About half of the world population suffers from the malnutrition of iron, zinc, calcium, iodine and selenium. Most of the major staple crops of the world, such as rice, wheat, cassava, beans, sweet potato, pearl millet or maize are often deficient in some of these mineral elements. Hence, increasing the concentration of bioavailable micronutrients in edible crop tissues (biofortification) has become a promising strategy in modern agriculture, allowing the access of more nutritious foods, to more people, with the use of fewer resources. Traditional agricultural practices can partly enhance the nutritional value of plant foods, but the advances in the ‘omics’ technologies are rapidly being exploited to engineer crops with enhanced key nutrients. Ionomics, or the study of the ionome (which can be defined as the mineral trace element composition of a particular organism), is a modern functional genomics tool that can provide high throughput information about the broad spectrum nutrient composition of a given plant food. In alliance with other ‘omics’ technologies, such as genomics, transcriptomics and proteomics it can be used to identify numerous genes with important roles in the uptake, transport and accumulation of mineral nutrients in plant foods, in particular in their edible parts. This review provides a critical comparison of the strategies that have been developed to diminish nutrient deficiencies in plant-based foods (SWOT analysis) and a summary of the gene families involved in the mineral nutrient pathways. Finally, it also discusses how ‘omics’ techniques can be used in genetic engineering programs to increase mineral levels and bioavailability in the most important staple food crops and the socioeconomic implications of plant-based biofortified foods.

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1. Introduction

Humans and other animals require a multitude of nutrients in order to have a properly functioning body, in terms of growth, development and metabolism. Plant based foods constitute one of the most important nutrient sources in human diet since the beginning of mankind. From a health perspective, plant nutrients of dietary interest can be divided into five main categories, the first three to be enhanced and the latter two to be limited or removed: macronutrients, micronutrients, ‘promoters’, anti-nutrients, and allergens (Newell-McGloughlin, 2008). There is a strong evidence that the consumption of plant-based foods is associated with reduced risk of many chronic diseases (McEvoy, Temple, & Woodside, 2012; Sabaté, 2003), cardiovascular disease (Tomé-Carneiro et al., 2012), and certain types of cancer (Magee, Owusu-Apenten, McCann, Gill, & Rowland, 2012; Olsen, Glimmer, Aaby, Saha, & Borge, 2012). Therefore, there is a strong recommendation for increasing the amount of vegetable, fruits and cereals intake.

The Food and Agriculture Organization (FAO) estimates that by 2050 the world’s population will reach 9.1 billion (FAO, 2009). Most of this population growth will occur in developing countries and will be accompanied with an increasing rural flight. In order to feed this larger, more urban population, food production must increase by 70% (FAO, 2009). However, the amount of arable land is being reduced and much of the natural resources already in use show signs of degradation. Moreover, staple crops (e.g. plants that constitute the major food item in the diets of people in developing countries; e.g. rice, wheat, cassava and maize) regrettably contain low levels of micronutrients, making them insufficient to meet the minimum daily requirements. Deficiencies in mineral micronutrients, including iron (Fe), zinc (Zn), selenium (Se), and iodine (I), are affecting more than half of the world population (Zhao & McGrath, 2009). For instance, Fe deficiency is the most serious nutritional problem in the world today, affecting approximately 2.7 billion people (Hirschi, 2009). Other minerals, such as calcium (Ca), magnesium (Mg), and copper (Cu), can also be deficient in the diets of some populations (White & Broadley, 2005). Widespread micronutrient malnutrition (MMN) results in an enormous negative socio-economic impact at the individual, community, and national levels (Darnton-Hill et al., 2005). This problem – also known as hidden hunger – is considered one of the most serious global challenges of the humankind (Copenhagen Consensus 2004; http://www.copenhagenconsensus.com). MMN has been significantly mitigated in some countries thanks to the programs that fortify processed foods. Nevertheless, fortification efforts has only a partial success in countries with limited industrial agriculture, food processing and distribution networks (Pfeiffer & McClafferty, 2007). In this scenario, it is vital to develop strategies that allow us to produce plant foods more efficiently, and with higher micronutrient concentration and bioavailability in their edible tissues. The major goal of the plant-based foods biofortification programs is to provide a continuous supply of nutrient-dense plant foods to large numbers of people (for guaranteeing ‘nutrient security’) with making use of few resources (cost-effective strategies with one-off cost and reduced logistics).

The Harvest Plus Challenge Program is a large biofortification platform that was launched in 2004 with the goal of reducing micronutrient malnutrition in Asia and Africa. The program has focused on seven staple food crops (rice, beans, cassava, maize, sweet potatoes, pearl millet and wheat) and targeted three major nutrients: Fe, Zn and vitamin A. However, enrichment of these three micronutrients only scratches the surface of what is currently being done in biofortification programs worldwide. Research also includes other nutrients and secondary metabolites such as antioxidants, anthocyanins, lycopene, vitamin E, essential amino acids, essential and very long chain fatty acids or folate. Biofortification programs have also been diversified to other crops such as apple (Szankowski et al., 2003), canola (Roesler, Shintani, Savage, Boddupalli, & Ohirogge, 1997; Shewmaker, Sheehy, Daley, Colburn, & Ke, 1999), carrots (Morris, Hawthorne, Hotze, Abrams, & Hirschi, 2008), lettuce (Goto, Yoshihara, & Saiki, 2000), potato (Diretto et al., 2006), amongst many others.

In order to accomplish the biofortification challenges, novel technologies have started to provide valuable information: the so-called ‘omics’ era. Genomics has given researchers the complete genome from an increasing number of plant foods, and has provided single nucleotide polymorphisms (SNPs) information of important nutrient-related genes. The field of transcriptomics has delivered holistic, non-targeted identification of genes that are expressed by the plant in response to nutritional changes. Proteomics has helped in identifying which transcripts will actually be utilized for protein synthesis, whereas metabolomics has gone a step forward and provided information on the metabolites produced by the plant that may be involved in plant nutrition. Finally, the field of ionomics has integrated the nutrient profiles of a given plant food, incorporating information from the previous ‘omics’ sciences, and accounting for plant tissue, environmental, and developmental factors.

This review will comprehensively describe the challenges, limitations, and potentials of biofortified crops. It will also explain the different types of biofortification strategies and the principal gene families involved in plant nutrition. Finally, it will focus on how the different ‘omics’ fields can be important tools to achieve the goal of producing plant foods that are nutritionally richer and environmentally sound.

2. Strategies for biofortification of food crops

There are three main strategies currently in practice which have been successfully adopted for improving the nutritional content of plant-based foods: agronomic biofortification, conventional plant breeding and genetic engineering. The agronomical approach is mainly focused on optimizing the application of mineral fertilizers and/or the improvement of the solubilization and mobilization of mineral elements in the soil (White & Broadley, 2009). The other two approaches aim at improving plant varieties for a higher capacity to accumulate micronutrients in the edible plant tissues and to increase their bioavailability to humans (through higher concentrations of ‘promoter’ substances and lower concentrations of ‘antinutrients’). The choices of conventional breeding or of the use of genetically modified crops are two possible approaches when breeding for this type of biofortified crops. Table 1 summarizes the strengths, weaknesses, opportunities and threats (SWOT analysis) of each biofortification strategy allowing a critical comparison between them.

2.1. Agronomic biofortification

Mineral elements in the soil can be present as free ions, as surface-adsorbed ions, as dissolved compounds or precipitates, as part of lattice structures or contained within the soil biota (White & Broadley, 2009). A common limitation for biofortification is the generally low phytoavailability of mineral micronutrients in the soil. Thus, the agronomic efforts have been directed toward the application of mineral fertilizers and the improvement of the solubilization and mobilization of mineral elements in the soil.

Although relatively simple and with immediate results, this strategy can only be used for fortifying plants with mineral elements and not with organic nutrients (e.g. vitamins) which must be synthesized by the plant itself. Moreover, the viability of this biofortification strategy depends on several factors, including soil composition, mineral mobility in the soil and in the plant, and its accumulation site (Hirschi, 2009; Zhu et al., 2007). Therefore, the application of fertilizers containing essential mineral micronutrients cannot be seen as an universal approach for enhancing the micronutrient levels in edible crop tissues. In general, mineral elements with a good mobility in the soil and in the plant are good candidates for a successful agronomic biofortification (reviewed by White & Broadley, 2009).
the case of Se, I and Zn where the use of inorganic fertilizers have been particularly successful (Dai, Zhu, Zhang, & Huang, 2004; Hartikainen, 2005; White & Broadley, 2005). For instance, the application of inorganic Se fertilizers to increase crop Se concentration had a great impact in several countries such as Finland, New Zealand and France. In many indigenous Finnish plant-food items it resulted in over 10-fold increase in Se concentrations (Eurola, Efklholm, Vilen, Koivistoinen, & Varo, 1989, 1991). Similarly, the use of inorganic I and Zn also had an impact on plant enrichment at a country scale, respectively, in China and Thailand (Winkler, 2011). In contrast, Fe shows a low mobility in soil because FeSO₄ is rapidly bound by soil particles and converted into Fe(III), becoming unavailable to plant roots. Consequently, Fe fertilization has not been successful in biofortification efforts (Grusak & Dellapenna, 1999).

To partly overcome the phytoavailability bottleneck, synthetic metal chelators (e.g. EDTA) have been used to enhance the efficiency of micro-nutrient fertilizers applied to deficient soils (Shuman, 1998; Zhao & McGrath, 2009). This is the case, for example, of Fe- and Zn-chelates which were effective in increasing mineral concentration in edible cereal, vegetable and fruit tissues (Rengel, Batten, & Crowley, 1999; Shuman, 1998). Foliar fertilization is an alternative method for applying soluble inorganic fertilizers in situations where mineral elements are not readily translocated to edible tissues. This method is commonly used in horticultural crops to prevent Ca-deficiency disorders (Ho & White, 2005) and also for the application of MgSO₄ in some crops (Metson, 1974). Soil micro-organisms (e.g. mycorrhizal fungi and N₂-fixing bacteria) can also be exploited to increase the volume of the soil that can be explored and the phytoavailability of mineral elements (Rengel et al., 1999). Several studies have found that the mycorrhizal associations increase Fe, Se, Zn and Cu concentrations in crop plants (Cavagnaro, 2008; Rengel et al., 1999).

A major drawback of the fertilization strategy for plant biofortification is the frequent need for regular applications, which makes this approach costly, difficult in logistic terms (heavy and bulky products) and potentially negative for the environment (Hirsch, 2009; White & Broadley, 2009; Winkler, 2011). Additionally, the availability of certain mineral elements may become limited due to the risk of reserves exhaustion (reviewed by White & Broadley, 2009). This seems particularly problematic for Se since its reserves are expected to be exhausted in less than 40 years.

Taking into account all the aforementioned limitations, inherent to the agronomic biofortification through fertilizer application, it is important to explore more cost-effective and long-term strategies to improve micronutrient density in edible plant portions. Nonetheless, it has been advocated that this strategy should be combined with other biofortification approaches, particularly when the phytoavailability of minerals can restrict their concentration in the edible plant portions (Graham et al., 2007; White & Broadley, 2009).

2.2. Conventional plant breeding

Traditional plant breeding has been mostly focused on increasing crop yields, enhancing crop resistance to pests and diseases and having tolerance to abiotic stresses such as drought and salinity. Several studies that performed an analysis of historical data have shown that the increases in crop yield over the last four decades have been accompanied by decreases in the mineral concentrations of the edible plant tissues (Fan et al., 2008; Garvin, Welch, & Finley, 2006). Examples of minerals that their mean concentration in the dry matter has declined in several plant-based foods are Fe, Zn, Cu and Mg (reviewed by White & Broadley, 2009). Thus, more recently an effort has been put into conventional plant breeding for enhancing the micronutrient concentrations of both minerals and vitamins in the edible parts of the plant. Since conventional plant breeding explores the intrinsic properties of the different crop varieties, this strategy finds a widespread public acceptance and a simple legal framework (Bouis, 2000; Hirschi, 2009; Winkler, 2011). Additionally, this biofortification approach only represents a one-off cost since it involves just one initial subsidized distribution and the seeds can be further harvested and used for future years (Winkler, 2011). For these reasons, this cost-effective and long-term biofortification strategy is considered as possibly the most expedient solution for enhancing the micronutrient density of edible plant tissues (Hirsch, 2009).

The major weaknesses and threats to the conventional plant breeding approach are: the long-development time, the dependence on the phytoavailability of the mineral nutrients in the soil and the need for sufficient genetic variation of a given trait within species. Nonetheless, many traits needed in biofortification programs can be found by exploring the genetic variation in germplasm collections or by exploiting transgressive segregation or heterosis (Mayer, Pfeiffer, & Bouis, 2008).

An extensive number of studies have showed that a large within-species genetic variation does exist in various crops, both in terms of the concentration of minerals and vitamins in the edible tissues as well as in terms of their bioavailability to human gut (reviewed by White & Broadley, 2009). For instance, different rice genotypes show a 4-fold variation in Fe and Zn levels and up to a 6.6-fold variation has been reported in beans and peas (Gregorio, Sendhira, Htut, & Graham, 2000). Apparently, this genotypic variation is generally more reduced in tubers (White & Broadley, 2009) and in fruits (e.g. Fe, Zn,
Ca and Mg concentrations in strawberry differed less than 2-fold; Hakala, Lapveteläinen, Houpalahlit, Kallio, & Talhonen, 2003). Wheat is a successful example of a crop with limited genetic variation in the Se content, within the cultivated germplasm, which has been overcome by crossing to a distant wild relative (Lyons et al., 2005). Therefore, seed banks can have an important role in the biofortification process when screening germplasm for a given trait. The new Svalbard Global Seed Vault (inside the Arctic Circle) will become the most comprehensive one.

Due to the strengths and opportunities associated with the biofortification through conventional breeding (Table 1), many international programs are underway to enrich the nutrient content of several crops, both to improve health and to prevent MNN. Among them the HarvestPlus is the most significant, systematic and symbolic breeding program (see Introduction of this review).

2.3. Genetic engineering

In the absence of sufficient genotypic variation for the desired trait within the species, or when the crop itself is not amenable to conventional breeding (due to a lack of sexuality; e.g. banana), then genetic engineering offers a valid alternative for increasing the concentration and bioavailability of micronutrients in the edible crop tissues (Mayer et al., 2008). The increasing number of fully sequenced genomes in staple crops [e.g. rice (I.R.G.S, Project, 2005), sorghum (Paterson et al., 2009), and soybean (Schmutz et al., 2010)] and the powerful new molecular and genomic tools open new opportunities for this biofortification strategy. Thus, a fast development in this field is expected in the coming years (Waters & Sankaran, 2011).

In general, this biofortification strategy shares several strengths and weaknesses with the conventional plant breeding (as shown in Table 1). However, in contrast to the later, the genetic engineering approach faces the threat of a low public acceptance (especially in Europe; see below Section 5), and consequently a complex legal framework. One main concern is the so-called ‘gene flow’ environmental problem, i.e. the concern of transfer of inserted genes to non-target species (Winkler, 2011). Nevertheless, this potential drawback will vary according to the reproductive strategy of the plant, and the environmental effects remains to be conclusively proven.

Targets for transgenes include redistributing micronutrients between tissues, increasing the efficiency of biochemical pathways in edible tissues, or even the reconstruction of selected pathways. Some strategies, rather than increasing production or accumulation of micronutrients, might involve the removal of ‘antinutrients’ or inclusion of ‘promoter’ substances enhancing the bioavailability of micronutrients (Section 3 of this review).

Genetic engineering strategies to increase the mineral content of plants have focused primarily on Fe and Zn, which are the most frequently deficient micronutrients in human diets (Curie & Briat, 2003; Palmgren et al., 2008). For instance, the Fe level in rice increased by 3- to 4-fold due to the expression of ferritin, an iron-storage protein (Goto et al., 2000; Vasconcelos et al., 2003). Although rice polishing leads to a decrease in the mineral levels, the iron concentration and bioavailability (tested in rats) of transgenic polished rice was still significantly higher in ferritin-enhanced lines (Murray-Kolb et al., 2002). Additionally, there are many successful examples of transgenic crops that showed an increased concentration of vitamins or mineral elements (for details see recent reviews: Hirsch, 2009; White & Broadley, 2009). The most well-known is the ‘golden rice’, which is a variety of genetically engineered (GE) rice where the carotenoid biosynthetic pathway has been reconstituted in non-carotenogenic endosperm tissue in order to produce β-carotene (pro-vitamin A) to help combat vitamin A deficiency (Paine et al., 2005). It is possible that these GE rice varieties will be capable of providing the recommended daily allowance of vitamin A (in the form of β-carotene) in 100 to 200 g of rice. This approach is also being applied to other crops, including maize, orange cauliflower, tomato, yellow potatoes and golden

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directed to the most limiting steps (Waters & Sankaran, 2011). Plants do not all use the same strategies to uptake nutrients. For instance, in the case of Fe dicots and non-graminaceous monocots species respond to Fe limiting environments by induction of strategy I for Fe acquisition. This involves the expression of an active proton pump to increase solubility of Fe(III), a ferric chelate reductase to generate the more soluble Fe(II), and an Fe transporter. In contrast, graminaceous monocots respond to Fe limiting environments by induction of strategy I for Fe acquisition. Case of Fe dicots and non-graminaceous monocots species respond to Fe limiting environments by induction of strategy I for Fe acquisition.

Once in the xylem, nutrients will be “pulled” upwards through transpiration forces, transported to the shoots, and deposited in the leaves (second step; Fig. 1). At this point, leaves are working as sinks for nutrients and carbohydrates. The concentrations of mineral elements in edible tissues are also influenced by their mobility in the vascular system.

All leaves on a plant undergo a transition from a sink (a carbon and micronutrient importer) to a source (a carbon and nutrient exporter) during their development. The transition happens when the amount of carbon accumulated by photosynthesis is greater than the requirement of respiration and growth. Since xylem unloading and phloem loading are not well characterized, augmenting translocation of nutrients from the source leaves to the sink tissues (grains and younger leaves) has been one of the major bottlenecks in biofortification programs. Although nutrients such as Se and Mg are transported readily in the phloem, Fe, Zn, Cu and I are not, and Ca has little phloem mobility (White & Broadley, 2003). For this reason, phloem-fed tissues such as fruits, seeds and tubers are often poor sources of these nutrients while leafy vegetables are richer sources as the nutrient transport of the later is via the xylem vessels (White & Broadley, 2009). Several genes have been identified that are responsible for Fe (FROs; Wu et al., 2005), Zn (ZIPs; Wintz et al., 2003) and Cu (COPs; del Pozo et al., 2010) transport in the vascular system.

Fe and Zn tend to move in the phloem stream in a chelated form (Blindauer & Schmid, 2010). Two major candidates for the ligand that transports Fe are a protein called ITP (iron transport protein), which was identified in the phloem of Ricinus communis (Kruger, Berkowitz, Stephan, & Hell, 2002), and nicotianamine (NA), a peptide which was first identified in the monocots (Higuchi et al., 1999) but

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that is also present in dicots (Suzuki, Higuchi, Nakanishi, Nishizawa, & Mori, 1999). Besides binding Fe, NA is also capable of binding and presumably transporting other nutrients such as Mn, Zn, Cu, Co and Ni (Higuchi et al., 1999; Sancenón, Puig, Mira, Thiele, & Peñarrubia, 2003). The genes responsible for micronutrient–NA transport belong to the yellow stripe-like (YSL) family (Chu et al., 2010; Le Jean, Schikora, Mari, Briat, & Curie, 2005). These genes were mostly identified in Arabidopsis thaliana (DiDonato, Roberts, Sanderson, Eisley, & Walker, 2004; Stacey, Koh, Becker, & Stacey, 2002; Stacey, Osawa, Patel, Gassmann, & Stacey, 2006; Stacey et al., 2008; Waters et al., 2006), maize (Roberts, Pierson, Panaviene, & Walker, 2004; Ueno, Patel, Gassmann, & Stacey, 2006; Stacey et al., 2008; Waters et al., 2006), rice (Ueno et al., 2009) and barley (Ueno et al., 2009) and rice (Ishimaru et al., 2010; Vasconcelos, Li, Lubkowitz, & Grusak, 2008).

The next step of the nutrient biofortification pathway is nutrient deposition and storage in the grains (third step; Fig. 1). The grain is connected to the maternal plant by a single vascular bundle which provides the nutrients to the seed coat (Zhang et al., 2007), and are later on channeled to the developing endosperm. Genes from the HMA, ZIP, Nramp, NAS, and YSL may be involved in the process (Tauris, Borg, Gregersen, & Holm, 2009). In terms of nutrient localization in the grain, Fe and Zn are mostly stored in the embryo and the aleurone layer (Vasconcelos et al., 2003), and the endosperm itself is very poor in terms of micronutrient content. It seems that Fe is loaded into seed vacuoles by the VITI1 protein (Kim et al., 2006) and it is remobilized from the vacuole by the Nramp3 and Nramp4 transporters (Lanquar et al., 2005). Ferritin in the leaves (Liao et al., 2012), and globulins, albumins and glutelins in the seeds (Aguirre, Torres, Mendoza-Hernández, Garcia-Gasca, & Blanco-Labra, 2012) are important nutrient storage proteins in the plant that can also be modulated in biofortification programs.

From the description above, it is visible that the movement of nutrients within the plant is a tightly regulated molecular mechanism, and that in order to overcome nutrient transport barriers the plant needs to be “tricked” in order to uptake, transport or accumulate higher concentrations of these nutrients. Also, caution must be made, because in certain instances, accumulating higher levels of a particular element may have negative side effects to the plant food and to the ultimate consumer due to accumulation of undesirable metals (Park et al., 2005).

3.2. Increasing nutrient bioavailability

From a nutritional standpoint, bioavailability is a term that designates the quantity or fraction of an ingested dose that is actually absorbed by the human gut (Heaney, 2001), and it can be a target for gene manipulation in biofortification programs (fourth step; Fig. 1). Several nutrients can interact with each other, modulating their respective absorption rates. Thus, efforts have also been directed toward increasing the concentrations of ‘promoter’ substances (stimulating the absorption of essential mineral elements) and reducing the concentrations of ‘antinutrients’ (interfering with their absorption) of the biofortified crops (White & Broadley, 2009). With regard to the ‘promoter’ substances, the best described ones are certain vitamins, inulin and cystein amino acid. Vitamin E, vitamin D, choline, nicotinic acid and provitamin A can promote the absorption of Se, Ca P, Fe and Zn, methionine and tryptophan (Brinch-Pedersen, Borg, Tauris, & Holm, 2007). Several transgenic approaches have been successful at enhancing the vitamin content of plant foods. One example is over-expression of a wheat dehydroascorbate reductase (DHAR) in maize and tobacco (Chen, Young, Ling, Chang, & Gallie, 2003), leading to an increase of ascorbic acid levels by two-to-four-fold. For a comprehensive review on this topic please see DellaPenna and Pogson (2006). The role of inulin in enhancing Fe absorption is controversial, as certain clinical trials did not show an increase in Fe absorption in women with low Fe status (Petry, Egli, Chassard, Lacroix, & Hurrell, 2012) or in the porcine model (Patterson et al., 2009). Short-chain fatty acids produced during fermentation of fructans and inulin can lower intestinal lumen pH and improve mineral solubility and absorption. In cereals, the fructan level in maize kernels was increased 9-fold via expression of the gene SacB from Bacillus amyloliquefaciens (Caimi, McCole, Klein, & Kerr, 1996).

Concerning the ‘antinutrients’ the most well-known are phytate, certain polyphenols and oxalate (for review, please see White & Broadley, 2009). Phytate is a highly indigestible compound (for all monogastric animals) and is considered the most deleterious absorption factor for mineral in cereals and legumes (Bouis, 2000). The concentrations of phytate can be very diverse between plant foods, and even within cultivars of the same plant species (Devappa, Makkar, & Becker, 2012), allowing low-phytate germplasm screenings. This strategy has been successful at identifying wheat lines with low phytate concentrations (Shewry & Ward, 2012). Another strategy, based on manipulating the biosynthesis of phytate via mutation of a myo-inositol kinase (MIK) gene, has successfully produced rice with lower phytate concentrations (Shi, Wang, Hazebroek, Ertl, & Harp, 2005). A third and final strategy has been to overexpress phytase – a phytase degrading enzyme (Brinch-Pedersen, Sørensen, & Holm, 2002) – in edible tissues. A number of studies have introduced microbiological phytase genes in diverse plant foods such as tobacco (Ullah, Sethumadhavan, Mullaney, Ziegelhofer, & Austin-Phillips, 1999), soybean (Denbow et al., 1998) or rice (Lucca, Hurrell, & Potrykus, 2001). Cysteine and cysteine-containing peptides have the ability to enhance the absorption of non-haem Fe in man. Lucca et al. (2001) transformed rice with an endogenous cysteine-rich metallothionine-like gene (rgMT), alongside a phytase from Aspergillus niger and the ferritin gene from Phaseolus vulgaris, under an endosperm specific promoter, and obtained complete degradation of phytate in the seeds.

Polyphenols are a group of chemical substances found in plants foods characterized by the presence of more than one phenol unit or building block per molecule. More than 4000 flavonoids have been identified (Chang, Luo, & He, 2009), and fruits, vegetables, and beverages are the main sources of phenolic compounds in the human diet (Andjelković et al., 2006). The polyphenol levels of cereal seeds have been reduced via incubation with polyphenol oxidase which, when combined with a phytase-mediated phytate reduction, has shown to significantly increase the availability of Fe (Matuschek, Towo, & Svanberg, 2001). In spite of the negative impact of the ‘antinutrients’ on the absorption of essential mineral elements care should be made while modulating these substances, as certain ‘antinutrients’ have important beneficial roles in human health. It is also the case of fiber, which has become an increasingly significant area of controversy (Dakhar, Anajwala, & Selote, 2012). Dietary fiber is an important element in human diet which is present in plant foods. However, it acts as an ‘antinutrient’ by binding minerals and preventing their absorption. To our knowledge, there is no plant with GE fiber concentration for increased nutrient bioavailability, but recently whole-genome shotgun sequencing (WGS) was performed in flax (Linum usitatissimum), an ancient crop that is widely cultivated as a source of fiber (Wang et al., 2012). This will certainly accelerate crop improvement and stimulate further studies that aim at altering fiber content of plant foods.

4. ‘Omics’ technologies as biofortification tools

Understanding the functional connections between genes, transcripts, proteins, metabolites and nutrients is one of biology’s greatest challenges in the postgenomic era, and recent technological advances are allowing great advances in this area. This knowledge will highly benefit the plant biofortification processes. Fig. 2 shows a schematic representation of the various technologies associated with the well-developed ‘omics’ and the newly emerging systems biology. The integration of this knowledge can be used to help defining the appropriate biofortification strategies. However, the environment at
which the plant will ultimately be grown (with special focus on mineral availability and concentration in the soil), the plant tissue that needs to be targeted (e.g., grain, leaf, tuber), and the developmental stage of the plant (as nutrient uptake is a cumulative process over time and is likely to be influenced by tissue age) are of utmost importance when defining the most suitable biofortification tools.

As seen before, nutrient storage in distinct plant organs such as roots, leaves, or seeds is regulated by a specific subset of genes which control whole plant concentrations for numerous elements. The field of genomics has greatly contributed to this information, as it has identified the genes and genomes of several plant foods. It is also clear from the descriptions above that some genes are responsible for the uptake and transport of more than one mineral, affecting the mineral homoeostasis in a coordinated way. Therefore, alternate tools for expediting the prioritization of gene candidates that control elemental accumulation in plants are desirable (Conn, Berninger, Broadley, & Gilliham, 2012). One of such methods relies on germplasm screening, and defines the available genetic variation. Germplasm variations occurring among and within natural populations of a given plant species can be used as an important tool for gene discovery (Karley & White, 2009).

Identifying the genes that regulate nutrient uptake, transport, storage and bioavailability (described on Section 3) has been a challenging and lengthy procedure, commonly involving large natural breeding populations or screens of laboratory-induced mutants, and individual gene functional analysis. For this reason, the field of high throughput sequencing/transcriptomics has been pivotal in accelerating gene discovery. It provides a non-targeted, full spectrum analysis of all the genes expressed by a plant’s tissue at a given time point, and commonly used technologies include: 454 pyrosequencing, that gave the transcriptome and contributed greatly to gene identification in wheat (Lai et al., 2012); microarray, which has been used to understand Fe nutrition mechanisms in soybean (O’Rourke et al., 2009); serial analysis of gene expression (SAGE), which was used to study gene expression in the barley (Hordeum vulgare) (White et al., 2006); suppression subtractive hybridization (SSH), which has identified mineral upregulated genes in rice (Sperotto et al., 2009); and macroarray technology, which has been used to identify Fe-related genes also in rice (Narayanan, Vasconcelos, & Grusak, 2007).

The field of proteomics has helped researchers understand the effects of proteins on plant mineral nutrient homeostasis. It seeks to monitor the protein fluctuations under variable developmental and environmental influences, as programmed by the genome, and mediated by the transcriptome. Consequently, it is necessary to measure changes in protein abundance and turnover rate as faithfully as possible. A recent proteomics technology termed iTRAQ (isobaric tags for relative and absolute quantification)-based quantitative proteomics analyzed the microsomal proteins from Arabidopsis roots and 521 proteins were identified (Fukao et al., 2011). Other more routinely used technologies such as SDS-PAGE can be used in proteomics studies that are useful for plant biofortification programs (Fig. 2) (for a review on this topic please see Kussman, Panchaud, & Affolter, 2010).

Allied to these the ‘omics’ technologies (genomics, transcriptomics and proteomics), metabolomics is also an emerging and required science. A better understanding of the pathways responsible for the biosynthesis of nutritionally relevant metabolites is key to gaining more effective control of the absence/level of presence of such components in our food (Hall, Brouwer, & Fitzgerald, 2008). Plants are very rich in chemically diverse metabolites, which are usually present in a large range of concentrations (De Vos et al., 2007), and no single analytical method is currently capable of extracting and detecting all metabolites. However, different extraction and detection techniques, such as gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and nuclear magnetic resonance (NMR) can be utilized.

Fig. 2. Schematic representation of the most commonly used techniques associated with each ‘omics’ discipline, and the importance of integrating environment, plant tissue, and developmental stage data when defining plant biofortification strategies.

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The proteins identified via proteomics will commonly represent transporters and enzymes involved in mineral uptake and transport. It is important to take into account that plants require at least 17 elements for proper growth and development and that there are 92 elements identified on earth (Karley & White, 2009). Ionomics studies all these elements and identifies the mechanisms that coordinately regulate these elements in response to genetic and environmental factors (reviewed in Salt, Baxter, & Lahner, 2008; Williams & Salt, 2009). To conduct this holistic analysis of mineral changes in the plant, and in the environment, one must use specialized techniques that allow the determination of numerous elements, preferably with a small amount of sample. Two of the best known and most utilized techniques are high-throughput inductively coupled plasma optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS). Recently, ICP-OES has been used to analyse the mineral partitioning and remobilization in rice plants grown under different Fe concentrations (Sperotto, Vasconcelos, Grusk, & Fett, 2012). The authors suggested that a continued uptake and translocation of mineral nutrients from the roots during seed fill are perhaps more important than mineral remobilization. Buescher et al. (2010) utilized ICP-MS to analyze the concentrations of 17 different elements in Arabidopsis grown in several environments using ICP-MS and the authors found significant differences between accessions for most elements. ‘Omics’ research has started generating data at a pace never seen before. A big constraint that is currently posed to researchers worldwide is the lack of computer power to analyze their data. With this problem in mind, one of the most recent solutions is called cloud computing (Shanker, 2012), which consists in making better use of multiple computers and processors at a time via internet. Additionally, in order to interpret this increasingly amount of data, each ‘omic’ discipline uses its own set of bioinformatics software tools, either proprietary or freely available. ExPASy (http://www.expasy.org/) provides access to a compilation of scientific databases and bioinformatic tools in different ‘omic’ areas such as genomics, transcriptomics, proteomics or systems biology. As an example, sequencing and transcriptomic studies rely on tools such as MIRA (Chevreux et al., 2004), Myrna (Langmead, Hansen, & Leek, 2010), MG-RAST (Meyer et al., 2008) or Blast2Go (ZBG) (Conesa et al., 2005), whereas proteomic studies frequently utilize InterPro (Hunter et al., 2008) and Mascot (Perkins, Pappin, Creasy, & Cottrell, 1999).

Systems biology aims at integrating the data from the individual ‘omics’ sciences, in a concatenated way (Fig. 2). With the fast development in these research fields we expect that in the near future a successful biofortification strategy should utilize this type of approach, because it enables one to predict the secondary effects from manipulating a particular metabolic pathway. To study the interactions among metabolites, proteins and transcripts the data generated has to be pre-processed to achieve normalization and scaling of the multidimensional ‘omics’ data. This step is necessary since data originating from different ‘omic’ techniques have different formats and scales (multidimensional), therefore needing normalization before any analysis can be made. After pre-processing, data can be statistically analyzed. Particularly, correlation analysis and network visualization can be made using available tools such as MapMan (http://mapman.gabipd.org/web/guest/home), R (http://www.r-project.org/), and KEGG (Winter & Huber, 2010) which will allowing emphasizing links between and within the different types of data.

5. Socioeconomic considerations of plant-based biofortified foods

The global importance of MNM (described in the Introduction) has attracted the attention of the nutrition community and has also become a mandate among development agencies and governments. Possible ways of mitigating MNM is to increase dietary diversification, mineral supplementation, food fortification and increasing the concentration and bioavailability of plant-based foods. The first three approaches have not always been successful (White & Broadley, 2009). Since changing people’s diet is something difficult to achieve, the concept that the fortification process must take place in the field rather than in the factory was well received (Winkler, 2011). As mentioned earlier, many international biofortification programs to enrich the nutrient content of plant-based foods are being conducted. The main targets of these programs are clearly the staple crops in order to reduce MNM in developing countries. However, the benefits of plant-food biofortification can be extended beyond this target audience to a worldwide scale, to increase health and prevent diseases—assuming that these added value food products are accepted and distributed to the market at competitive prices.

The cost effectiveness of biofortification process has been calculated for a number of target countries and crops (e.g. Neidecker-Gonzales, Nestel, & Bouis, 2007; Stein, Sachdev, & Qaim, 2006). In all circumstances, the costs of biofortification represent only a small fraction of a classical public health intervention via supplementation—with the costs of the conventional breeding being significantly lower than the ones of the genetic engineering approach. From the farmer point of view, the major biofortification programs promise to supply their seeds free of charge to the governments of developing countries for distributing them through the national system. Moreover, after the initial distribution phase, the use of biofortified crops requires little more than the costs for reliable seed production (Mayer et al., 2008).

In spite of the clear positive effects of the biofortification approach in developed countries, demand for any biofortified plant-food must be strong enough to drive the product through a complex process with several stages (discovery, development, dissemination and outcomes; Winkler, 2011), and the health benefits of the foods must be readily apparent to the consumers. The controversy that has surrounded the transgenic plants, especially in Europe in the late 90s, and regulatory hurdles associated with this technology make commercial applications more difficult (Johnson, Raybould, Hudson, & Poppy, 2007). There are marked differences in the regulation of GE plant-foods between countries. For instance, the European Union, Australia, China, and other countries require GMO labeling (genetically modified organisms), while in the United States the bio-technology labeling is not mandatory. The main risks that have been discussed are related to the potential negative impact on the environment and on public health (Hunt, 2004). According to the ‘Cartagena Protocol on Biosafety’ one should follow the ‘precaution’ principle. Therefore, the experimental parameters used to test GE plant-foods should be similar to the clinical trials performed with novel pharmacological agents. Thus, if the GE plant-foods want to penetrate into new markets, information from neutral sources or information from a variety of sources needs to be provided to the consumers. Additionally, the safety of this type of foods should be tested by independent researchers. The profiling techniques mentioned in the previous section (transcriptomics, proteomics, and metabolomics) have been increasingly applied to the analysis of GE crop plants with regard to test their food safety and nutritional equivalence. In a recent literature review survey on 44 recent “omic” comparisons between GE and non-GE crop lines, the authors concluded that none of the published “omic” assessments raised safety concerns about GE cultivars (Ricroch, Bergé, & Kuntz, 2011).

Interactions with other nutrients in the plant matrices, potential allergic responses of the consumer, and alterations of plant stress responses are some of the parameters that need to be determined (Hirschi, 2009). However, the results from Ricroch et al. (2011) show that genetic engineering has less impact on the expression of RNA or on protein and metabolite levels than conventional breeding or environmental conditions (e.g. drought). Measuring the ionome (i.e. the mineral nutrients and trace elements found in the biological tissues) can be an important tool when analyzing the effectiveness of the plant biofortification strategy, and could be used as a validation tool in biofortification programs.
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