

## Review

# Enhancing Mineral Content in Plant Food Products

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Plant foods can serve as dietary sources of all essential minerals required by humans. Unfortunately, mineral concentrations are low in some plants, especially many staple food crops; thus, efforts are underway to increase the mineral content of these foods as a means to ensure adequate attainment of dietary minerals in all individuals. While these efforts have included classical breeding approaches in the past, it is clear that future progress can be made by utilizing the tools of biotechnology to effect directed changes in plant mineral status. Reviewed are the short- and long-distance mineral transport mechanisms responsible for the root acquisition and whole-plant partitioning of mineral ions in crop plants. This background is used to discuss different transgenic strategies with the potential to enhance mineral content in vegetative and/or reproductive tissues. Due to various constraints imposed by plant transport systems on whole-plant mineral movement, it is argued that modifications designed to increase the supply of minerals to edible organs should have the highest chance for success. Examples of previous efforts to manipulate plant mineral nutrition through the introduction of novel transgenes are presented to demonstrate the utility of these approaches.

### Key teaching points:

- Plants are capable of absorbing a wide range of mineral ions with relevance to human nutrition and health.
- Mineral concentrations in many plant foods are low, relative to human requirements; this has elicited efforts to enhance plant mineral content by using the tools of plant biotechnology.
- To be effective, transgenic strategies for mineral improvement must be designed to complement or enhance the existing mechanisms and regulatory processes that govern plant mineral nutrition.
- Processes relevant to plant nutrient content include mineral availability in the soil environment, short-distance ion movement using membrane transporters and long-distance mineral movement utilizing the xylem or phloem pathways.
- Different strategies are necessary to enhance the mineral content of xylem-fed tissues such as leaves *versus* phloem-fed tissues such as seeds.
- For seed crops, transgene expression designed to increase mineral loading into the phloem pathway is envisioned as a primary strategy to effectively enhance seed mineral content.

## INTRODUCTION

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well-being [1,2]. Most minerals are obtained as inorganic ions, except for N and S, which are obtained predominantly as components of amino acids and other organic molecules, or for P, which is obtained in nucleotides, nucleic acids and phospholipids. Plant-derived foods have the potential to serve as dietary

sources for all human-essential minerals [3], and with a well-balanced diet that includes mixed sources of grains, fruits and vegetables, plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle [4–6]. Unfortunately, consumption patterns are not always ideal, and many individuals both in developed and developing countries are failing to attain recommended mineral intakes [7–10]. Whereas an increased consumption of plant food products would be beneficial, it appears that behavioral and/or environmental factors will

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continue to limit their consumption [11,12]. Thus, as an alternative strategy, efforts are underway to increase the nutrient composition of those plant foods which people do eat, as an attempt to ensure adequate attainment of dietary nutrients in all individuals [13–16].

Currently, the achievable densities of minerals in our existing agricultural crops means that few individual plant foods are able to supply the daily recommended intake for any given mineral in an average or reasonable serving size. This problem of low mineral density is particularly troublesome in staple foods, such as cereal grains and tuber crops, which make up a large proportion of daily food intake in the developing world [17]. Fortunately, plant mineral concentrations do vary both among plant sources (i.e., species, cultivars) and among plant tissues (e.g., leafy structures *versus* seeds) [3,18–20], thereby demonstrating that genetic differences exist, which can contribute to the plant's ability to acquire and sequester minerals. Plant scientists are currently working to decipher the molecular components and physiological processes that underlie plant mineral nutrition, such that this understanding can be used to enhance the mineral content of agronomically important crop species. These efforts include classical breeding approaches designed to characterize and exploit genetic variation for mineral content [13,21,22], as well as new approaches involving gene discovery and directed genetic modification to create new plant lines with improved mineral qualities [15,23,24]. In this paper we will focus on the latter approaches, as we review the factors that control plant mineral nutrition and discuss the current and future potential of biotechnology as a tool to enhance mineral content in our plant-based food supply. It should be noted that manipulating plants to improve the bioavailability of minerals is an alternative option for enhancing mineral nutrient *quality* [14], but this strategy will not be addressed in the current review.

### Which Minerals Can Plants Provide?

When designing transgenic approaches to enhance plant quality traits, there often is a tendency to look for one gene that will help complete a biochemical pathway and thereby serve to synthesize a nutrient of interest [23,25]. Of course, for mineral enhancements, no gene products exist which allow a plant (or any other organism) to synthesize minerals. All mineral elements must be acquired from the external environment, and because plants are non-mobile organisms, any minerals that a plant might acquire must be present in the local soil volume that can be reached by its root system. If the mineral occurs in a soluble form (usually as an ionized inorganic ion), it potentially can be absorbed into the plant and be mobilized from the roots to various vegetative and reproductive tissues.

Which minerals can plants absorb, and thus provide in the diet? An obvious initial list includes the 14 mineral elements defined as essential for plant growth and reproductive success [26]. These are N, S, P, K, Ca, Mg, Cl, Fe, Zn, Mn, Cu, B, Mo,

and Ni. Because of their essentiality, all plant foods contain some level of each of these elements, and it should come as no surprise that plants have developed various forms of molecular machinery (i.e., membrane transporters) to acquire these mineral nutrients from their soil environment [27–29]. Of these 14 elements, human essentiality has been confirmed for all but B and Ni, although circumstantial evidence for their essentiality has been reported [30]. Na, Cr, I and Se also are required by humans, but not by plants. Fortunately for humans, however, plants can acquire these other elements through non-specific influx processes using existing transporters localized to their roots [31]. The overall uptake of these plant non-essential elements depends on their availability in the soil, in conjunction with the extent of their influx through non-specific transporters. In fact, a wide range of plant non-essential elements (both benign and detrimental) have been measured in plant tissues, with concentrations sometimes reaching dramatic levels if soil availability is high (e.g., Cr, Se, Ar) [31]. A number of these elements, also referred to as the ultratrace elements, have been demonstrated to provide various health benefits in humans [30], and thus their incorporation into plant tissues is of dietary relevance.

### What Factors Control Plant Mineral Content?

Before discussing possible transgenic strategies related to mineral improvement, it is necessary to understand the pathways and constraints involved in the delivery of minerals to various plant structures. Unlike organic nutrients, whose biochemical pathways can be identified as enzymes localized to specific cellular or subcellular compartments [32], when we discuss mineral pathways, we are referring to transport proteins localized to various membranes, as well as long-distance systems used to move water and nutrients throughout the plant. Thus, for minerals, a complex, integrated system of tissues and membrane processes is required to move any given mineral from the root-soil interface to each cell throughout the organism.

Assuming a mineral ion is available in the soil, an appropriate transport protein must be present to facilitate its movement into the root symplasm. Once it enters this compartment, the mineral either can provide for the local nutritional needs of the root cells, or it can be moved to the xylem pathway. The xylem transport system is composed of columns of non-living cells (i.e., devoid of membranes) that provide an open route for the bulk flow of water and mineral ions [33]. This solution (xylem sap) moves up the plant in response to a reduction in water potential that is created primarily by the evaporative loss of water (i.e., transpiration) from various exposed structures. Most water leaves these structures through small pores known as stomata. Thus, minerals within the xylem sap are delivered non-selectively as columns of water are pulled up through the plant and are accumulated preferentially in those tissues with high rates of water loss. These tissues include most leaves and certain reproductive structures, such as the pod walls of legume

species or the leaf-like organs encompassing certain cereal grains (e.g., rice, wheat). It should be noted that non-exposed structures (e.g., most developing seeds, closely packed leaves) or organs with low densities of stomatal pores (e.g., many fruit) can exhibit very low, or even no rate of transpiration [33,34]. Consequently, mineral delivery via the xylem pathway to these types of organs will be low to non-existent [35].

How do these poorly transpiring structures get minerals? A second long-distance pathway, known as the phloem transport system, is responsible for the movement of sugars, amino acids, certain minerals and various secondary metabolites from photosynthetic "source" organs (primarily leaves) to several "sink" regions [26]. Sinks include such things as reproductive tissues (seeds, fruit), young developing leaves and roots. The phloem transport pathway is composed of columns of cells, but unlike the situation in the xylem pathway, these are living cells bounded by membranes. Phloem sap moves from cell to cell through membrane-lined pores; the driving force is a hydrostatic pressure gradient which is established as solutes enter the phloem symplasm in source regions (causing an osmotic influx of water) and exit the symplasm in sink regions (with a contrasting osmotic efflux of water). Solute movement into the phloem symplasm can occur either via membrane transporters or through symplastic connections between phloem and surrounding cells [36]. In either case, the predominant solute in phloem sap is a sugar molecule (usually sucrose, but it could be various oligosaccharides), and, except for potassium, most minerals are minor solutes in this system [37]. Thus, the ability of minerals to contribute osmotically to the hydrostatic driving force is quite minimal, and their movement to sink tissues is controlled not by the mineral needs of sink tissues, but by the overriding whole-plant dynamics of source-sink carbohydrate partitioning. In other words, movement in the phloem pathway is rather non-directed, at least from the standpoint of most minerals.

To be fair, it should be stated that the plant does have some directed control over mineral movement in the phloem, through the extent to which each mineral is initially loaded into the pathway. With a higher flux of any given mineral into the phloem system, more of that mineral will be delivered to distant sink tissues, albeit in a non-directed manner. Our understanding of phloem mineral loading is quite limited, but it is clear that a pool of mineral ions must be present in source tissues and that some type of transport capacity must be present for their delivery into the phloem symplasm. Interestingly, this is a similar set of constraints as those described for mineral acquisition at the root-soil interface.

### Transgenic Strategies to Enhance Mineral Content

In light of the mechanisms described above, it is clear that transgenic efforts to enhance mineral content in specific plant tissues must contend not only with transmembrane events at

various points in the whole-plant continuum, but also the long-distance pathways that serve to move absorbed minerals throughout the plant. Furthermore, because minerals must be transported to various organs, the more successful (and quantitatively significant) transgenic strategies should be those directed towards enhancing the supply processes, rather than manipulating the receiving organ itself. With respect to the supply of minerals, different approaches are needed if one wishes to enhance the mineral content of a xylem-fed organ, as opposed to that of a phloem-fed organ [38]. Specifically, for most vegetable crops, genetic modifications that lead to enhanced root uptake should result in more of a given mineral being transported (via the xylem) to shoot vegetative tissues. For seed, tuber and some fruit crops, transgene expression that leads to enhanced phloem mineral loading is required, although if the pool of available minerals is limited in leaves (and other source regions) then both phloem loading *and* root influx may need to be manipulated.

In recent years, plant scientists have begun to identify various genes which encode proteins of relevance to the membrane transport of different mineral nutrients. These protein products include membrane transporters, as well as proteins that facilitate the availability of minerals at the root-soil interface. In the first category, data are now available for genes encoding various divalent metal transporters (e.g., for  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and others) [28,39,40],  $\text{Ca}^{2+}/\text{H}^+$  - or  $\text{Mg}^{2+}/\text{H}^+$  - antiporters [41,42], nitrate co-transport proteins [43] or phosphate transporters [44,45], to name a few. Both molecular and functional analyses are ongoing to better understand how the expression of these genes/proteins are regulated and to characterize the selectivity and capacity of plant transporters [e.g., 46,47]. As this information unfolds, we should be well positioned to design effective gene constructs that will enhance root mineral uptake and increase plant mineral content, at least in vegetative tissues. Thus far, overexpression of a  $\text{Ca}^{2+}/\text{H}^+$  - antiporter in the model plant, *Arabidopsis thaliana*, has led to a moderate increase in leaf Ca concentration [48], thus demonstrating the potential utility of this approach.

For some minerals such as Fe, the membrane transport event is not the only process, or even the rate-limiting process, in root acquisition. In certain species (e.g., legumes, tree crops), Fe must be reduced from its ferric to ferrous redox state prior to uptake; this occurs predominantly through the action of a transmembrane reductase mechanism [49]. In other species (e.g., cereals), ferric Fe is chelated to one of several organic molecules, known as phytosiderophores, which are synthesized and secreted by the roots prior to their reabsorption as an Fe-phytosiderophore complex [50]. Recent work has led to the identification of genes for ferric reductases [51], for enzymes involved in phytosiderophore biosynthetic pathways [24] and possibly for metal-phytosiderophore membrane transporters [52]. Overexpression of a yeast ferric reductase gene in tobacco roots led to enhanced constitutive reductase activity [53], and the overexpression of barley nicotianamine aminotransferase

genes [54] in rice roots resulted in enhanced phytosiderophore synthesis by these roots [55]. By increasing the availability of Fe at the root surface, both strategies enabled the plants to accumulate more Fe in leaf (and root) tissues.

Strategies to manipulate soil availability are possible for other minerals. Phosphorus is an essential plant nutrient that forms insoluble compounds, both in acid and alkaline soils, thus limiting its availability to the plant [56]. Tobacco plants engineered to overproduce and excrete citrate, which helps to solubilize phosphates, have been shown to accumulate more P and yield more leaf and fruit biomass [57]. Although phosphorus is not necessarily limiting in the human diet, by improving plant P content, general plant health can be enhanced along with the overall absorption and storage of other mineral nutrients.

As noted above, strategies focused on the root acquisition of minerals have a direct influence on xylem-fed organs, but not necessarily on phloem-fed organs, such as seeds. Unfortunately, few studies have addressed the mechanisms or regulation of phloem mineral loading [58–60], and there is a great need for gene discovery of phloem-specific genes and promoter sequences [61], especially those with relevance to mineral transport processes. Other information that is urgently needed is an understanding of how or where minerals are stored in source tissues prior to phloem loading, what regulates their storage in these pools and what factors determine their ultimate partitioning to the phloem pathway. As any of these processes could limit the availability of specific minerals for phloem loading, they would be potential targets for genetic manipulation in addition to targeted enhancements of phloem loading itself. Clearly, our ability to devise sensible transgenic strategies to enhance the mineral content of seeds and other phloem-fed tissues will be quite limited until we have more of this information.

Although we have stressed the importance of manipulating the supply side of plant mineral nutrition, there have been efforts to manipulate storage tissues directly in order to enhance their mineral content. Overexpression of the Fe-storage protein ferritin in rice grains has been reported to result in a threefold [62], a twofold [63] or in no increase [64] in seed Fe concentration. How this transgenic strategy enabled more total Fe to move to the grains in some cases is unclear, although one possibility stems from the fact that in rice, Fe can move to the base of grains (rachilla region) via xylem flow [65]. With increased ferritin protein providing an added sink for Fe, more of this mineral could have moved to seeds by diffusional routes. Ferritin overexpression in vegetative tissues also leads to increased leaf Fe concentrations; this has been shown in tobacco, wheat and rice [64,66,67]. It appears that by sequestering Fe in leaf tissues, a functional Fe-deficiency is induced, and the endogenous homeostatic mechanisms that upregulate root Fe acquisition are activated to increase total plant Fe [66,68]. Increased Fe transport to leaves, however, does not necessarily lead to elevated seed Fe content [59,64,66,69].

## CONCLUSIONS

Plants are complex organisms whose mineral needs are determined by a number of molecular, cellular and whole-plant events. Transgenic manipulation to improve mineral content is clearly a viable option, but we still need to identify many of the molecular players relevant to mineral transport before this technology will have widespread utility. Our knowledge base is most lacking in the area of mineral loading into the phloem pathway, which is a critical process in the delivery of mineral nutrients to developing seeds. Even with this knowledge, significant improvements in the content of certain minerals likely will require the insertion of multiple transgenes, especially if the pool of minerals in source tissues must also be enhanced. This will not be an easy task, but for some minerals it may be a necessary approach to ensure their enhanced transport through multiple membrane and tissue systems. Fortunately, research in plant transformation technologies and plant mineral nutrition is ongoing and will continue to receive much attention, because efforts to improve plant mineral status are important not only for the nutritional value of our food supply, but also for the health and reproductive output of our agronomic crops.

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