase their Fe concentrations (Lanquar *et al.*, 2005; Kim *et al.*, 2006b; Puig *et al.*, 2007a), and expressing plant ferritin or human lactoferrin genes has increased Fe, Zn and Cu concentrations in seeds of rice (Goto *et al.*, 1999; Lucca *et al.*, 2001, 2002; Nandi *et al.*, 2002; Krishnan *et al.*, 2003; Vasconcelos *et al.*, 2003; Qu *et al.*, 2005; Sivaprakash *et al.*, 2006), and Fe concentrations in maize seeds (Drakakaki *et al.*, 2005), tomato fruits and potato tubers (Chong & Langridge, 2000). The overexpression of plant ferritin genes has also been reported to increase Fe concentrations in lettuce leaves (Goto *et al.*, 2000).

The misexpression of genes affecting Zn and Cu uptake and movement within the plant can increase the concentration of these elements in edible portions. For example, pea *bronze* (*brz*) and *degenerated leaflet* (*dgl*) and Arabidopsis *ferric reductase defective 3/manganese accumulator 1* (*frd3* = *man1*) mutants constitutively expressing rhizosphere Fe(III) reductase activity have greater shoot Zn and Cu concentrations than wild-type plants (Delhaize, 1996; Grusak, 2000; Rogers & Guerinot, 2002), and transgenic barley plants expressing *AtZIP1* produce smaller seeds with higher Zn concentrations than wild-type plants (Ramesh *et al.*, 2004). It has also been suggested that, because AtCAX1 can be modified to transport Zn^{2+} into the vacuole, modified CAX transporters could also be used to increase Zn concentrations in the edible tissues of transgenic plants (Shigaki *et al.*, 2005). Zinc concentrations in Arabidopsis leaves can be increased by overexpressing genes encoding AtHMA4 (Verret *et al.*, 2004) or AtMTP3 (Arrivault *et al.*, 2006), and by reducing the expression of *AtHMA2* (Eren & Argüello, 2004) or *AtOPT3* (Stacey *et al.*, 2008). Seeds of the *opt3-2* mutant have higher Zn and Cu concentrations than seeds of wild-type plants (Stacey *et al.*, 2008). Seed Fe and Zn concentrations can be increased in wheat by expressing RNA interference (RNAi) constructs of an NAC transcription factor (NAM-B1) that accelerates senescence and increases remobilization of mineral elements from leaves to developing grain (Uauy *et al.*, 2006). The accumulation of Cu in plant tissues can be increased by the overexpression of genes encoding Cu transporters of the HMA family and/or Cu-binding metallotheioneins (Puig *et al.*, 2007a). Similarly, Indian mustard (*B. juncea*) genetically engineered to produce more glutathione, phytochelatins and total thiols had greater concentrations of Zn and Cu than nontransformed controls, when grown on soils containing high concentrations of these elements (Bennett *et al.*, 2003). It has been observed that manipulation of the concentrations of plant growth regulators can also increase the accumulation of Zn and Cu. For example, transgenic tomato plants expressing bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which produce less ethylene, have greater concentrations of Zn and Cu in their leaves than nontransgenic plants (Grichko *et al.*, 2000).

Interestingly, the misexpression of neither genes encoding Ca^{2+}-permeable channels of the plasma membrane nor genes encoding any Ca^{2+}-ATPases appears to increase shoot Ca concentrations (Kim *et al.*, 2001; Hampton *et al.*, 2005; White, 2005; Kaplan *et al.*, 2007). However, plants overexpressing genes encoding vacuolar Ca^{2+}/H^{+} antiporters have greater shoot Ca concentrations than wild-type plants (Hirschi, 2001; Hirschi *et al.*, 2001) and the expression of genes encoding AtCAX1 lacking its autoinhibitory domain (*sCAX1*), a modified AtCAX2 (*sCAX2*) or AtCAX4 increases Ca concentrations and, potentially, dietary Ca delivery, in edible portions of transgenic carrot (Park *et al.*, 2004; Morris *et al.*, 2008), lettuce (Park *et al.*, 2009), tomato (Park *et al.*, 2005a) and potato (Park *et al.*, 2005b; Kim *et al.*, 2006a). Remarkably, Arabidopsis mutants lacking AtSKOR, a Ca^{2+}-permeable outward-rectifying K^{+} channel expressed in the pericycle and xylem parenchyma of the root, also have greater shoot Ca concentrations than wild-type plants, which is consistent with AtSKOR removing Ca^{2+} from the xylem sap (Gaymard *et al.*, 1998). Shoots of transgenic plants overexpressing calreticulin, the main Ca^{2+}-binding protein in the endoplasmic reticulum, have higher Ca concentrations than wild-type plants (Wyatt *et al.*, 2002) and it has been speculated that the overexpression of vacuolar Ca^{2+}-binding proteins might also increase Ca concentrations in edible produce (White, 2005).

Selenium concentrations in produce depend upon the ability of crop plants to take up Se, redistribute it within the plant, and accumulate it in nontoxic forms in edible tissues. Differences between Se-accumulator and nonaccumulator plants in their Se uptake capacity and tissue Se/S quotients appear to be related to the selectivity of Se/S transporters in the plasma membrane of root cells. It has, therefore, been speculated that appropriate allelic variation in the domain(s) conferring selenate/sulphate selectivity in HASTs, combined with the constitutive expression of Se-selective HASTs, could be used to produce crops with increased Se concentrations

and tissue Se/S quotients (Terry *et al.*, 2000; White *et al.*, 2004, 2007a,b; Sors *et al.*, 2005b; Broadley *et al.*, 2006b). Differences in the ability of plant species to tolerate high tissue Se concentrations are thought to be a consequence of differences in their Se metabolism, and, in particular, the production of nontoxic, sink metabolites (Sors *et al.*, 2005a,b). Consistent with this hypothesis, the leaves of transgenic plants overexpressing genes encoding SeCys methyltransferase and/or ATP sulphurylase have greater Se tolerance, and higher concentrations of methylcysteine, selenomethylcysteine, and total Se, than wild-type plants (Pilon-Smits *et al.*, 1999; Montes-Bayón *et al.*, 2002; Ellis *et al.*, 2004; LeDuc *et al.*, 2004, 2006; Van Huysen *et al.*, 2004; Bañuelos *et al.*, 2005; Sors *et al.*, 2005a). Similarly, transgenic plants expressing a mouse SeCys lyase or the chloroplast protein CpNifS, which both catalyse the conversion of selenocysteine to alanine and elemental Se, also have greater Se tolerance and higher leaf Se concentrations than wild-type plants (Garifullina *et al.*, 2003; Pilon *et al.*, 2003; Van Hoewyk *et al.*, 2005).

The bioavailability and benefits to human health of dietary Se depend upon not only the amounts but also the chemical forms of Se supplied (Combs, 2001; Rayman, 2004; Finlay, 2007). The range between Se deficiency and Se toxicity in human diets is narrow (Combs, 2001; Rayman, 2004; White & Broadley, 2005a), but supplying Se in forms with greater bioavailability, such as SeMet and SeCys, will most improve the Se status of populations with low dietary Se intakes (Rayman, 2004; Thomson, 2004). The dominant organic form of Se in cereals is SeMet (Broadley *et al.*, 2006b; Hawkesford & Zhao, 2007). By contrast, members of the *Brassica* and *Allium* genera contain high concentrations of SeMSeCys (Broadley *et al.*, 2006b; Finlay, 2007). This compound may be effective at reducing the incidence of various cancers and has a low potential for Se toxicity (Whanger, 2004; Finlay, 2007). Thus, increasing SeMet, SeCys and/or SeMSeCys are attractive targets for transgenic strategies to improve Se bioavailability from edible crops.

Manipulating concentrations of antinutrients and promoter substances

Transgenic approaches to increase the bioavailability of Fe, Zn and Ca in food have focused on reducing the concentrations of antinutrients, such as oxalate, polyphenolics and phytate, and increasing the concentrations of promoter substances, such as ascorbate, β-carotene and cysteine-rich polypeptides, in edible produce (Lönnerdal, 2003; White & Broadley, 2005a; Kopsell & Kopsell, 2006; Lucca *et al.*, 2006; Davies, 2007; Zhu *et al.*, 2007).

Two transgenic strategies have been adopted to reduce phytate concentrations in edible produce (Lönnerdal, 2003; Raboy, 2003, 2007). The first strategy is to reduce the expression of genes encoding enzymes involved in the synthesis or sequestration of IP_6, which has successfully reduced phytate concentrations in seeds of maize, soybean and rice (Kuwano *et al.*, 2006; Nunes *et al.*, 2006; Shi *et al.*, 2005, 2007). The second strategy is to overexpress phytases in edible tissues. This strategy has proved successful in reducing endogenous phytate concentrations in seeds and/or increasing phytase activity in feed from rice (Lucca *et al.*, 2001, 2002; Hong *et al.*, 2004), wheat (Brinch-Pedersen *et al.*, 2006), maize (Drakakaki *et al.*, 2005; Chen *et al.*, 2008), soybean (Chiera *et al.*, 2004; Bilyeu *et al.*, 2008), alfalfa (*Medicago sativa*; Flachowski *et al.*, 2005) and canola (*Brassica napus*; Ponstein *et al.*, 2002).

Several crop plants have been genetically modified to contain greater concentrations of β-carotene, the precursor of vitamin A, in their edible tissues. These include golden rice (Al-Babili & Beyer, 2005; Paine *et al.*, 2005) and maize (Aluru *et al.*, 2008), golden canola (Ravanello *et al.*, 2003), orange cauliflower (Lu *et al.*, 2006), tomato (*Solanum lycopersicum*; Fraser & Bramley, 2004; Davuluri *et al.*, 2005; Taylor & Ramsay, 2005; Botella-Pavía & Rodríguez-Concepción, 2006; Kopsell & Kopsell, 2006; Davies, 2007; Wurbs *et al.*, 2007; Zhu *et al.*, 2007) and yellow potatoes (*Solanum tuberosum* and *S. phureja*; Ducreux *et al.*, 2005; Lu *et al.*, 2006; Morris *et al.*, 2006; Diretto *et al.*, 2007; Lopez *et al.*, 2008). The strategy has been either to overexpress carotenogenic transgenes from nuclear or plastid genomes or to alter the expression of genes controlling plastid development. Similarly, genetic modification (GM) approaches have been used to increase ascorbate concentrations in lettuce leaves (Jain & Nessler, 2000) and maize kernels (Chen *et al.*, 2003). Attempts to increase protein cysteine concentrations in edible tissues have also proved successful on occasion (Chakraborty *et al.*, 2000; Lucca *et al.*, 2001, 2002; Sun & Liu, 2004; Welch & Graham, 2004), but not always (Tabe & Droux, 2002; Hagan *et al.*, 2003; Zhang *et al.*, 2003a; Chiaiese *et al.*, 2004). Although genes encoding proteins with abundant methionine and cysteine residues are readily expressed in transgenic plants, and protein methionine concentrations can be increased greatly, a concomitant decrease in the expression of genes encoding endogenous cysteine-rich proteins results in an apparent inability to raise protein cysteine concentrations (Tabe & Droux, 2002; Chiaiese *et al.*, 2004; Ufaz & Galili, 2008). It has been suggested that this might be addressed by the application of S fertilizers, as the down-regulation of the endogenous genes is a characteristic response to S starvation.

Is biofortification of edible produce a solution to the 'hidden hunger'?

The term 'hidden hunger' has been used to describe the micronutrient malnutrition inherent in human diets that are adequate in calories but lack vitamins and/or mineral elements. The diets of a large proportion of the world's population are deficient in Fe, Zn, Ca, Mg, Cu, Se or I, which affects human health and longevity, and therefore national economies. Mineral

malnutrition can be addressed by increasing the amount of fish and animal products in diets, mineral supplementation, food fortification and/or increasing the bioavailability of mineral elements in edible crops. However, as observed in the introduction, strategies to increase dietary diversification, mineral supplementation and food fortification have not always proved successful. For this reason, the biofortification of crops through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements, has been advocated. To determine whether biofortification strategies can address mineral malnutrition of humans, decision-makers have posed six key questions (Bouis, 2000; Bouis *et al.*, 2000; Nestel *et al.*, 2006).

- Is breeding (combined with appropriate agronomy) for high nutrient content scientifically feasible?
- Will farmers adopt the new genotypes?
- What is the target nutrient content for breeding?
- What is the impact on nutritional status?
- Is it cost-effective?
- Will consumers accept the biofortified foods?

It should be clear from the foregoing sections that breeding for edible produce containing higher concentrations of all the mineral elements most often lacking in human diets is possible without affecting yields. However, it is also apparent that, although many soils contain ample mineral elements, plant breeding must be combined with appropriate agronomy and the application of mineral fertilizers when the phytoavailability of mineral elements restricts plant growth or concentrations of mineral elements in edible portions. Although the use of mineral fertilizers is evidently feasible in the developed world, as exemplified by the success of Se fertilization of crops in Finland (Lyons *et al.*, 2003; Hartikainen, 2005; Broadley *et al.*, 2006b), Zn fertilization in Turkey (Cakmak, 2004) and I fertilization in China (Jiang *et al.*, 1997), the distribution of mineral fertilizers requires appropriate social infrastructures, stable political policies and continued investment, which has stymied previous attempts at mineral supplementation and food fortification in developing countries. It is likely that farmers in both developed and developing countries will adopt new genotypes that acquire mineral elements more efficiently, particularly if biofortified produce demands a premium price and crops can be grown on soils with low phytoavailability of mineral elements with reduced fertilizer inputs, better germination, seedling vigour and resistance to pathogens (Rengel & Graham, 1995; Yilmaz *et al.*, 1998; Welch, 1999; Bouis *et al.*, 2000; Genc *et al.*, 2000; Cakmak, 2004, 2008; Graham *et al.*, 2007).

The target concentration for a specific mineral element in the edible portion of a biofortified crop will be determined by the amount of that element required in the human diet, the deficit of the mineral element in the diet of an affected population, the number of crops that will be biofortified, the bioavailability of the mineral element following processing and cooking, and the contributions of each biofortified crop to the diet of the affected population. Thus, strategies for addressing mineral malnutrition through biofortification and, therefore, target concentrations of mineral elements in edible produce will depend greatly upon local diet and culinary customs. When more than one mineral element is lacking in the diet, biofortification strategies must deliver all of them to the affected population. However, when a mineral or vitamin deficiency is induced by the lack of another mineral or vitamin, as occurs among Fe, Zn and provitamin A carotenoid deficiencies (Hess *et al.*, 2005) and between Se and I deficiencies (Lyons *et al.*, 2004), it can be corrected by the biofortification of edible crops with the appropriate mineral and/or vitamin that is lacking in the diet.

In developing countries, it has been suggested that biofortification strategies should focus on the staple foods that dominate people's diets (Bouis, 2000; Pfeiffer & McClafferty, 2007). The argument is simple: if the concentrations of mineral elements in staple foods can be increased, then the delivery of mineral elements to vulnerable populations can be increased *pro rata* to their contribution to the diet, without a change in behaviour (Bouis, 1999; Bouis *et al.*, 2000; Graham *et al.*, 2007). Target staples include rice and wheat, which are staple foods for over half the world's population, maize, which is the staple food in much of sub-Saharan Africa and in Mesoamerica, common bean, which supplies significant amounts of minerals to populations in Africa and Latin America, and cassava, which is the main source of dietary carbohydrates in sub-Saharan Africa. Theoretical studies indicate that a strategy based on the biofortification of staple crops should increase the delivery of mineral elements to human diets and dramatically improve the nutritional status of vulnerable populations in developing countries (Bouis *et al.*, 2000). In this context, a doubling of the Fe and Zn concentrations in cereal grains and legume seeds would be an appropriate and achievable target. The HarvestPlus consortium has suggested an absolute target for the additional Zn and Fe in biofortified crops of between 30 and 40% of the estimated average dietary requirement for humans (Holtz, 2007). Low-phytate crops could also be used to increase the bioavailability of Fe and Zn in plant foods to achieve these targets (Pfeiffer & McClafferty, 2007), but there is much debate about this strategy because high dietary phytate has been linked to various health benefits (Vucenik & Shamsuddin, 2006). Similarly, although reducing the concentrations of specific polyphenolic compounds could increase the bioavailability of Fe in the diet, it could also compromise the beneficial effects of these compounds on human health (Scalbert *et al.*, 2005).

The impact of biofortified produce on the nutritional status of humans has rarely been tested. However, it is evident that the application of mineral fertilizers containing Se, I or Zn can have a significant impact on the nutritional status of a vulnerable population (Jiang *et al.*, 1997; Cakmak, 2004; Rayman, 2008). In addition, it was found that the consumption of Fe-biofortified rice improved the Fe status of nonanaemic

Filipino women (Haas *et al.*, 2005) and that replacing conventional varieties with *lpa* mutants in people's diets improved their Fe, Zn and Ca status, especially when consumption of dietary minerals was low (Mendoza *et al.*, 1998, 2001; Adams *et al.*, 2002; Hambidge *et al.*, 2004, 2005; Mazariegos *et al.*, 2006). These data suggest that the biofortification of edible produce can improve the nutritional status of humans.

Biofortification of edible produce through genetic strategies is potentially cost effective and will deliver most benefits to the 40% of the world's population who rely primarily on their own food for sustenance. It has been suggested that a one-time financial investment in seeds of cereal staples that acquire mineral elements more effectively from the soil could support the Fe, Zn, Ca and Mg requirements of rural populations in remote areas. Similarly, a one-time financial investment would suffice for vegetatively propagated crops, such as cassava, potato and banana (Johns & Eyzaguirre, 2007). Most economic analyses suggest that genetic strategies towards biofortification are more cost effective than dietary diversification, supplementation or food fortification programmes (Bouis, 1999; Bouis *et al.*, 2000; Horton, 2006; Stein *et al.*, 2007; Ma *et al.*, 2008). Early economic analyses for Zn biofortification of wheat in Turkey suggested a cost-to-benefit quotient of greater than 20 over two decades (Bouis, 1999), and cost-to-benefit quotients of between 20 and 30 for Fe biofortification of rice in South Asia and for Fe biofortification of rice and wheat in Bangladesh and India over the same period (Bouis *et al.*, 2003). Informal estimates of cost-to-benefit quotients for fertilization with Se and/or I also suggest high returns on financial investments (Lyons *et al.*, 2005; Horton, 2006). More recently, the potential impact of biofortification has been quantified as the saving of disability-adjusted life years (DALYs; Stein *et al.*, 2005). It has been estimated that the annual burden of Fe-deficiency anaemia in India is 4 million lost DALYs and that Fe biofortification may reduce this burden significantly. Similarly, it is estimated that the annual burden of Zn deficiency in India is 2.8 million lost DALYs and Zn biofortification of rice and wheat may reduce this burden by 20–51% (Stein *et al.*, 2007). The cost of saving 1 DALY from Zn biofortification of rice and wheat in India was estimated as $US 0.73–7.31 (Stein *et al.*, 2007). The cost of saving 1 DALY from the biofortification of beans/pearl millet/potatoes with Fe and Zn in Africa has been estimated as $US 2–20.

It is thought that consumers in both developed and developing countries will accept foods prepared from biofortified crops provided that they are not appreciably more expensive than the alternatives and that biofortification does not alter the appearance, taste, texture or cooking quality of foods (Bouis *et al.*, 2003). It is thought unlikely that small quantities of mineral elements will alter these properties of foods, but manipulating the concentrations of promoters and antinutrients might affect both taste and colour. If it can be demonstrated that foods prepared using biofortified produce are more beneficial to human health, this will, of course, influence consumer choice in both developed and developing countries.

In conclusion, biofortification strategies based on crop breeding, targeted genetic manipulation and/or the application of mineral fertilizers hold great potential for addressing mineral malnutrition in humans. The questions posed by decision-makers have been answered positively, and international initiatives, such as the HarvestPlus programme, have begun to deliver crops with the potential to increase both the amounts and bioavailability of essential mineral elements in human diets.

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References

Abadía J, López-Millán A-F, Rombolà A, Abadía A. 2002. Organic acids and Fe deficiency: a review. *Plant and Soil* **241**: 75–86.

Abbo S, Grusak MA, Tzuk T, Reifen R. 2000. Genetic control of seed weight and calcium concentration in chickpea seed. *Plant Breeding* **119**: 427–431.

Abbo S, Molina C, Jungmann R, Grusak MA, Berkovitch Z, Reifen R, Kahl G, Winter P, Reifen R. 2005. Quantitative trait loci governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* **111**: 185–195.

Abdalla AA, El Tinay AH, Mohamed BE, Abdalla AH. 1998. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. *Food Chemistry* **63**: 243–246.

Abdel-Ghany SE, Müller-Moulé P, Niyogi KK, Pilon M, Shikanai T. 2005. Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. *Plant Cell* **17**: 1233–1251.

Adams CL, Hambidge M, Raboy V, Dorsch JA, Sian L, Westcott JL, Krebs NF. 2002. Zinc absorption from a low-phytic acid maize. *American Journal of Clinical Nutrition* **76**: 556–559.

Agbor-Egbe T, Trèche S. 1995. Evaluation of the chemical composition of Cameroonian yam germplasm. *Journal of Food Composition and Analysis* **8**: 274–283.

Aitken RL, Dickson T, Hailes KJ, Moody PW. 1999. Response of field-grown maize to applied magnesium in acidic soils in north-eastern Australia. *Australian Journal of Agricultural Research* **50**: 191–198.

Al-Babili S, Beyer P. 2005. Golden Rice – five years on the road – five years to go? *Trends in Plant Science* **10**: 565–573.

Albihn PBE, Savage GP. 2001. The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand-grown oca (*Oxalis tuberosa* Mol). *Journal of the Science of Food and Agriculture* **81**: 1027–1033.

Alderman G, Jones DIH. 1967. The iodine content of pastures. *Journal of the Science of Food and Agriculture* **18**: 197–199.

Alloway BJ. 2004. *Zinc in soils and crop nutrition*. Brussels, Belgium: International Zinc Association.

Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Wang K, Rodermel S. 2008. Generation of transgenic maize with enhanced provitamin A content. *Journal of Experimental Botany* **59**: 3551–3562.

Alvarez J, Marcó LM, Arroyo J, Greaves ED, Rivas R. 2003. Determination of calcium, potassium, manganese, iron, copper and zinc levels in representative samples of two onion cultivars using total reflection X-ray fluorescence and ultrasound extraction procedure. *Spectrochimica Acta Part B* **58**: 2183–2189.

Andrés-Colás N, Sancenón V, Rodríguez-Navarro S, Mayo S, Thiels DJ, Ecker J, Puig S, Peñarrubia L. 2006. The Arabidopsis heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification in roots. *Plant Journal* **45**: 225–236.

Ariza-Nieto M, Blair MW, Welch RM, Glahn RP. 2007. Screening of iron bioavailability patterns in eight bean (*Phaseolus vulgaris* L.) genotypes using the Caco-2 cell in vitro model. *Journal of Agricultural and Food Chemistry* **55**: 7950–7956.

Arrivault S, Senger T, Krämer U. 2006. The Arabidopsis metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. *Plant Journal* **46**: 861–879.

Assunção AGL, Da Costa Martins P, De Folter S, Vooijs R, Schat H, Aarts MGM. 2001. Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant, Cell & Environment* **24**: 217–226.

Baierová V, Baier J. 1993. Uptake of nutrients by sugar beet. *Rostlinná Výroba* **39**: 1095–1101.

Bálint AF, Kovacs G, Erdei L, Sutka J. 2001. Comparisons of the Cu, Zn, Fe, Ca and Mg contents of the grains of wild, ancient and cultivated wheat species. *Cereal Research Communications* **29**: 375–382.

Bañuelos G, Terry N, LeDuc DL, Pilon-Smits EAH, Mackey B. 2005. Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium-contaminated sediment. *Environmental Science & Technology* **39**: 1771–1777.

Bänziger M, Long J. 2000. The potential for increasing the iron and zinc density of maize through plant-breeding. *Food and Nutrition Bulletin* **21**: 397–400.

Baranska M, Baranski R, Schulz H, Nothnagel T. 2006. Tissue-specific accumulation of carotenoids in carrot roots. *Planta* **224**: 1028–1037.

Barber SA. 1995. *Soil nutrient bioavailability: a mechanistic approach*. New York, NY, USA: Wiley.

Barbier-Brygoo H, Vinauger M, Colcombet J, Ephritikhine G, Frachisse J, Maurel C. 2000. Anion channels in higher plants: functional characterization, molecular structure and physiological role. *Biochimica et Biophysica Acta* **1465**: 199–218.

Barea JM, Pozo MJ, Azcón R, Azcón-Aguilar C. 2005. Microbial co-operation in the rhizosphere. *Journal of Experimental Botany* **56**: 1761–1778.

Bashir K, Inoue H, Nagasaka S, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. 2006. Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. *Journal of Biological Chemistry* **281**: 32395–32402.

Baxter I, Tchieu J, Sussman MR, Boutry M, Palmgren MG, Gribskov M, Harper JF, Axelsen KB. 2003. Genomic comparison of P-type ATPase ion pumps in Arabidopsis and rice. *Plant Physiology* **132**: 618–628.

Beebe S, Gonzalez AV, Rengifo J. 2000. Research on trace minerals in the common bean. *Food and Nutrition Bulletin* **21**: 387–391.

Bennett LE, Burkhead JL, Hale KL, Terry N, Pilon M, Pilon-Smits EAH. 2003. Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. *Journal of Environmental Quality* **32**: 432–440.

Bereczky Z, Wang H-Y, Schubert V, Ganal M, Bauer P. 2003. Differential regulation of *NRAMP* and *IRT* metal transporter genes in wild type and iron uptake mutants of tomato. *Journal of Biological Chemistry* **278**: 24697–24704.

Bhaskarachary K, Ananthan R, Longvah T. 2007. Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. *Food Chemistry* **106**: 85–89.

Bhaskarachary K, Rao DSS, Deosthale YG, Reddy V. 1995. Carotene content of some common and less familiar foods of plant origin. *Food Chemistry* **54**: 189–193.

Bilyeu KD, Zeng P, Coello P, Zhang ZJ, Krishnan HB, Bailey A, Beuselinck PR, Polacco JC. 2008. Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. *Plant Physiology* **146**: 468–477.

Bino RJ, de Vos CHR, Lieberman M, Hall RD, Bovy A, Jonker HH, Tikunov Y, Lommen A, Moco S, Levin I. 2005. The light-hyperresponsive *high pigment-2dg* mutation of tomato: alterations in the fruit metabolome. *New Phytologist* **166**: 427–438.

Blasco B, Rios JJ, Cervilla LM, Sánchez-Rodrigez E, Ruiz JM, Romero L. 2008. Iodine biofortification and antioxidant capacity of lettuce: potential benefits for cultivation and human health. *Annals of Applied Biology* **152**: 289–299.

Bohn L, Meyer AS, Rasmussen SK. 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science B* **9**: 165–191.

Botella-Pavía P, Rodríguez-Concepción M. 2006. Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum* **126**: 369–381.

Bouis HE. 1999. Economics of enhanced micronutrient density in food staples. *Field Crops Research* **60**: 165–173.

Bouis HE. 2000. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition* **16**: 701–704.

Bouis HE, Chassy BM, Ochanda O. 2003. Genetically modified food crops and their contribution to human nutrition and food quality. *Trends in Food Science and Technology* **14**: 191–209.

Bouis HE, Graham RD, Welch RM. 2000. The Consultative Group on International Agricultural Research (CGIAR) micronutrients project: justification and objectives. *Food and Nutrition Bulletin* **21**: 374–381.

Bradshaw HD. 2005. Mutations in *CAX1* produce phenotypes characteristic of plants tolerant to serpentine soils. *New Phytologist* **167**: 81–88.

Branch WD, Gaines TP. 1983. Seed mineral composition of diverse peanut germplasm. *Peanut Science* **10**: 5–8.

Bregitzer P, Raboy V. 2006. Effects of four independent low-phytate mutations on barley agronomic performance. *Crop Science* **46**: 1318–1322.

Briat JF, Lobréaux S, Grignon N, Vansuyt G. 1999. Regulation of plant ferritin synthesis: how and why. *Cellular and Molecular Life Sciences* **56**: 155–166.

Brinch-Pedersen H, Hatzack F, Stöger E, Arcalis E, Pontopidan K, Holm PB. 2006. Heat-stable phytases in transgenic wheat (*Triticum aestivum* L.): deposition pattern, thermostability, and phytate hydrolysis. *Journal of Agricultural and Food Chemistry* **54**: 4624–4632.

Broadley MR, Bowen HC, Cotterill HL, Hammond JP, Meacham MC, Mead A, White PJ. 2003. Variation in the shoot calcium content of angiosperms. *Journal of Experimental Botany* **54**: 1431–1446.

Broadley MR, Bowen HC, Cotterill HL, Hammond JP, Meacham MC, Mead A, White PJ. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. *Journal of Experimental Botany* **55**: 321–336.

Broadley MR, Hammond JP, King GJ, Astley D, Bowen HC, Meacham MC, Mead A, Pink DAC, Teakle GR, Hayden RM *et al.* 2008. Shoot calcium and magnesium concentrations differ between subtaxa, are highly heritable, and associate with potentially pleiotropic loci in *Brassica oleracea*. *Plant Physiology* **146**: 1707–1720.

Broadley MR, Mead A, White PJ. 2006a. Reply to Davis (2006) Commentary. *Journal of Horticultural Science and Biotechnology* **81**: 554–555.

Broadley MR, White PJ, Bryson RJ, Meacham MC, Bowen HC, Johnson SE, Hawkesford MJ, McGrath SP, Zhao F-J, Breward N *et al.* 2006b. Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society* **65**: 169–181.

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. 2007. Zinc in plants. *New Phytologist* **173**: 677–702.

Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A, White PJ. 2001. Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytologist* **152**: 9–27.

Brown JC, Chaney RL. 1971. Effect of iron on the transport of citrate into the xylem of soybeans and tomatoes. *Plant Physiology* **47**: 836–840.

Brown TA, Shrift A. 1982. Selenium: toxicity and tolerance in higher plants. *Biological Reviews* **57**: 59–84.

Bruce LJ, Pan RJ, Cope DL, Uchikawa M, Gunn RB, Cherry RJ, Tanner MJA. 2004. Altered structure and anion transport properties of band 3 (AE1, SLC4A1) in human red cells lacking glycophorin A. *Journal of Biological Chemistry* **279**: 2414–2420.

Brune M, Rossander L, Hallberg L. 1989. Iron absorption and phenolic compounds: importance of different phenolic structures. *European Journal of Clinical Nutrition* **43**: 547–557.

Bryant RJ, Dorsch JA, Peterson KL, Rutger JN, Raboy V. 2005. Phosphorus and mineral concentrations in whole grain and milled low phytic acid (lpa) 1-1 rice. *Cereal Chemistry* **82**: 517–522.

Bunzl K, Trautmannscheimer M, Schramel P, Reifenhäuser W. 2001. Availability of arsenic, copper, lead, thallium, and zinc to various vegetables grown in slag-contaminated soils. *Journal of Environmental Quality* **30**: 934–939.

Butler GW, Glenday AC. 1961. Iodine content of pasture plants. II. Inheritance of leaf iodine content of perennial ryegrass (*Lolium perenne*). *Australian Journal of Biological Science* **15**: 183–187.

Cakmak I. 2002. Plant nutrition research: priorities to meet human needs for food in sustainable ways. *Plant and Soil* **247**: 3–24.

Cakmak I. 2004. *Proceedings of the International Fertiliser Society 552. Identification and correction of widespread zinc deficiency in Turkey – a success story*. York, UK: International Fertiliser Society.

Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and Soil* **302**: 1–17.

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Özkan H. 2004. *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Science and Plant Nutrition* **50**: 1047–1054.

Caris C, Hordt W, Hawkins HJ, Romheld V, George E. 1998. Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. *Mycorrhiza* **8**: 35–39.

Cavagnaro TR. 2008. The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. *Plant and Soil* **304**: 315–325.

Chakraborty S, Chakraborty N, Datta A. 2000. Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene from *Amaranthus hypochondriacus*. *Proceedings of the National Academy of Sciences, USA* **97**: 3724–3729.

Chávez AL, Bedoya JM, Sánchez T, Iglesias C, Ceballos H, Roca W. 2000. Iron, carotene, and ascorbic acid in cassava roots and leaves. *Food and Nutrition Bulletin* **21**: 410–413.

Chávez AL, Sánchez T, Jaramillo G, Bedoya JM, Echeverry J, Bolaños EA, Ceballos H, Iglesias CA. 2005. Variation of quality traits in cassava roots evaluated in landraces and improved clones. *Euphytica* **143**: 125–133.

Chen R, Xue G, Chen P, Yao B, Yang W, Ma Q, Fan Y, Zhao Z, Tarczynski MC, Shi J. 2008. Transgenic maize plants expressing a fungal phytase gene. *Transgenic Research* **17**: 633–643.

Chen Z, Young TE, Ling J, Chang S-C, Gallie DR. 2003. Increasing vitamin C content of plants through enhanced ascorbate recycling. *Proceedings of the National Academy of Sciences, USA* **100**: 3525–3530.

Chhuneja P, Dhaliwal HS, Bains NS, Singh K. 2006. *Aegilops kotschyi* and *Aegilops tauschii* as sources for higher levels of grain iron and zinc. *Plant Breeding* **125**: 529–531.

Chiaiese P, Ohkama-Ohtsu N, Molvig L, Godfree R, Dove H, Hocart C, Fujiwara T, Higgins TJV, Tabe LM. 2004. Sulphur and nitrogen nutrition influence the response of chickpea seeds to an added, transgenic sink for organic sulphur. *Journal of Experimental Botany* **55**: 1889–1901.

Chiba Y, Ishikawa M, Kijima F, Tyson RH, Kim J, Yamamoto A, Nambara E, Leustek T, Wallsgrove RM, Naito S. 1999. Evidence for autoregulation of cystathionine γ-synthase mRNA stability in *Arabidopsis*. *Science* **286**: 1371–1374.

Chiera JM, Finer JJ, Grabau EA. 2004. Ectopic expression of a soybean phytase in developing seeds of *Glycine max* to improve phosphorus availability. *Plant Molecular Biology* **56**: 895–904.

Chong DKX, Langridge WHR. 2000. Expression of full-length bioactive antimicrobial human lactoferrin in potato plants. *Transgenic Research* **9**: 71–78.

Cichy KA, Forster S, Grafton KF, Hosfield GL. 2005. Inheritance of seed zinc accumulation in navy bean. *Crop Science* **45**: 864–870.

Clemens S, Antiosiewicz DM, Ward JM, Schachtman DP, Schroeder JI. 1998. The plant cDNA *LCT1* mediates the uptake of calcium and cadmium in yeast. *Proceedings of the National Academy of Sciences, USA* **95**: 12043–12048.

Coelho CMM, Santos JCP, Tsai SM, Vitorello VA. 2002. Seed phytate content and phosphorus uptake and distribution in dry bean genotypes. *Brazilian Journal of Plant Physiology* **14**: 51–58.

Cohen D. 2007. Earth's natural wealth: an audit. *New Scientist* **2605**: 34–41.

Colangelo EP, Guerinot ML. 2004. The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. *Plant Cell* **16**: 3400–3412.

Colangelo EP, Guerinot ML. 2006. Put the metal to the petal: metal uptake and transport throughout plants. *Current Opinion in Plant Biology* **9**: 322–330.

Colmenero-Flores JM, Martínez G, Gamba G, Vázquez N, Iglesias DJ, Brumós J, Talón M. 2007. Identification and functional characterization of cation-chloride cotransporters in plants. *Plant Journal* **50**: 278–292.

Combs GF. 2001. Selenium in global food systems. *British Journal of Nutrition* **85**: 517–547.

Courtney M, Mcharo M, La Bonte D, Gruneberg W. 2008. Heritability estimates for micronutrient composition of sweetpotato storage roots. *HortScience* **43**: 1382–1384.

Curie C, Alonso JM, Le Jean M, Ecker JR, Briat J-F. 2000. Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. *Biochemical Journal* **347**: 749–755.

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat J-F, Walker EL. 2001. Maize *yellow stripe1* encodes a membrane protein directly involved in Fe(III) uptake. *Nature* **409**: 346–349.

Curien G, Job D, Douce R, Dumas R. 1998. Allosteric activation of *Arabidopsis* threonine synthase by S-adenosylmethionine. *Biochemistry* **37**: 13212–13221.

Dai F, Wang J, Zhang S, Xu Z, Zhang G. 2007. Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley. *Food Chemistry* **105**: 606–611.

Dai JL, Zhu YG, Huang YZ, Zhang M, Song JL. 2006. Availability of iodide and iodate to spinach (*Spinacia oleracea* L.) in relation to total iodine in soil solution. *Plant and Soil* **289**: 301–308.

Dai J-L, Zhu Y-G, Zhang M, Huang Y-Z. 2004. Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. *Biological Trace Element Research* **101**: 265–276.

Davey MW, Stals E, Ngoh-Newilah G, Tomekpe K, Lusty C, Markham R, Swennen R, Keulemans J. 2007. Sampling strategies and variability in fruit pulp micronutrient contents of West and Central African bananas and plantains (*Musa* species). *Journal of Agricultural and Food Chemistry* **55**: 2633–2644.

David-Assael O, Berezin I, Shoshani-Knaani N, Saul H, Mizrachy-Dagri T, Chen J, Brook E, Shaul O. 2006. AtMHX is an auxin and ABA-regulated transporter whose expression pattern suggests a role in metal homeostasis in tissues with photosynthetic potential. *Functional Plant Biology* **33**: 661–672.

Davies KM. 2007. Genetic modification of plant metabolism for human health benefits. *Mutation Research* **622**: 122–137.

Davis DR. 2006. Commentary on 'Historical variation in the mineral composition of edible horticultural products'. *Journal of Horticultural Science and Biotechnology* **81**: 553–554.

Davis DR. (in press). Declining fruit and vegetable nutrient composition – What is the evidence? *HortScience*.

Davis DR, Epp MD, Riordan HD. 2004. Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *Journal of the American College of Nutrition* **23**: 669–682.

Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR. Palys J, Uhlig J *et al.* 2005. Fruit-specific RNAi-mediated suppression of *DET1* enhances carotenoid and flavonoid content in tomatoes. *Nature Biotechnology* **23**: 890–895.

De Angeli A, Monachello D, Ephritikhine G, Frachisse JM, Thomine S, Gambale F, Barbier-Brygoo H. 2006. The nitrate/proton antiporter AtCLCa mediates nitrate accumulation in plant vacuoles. *Nature* **442**: 939–942.

Degryse F, Verma VK, Smolders E. 2008. Mobolization of Cu and Zn by root exudates of dicotyledonous plants in resin-buffered solutions and in soil. *Plant and Soil* **306**: 69–84.

Delhaize E. 1996. A metal-accumulator mutant of *Arabidopsis thaliana*. *Plant Physiology* **111**: 849–855.

Demidchik V, Bowen HC, Maathuis FJM, Shabala SN, Tester MA, White PJ, Davies JM. 2002. *Arabidopsis thaliana* root nonselective cation channels mediate calcium uptake and are involved in growth. *Plant Journal* **32**: 799–808.

Demidchik V, Maathuis FJM. 2007. Physiological roles of nonselective cation channels in plants: from salt stress to signaling and development. *New Phytologist* **175**: 387–404.

Deosthale YG. 1981. Trace element composition of common oilseeds. *Journal of the American Oil Chemists Society* **58**: 988–990.

Dicko MH, Hilhorst R, Gruppen H, Traore AS, Laane C, Van Berkel WJH, Voragen AGJ. 2002. Comparison of content in phenolic compounds, polyphenol oxidase, and peroxidase in grains of fifty sorghum varieties from Burkina Faso. *Journal of Agricultural and Food Chemistry* **50**: 3780–3788.

DiDonato RJ, Roberts LA, Sanderson T, Eisley RB, Walker EL. 2004. Arabidopsis *Yellow Stripe-Like2* (*YSL2*): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant Journal* **39**: 403–414.

Diretto G, Welsch R, Tavazza R, Mourgues F, Pizzichini D, Beyer P, Giuliano G. 2007. Silencing of beta-carotene hydroxylase increases total carotenoid and beta-carotene levels in potato tubers. *BMC Plant Biology* **7**: 11.

Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T. 2007. Multiple QTL-effects of wheat *Gpc-B1* locus on grain protein and micronutrient concentrations. *Physiologia Plantarum* **129**: 635–643.

Douchkov D, Gryczka C, Stephan UW, Hell R, Bäumlein H. 2005. Ectopic expression of nicotianamine synthase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant, Cell & Environment* **28**: 365–374.

Drakakaki G, Marcel S, Glahn RP, Lund EK, Pariagh S, Fischer R, Christou P, Stoger E. 2005. Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus* phytase in maize results in significant increases in the levels of bioavailable iron. *Plant Molecular Biology* **59**: 869–880.

Draycott P, Allison M. 1998. *Proceedings of the International Fertiliser Society 412. Magnesium fertilisers in soil and plants: comparisons and usage*. York, UK: International Fertiliser Society.

Drummond RSM, Tutone A, Li Y-C, Gardner RC. 2006. A putative magnesium transporter AtMRS2-11 is localized to the plant chloroplast envelope membrane system. *Plant Science* **170**: 78–89.

Ducreux LJM, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA. 2005. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. *Journal of Experimental Botany* **56**: 81–89.

Durrett TP, Gassmann W, Rogers EE. 2007. The FRD3-nediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiology* **144**: 197–205.

Duy D, Wanner G, Meda AR, von Wirén N, Soll J, Philippar K. 2007. PIC1, an ancient permease in arabidopsis chloroplasts, mediates iron transport. *Plant Cell* **19**: 986–1006.

Ekholm P, Reinivuo H, Mattila P, Pakkala H, Koponen J, Happonen A, Hellstrom J, Ovaskainen ML. 2007. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *Journal of Food Composition and Analysis* **20**: 487–495.

Elbaz B, Shoshani-Knaani N, David-Assael O, Mizrachy-Dagri T, Mizrahi K, Saul H, Brook E, Berezin I, Shaul O. 2006. High expression in leaves of the zinc hyperaccumulator *Arabidopsis halleri* of *AhMHX*, a homolog of an A*rabidopsis thaliana* vacuolar metal/proton exchanger. *Plant, Cell & Environment* **29**: 1179–1190.

Ellis DR, Sors TG, Brunk DG, Albrecht C, Peer W, Pickering IJ, Salt DE. 2004. Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase. *BMC Plant Biology* **4**: 1.

Enomoto Y, Hodoshima H, Shimada H, Shoji K, Yoshihara T, Goto F. 2007. Long-distance signals positively regulate the expression of iron uptake genes in tobacco roots. *Planta* **227**: 81–89.

Epstein E. 1972. *Mineral nutrition of plants: principles and perspectives*. New York, NY, USA: John Wiley.

Erdal I, Yilmaz A, Taban S, Eker S, Torun B, Cakmak I. 2002. Phytic acid and phosphorus concentrations in seeds of wheat cultivars grown with and without zinc fertilization. *Journal of Plant Nutrition* **25**: 113–127.

Eren E, Argüello JM. 2004. Arabidopsis HMA2, a divalent heavy metal-transporting P_{IB}-type ATPase, is involved in cytoplasmic Zn^{2+} homeostasis. *Plant Physiology* **136**: 3712–3723.

Ertl DS, Yuong KA, Raboy V. 1998. Plant genetic approaches to phosphorus management in agricultural production. *Journal of Environmental Quality* **27**: 299–304.

Eurola M, Ekholm P, Ylinen M, Koivistoinen P, Varo P. 1989. Effects of selenium fertilization on the selenium content of selected Finnish fruits and vegetables. *Acta Agriculturae Scandinavica* **39**: 345–350.

Eurola M, Hietaniemi V, Kontturi M, Tuuri H, Kangas A, Niskanen M, Saastamoinen M. 2004. Selenium content of Finnish oats in 1997–1999: effect of cultivars and cultivation techniques. *Agricultural and Food Science* **13**: 46–53.

Eurola MH, Ekholm PI, Ylinen ME, Koivistoinen PE, Varo PT. 1991. Selenium in Finnish foods after beginning the use of selenate-supplemented fertilisers. *Journal of the Science of Food and Agriculture* **56**: 57–70.

Fan M-S, Zhao F-J, Fairweather-Tait SJ, Poulton PR, Dunham SJ, McGrath SP. 2008. Evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology* **22**: 315–324.

Fang Y, Wang L, Xin Z, Zhao LY, An XX, Hu QH. 2008. Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients

concentration and yield of rice grain in China. *Journal of Agricultural and Food Chemistry* **56**: 2079–2084.

Farnham MW, Grusak MA, Wang M. 2000. Calcium and magnesium concentration of inbred and hybrid broccoli heads. *Journal of the American Society for Horticultural Science* **125**: 344–349.

Farnham MW, Hale AJ, Grusak MA, Finley JW. 2007. Genotypic and environmental effects on selenium concentration of broccoli heads grown without supplemental selenium fertilizer. *Plant Breeding* **126**: 195–200.

Feil B, Fossati D. 1995. Mineral composition of triticale grains as related to grain yield and grain protein. *Crop Science* **35**: 1426–1431.

Feil B, Fossati D. 1997. Phytic acid in triticale grains as affected by cultivar and environment. *Crop Science* **37**: 916–921.

Filatov V, Dowdle J, Smirnoff N, Ford-Lloyd B, Newbury HJ, Macnair MR. 2006. Comparison of gene expression in segregating families identifies genes and genomic regions involved in a novel adaptation, zinc hyperaccumulation. *Molecular Ecology* **15**: 3045–3059.

Finlay JW. 2007. Increased intakes of selenium-enriched foods may benefit human health. *Journal of the Science of Food and Agriculture* **87**: 1620–1629.

Flachowski G, Chesson A, Aulrich K. 2005. Animal nutrition with feeds from genetically modified plants. *Archives of Animal Nutrition* **59**: 1–40.

Food and Agriculture Organization of the United Nations (FAO). 2006. *Agricultural production statistics (FAOSTAT: ProdSTAT module)*. http://faostat.fao.org/site/291/default.aspx, last accessed August 2008.

Food Standards Agency (FSA). 2002. *McCance and Widdowson's the composition of foods*. Sixth summary edition. Cambridge, UK: Royal Society of Chemistry.

Franceschi VR, Nakata PA. 2005. Calcium oxalate in plants: formation and function. *Annual Review of Plant Biology* **56**: 41–71.

Fraser PD, Bramley PM. 2004. The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research* **43**: 228–265.

Frommer WB, von Wirén N. 2002. Ping-pong with boron. *Nature* **410**: 282–283.

Frossard E, Bucher M, Mächler F, Mozafar A, Hurrell R. 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture* **80**: 861–879.

Fuge R, Johnson CC. 1986. The geochemistry of iodine – a review. *Environmental Geochemistry and Health* **8**: 31–54.

Gardner RC. 2003. Genes for magnesium transport. *Current Opinion in Plant Biology* **6**: 263–267.

Garifullina GF, Owen JD, Lindblom SD, Tufan H, Pilon M, Pilon-Smits EAH. 2003. Expression of a mouse selenocysteine lyase in *Brassica juncea* chloroplasts affects selenium tolerance and accumulation. *Physiologia Plantarum* **118**: 538–544.

Garvin DF, Welch RM, Finley JW. 2006. Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *Journal of the Science of Food and Agriculture* **86**: 2213–2220.

Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferrière N, Thibaud J-B, Sentenac H. 1998. Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. *Cell* **94**: 647–655.

Gelin JP, Forster S, Grafton KF, McClean PE, Kooas-Cifuentes GA. 2007. Analysis of seed zinc and other minerals in a recombinant inbred population of navy bean (*Phaseolus vulgaris* L.). *Crop Science* **47**: 1361–1366.

Genc Y, Humphries JM, Lyons GH, Graham RD. 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. *Journal of Trace Elements in Medicine and Biology* **18**: 319–324.

Genc Y, McDonald GK, Graham RD. 2000. Effect of seed zinc content on early growth of barley (*Hordeum vulgare* L.) under low and adequate soil zinc supply. *Australian Journal of Agricultural Science* **51**: 37–46.

George L, Romanowsky SM, Harper JF, Sharrock RA. 2008. The ACA10 Ca^{2+}-ATPase regulates adult vegetative development and inflorescence architecture in Arabidopsis. *Plant Physiology* **146**: 716–728.

Gibson RS. 2006. Zinc: the missing link in combating micronutrient malnutrition in developing countries. *Proceedings of the Nutrition Society* **65**: 51–60.

Gissel-Nielsen G. 1998. Effects of selenium supplementation of field crops. In: Frankenberger WT, Engberg RA, eds. *Environmental chemistry of selenium*. New York, NY, USA: Dekker, 99–112.

Glahn RP, Cheng Z, Welch RM. 2002. Comparison of iron bioavailability from 15 rice genotypes: studies using an in vitro digestion/Caco-2 cell culture model. *Journal of Agricultural and Food Chemistry* **50**: 3586–3591.

Goto F, Yoshihara T, Saiki H. 2000. Iron accumulation and enhanced growth in transgenic lettuce plants expressing the iron-binding protein ferritin. *Theoretical and Applied Genetics* **100**: 658–664.

Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F. 1999. Iron fortification of rice seed by the soybean ferritin gene. *Nature Biotechnology* **17**: 282–286.

Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I. 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Research* **60**: 57–80.

Graham RD, Welch RM, Bouis HE. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Advances in Agronomy* **70**: 77–142.

Graham RD, Welch RM, Saunders DA, Ortiz-Monasterio I, Bouis HE, Bonierbale M, de Haan S, Burgos G, Thiele G, Liria R *et al.* 2007. Nutritious subsistence food systems. *Advances in Agronomy* **92**: 1–74.

Gregorio GB, Senadhira D, Htut H, Graham RD. 2000. Breeding for trace mineral density in rice. *Food and Nutrition Bulletin* **21**: 382–386.

Grichko VP, Filby B, Glick BR. 2000. Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. *Journal of Biotechnology* **81**: 45–53.

Gross J, Stein RJ, Fett-Neto AG, Fett JP. 2003. Iron homeostasis related genes in rice. *Genetics and Molecular Biology* **26**: 477–497.

Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D. 1998. Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. *Proceedings of the National Academy of Sciences, USA* **95**: 7220–7224.

Grotz N, Guerinot ML. 2006. Molecular aspects of Cu, Fe and Zn homeostasis in plants. *Biochimica et Biophysica Acta* **1763**: 595–608.

Grusak MA. 2000. Strategies for improving the iron nutritional quality of seed crops: lessons learned from the study of unique iron-hyperaccumulating pea mutants. *Pisum Genetics* **32**: 1–5.

Grusak MA, Cakmak I. 2005. Methods to improve the crop-delivery of minerals to humans and livestock. In: Broadley MR, White PJ, eds. *Plant nutritional genomics*. Oxford, UK: Blackwell, 265–286.

Guo WJ, Bundithya W, Goldsbrough PB. 2003. Characterization of the *Arabidopsis thaliana* metallothionein gene family: tissue-specific expression and induction during senescence and in response to copper. *New Phytologist* **159**: 369–381.

Guo W-J, Meetam M, Goldsbrough PB. 2008. Examining the specific contributions of individual arabidopsis metallothioneins to copper distribution and metal tolerance. *Plant Physiology* **146**: 1697–1706.

Gupta UC. 1979. Copper in agricultural crops. In: Nriagu JO, ed. *Copper in the environment*. New York, NY, USA: John Wiley, 255–287.

Gupta UC, Gupta SC. 2002. Quality of animal and human life as affected by selenium management of soils and crops. *Communications in Soil Science and Plant Analalysis* **33**: 2537–2555.

Guttieri M, Bowen D, Dorsch JA, Raboy V, Souza E. 2004. Identification and characterization of a low phytic acid wheat. *Crop Science* **44**: 418–424.

Guttieri MJ, Peterson KM, Souza EJ. 2006a. Agronomic performance of low phytic acid wheat. *Crop Science* **46**: 2623–2629.

Guttieri MJ, Peterson KM, Souza EJ. 2006b. Mineral distributions in milling fractions of low phytic acid wheat. *Crop Science* **46**: 2692–2698.

Guzmán-Maldonado SH, Acosta-Gallegos J, Paredes-López O. 2000. Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L.). *Journal of the Science of Food and Agriculture* **80**: 1874–1881.

Guzmán-Maldonado SH, Martínez O, Acosta-Gallegos JA, Guevara-Lara F, Paredes-López O. 2003. Putative quantitative trait loci for physical and chemical components of common bean. *Crop Science* **43**: 1029–1035.

Haas JD, Beard JL, Murray-Kolb LE, del Mundo AM, Felix A, Gregorio GB. 2005. Iron-biofortified rice improves the iron stores of nonanemic Filipino women. *Journal of Nutrition* **135**: 2823–2830.

Hacisalihoglu G, Kochian LV. 2003. How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytologist* **159**: 341–350.

Hagan ND, Upadhyaya N, Tabe LM, Higgins TJV. 2003. The redistribution of protein sulfur in transgenic rice expressing a gene for a foreign sulfur-rich protein. *Plant Journal* **34**: 1–11.

Hailes KJ, Aitken RL, Menzies NW. 1997. Magnesium in tropical and subtropical soils from north-eastern Australia. II. Response by glasshouse-grown maize to applied magnesium. *Australian Journal of Soil Research* **35**: 629–641.

Hakala M, Lapveteläinen A, Houpalahti R, Kallio H, Tahvonen R. 2003. Effects of varieties and cultivation conditions on the composition of strawberries. *Journal of Food Composition and Analysis* **16**: 67–80.

Hall JL, Williams LE. 2003. Transition metal transporters in plants. *Journal of Experimental Botany* **54**: 2601–2613.

Hambidge KM, Huffer JW, Raboy V, Grunwald GK, Westcott JL, Sian L, Miller LV, Dorsch JA, Krebs NF. 2004. Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids. *American Journal of Clinical Nutrition* **79**: 1053–1059.

Hambidge KM, Krebs NF, Westcott JL, Sian L, Miller LV, Peterson KL, Raboy V. 2005. Absorption of calcium from tortilla meals prepared from low-phytate maize. *American Journal of Clinical Nutrition* **82**: 84–87.

Hammond JP, Bowen HC, White PJ, Mills V, Pyke KA, Baker AJM, Whiting SN, May ST, Broadley MR. 2006. A comparison of the *Thlaspi caerulescens* and *Thlaspi arvense* shoot transcriptomes. *New Phytologist* **170**: 239–260.

Hampton CR, Broadley MR, White PJ. 2005. Short Review: the mechanisms of radiocaesium uptake by Arabidopsis roots. *Nukleonika* **50**: S3–S8.

Hardarson G, Broughton WJ. 2003. *Maximising the use of biological nitrogen fixation in agriculture*. Dordrecht, the Netherlands: Kluwer.

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET *et al.* 2008. Natural genetic variation in *lycopene epsilon cyclase* tapped for maize biofortification. *Science* **319**: 330–333.

Harrier LA, Watson CA. 2003. The role of arbuscular mycorrhizal fungi in sustainable cropping systems. *Advances in Agronomy* **20**: 185–225.

Harris D, Rashid A, Miraj G, Arif M, Shah H. 2007. 'On-farm' seed priming with zinc sulphate solution – A cost-effective way to increase the maize yields of resource-poor farmers. *Field Crops Research* **102**: 119–127.

Hartikainen H. 2005. Biogeochemistry of selenium and its impact on food chain quality and human health. *Journal of Trace Elements in Medicine and Biology* **18**: 309–318.

Haslett BS, Reid RJ, Rengel Z. 2001. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves and roots. *Annals of Botany* **87**: 379–386.

Haswell ES. 2007. MscS-like proteins in plants. *Current Topics in Membranes* **58**: 329–359.

Hatzack F, Johansen KS, Rasmussen SK. 2000. Nutritionally relevant parameters in low-phytate barley (*Hordeum vulgare* L.) grain mutants. *Journal of Agricultural and Food Chemistry* **48**: 6074–6080.

Hawkesford MJ, De Kok LJ. 2006. Managing sulphur metabolism in plants. *Plant, Cell & Environment* **29**: 382–395.

Hawkesford MJ, Zhao F-J. 2007. Strategies for increasing the selenium content of wheat. *Journal of Cereal Science* **46**: 282–292.

Haydon MJ, Cobbett CS. 2007a. Transporters of ligands for essential metal ions in plants. *New Phytologist* **174**: 499–506.

Haydon MJ, Cobbett CS. 2007b. A novel major facilitator superfamily protein at the tonoplast influences zinc tolerance and accumulation in Arabidopsis. *Plant Physiology* **143**: 1705–1719.

He X, Nara K. 2007. Element biofortification: can mycorrhizas potentially offer a more effective and sustainable pathway to curb human malnutrition? *Trends in Plant Science* **12**: 331–333.

Hess SY, Thurnham DI, Hurrell RF. 2005. *Technical monograph 6. Influence of provitamin A carotenoids on iron, zinc, and vitamin A status*. Washington, WA, USA: HarvestPlus.

Hesse H, Hoefgen R. 2003. Molecular aspects of methionine biosynthesis. *Trends in Plant Science* **8**: 259–262.

Hides DH, Thomas TA. 1981. Variation in the magnesium content of grasses and its improvement by selection. *Journal of the Science of Food and Agriculture* **32**: 990–991.

Hirschi K. 2001. Vacuolar H^+/Ca^{2+} transport: who's directing the traffic? *Trends in Plant Science* **6**: 100–104.

Hirschi KD, Miranda ML, Wilganowski NL. 2001. Phenotypic changes in *Arabidopsis* caused by expression of a yeast Ca^{2+}/H^+ antiporter. *Plant Molecular Biology* **46**: 57–65.

Ho L, White PJ. 2005. A cellular hypothesis for the induction of blossom-end rot in tomato fruit. *Annals of Botany* **95**: 571–581.

Hoffland E, Wei C, Wissuwa M. 2006. Organic anion exudation by lowland rice (*Oryza sativa* L.) at zinc and phosphorus deficiency. *Plant and Soil* **283**: 155–162.

Holden J, Gebhardt S, Haytowitz D, Bhagwat S. (in press). USDA's composition data for fruits and vegetables – Sources, measurement, and variation. *HortScience*.

Holland B, Unwin ID, Buss DH. 1991. *Vegetables, herbs and spices. Fifth Supplement to the Fourth Edition of McCance and Widdowson's the Composition of Foods*. London, UK: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Holland B, Unwin ID, Buss DH. 1992. *Fruit and nuts. First Supplement to the Fifth Edition of McCance and Widdowson's the Composition of Foods*. London, UK: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Holtz C. 2007. An overview of HarvestPlus nutrition research and key findings. In: *BioAsia 2007, Bangkok, Thailand, 7–9th November 2007*. http://www.biotec.or.th/BioAsia2007/home/Conference-Program.asp, last accessed 19th August 2008.

Hong C-Y, Cheng K-J, Tseng T-H, Wang C-S, Liu L-F, Yu S-M. 2004. Production of two highly active bacterial phytases with broad pH optima in germinating transgenic rice seeds. *Transgenic Research* **13**: 29–39.

van Hoof NALM, Hassinen VH, Hakvoort HWJ, Ballintijn KF, Schat H, Verkleij JAC, Ernst WHO, Karenlampi SO, Tervahauta AI. 2001. Enhanced copper tolerance in *Silene vulgaris* (Moench) Garcke populations from copper mines is associated with increased transcript levels of a 2b-type metallothionein gene. *Plant Physiology* **126**: 1519–1526.

Horner HT, Cervantes-Martinez T, Healy R, Reddy MB, Deardorff BL, Bailey TB, Al-Wahsh I, Massey LK, Palmer RG. 2005. Oxalate and phytate concentrations in seeds of soybean cultivars (*Glycine max* L.). *Journal of Agricultural and Food Chemistry* **53**: 7870–7877.

Horton S. 2006. The economics of food fortification. *Journal of Nutrition* **136**: 1068–1071.

Hotz C, Brown KH. 2004. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin* **25**: 94–204.

Houlton BZ, Wang Y-P, Vitousek PM, Field CB. 2008. A unifying

framework for dinitrogen fixation in the terrestrial biosphere. *Nature* **454**: 327–330.

House WA, Welch RM, Beebe S, Cheng Z. 2002. Potential for increasing the amounts of bioavailable zinc in dry beans (*Phaseolus vulgaris* L.) through plant breeding. *Journal of the Science of Food and Agriculture* **82**: 1452–1457.

Howard LR, Talcott ST, Brenes CH, Villalon B. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *Journal of Agricultural and Food Chemistry* **48**: 1713–1720.

Howitt CA, Pogson BJ. 2006. Carotenoid accumulation and function in seeds and nongreen tissues. *Plant, Cell & Environment* **29**: 435–445.

Hulke BS, Fehr WR, Welke GA. 2004. Agronomic and seed characteristics of soybean with reduced phytate and palmitate. *Crop Science* **44**: 2027–2031.

Hulshof PJM, Kosmeijer-Schuil T, West CE, Hollman PCH. 2007. Quick screening of maize kernels for provitamin A content. *Journal of Food Composition and Analysis* **20**: 655–661.

Hurrell RF. 2003. Influence of vegetable protein sources on trace element and mineral bioavailability. *Journal of Nutrition* **133**: S2973–S2977.

Hurrell RF, Reddy M, Cook JD. 1999. Inhibition of nonhaem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition* **81**: 289–295.

Ibrikci H, Knewtson SJB, Grusak MA. 2003. Chickpea leaves as a vegetable green for humans: evaluation of mineral composition. *Journal of the Science of Food and Agriculture* **83**: 945–950.

Inal A, Gunes A, Zhang F, Cakmak I. 2007. Peanut/maize intercropping induced changes in rhizosphere and nutrient concentrations in shoots. *Plant Physiology and Biochemistry* **45**: 350–356.

Ishikawa T, Dowdle J, Smirnoff N. 2006. Progress in manipulating ascorbic acid biosynthesis and accumulation in plants. *Physiologia Plantarum* **126**: 343–355.

Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. 2005. OsZIP4, a novel zinc-regulated zinc transporter in rice. *Journal of Experimental Botany* **56**: 3207–3214.

Ishimaru Y, Suzuki M, Tsukamoto T, Suzuki K, Nakazono M, Kobayashi T, Wada Y, Watanabe S, Matsuhashi S, Takahashi M *et al.* 2006. Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. *Plant Journal* **45**: 335–346.

Islam FMA, Basford KE, Jara C, Redden RJ, Beebe S. 2002. Seed compositional and disease resistance differences among gene pools in cultivated common bean. *Genetic Resources and Crop Evolution* **49**: 285–293.

Islam FMA, Beebe S, Muñoz M, Tohme J, Redden RJ, Basford KE. 2004. Using molecular markers to assess the effect of introgression on quantitative attributes of common bean in the Andean gene pool. *Theoretical and Applied Genetics* **108**: 243–52.

Ismail AM, Heuer S, Thomson MJ, Wissuwa M. 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant and Soil* **65**: 547–570.

Iwane A. 1991. Effect of cultivar and year on mineral components of apples. *Journal of the Japanese Society for Food Science and Technology* **38**: 329–336.

Jain AK, Nessler CL. 2000. Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants. *Molecular Breeding* **6**: 73–78.

Jakoby M, Wang HY, Reidt W, Weisshaar B, Bauer P. 2004. *FRU* (*BHLH029*) is required for induction of iron mobilization genes in *Arabidopsis thaliana*. *FEBS Letters* **577**: 528–534.

Jarrell WM, Beverly RB. 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* **34**: 197–224.

Jefferson PG, Mayland HF, Asay KH, Berdahl JD. 2001. Variation in mineral concentration and grass tetany potential among Russian wildrye accessions. *Crop Science* **41**: 543–548.

Jiang X-M, Cao X-Y, Jiang J-Y, Ma T, James DW, Rakeman MA, Dou Z-H, Mamette M, Amette K, Zhang M-L *et al.* 1997. Dynamics of environmental supplementation of iodine: four years' experience in iodination of irrigation water in Hotien, Xinjiang, China. *Archives of Environmental Health* **52**: 399–408.

Johns T, Eyzaguirre PB. 2007. Biofortification, biodiversity and diet: a search for complementary applications against poverty and malnutrition. *Food Policy* **32**: 1–24.

Jolley VD, Hansen NC, Shiffler AK. 2004. Nutritional and management related interactions with iron-deficiency stress response mechanisms. *Soil Science and Plant Nutrition* **50**: 973–981.

Joyce C, Deneau A, Peterson K, Ockenden I, Raboy V, Lott JNA. 2005. The concentrations and distributions of phytic acid phosphorus and other mineral nutrients in wild-type and *low phytic acid* Js-12-LPA wheat (*Triticum aestivum*) grain parts. *Canadian Journal of Botany* **83**: 1599–1607.

Kaplan B, Sherman T, Fromm H. 2007. Cyclic nucleotide-gated channels in plants. *FEBS Letters* **581**: 2237–2246.

Kapoor HC, Naik MS. 1970. Effects of soil and spray applications of urea and storage on the β-carotene content of yellow endosperm sorghum and pearl millet grains. *Indian Journal of Agricultural Science* **40**: 942–947.

Kashparov V, Colle C, Zvarich S, Yoschenko V, Levchuk S, Lundin S. 2005. Soil-to-plant halogens transfer studies 1. Root uptake of radioiodine by plants. *Journal of Environmental Radioactivity* **79**: 187–204.

Kawazu Y, Okimura M, Ishii T, Yui S. 2003. Varietal and seasonal differences in oxalate content of spinach. *Scientia Horticulturae* **97**: 203–210.

Kesler SE. 2007. Mineral supply and demand into the 21st century. In: Briskey JA, Schulz KJ, eds. *U.S. Geological Survey circular 1294: proceedings for a workshop on deposit modeling, mineral resource assessment, and their role in sustainable development*. Reston, VA, USA: U.S. Geological Survey, 55–62.

Kim CK, Han J-S, Lee H-S, Oh J-Y, Shigaki T, Park SH, Hirschi K. 2006a. Expression of an *Arabidopsis CAX2* variant in potato tubers increases calcium levels with no accumulation of manganese. *Plant Cell Reports* **25**: 1226–1232.

Kim DJ, Kim H, Kim M, Lee J. 2007. Analysis of oxalic acid of various vegetables consumed in Korea. *Food Science and Biotechnology* **16**: 650–654.

Kim SA, Kwak JM, Jae S-K, Wang M-H, Nam HG. 2001. Overexpression of the *AtGluR2* gene encoding an *Arabidopsis* homolog of mammalian glutamate receptors impairs calcium utilisation and sensitivity to ionic stress in transgenic plants. *Plant and Cell Physiology* **42**: 74–84.

Kim SA, Punshon T, Lanzirotti A, Li L, Alonso JM, Ecker JR, Kaplan J, Guerinot ML. 2006b. Localization of iron in *Arabidopsis* seed requires the vacuolar membrane transporter VIT1. *Science* **314**: 1295–1298.

Kinzel H. 1982. *Pflanzenökologie und Mineralstoffwechsel*. Stuttgart, Germany: Ulmer.

Kinzel H, Lechner I. 1992. The specific mineral metabolism of selected plant species and its ecological implications. *Botanica Acta* **105**: 355–361.

Kirkby EA, Johnston AE. 2008. Soil and fertilizer phosphorus in relation to crop nutrition. In: Hammond JP, White PJ, eds. *The ecophysiology of plant-phosphorus interactions*. Dordrecht, the Netherlands: Springer, 177–223.

Kitchen JW, Burns EE, Perry BA. 1964. Calcium oxalate content of spinach (*Spinacia oleracea* L.). *Journal of the American Society for Horticultural Science* **84**: 441–45.

Kobayashi T, Ogo Y, Itai RN, Nakanishi H, Takahashi M, Mori S, Nishizawa NK. 2007. The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants. *Proceedings of the National Academy of Sciences, USA* **104**: 19150–19155.

Kodama S, Takahashi Y, Okumura K, Uruga T. 2006. Speciation of iodine in solid environmental samples by iodine K-edge XANES: application to soils and ferromanganese oxides. *Science of the Total Environment* **363**: 275–284.

Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. 2004. *OsYSL2* is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant Journal* **39**: 415–424.

Kopsell DA, Kopsell DE, Lefsrud MG, Curran-Celentano J, Dukach LE. 2004a. Variation in lutein, β-carotene, and chlorophyll concentrations among *Brassica oleracea* cultigens and seasons. *HortScience* **39**: 361–364.

Kopsell DA, Kopsell DE. 2006. Accumulation and bioavailability of dietary carotenoids in vegetable crops. *Trends in Plant Science* **11**: 499–507.

Kopsell DA, Randle WM. 1997. Selenate concentration affects selenium and sulfur uptake and accumulation by 'Granex 33' onions. *Journal of the American Society for Horticultural Science* **122**: 721–726.

Kopsell DA, Randle WM. 2001. Genetic variances and selection potential for selenium accumulation in a rapid-cycling *Brassica oleracea* population. *Journal of the American Society for Horticultural Science* **126**: 329–335.

Kopsell DE, Kopsell DA, Lefsrud MG, Curran-Celentano J. 2004b. Variability in elemental accumulations among leafy *Brassica oleracea* cultivars and selections. *Journal of Plant Nutrition* **27**: 1813–1826.

Kothari SK, Marschner H, Romheld V. 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant and Soil* **131**: 177–185.

Krämer U, Talke IN, Hanikenne M. 2007. Transition metal transport. *FEBS Letters* **581**: 2263–2272.

Krinke O, Novotná Z, Valentová O, Martinec J. 2007. Inositol trisphosphate receptor in higher plants: is it real? *Journal of Experimental Botany* **58**: 361–376.

Krishnan S, Datta K, Baisakh N, de Vasconcelos M, Datta SK. 2003. Tissue-specific localization of β-carotene and iron in transgenic rice (*Oryza sativa* L.). *Current Science* **84**: 1232–1234.

Krüger C, Berkowitz O, Stephan UW, Hell R. 2002. A metal-binding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis* L. *Journal of Biological Chemistry* **277**: 25062–25069.

Kung C-CS, Huang W-N, Huang Y-C, Yeh K-C. 2006. Proteomic survey of copper-binding proteins in *Arabidopsis* roots by immobolized metal affinity chromatography and mass spectrometry. *Proteomics* **6**: 2740–2758.

Kurilich AC, Juvik JA. 1999. Quantification of carotenoid and tocopherol antioxidants in *Zea mays*. *Journal of Agricultural and Food Chemistry* **47**: 1948–1955.

Kurilich AC, Tsau GJ, Brown A, Howard L, Klein BP, Jeffery EH, Kushad M, Wallig MA, Juvik JA. 1999. Carotene, tocopherol, and ascorbate in subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry* **47**: 1576–1581.

Kuwano M, Ohyama A, Tanaka Y, Mimura T, Takaiwa F, Yoshida KT. 2006. Molecular breeding for transgenic rice with low phytic-acid phenotype through manipulating myo-inositol 3-phosphate synthase gene. *Molecular Breeding* **18**: 263–272.

Lanquar V, Lelièvre F, Bolte S, Hamès C, Alcon C, Neumann D, Vansuyt G, Curie C, Schröder A, Krämer U *et al.* 2005. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO Journal* **24**: 4041–4051.

Larsen EH, Lobinski R, Burger-Meyer K, Hansen M, Ruzik R, Mazurowska L, Rasmussen PH, Sloth JJ, Scholten O, Kik C. 2006. Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate. *Analytical and Bioanalytical Chemistry* **385**: 1098–1108.

LeDuc DL, AbdelSamie M, Móntes-Bayon M, Wu CP, Reisinger SJ, Terry N. 2006. Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. *Environmental Pollution* **144**: 70–76.

LeDuc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF, Wu CP, AbdelSamie M, Chiang C-Y, Tagmount A, deSouza M *et al.* 2004. Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian mustard increases selenium tolerance and accumulation. *Plant Physiology* **135**: 377–383.

Lee S, Kim Y-Y, Lee Y, An G. 2007. Rice P_{1B}-type heavy-metal ATPase, OsHMA9, is a metal efflux protein. *Plant Physiology* **145**: 831–842.

Li H-F, McGrath SP, Zhao F-J. 2008. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytologist* **178**: 92–102.

Li L, Cheng X, Ling H-Q. 2004. Isolation and characterization of Fe(III)-chelate reductase gene *LeFRO1* in tomato. *Plant Molecular Biology* **54**: 125–136.

Li L, Paolillo DJ, Parthasarathy MV, DiMuzio EM, Garvin DF. 2001a. A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *Plant Journal* **26**: 59–67.

Li L, Tutone AF, Drummond RSM, Gardner RC, Luan S. 2001b. A novel family of magnesium transport genes in Arabidopsis. *Plant Cell* **13**: 2761–2775.

Libert B. 1987. Breeding a low-oxalate rhubarb (*Rheum* sp. L.). *Journal of Horticultural Science* **62**: 523–529.

Libert B, Creed C. 1985. Oxalate content of seventy-eight rhubarb cultivars and its relation to some other characters. *Journal of Horticultural Science* **60**: 257–261.

Libert B, Franceschi VR. 1987. Oxalate in crop plants. *Journal of Agriculture and Food Chemistry* **35**: 926–938.

Lin L, Ockenden I, Lott JNA. 2005. The concentrations and distribution of phytic acid-phosphorus and other mineral nutrients in wild-type and *low phytic acid1-1* (*lpa1-1*) corn (*Zea mays* L.) grains and grain parts. *Canadian Journal of Botany* **83**: 131–141.

Ling H-Q, Bauer P, Bereczky Z, Keller B, Ganal M. 2002. The tomato *fer* gene encoding a bHLH protein controls iron-uptake responses in roots. *Proceedings of the National Academy of Sciences, USA* **99**: 13938–13943.

Liu JC, Ockenden I, Truax M, Lott JNA. 2004a. Phytic acid-phosphorus and other nutritionally important mineral nutrient elements in grains of wild-type and *low phytic acid* (*lpa1-1*) rice. *Soil Science Research* **14**: 109–116.

Liu K, Peterson KL, Raboy V. 2007. Comparison of the phosphorus and mineral concentrations in bran and abraded kernel fractions of a normal barley (*Hordeum vulgare*) cultivar versus four low phytic acid isolines. *Journal of Agriculture and Food Chemistry* **55**: 4453–4460.

Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler C, Giovannoni J. 2004b. Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proceedings of the National Academy of Sciences, USA* **101**: 9897–9902.

Loladze I. 2002. Rising atmospheric CO_2 and human nutrition: toward globally imbalanced plant stoichiometry. *Trends in Ecology and Evolution* **17**: 457–461.

Lolas GM, Markakis P. 1975. Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* **28**: 1313–1315.

Lolas GM, Palamidis N, Markakis P. 1976. The phytic acid–total phosphorus relationship in barley, oats, soybeans, and wheat. *Cereal Chemistry* **53**: 867–871.

Loneragan JF. 1997. Plant nutrition in the 20th and perspectives for the 21st century. *Plant and Soil* **196**: 163–174.

Long JK, Bänzinger M, Smith ME. 2004. Diallel analysis of grain iron and zinc density in southern African-adapted maize inbreds. *Crop Science* **44**: 2019–2026.

Lönnerdal B. 2002. Phytic acid-trace element (Zn, Cu, Mn) interactions. *International Journal of Food Science and Technology* **37**: 749–758.

Lönnerdal B. 2003. Genetically modified plants for improved trace element nutrition. *Journal of Nutrition* **133**: 1490–1493.

Lopez AB, Van Eck J, Conlin BJ, Paolillo DJ, O'Neill J, Li L. 2008. Effect of the cauliflower Or transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. *Journal of Experimental Botany* **59**: 213–223.

López-Millán A-F, Ellis DR, Grusak MA. 2004. Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago trunculata*. *Plant Molecular Biology* **54**: 583–596.

Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran DM, Cosman KM, Conlin BJ, Paolillo DJ, Garvin DF, Vrebalov J *et al*. 2006. The cauliflower Or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. *Plant Cell* **18**: 3594–3605.

Lucca P, Hurrell R, Potrykus I. 2001. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theoretical and Applied Genetics* **102**: 392–397.

Lucca P, Hurrell R, Potrykus I. 2002. Fighting iron deficiency anemia with iron-rich rice. *Journal of the American College of Nutrition* **21**: 184S–190S.

Lucca P, Poletti S, Sautter C. 2006. Genetic engineering approaches to enrich rice with iron and vitamin A. *Physiologia Plantarum* **126**: 291–303.

Lynch JP. 2007. Roots of the second green revolution. *Australian Journal of Botany* **55**: 493–512.

Lyons G, Stangoulis J, Graham R. 2003. High-selenium wheat: biofortification for better health. *Nutrition Research Reviews* **16**: 45–60.

Lyons GH, Judson GJ, Ortiz-Monasterio I, Genc Y, Stangoulis JCR, Graham RD. 2005. Selenium in Australia: selenium status and biofortification of wheat for better health. *Journal of Trace Elements in Medicine and Biology* **19**: 75–82.

Lyons GH, Stangoulis JCR, Graham RD. 2004. Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the *HarvestPlus* program. *Nutrition Reviews* **62**: 247–252.

Ma G, Jin Y, Li Y, Zhai F, Kok FJ, Jacobsen E, Yang X. 2008. Iron and zinc deficiencies in China: what is a feasible and cost-effective strategy? *Public Health Nutrition* **11**: 632–638.

Ma JF, Higashitani A, Sato K, Takeda K. 2004. Genotypic variation in Fe concentration of barley grain. *Soil Science and Plant Nutrition* **50**: 1115–1117.

Mackowiak CL, Grossl PR. 1999. Iodate and iodide effects on iodine uptake and partitioning in rice (*Oryza sativa* L.) grown in solution culture. *Plant and Soil* **212**: 135–143.

Mackowiak CL, Grossl PR, Cook KL. 2005. Iodine toxicity in a plant-solution system with and without humic acid. *Plant and Soil* **269**: 141–150.

Marmagne A, Vinauger-Douard M, Monachello D, de Longevialle AF, Charon C, Allot M, Rappaport F, Wollman FA, Barbier-Brygoo H, Ephritikhine G. 2007. Two members of the *Arabidopsis* CLC (chloride channel) family, AtCLCe and AtCLCf, are associated with thylakoid and Golgi membranes, respectively. *Journal of Experimental Botany* **58**: 3385–3393.

Marschner H. 1995. *Mineral nutrition of higher plants*. London, UK: Academic Press.

Massey LK. 2007. Food oxalate: factors affecting measurement, biological variation, and bioavailability. *Journal of the American Dietetic Association* **107**: 1191–1194.

Massey LK, Palmer RG, Horner HT. 2001. Oxalate content of soybean seeds (*Glycine max:* Leguminosae), soyfoods, and other edible legumes. *Journal of Agricultural and Food Chemistry* **49**: 4262–66.

Mazariegos M, Hambidge KM, Krebs NF, Westcott JE, Lei S, Grunwald GK, Campos R, Barahona B, Raboy V, Solomons NW. 2006. Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize. *American Journal of Clinical Nutrition* **83**: 59–64.

Maziya-Dixon B, Kling JG, Menkir A, Dixon A. 2000. Genetic variation in total carotene, iron, and zinc contents of maize and cassava genotypes. *Food and Nutrition Bulletin* **21**: 419–422.

McConn MM, Nakata PA. 2002. Calcium oxalate crystal morphology mutants from *Medicago truncatula*. *Planta* **215**: 380–386.

McDonald GK, Genc Y, Graham RD. 2008. A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yield. *Plant and Soil* **306**: 49–55.

McGrath SP. 1985. The effects of increasing yields on the macro- and microelement concentrations and offtakes in the grain of winter wheat. *Journal of the Science of Food and Agriculture* **36**: 1073–1083.

McLaughlin SB, Wimmer R. 1999. Calcium physiology and terrestrial ecosystem processes. *New Phytologist* **142**: 373–417.

Mendoza C. 2002. Effect of genetically modified low phytic acid plants on mineral absorption. *International Journal of Food Science and Technology* **37**: 759–767.

Mendoza C, Viteri FE, Lönnerdal B, Raboy V, Young KA, Brown KH. 2001. Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. *American Journal of Clinical Nutrition* **73**: 80–85.

Mendoza C, Viteri FE, Lönnerdal B, Young KA, Raboy V, Brown KH. 1998. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *American Journal of Clinical Nutrition* **68**: 1123–1128.

Mengoni A, Gonnelli C, Hakvoort HWJ, Galardi F, Bazzicalupo M, Gabbrielli R, Schat H. 2003. Evolution of copper-tolerance and increased expression of a 2b-type metallothionein gene in *Silene paradoxa* L. populations. *Plant and Soil* **257**: 451–457.

Metson AJ. 1974. Magnesium in New Zealand soils. I. Some factors governing the availability of soil magnesium: a review. *New Zealand Journal of Experimental Agriculture* **2**: 277–319.

Miedema H, Demidchik V, Véry A-A, Bothwell JHF, Brownlee C, Davies JM. 2008. Two voltage-dependent calcium channels co-exist in the apical plasma membrane of *Arabidopsis thaliana* root hairs. *New Phytologist* **179**: 378–385.

Milner MJ, Kochian LV. 2008. Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. *Annals of Botany* **102**: 3–13.

Mira H, Martínez N, Peñarrubia L. 2002. Expression of a vegetative-storage-protein from *Arabidopsis* is regulated by copper, senescence and ozone. *Planta* **214**: 939–946.

Mira H, Martínez-Garcia F, Peñarrubia L. 2001. Evidence for plant-specific intercellular transport of the Arabidopsis copper chaperone CCH. *Plant Journal* **25**: 521–528.

Monasterio I, Graham RD. 2000. Breeding for trace minerals in wheat. *Food and Nutrition Bulletin* **21**: 392–396.

Montes-Bayón M, LeDuc DL, Terry N, Caruso JA. 2002. Selenium speciation in wild-type and genetically modified Se accumulating plants with HPLC separation and ICP-MS/ES-MS detection. *Journal of Analytical Atomic Spectrometry* **17**: 872–879.

Moore CA, Bowen HC, Scrase-Field S, Knight MR, White PJ. 2002. The deposition of suberin lamellae determines the magnitude of cytosolic Ca^{2+} elevations in root endodermal cells subjected to cooling. *Plant Journal* **30**: 457–466.

Moraghan JT, Padilla J, Etchevers JD, Grafton K, Acosta-Gallegos JA. 2002. Iron accumulation in seed of common bean. *Plant and Soil* **246**: 175–183.

Morgan JAW, Bending GD, White PJ. 2005. Biological costs and benefits to plant-microbe interactions in the rhizosphere. *Journal of Experimental Botany* **56**: 1729–1739.

Morris J, Hawthorne KM, Hotze T, Abrams SA, Hirschi KD. 2008. Nutritional impact of elevated calcium transport activity in carrots. *Proceedings of the National Academy of Sciences, USA* **105**: 1431–1435.

Morris J, Nakata P, McConn M, Brock A, Hirschi KD. 2007. Increased

calcium bioavailability in mice fed genetically engineered plants lacking calcium oxalate. *Plant Molecular Biology* **64**: 613–618.

Morris WL, Ducreux L, Griffiths DW, Stewart D, Davies HV, Taylor MA. 2004. Carotenogenesis during tuber development and storage in potato. *Journal of Experimental Botany* **55**: 975–982.

Morris WL, Ducreux LJM, Fraser PD, Millam S, Taylor MA. 2006. Engineering ketocarotenoid biosynthesis in potato tubers. *Metabolic Engineering* **8**: 253–263.

van de Mortel JE, Villanueva LA, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, Ver Loren van Themaat E, Koornneef M, Aarts MGM. 2006. Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology* **142**: 1127–1147.

Mortimer JC, Laohavisit A, Macpherson N, Webb A, Brownlee C, Battey NH, Davies JM. 2008. Annexins: multifunctional components of growth and adaptation. *Journal of Experimental Botany* **59**: 533–544.

Mou B. 2005. Genetic variation of beta-carotene and lutein contents in lettuce. *Journal of the American Society for Horticultural Science* **130**: 870–876.

Mou B. 2008. Evaluation of oxalate concentration in the U.S. spinach germplasm collection. *HortScience* **43**: 1690–1693.

Mukherjee I, Campbell NH, Ash JS, Connolly EL. 2006. Expression profiling of the Arabidopsis ferric chelate reductase (FRO) gene family reveals differential regulation by iron and copper. *Planta* **223**: 1178–1190.

Muramatsu Y, Uchida S, Ohmomo Y. 1993. Root uptake of radioiodine by rice plants. *Journal of Radiation Research* **34**: 214–220.

Muramatsu Y, Yoshida S, Bannai T. 1995. Tracer experiments on the behaviour of radioiodine in the soil-plant-atmosphere system. *Journal of Radioanalytical and Nuclear Chemistry – Articles* **194**: 303–310.

Murphy A, Taiz L. 1995. Comparison of metallothionein gene expression and nonprotein thiols in ten Arabidopsis ecotypes. Correlation with copper tolerance. *Plant Physiology* **109**: 945–954.

Nagata T, Iizumi S, Satoh K, Ooka H, Kawai J, Carninci P, Hayashizaki Y, Otomo Y, Murakami K, Matsubara K *et al.* 2004. Comparative analysis of plant and animal calcium signal transduction element using plant full-length cDNA data. *Molecular Biology and Evolution* **21**: 1855–1870.

Nakamura A, Fukuda A, Sakai S, Tanaka Y. 2006. Molecular cloning, functional expression and subcellular localization of two putative vacuolar voltage-gated chloride channels in rice (*Oryza sativa* L.). *Plant and Cell Physiology* **47**: 32–42.

Nakata PA, McConn MM. 2000. Isolation of *Medicago truncatula* mutants defective in calcium oxalate crystal formation. *Plant Physiology* **124**: 1097–1104.

Nakata PA, McConn MM. 2006. A genetic mutation that reduces calcium oxalate increases calcium availability in *Medicago truncatula*. *Functional Plant Biology* **33**: 703–706.

Nakata PA, McConn MM. 2007. Calcium oxalate content affects the nutritional availability of calcium from *Medicago truncatula* leaves. *Plant Science* **172**: 958–961.

Nandi S, Suzuki YA, Huang J, Yalda D, Pham P, Wu L, Bartley G, Huang N, Lönnerdal B. 2002. Expression of human lactoferrin in transgenic rice grains for the application in infant formula. *Plant Science* **163**: 713–722

Nergiz C, Yildiz H. 1997. Research on chemical composition of some varieties of European plums (*Prunus domestica*) adapted to the Aegean district of Turkey. *Journal of Agricultural and Food Chemistry* **45**: 2820–2823.

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W. 2006. Biofortification of staple food crops. *Journal of Nutrition* **136**: 1064–1067.

Nesterenko S, Sink KC. 2003. Carotenoid profiles of potato breeding lines and selected cultivars. *HortScience* **38**: 1173–1177.

Nicolle C, Carnat A, Fraisse D, Lamaison J-L, Rock E, Michel H, Amouroux P, Remesy C. 2004a. Characterisation and variation of antioxidant micronutrients in lettuce (*Lactuca sativa* folium). *Journal of the Science of Food and Agriculture* **84**: 2061–2069.

Nicolle C, Simon G, Rock E, Amouroux P, Rémésy C. 2004b. Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars. *Journal of the American Society for Horticultural Science* **129**: 523–529.

Noctor G. 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. *Plant Cell & Environment* **29**: 409–425.

Nunes ACS, Vianna GR, Cuneo F, Amaya-Farfán J, de Capdeville G, Rech EL, Aragão FJL. 2006. RNAi-mediated silencing of the myo-inositol-1-phosphate synthase gene (*GmMIPS1*) in transgenic soybean inhibited seed development and reduced phytate content. *Planta* **224**: 125–132.

O'Dell RE, James JJ, Richards JH. 2006. Congeneric serpentine and nonserpentine shrubs differ more in leaf Ca:Mg than in tolerance of low N, low P, or heavy metals. *Plant and Soil* **280**: 49–64.

Ockenden I, Dorsch JA, Reid MM, Lin L, Grant LK, Raboy V, Lott JNA. 2004. Characterisation of the storage of phosphorus, inositol phosphate and cations in grain tissues of four barley (*Hordeum vulgare* L.) low phytic acid genotypes. *Plant Science* **167**: 1131–1142.

Ogo Y, Itai RN, Nakanishi H, Kobayashi T, Takahashi M, Mori S, Nishizawa NK. 2007. The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. *Plant Journal* **51**: 366–377.

Oikeh SO, Menkir A, Maziya Dixon B, Welch R, Glahn RP. 2003a. Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. *Journal of Plant Nutrition* **26**: 2307–2319.

Oltmans SE, Fehr WR, Welke GA, Raboy V, Peterson KL. 2005. Agronomic and seed traits of soybean lines with low-phytate phosphorus. *Crop Science* **45**: 593–598.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Peña RJ. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science* **46**: 293–307.

Oury F-X, Leenhardt F, Rémésy C, Chanliaud E, Duperrier B, Balfourier F, Charmet G. 2006. Genetic variability and stability of grain magnesium, zinc and iron concentration in bread wheat. *European Journal of Agronomy* **25**: 177–185.

Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, Ozkan H, Braun H-J, Sayers Z, Cakmak I. 2006. Concentration and localization of zinc during seed development and germination in wheat. *Physiologia Plantarum* **128**: 144–152.

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL *et al.* 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology* **23**: 482–487.

Palaisa KA, Morgante M, Williams M, Rafalski A. 2003. Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. *Plant Cell* **15**: 1795–1806.

Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK, Sanders D. 2008. Zinc biofortification of cereals: problems and solutions. *Trends in Plant Science* **13**: 464–473.

Papoyan A, Kochian LV. 2004. Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. *Plant Physiology* **136**: 3814–3823.

Park S, Cheng NH, Pittman JK, Yoo KS, Park J, Smith RH, Hirschi KD. 2005a. Increased calcium levels and prolonged shelf life in tomatoes expressing Arabidopsis H^+/Ca^{2+} transporters. *Plant Physiology* **139**: 1194–1206.

Park S, Elless MP, Park J, Jenkins A, Lim W, Chambers E, Hirschi KD. 2009. Sensory analysis of calcium-biofortified lettuce. *Plant Biotechnology Journal* **7**: 106–117.

Park S, Kang TS, Kim CK, Han JS, Kim S, Smith RH, Pike LM, Hirschi KD. 2005b. Genetic manipulation for enhancing calcium

content in potato tuber. *Journal of Agricultural and Food Chemistry* **53**: 5598–5603.

Park S, Kim C, Pike L, Smith R, Hirschi K. 2004. Increased calcium in carrots by expression of an Arabidopsis H^+/Ca^{2+} transporter. *Molecular Breeding* **14**: 275–282.

Parsons SA, Smith JA. 2008. Phosphorus removal and recovery from municipal wastewaters. *Elements* **4**: 109–112.

Peiter E, Maathuis FJM, Mills LM, Knight H, Pelloux J, Hetherington AM, Sanders D. 2005. The vacuolar Ca^{2+}-activated channel TPC1 regulates germination and stomatal movement. *Nature* **434**: 404–408.

Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences, USA* **97**: 4956–4960.

Peterson CJ, Johnson VA, Mattern PJ. 1986. Influence of cultivar and environment on mineral and protein concentrations of wheat flour, bran, and grain. *Cereal Chemistry* **63**: 183–186.

Petit J-M, Briat J-F, Lobréaux S. 2001. Structure and differential expression of the four members of the *Arabidopsis thaliana* ferritin gene family. *Biochemical Journal* **359**: 575–582.

Pezzarossa B, Piccotino D, Shennan C, Malorgio F. 1999. Uptake and distribution of selenium in tomato plants as affected by genotype and sulphate supply. *Journal of Plant Nutrition* **22**: 1613–1635.

Pfeiffer WH, McClafferty B. 2007. HarvestPlus: breeding crops for better nutrition. *Crop Science* **47**: S88–S105.

Pich A, Manteuffel R, Hillmer S, Scholz G, Schmidt W. 2001. Fe homeostasis in plant cells: does nicotianamine play multiple roles in the regulation of cytoplasmic Fe concentration? *Planta* **213**: 967–976.

Pilon M, Owen JD, Garifullina GF, Kurihara T, Mihara H, Esaki N, Pilon-Smits EAH. 2003. Enhanced selenium tolerance and accumulation in transgenic Arabidopsis expressing a mouse selenocysteine lyase. *Plant Physiology* **131**: 1250–1257.

Pilon-Smits EAH, Hwang S, Lytle CM, Zhu Y, Tai JC, Bravo RC, Chen Y, Leustek T, Terry N. 1999. Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. *Plant Physiology* **119**: 123–132.

Pilu R, Panzeri D, Gavazzi G, Rasmussen SK, Consonni G, Nielsen E. 2003. Phenotypic, genetic and molecular characterization of a maize low phytic acid mutant (*lpa241*). *Theoretical and Applied Genetics* **107**: 980–987.

Poletti S, Gruissem W, Sautter C. 2004. The nutritional fortification of cereals. *Current Opinion in Biotechnology* **15**: 162–165.

Ponstein AS, Bade JB, Verwoerd TC, Molendijk L, Storms J, Beudeker RF, Pen J. 2002. Stable expression of phytase (*phyA*) in canola (*Brassica napus*) seeds: towards a commercial product. *Molecular Breeding* **10**: 31–44.

Pottosin II, Schönknecht G. 2007. Vacuolar calcium channels. *Journal of Experimental Botany* **58**: 1559–1569.

Prom-u-thai C, Fukai S, Godwin ID, Huang L. 2007. Genotypic variation of iron partitioning in rice grain. *Journal of the Science of Food and Agriculture* **87**: 2049–2054.

Prychid CJ, Rudall PJ. 1999. Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of Botany* **84**: 725–739.

Puig S, Andrés-Colás N, García-Molina A, Peñarrubia L. 2007a. Copper and iron homeostasis in *Arabidopsis*: responses to metal deficiencies, interactions and biotechnological applications. *Plant, Cell & Environment* **30**: 271–290.

Puig S, Mira H, Dorcey E, Sancenón V, Andrés-Colás N, García-Molina A, Burkhead JL, Gogolin KA, Abdel-Ghany SE, Thiele DJ *et al.* 2007b. Higher plants possess two different types of ATX1-like copper chaperones. *Biochemical and Biophysical Research Communications* **354**: 385–390.

Qu LQ, Yoshihara T, Ooyama A, Goto F, Takaiwa F. 2005. Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. *Planta* **222**: 225–233.

Raboy V, Dickinson DB, Below FE. 1984. Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium and protein among lines of *Glycine max* and *G. soja*. *Crop Science* **24**: 431–434.

Raboy V. 2003. myo-Inositol-1–6-*hexakisphosphate*. *Phytochemistry* **64**: 1033–1043.

Raboy V. 2007. Seed phosphorus and the development of low-phytate crops. In: Turner BL, Richardson AE, Mullaney EJ, eds. *Inositol phosphates: linking agriculture and the environment*. Wallingford, UK: CABI, 111–132.

Raboy V, Noaman MM, Taylor GA, Pickett SG. 1991. Grain phytic acid and protein are highly correlated in winter wheat. *Crop Science* **31**: 631–635.

Ramesh SA, Choimes S, Schachtman DP. 2004. Over-expression of an *Arabidopsis* zinc transporter in *Hordeum vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. *Plant Molecular Biology* **54**: 373–385.

Ravanello MP, Ke D, Alvarez J, Huang B, Shewmaker CK. 2003. Coordinate expression of multiple bacterial carotenoid genes in canola leading to altered carotenoid production. *Metabolic Engineering* **5**: 255–263.

Rayman MP. 2004. The use of high-selenium yeast to raise selenium status: how does it measure up? *British Journal of Nutrition* **92**: 557–573.

Rayman MP. 2008. Food-chain selenium and human health: emphasis on intake. *British Journal of Nutrition* **100**: 254–268.

Reddy BVS, Ramesh S, Longvah T. 2005. Prospects of breeding for micronutrients and β-carotene-dense sorghums. *International Sorghum and Millets Newsletter* **46**: 10–14.

Reddy NR. 2002. Occurence, distribution, content, and dietary intake of phytate. In: Reddy NR, Sathe SK, eds. *Food phytates*. Boca Raton, FL, USA: CRC Press, 25–51.

Reddy NR, Pierson MD, Sathe SK, Salunkhe DK. 1989. *Phytates in cereals and legumes*. Boca Raton, FL, USA: CRC Press.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York, NY, USA: John Wiley & Sons, 193–229.

Rengel Z. 2001. Genotypic differences in micronutrient use efficiency in crops. *Communications in Soil Science and Plant Analysis* **32**: 1163–1186.

Rengel Z, Batten GD, Crowley DE. 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Research* **60**: 27–40.

Rengel Z, Graham RD. 1995. Importance of seed Zn content for wheat growth on Zn-deficient soil. II. Grain yield. *Plant and Soil* **173**: 267–274.

Richey KD, Silva JE, Costa UF. 1982. Calcium deficiency in in clayey B horizons of savanna oxisols. *Soil Science* **133**: 378–382.

Roberfroid MB. 2005. Introducing inulin-type fructans. *British Journal of Nutrition* **93**: S13–S25.

Roberfroid MB. 2007. Inulin-type fructans: functional food ingradients. *Journal of Nutrition* **137**: S2493–S2502.

Roberts LA, Pierson AJ, Panaviene Z, Walker EL. 2004. Yellow stripe1. Expanded roles for the maize iron-phytosiderophore transporter. *Plant Physiology* **135**: 112–120.

Roberts SK. 2006. Plasma membrane anion channels in higher plants and their putative functions in roots. *New Phytologist* **169**: 647–666.

Robinson NJ, Procter CM, Connolly EL, Guerinot ML. 1999. A ferric-chelate reductase for iron uptake from soils. *Nature* **397**: 694–697.

Rogers EE, Guerinot ML. 2002. FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in Arabidopsis. *Plant Cell* **14**: 1787–1799.

Rosa EAS, Haneklaus SH, Schug E. 2002. Mineral content of primary and secondary inflorescences of eleven broccoli cultivars grown in early and late seasons. *Journal of Plant Nutrition* **25**: 1741–1751.

Rosenfeld I, Beath OA. 1964. *Selenium: geobotany, biochemistry, toxicity, and nutrition*. New York, NY, USA: Academic Press.

Ross AB, Savage GP, Martin RJ, Vanhanen L. 1999. Oxalates in oca (New Zealand yam) (*Oxalis tuberosa* Mol.). *Journal of Agricultural and Food Chemistry* **47**: 5019–5022.

Roux SJ, Steinebrunner I. 2007. Extracellular ATP: an unexpected role as a signaler in plants. *Trends in Plant Science* **12**: 522–527.

Rude RK, Gruber HE. 2004. Magnesium deficiency and osteoporosis: animal and human observations. *Journal of Nutritional Biochemistry* **15**: 710–716.

Ryan MH, McInerney JK, Record IR, Angus JF. 2008. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *Journal of the Science of Food and Agriculture* **88**: 1208–1216.

Samuelsen AI, Martin RC, Mok DWS, Mok MC. 1998. Expression of the yeast FRE genes in transgenic tobacco. *Plant Physiology* **118**: 51–58.

Sancenón V, Puig S, Mateu-Andrés I, Dorcey E, Thiele DJ, Peñarrubia L. 2004. The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development. *Journal of Biological Chemistry* **279**: 15348–15355.

Sancenón V, Puig S, Mira H, Thiele DJ, Peñarrubia L. 2003. Identification of a copper transporter family in *Arabidopsis thaliana*. *Plant Molecular Biology* **51**: 577–587.

Sangketkit C, Savage GP, Martin RJ, Mason SL. 2001. Oxalate content of raw and cooked oca (*Oxalis tuberosa*). *Journal of Food Composition and Analysis* **14**: 389–397.

Scalbert A, Johnson IT, Saltmarsh M. 2005. Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition* **81**: S215–S217.

Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N. 2004. ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *Journal of Biological Chemistry* **279**: 9091–9096.

Schmidt W. 1999. Mechanisms and regulation of reduction-based iron uptake in plants. *New Phytologist* **141**: 1–26.

Schock I, Gregan J, Steinhauser S, Schweyen R, Brennicke A, Knoop V. 2000. A member of a novel *Arabidopsis thaliana* gene family of candidate Mg^{2+} ion transporters complements a yeast mitochondrial group II intron-splicing mutant. *Plant Journal* **24**: 489–501.

Schonhof I, Krumbeln A. 1996. Gehalt an wertgebenden Inhaltsstofen verschiedener Brokkolitypen (*Brassica oleracea* var. *italica* Plenck). *Gartenbauwissenschaft* **61**: 281–288.

Seigneurin-Berny D, Gravot A, Auroy P, Mazard C, Kraut A, Finazzi G, Grunwald D, Rappaport F, Vavasseur A, Joyard J *et al.* 2006. HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. *Journal of Biological Chemistry* **182**: 2882–2892.

Seiler GJ, Campbell LG. 2006. Genetic variability for mineral concentration in the forage of Jerusalem artichoke cultivars. *Euphytica* **150**: 281–288.

Shaul O, Hilgemann DW, de-Almeida-Engler J, Van Montagu M, Inzé D, Galili G. 1999. Cloning and characterization of a novel Mg^{2+}/H^{+} exchanger. *EMBO Journal* **18**: 3973–3980.

Shaul O. 2002. Magnesium transport and function in plants: the tip of the iceberg. *Biometals* **15**: 309–323.

Shear CB. 1975. Calcium-related disorders of fruits and vegetables. *HortScience* **10**: 361–365.

Shennan C, Schachtman DP, Cramer GR. 1990. Variation in [^{75}Se]selenate uptake and partitioning among tomato cultivars and wild species. *New Phytologist* **115**: 523–530.

Shi J, Wang H, Hazebroek J, Ertl DS, Harp T. 2005. The maize *low-phytic acid 3* encodes a myo-inositol kinase that plays a role in phytic acid biosynthesis in developing seeds. *Plant Journal* **42**: 708–719.

Shi J, Wang H, Schellin K, Li B, Faller M, Stoop JM, Meeley RB, Ertl DS, Ranch JP, Glassman K. 2007. Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. *Nature Biotechnology* **25**: 930–937.

Shigaki T, Barkla BJ, Miranda-Vergara MC, Zhao J, Pantoja O, Hirschi KD. 2005. Identification of a crucial histidine involved in metal transport activity in the *Arabidopsis* cation/H^{+} exchanger CAX1. *Journal of Biological Chemistry* **280**: 30136–30142.

Shigaki T, Hirschi KD. 2006. Diverse functions and molecular properties emerging for CAX cation/H^{+} exchangers in plants. *Plant Biology* **8**: 419–429.

Shikanai T, Müller-Moulé P, Munekage Y, Niyogi KK, Pilon M. 2003. PAA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. *Plant Cell* **15**: 1333–1346.

Shuman LM. 1998. Micronutrient fertilizers. *Journal of Crop Production* **1**: 165–195.

Simonne AH, Simonne EH, Eitenmiller RR, Mills HA, Green NR. 1997. Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). *Journal of Food Composition and Analysis* **10**: 299–311.

Simonne AH, Smith M, Weaver DB, Vail T, Barnes S, Wei CI. 2000. Retention and changes of soy isoflavones and carotenoids in immature soybean seeds (Edamame) during processing. *Journal of Agricultural and Food Chemistry* **48**: 6061–6069.

Sivaprakash KR, Krishnan S, Datta SK, Parida AK. 2006. Tissue-specific histochemical localization of iron and ferritin gene expression in transgenic *indica* rice Pusa Basmati (*Oryza sativa* L.). *Journal of Genetics* **85**: 157–160.

Sleper DA, Vogel KP, Asay KH, Mayland HF. 1989. Using plant breeding and genetics to overcome the incidence of grass tetany. *Journal of Animal Science* **67**: 3456–3462.

Slingerland MA, Traore K, Kayodé APP, Mitchikpe CES. 2006. Fighting Fe deficiency malnutrition in West Africa: an interdisciplinary programme on a food chain approach. *Netherlands Journal of Agricultural Science* **53**: 253–279.

Smirnoff N, Conklin PL, Loewus FA. 2001. Biosynthesis of ascorbic acid in plants: a renaissance. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 437–467.

Smith AG, Croft MT, Moulin M, Webb ME. 2007. Plants need their vitamins too. *Current Opinion in Plant Biology* **10**: 266–275.

Smith KF, Rebetzke GJ, Eagles HA, Anderson MW, Easton HS. 1999. Genetic control of mineral concentration and yield in perennial ryegrass (*Lolium perenne* L.), with special emphasis on minerals related to grass tetany. *Australian Journal of Agricultural Research* **50**: 79–86.

Smith SE, Read DJ. 2007. *Mycorrhizal symbiosis*, 3rd edn. London, UK: Elsevier.

Sors TG, Ellis DR, Na GN, Lahner B, Lee S, Leustek T, Pickering IJ, Salt DE. 2005a. Analysis of sulfur and selenium assimilation in *Astragalus* plants with varying capacities to accumulate selenium. *Plant Journal* **42**: 785–797.

Sors TG, Ellis DR, Salt DE. 2005b. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research* **86**: 373–389.

Stacey MG, Patel A, McClain WE, Mathieu M, Remley M, Rogers EE, Gassmann W, Blevins DG, Stacey G. 2008. The arabidopsis AtOPT3 protein functions in metal homeostasis and movement of iron to developing seeds. *Plant Physiology* **146**: 589–601.

Stangoulis JCR, Huynh BL, Welch RM, Choi EY, Graham RD. 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* **154**: 289–294.

Stein AJ, Meenakshi JV, Qaim M, Nestel P, Sachdev HPS, Bhutta ZA. 2005. *Technical monograph 4. Analysing the health benefits of biofortified staple crops by means of the disability-adjusted life years approach: A handbook focusing on iron, zinc and vitamin A*. Washington, WA, USA: HarvestPlus.

Stein AJ, Nestel P, Meenakshi JV, Qaim M, Sachdev HPS, Bhutta ZA. 2007. Plant breeding to control zinc deficiency in India: how cost-effective is biofortification? *Public Health Nutrition* **10**: 492–501.

Sterrett SB, Reynolds CW, Schales FD, Chaney RL, Douglass LW. 1983. Transplant quality, yield, and heavy-metal accumulation of tomato,

muskmelon, and cabbage grown in media containing sewage sludge compost. *Journal of the American Society for Horticultural Science* **108**: 36–41.

Sun SSM, Liu Q. 2004. Transgenic approaches to improve the nutritional quality of plant proteins. *In Vitro Cellular & Developmental Biology – Plant* **40**: 155–162.

Suzuki M, Takahashi T, Tsukamoto T, Watanabe S, Matsuhashi S, Yazaki J, Kishimoto N, Kikuchi S, Nakanishi H, Mori S *et al.* 2006. Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *Plant Journal* **48**: 85–97.

Tabe LM, Droux M. 2002. Limits to sulphur accumulation in transgenic lupin seeds expressing a foreign sulphur-rich protein. *Plant Physiology* **128**: 1137–1148.

Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S. 2001. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nature Biotechnology* **19**: 466–469.

Talke IN, Hanikenne M, Krämer U. 2006. Zn-dependent global transcriptional control, transcriptional de-regulation and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **142**: 148–167.

Tamoutsidis E, Papadopoulos I, Tokatlidis L, Zotis S, Mavropoulos T. 2002. Wet sewage sludge application effect on soil properties and element content of leaf and root vegetables. *Journal of Plant Nutrition* **25**: 1941–1955.

Tanaka M, Nakashima T, Mori K. 2003. Differences in density and size of idioblasts containing calcium oxalate crystals in petioles among cultivars of taro (*Colocasia esculenta* (L.) Schott. and *C. gigantea* Hook. f.). *Journal of the Japanese Society for Horticultural Science* **72**: 551–556.

Taylor M, Ramsay G. 2005. Carotenoid biosynthesis in plant storage organs: recent advances and prospects for improving plant food quality. *Physiologia Plantarum* **124**: 143–151.

Terry N, Zayed AM, de Souza MP, Tarun AS. 2000. Selenium in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**: 401–432.

Thacher TD, Fischer PR, Strand MA, Pettifor JM. 2006. Nutritional rickets around the world: causes and future directions. *Annals of Tropical Paediatrics* **26**: 1–16.

Thakkar SK, Maziya-Dixon B, Dixon AGO, Failla ML. 2007. β-carotene micellarization during in vitro digestion and uptake by Caco-2 cells is directly proportional to β-carotene content in different genotypes of cassava. *Journal of Nutrition* **137**: 2229–2233.

Thomine S, Lelièvre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H. 2003. AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. *Plant Journal* **34**: 685–695.

Thompson K, Parkinson JA, Band SR, Spencer RE. 1997. A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytologist* **136**: 679–689.

Thomson CD. 2004. Assessment of requirements for selenium and adequacy of selenium status: a review. *European Journal of Clinical Nutrition* **58**: 391–402.

Titchenal CA, Dobbs J. 2007. A system to assess the quality of food sources of calcium. *Journal of Food Composition and Analysis* **20**: 717–724.

Tiwari VK, Rawat N, Neelam K, Randhawa GS, Singh K, Chhuneja P, Dhaliwal HS. 2008. Development of *Triticum turgidum* subsp. *durum* – *Aegilops longissima* amphiploids with high iron and zinc content through unreduced gamete formation in F_1 hybrids. *Genome* **51**: 757–766.

Turakainen M, Hartikainen H, Seppänen MM. 2004. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. *Journal of Agricultural and Food Chemistry* **52**: 5378–5382.

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* **314**: 1298–1301.

Ufaz S, Galili G. 2008. Improving the content of essential amino acids in crop plants: goals and opportunities. *Plant Physiology* **147**: 954–961.

Umaly RC, Poel LW. 1971. Effects of iodine in various formulations on the growth of barley and pea plants in nutrient solution culture. *Annals of Botany* **35**: 127–131.

United States Department of Agriculture (USDA). 1984. *Oxalic acid content of selected vegetables. Agriculture handbook No. 8-11, Vegetables and vegetable products*. Washington, DC, USA: USDA.

United States Department of Agriculture, Agricultural Research Service (USDA-ARS). 2007. *USDA National nutrient database for standard reference, release 20*. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl, last accessed August 2008.

United States Geological Survey. 2007. *Mineral commodity summaries 2007*. Reston, VA, USA: US Geological Survey.

Van Hoewyk D, Garifullina GF, Ackley AR, Abdel-Ghany SE, Marcus MA, Fakra S, Ishiyama K, Inoue E, Pilon M, Takahashi H *et al.* 2005. Overexpression of AtCpNifS enhances selenium tolerance and accumulation in Arabidopsis. *Plant Physiology* **139**: 1518–1528.

Van Huysen T, Terry N, Pilon-Smits EAH. 2004. Exploring the selenium phytoremediation potential of transgenic *Brassica juncea* overexpressing ATP sulfurylase or cystathionine-γ-synthase. *International Journal of Phytoremediation* **6**: 111–118.

Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, Oliveira M, Goto F, Datta SK. 2003. Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Science* **164**: 371–378.

Vasconcelos M, Eckert H, Arahana V, Graef G, Grusak MA, Clemente T. 2006. Molecular and phenotypic characterization of transgenic soybean expressing the *Arabidopsis* ferric chelate reductase gene *FRO2*. *Planta* **224**: 1116–1128.

Vauclare P, Kopriva S, Fell D, Suter M, Sticher L, von Ballmoos P, Krähenbühl U, Op den Camp R, Brunold C. 2002. Flux control of sulphate assimilation in *Arabidopsis thaliana*: adenosine 5′-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols. *Plant Journal* **31**: 729–740.

Velu G, Rai KN, Muralidharan V, Kulkarni VN, Longvah T, Raveendran TS. 2007. Prospects of breeding biofortified pearl millet with high grain iron and zinc content. *Plant Breeding* **126**: 182–185.

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P. 2004. Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. *FEBS Letters* **576**: 306–312.

Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu Ü, Lee Y, Martinoia E *et al.* 2008. Plant ABC proteins – a unified nomenclature and updated inventory. *Trends in Plant Science* **13**: 151–159.

Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat J-F, Curie C. 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* **14**: 1223–1233.

Vreugdenhil D, Aarts MGM, Koorneef M. 2005. Exploring natural genetic variation to improve plant nutrient content. In: Broadley MR, White PJ, eds. *Plant nutritional genomics*. Oxford, UK: Blackwell, 201–219.

Vreugdenhil D, Aarts MGM, Koornneef M, Nelissen H, Ernst WHO. 2004. Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. *Plant, Cell & Environment* **27**: 828–839.

Vucenik I, Shamsuddin AM. 2006. Protection against cancer by dietary IP6 and inositol. *Nutrition and Cancer* **55**: 109–125.

Wall MM. 2006. Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. *Journal of Food Composition and Analysis* **19**: 434–445.

Watanabe T, Broadley MR, Jansen S, White PJ, Takada J, Satake K, Takamatsu T, Tuah SJ, Osaki M. 2007. Evolutionary control of leaf element composition in plants. *New Phytologist* **174**: 516–523.

Waters BM, Grusak MA. 2008a. Whole-plant mineral partitioning throughout the life cycle in *Arabidopsis thaliana* ecotypes Columbia, Landsberg *erecta*, Cape Verde Islands, and the mutant line *ysl1ysl3*. *New Phytologist* **177**: 389–405.

Waters BM, Grusak MA. 2008b. Quantitative trait locus mapping for seed mineral concentrations in two *Arabidopsis thaliana* recombinant inbred populations. *New Phytologist* **179**: 1033–1047.

Welch RM. 1995. Micronutrient nutrition of plants. *Critical Reviews in Plant Science* **14**: 49–82.

Welch RM. 1999. Importance of seed mineral nutrient reserves in crop growth and development. In: Rengel Z, ed. *Mineral nutrition of crops: Fundamental mechanisms and implications*. New York, NY, USA: Food Products Press, 205–226.

Welch RM, Graham RD. 2002. Breeding crops for enhanced micronutrient content. *Plant and Soil* **245**: 205–214.

Welch RM, Graham RD. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**: 353–364.

Welch RM, Graham RD. 2005. Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. *Journal of Trace Elements in Medicine and Biology* **18**: 299–307.

Welch RM, House WA, Beebe S, Senadhira D, Gregorio GB, Cheng Z. 2000. Testing iron and zinc bioavailability in genetically enriched beans (*Phaseolus vulgaris* L.) and rice (*Oryza sativa* L.) in a rat model. *Food and Nutrition Bulletin* **21**: 428–433.

Welch RM, House WA, Ortiz-Monasterio I, Cheng Z. 2005. Potential for improving bioavailable zinc in wheat grain (*Triticum* species) through plant breeding. *Journal of Agricultural and Food Chemistry* **53**: 2176–2180.

Welch RM, Smith ME, Van Campen DR, Schaefer SC. 1993. Improving the mineral reserves and protein quality of maize (*Zea mays* L.) kernels using unique genes. *Plant and Soil* **156**: 215–218.

Westermann DT. 2005. Nutritional requirements of potatoes. *American Journal of Potato Research* **82**: 301–307.

Whanger PD. 2004. Selenium and its relationship to cancer: an update. *British Journal of Nutrition* **91**: 11–28.

Wheeler GL, Brownlee C. 2008. Ca^{2+} signaling in plants and green algae – changing channels. *Trends in Plant Science* **13**: 506–514.

White PJ. 2000. Calcium channels in higher plants. *Biochimica et Biophysica Acta (Biomembranes)* **1465**: 171–189.

White PJ. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany* **52**: 891–899.

White PJ. 2003. Ion transport. In: Thomas B, Murphy DJ, Murray BG, eds. *Encyclopaedia of applied plant sciences*. London, UK: Academic Press, 625–634.

White PJ. 2005. Calcium. In: Broadley MR, White PJ, eds. *Plant nutritional genomics*. Oxford, UK: Blackwell, 66–86.

White PJ, Bowen HC, Demidchik V, Nichols C, Davies JM. 2002a. Genes for calcium-permeable channels in the plasma membrane of plant root cells. *Biochimica et Biophysica Acta (Biomembranes)* **1564**: 299–309.

White PJ, Bowen HC, Marshall B, Broadley MR. 2007a. Extraordinarily high leaf selenium to sulphur ratios define 'Se accumulator' plants. *Annals of Botany* **100**: 111–118.

White PJ, Bowen HC, Parmaguru P, Fritz M, Spracklen WP, Spiby RE, Meacham MC, Mead A, Harriman M, Trueman LJ *et al.* 2004. Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. *Journal of Experimental Botany* **55**: 1927–1937.

White PJ, Bradshaw JE, Dale MFB, Ramsay G, Hammond JP, Broadley MR. (in press). Relationships between yield and mineral concentrations in potato tubers. *HortScience*.

White PJ, Broadley MR. 2001. Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany* **88**: 967–988.

White PJ, Broadley MR. 2003. Calcium in plants. *Annals of Botany* **92**: 487–511.

White PJ, Broadley MR. 2005a. Biofortifying crops with essential mineral elements. *Trends in Plant Science* **10**: 586–593.

White PJ, Broadley MR. 2005b. Historical variation in the mineral composition of edible horticultural products. *Journal of Horticultural Science and Biotechnology* **80**: 660–667.

White PJ, Broadley MR, Bowen HC, Johnson SE. 2007b. Selenium and its relationship with sufur. In: Hawkesford MJ, de Kok LJ, eds. *Sulfur in plants – an ecological perspective*. London, UK: Springer, 225–252.

White PJ, Broadley MR, Greenwood DJ, Hammond JP. 2005. *Proceedings of the International Fertiliser Society 568. Genetic modifications to improve phosphorus acquisition by roots*. York, UK: International Fertiliser Society.

White PJ, Hammond JP. 2008. Phosphorus nutrition of terrestrial plants. In: White PJ, Hammond JP, eds. *The ecophysiology of plant-phosphorus interactions*. Dordrecht, the Netherlands: Springer, 51–81.

White PJ, Marshall J, Smith JAC. 1990. Substrate kinetics of the tonoplast H^+-translocating inorganic pyrophosphatase and its activation by free Mg^{2+}. *Plant Physiology* **93**: 1063–1070.

White PJ, Whiting SN, Baker AJM, Broadley MR. 2002b. Does zinc move apoplastically to the xylem in roots of *Thlaspi caerulescens*? *New Phytologist* **153**: 201–207.

Whiting SN, Broadley MR, White PJ. 2003. Applying a solute transfer model to phytoextraction: Zinc acquisition by *Thlaspi caerulescens*. *Plant and Soil* **249**: 45–56.

Whiting SN, de Souza MP, Terry N. 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. *Environmental Science and Technology* **35**: 3144–3150.

Wilkinson SR, Welch RM, Mayland HF, Grunes DL. 1990. Magnesium in plants: Uptake, distribution, function, and utilization by man and animals. *Metal Ions in Biological Systems* **26**: 33–56.

Wilson CW, Shaw PE, Knight RJ. 1982. Analysis of oxalic acid in carambola (*Averrhoa carambola* L.) and spinach by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* **30**: 1106–1108.

Wintz H, Fox T, Wu Y-Y, Feng V, Chen W, Chang H-S, Zhu T, Vulpe C. 2003. Expression profiles of *Arabidopsis thaliana* in mineral deficiencies reveal novel transporters involved in metal homeostasis. *Journal of Biological Chemistry* **278**: 47644–47653.

von Wirén N, Klair S, Bansal S, Briat J-F, Khodr H, Shioiri T, Leigh RA, Hider RC. 1999. Nicotianamine chelates both FeIII and FeII. Implications for metal transport in plants. *Plant Physiology* **119**: 1107–1114.

von Wirén N, Marschner H, Römheld V. 1995. Uptake kinetics of iron-phytosiderophores in two maize genotypes differing in iron efficiency. *Physiologia Plantarum* **93**: 611–616.

von Wirén N, Marschner H, Römheld V. 1996. Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. *Plant Physiology* **111**: 1119–1125.

Wissuwa M, Ismail AM, Yanagihara S. 2006. Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. *Plant Physiology* **142**: 731–741.

Wu H, Li L, Du J, Yuan Y, Cheng X, Ling H-Q. 2005. Molecular and biochemical characterization of the Fe(III) chelate reductase gene family in *Arabidopsis thaliana*. *Plant and Cell Physiology* **46**: 1505–1514.

Wu J, Schat H, Sun R, Koornneef M, Wang X, Aarts MGM. 2007. Characterization of natural variation for zinc, iron and manganese accumulation and zinc exposure response in *Brassica rapa* L. *Plant and Soil* **291**: 167–180.

Wu J, Yuan Y-X, Zhang X-W, Zhao J, Song X, Li Y, Li X, Sun R, Koornneef M, Aarts MGM *et al.* 2008. Mapping QTL for mineral accumulation and shoot dry biomass under different Zn nutritional conditions in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Plant and Soil* **310**: 25–40.

Wurbs D, Ruf S, Bock R. 2007. Contained metabolic engineering in tomatoes by expression of carotenoid biosynthesis genes from the plastid genome. *Plant Journal* **49**: 276–288.

Wyatt SE, Tsou P-L, Robertson D. 2002. Expression of the high capacity calcium-binding domain of calreticulin increases bioavailable calcium stores in plants. *Transgenic Research* **11**: 1–10.

Xue T, Hartikainen H, Piironen V. 2001. Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant and Soil* **237**: 55–61.

Yang F, Chen L, Hu Q, Pan G. 2003. Effect of the application of selenium on selenium content of soybean and its products. *Biological Trace Element Research* **93**: 249–256.

Yang X, Ye ZQ, Shi CH, Zhu ML, Graham RD. 1998. Genotypic differences in concentrations of iron, manganese, copper, and zinc in polished rice grains. *Journal of Plant Nutrition* **21**: 1453–1462.

Yilmaz A, Ekiz H, Gültekin I, Torun B, Barut H, Karanlik S, Cakmak I. 1998. Effect of seed zinc content on grain yield and zinc concentration of wheat grown in zinc-deficient calcareous soils. *Journal of Plant Nutrition* **21**: 2257–2264.

Yuan YX, Zhang J, Wang DW, Ling HQ. 2005. *AtbHLH29* of *Arabidopsis thaliana* is a functional ortholog of tomato *FER* involved in controlling iron acquisition in strategy I plants. *Cell Research* **15**: 613–621.

Yuasa K, Maeshima M. 2000. Purification, properties, and molecular cloning of a novel Ca^{2+}-binding protein in radish vacuoles. *Plant Physiology* **124**: 1069–1978.

Yuita K. 1992. Dynamics of iodine, bromine, and chlorine in soil. II. Chemical forms of iodine in soil solutions. *Soil Science and Plant Nutrition* **38**: 281–287.

Zhang P, Jaynes JM, Potrykus I, Gruissem W, Puonti-Kaerlas J. 2003a. Transfer and expression of an artificial storage protein (ASP1) gene in cassava (*Manihot esculenta* Crantz). *Transgenic Research* **12**: 243–250.

Zhang Y, Pan G, Chen J, Hu Q. 2003b. Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. *Plant and Soil* **253**: 437–443.

Zhao J, Jamar DCL, Lou P, Wang Y, Wu J, Wang X, Bonnema G, Koorneef M, Vreugdenhil D. 2008. Quantitative trait loci analysis of phytate and phosphate concentrations in seeds and leaves of *Brassica rapa*. *Plant, Cell & Environment* **31**: 887–900.

Zhao J, Paolo MJ, Jamar D, Lou P, van Eeuwijk F, Bonnema G, Vreugdenhil D, Koorneef M. 2007. Association mapping of leaf traits, flowering time, and phytate content in *Brassica rapa*. *Genome* **50**: 963–973.

Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P. 2007. Transgenic strategies for the nutritional enhancement of plants. *Trends in Plant Science* **12**: 548–555.

Zhu Y-G, Huang Y-Z, Hu Y, Liu Y-X. 2003. Iodine uptake by spinach (*Spinacia oleracea* L.) plants grown in solution culture: effects of iodine species and solution concentrations. *Environment International* **29**: 33–37.

Zia-Ul-Haq M, Iqbal S, Ahmad S, Imran M, Niaz A, Bhanger MI. 2007. Nutritional and compositional study of Desi chickpea (*Cicer arientinum* L.) cultivars grown in Punjab, Pakistan. *Food Chemistry* **105**: 1357–1363.

Zohlen A, Tyler G. 2004. Soluble inorganic tissue phosphorus and calcicole-calcifuge behaviour of plants. *Annals of Botany* **94**: 427–432.

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References S1–S12 Supplementary reference lists.

References S13 Alphabetical list of supplementary references.

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