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## Biofortification of Edible Plants

Set the Stage for Better Nutrition

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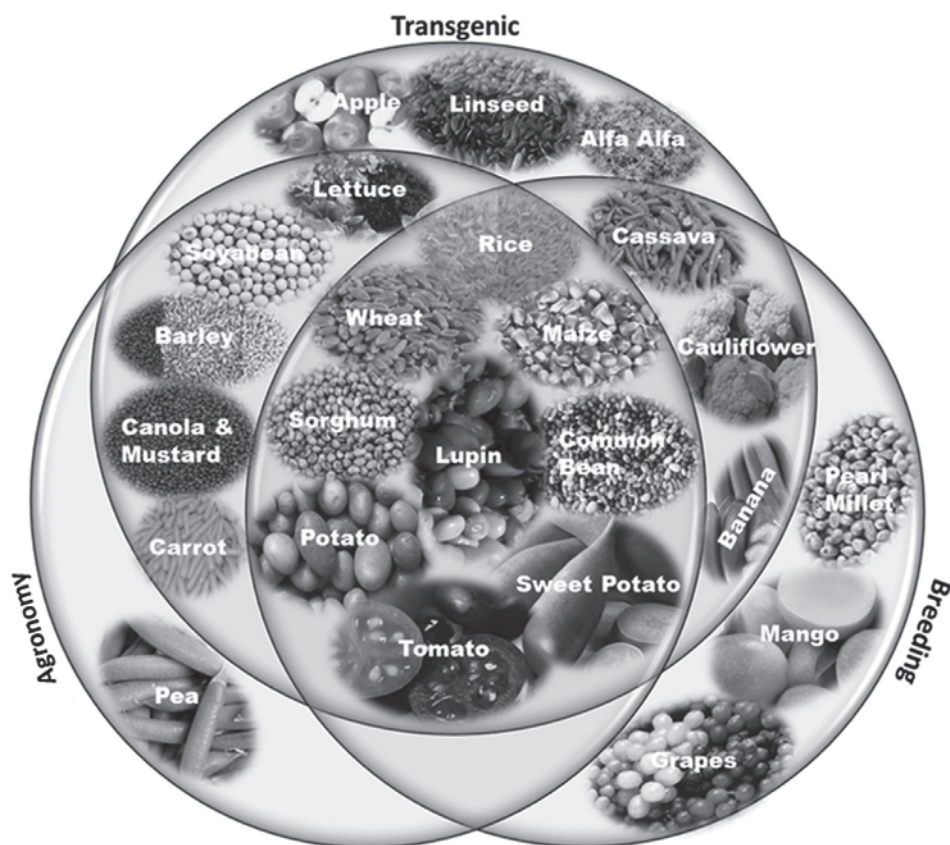
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### 1.1 Introduction

The human organism requires a large number of organic and mineral nutrients that are crucial for the growth, development, and the prevention of diseases and disorders. These nutrients, required at relatively high levels – macronutrients – or low to very low levels – micronutrients – are supplied either by our plant or animal food intakes. However, often the daily diet is balanced in calories and quantity, but does not provide the required amount of these nutrients, leading to malnutrition. Consequently, this malnutrition – or nutrients deficiency – can lead to a variety of metabolic and health problems such as digestive problems (Brodeur et al. 1993), skin disorders (Prendiville and Manfredi 1992), stunted or defective bone growth (Branca and Ferrari 2002) ... etc.

To compensate these low levels of nutrients in plants, biofortification of edible plants is becoming one of the most efficient strategies to overcome malnutrition, and many foods such as cereals, fruits, and vegetables are being fortified with nutrients that are needed to prevent mineral and vitamin deficiencies (Nestel et al. 2006).

From ancient times, agriculture has been the primary source of food and all nutrients for human, and the production systems have been subject to numerous changes to ensure quantitative and qualitative food supplies. With human development, the Industrial Revolution, and then the Green Revolution, food and nutrition turned to agriculture and agro-processing as a primary mean to mitigate, if not eradicating, nutrient deficiencies and malnutrition (Welch 2005). On the other hand, the development of novel life science technologies and biofortification of edible plants is regarded as a powerful tool to reduce malnutrition and improve dietary intake of essential minerals and vitamins in staple foods (Figure 1.1). New discoveries in biochemistry and molecular biology have led to the incredible development of advanced biotechnology and great promises for improving the output of bioavailable micronutrients from agricultural systems (Welch 2005). However, we need to pragmatically assess whether the biofortification is compatible with the diet diversification



**Figure 1.1** Biofortified crops generated by different approaches: transgenic, agronomic, and breeding. Staple cereals, most common vegetables, beans, and fruits have been targeted by all three approaches. Some crops have been targeted by only one or two approaches depending on its significance and prevalence in the daily human diet. (From Garg et al. 2018. Open access publication under Creative Commons Attribution License [CC BY] terms with free permission).

and how it might impact agricultural biodiversity for long-term sustainability (Bouis and Welch 2010; Johns and Eyzaguirre 2007).

This chapter provides a general overview of the different approaches and opportunities for the biofortification of edible plants to achieve the goal of improving human diet and setting the stage for the eradication or alleviation of malnutrition through sustainable agriculture.

## 1.2 Biofortification and Nutrition

To alleviate nutritional issues and nutrient deficiencies, biofortification of edible plants is considered the most appropriate approach. Modifying dietary customs of the population runs into likely resistance from communities. By contrast, biofortification focuses on

improving the nutritional content of region's current agricultural biodiversity, preserving its habits and customs (Johns and Eyzaguirre 2007).

Biofortification of food crops thus has the potential of reaching all the population and communities, particularly rural poor and vulnerable ones with no or limited access to industrially biofortified foods, or where conventional biofortification is difficult or cannot be implemented for technical, economic, or social reasons, and where large quantities of staple rather than nutrition foods crops is consumed.

In recent decades, action has been taken to address malnutrition and micronutrient intakes issues in developing countries. Biofortification technology has been identified as a priority initiative; however, it became evident that this technology might also benefit developed countries consumers as well. Therefore, biofortification became more common in developing countries and evidence of malnutrition mitigation nutrient, bioavailability enhancement and biofortified crops acceptability, are raising more interests. Recent data are showing that 150 different varieties of biofortified crops belonging to 10 different crop species have been released in 30 different countries, of which 27 are developing countries, while 12 other crop species are under evaluation for release in 21 other different countries (Bouis and Saltzman 2017; Bouis et al. 2006; Global Panel 2015). In this regard, many examples can be cited, and some of the studies have confirmed the nutritional value and cost-effectiveness of high-iron bean, orange-flesh sweet potato, cassava, maize, rice, and pearl millet (Global Panel 2015).

Vitamin A deficiency (VAD) is the most prevalent nutrient deficiency in young children in the developing countries, with more than 200 million children under the age five worldwide (WHO 2009). VAD has been rated as the first public health problem in more than 70 countries, and this deficiency affects about 33% of children aged between six months and five years in 2013, with 48% found in sub-Saharan Africa and 44% in South Asia (WHO 2009). In this regard, different studies showed the role of biofortified crops in alleviating VAD deficiency and improving the nutritional status of the population. In developing countries, a study carried out in sub-Saharan Africa showed that the daily vitamin A needs of young children can be covered by the intake of 100 g of orange-fleshed sweet potato (OFSP), a carotenoid-biofortified tuber (Low et al. 2017). Similar observation was noted in Zambia, where inadequate vitamin A intake prevalence was reduced by 3% (Lividini and Fiedler 2015), and diarrhea was reduced by biofortified crops as well (Jones and de Brauw 2015).

Mineral deficiencies are also a major concern, and studies have shown that biofortification might be one of the most cost-effective approaches in alleviating this public health issue (Broadley et al. 2008, 2009). Using Zn-biofortified wheat, valuable increases in Zn absorption have been achieved (Rosado et al. 2009). Feeding two-year-olds with zinc and iron biofortified pearl millet more than adequately enhanced the absorption of both minerals to meet the dietary requirement (Kodkany et al. 2013), and the iron status of schoolchildren (12 to 16 years old) and women fed iron-biofortified beans and pearl millet was significantly improved (Finkelstein et al. 2015; Haas et al. 2016).

Nevertheless, assessment of the efficacy of biofortified foods for enhancing human nutritional status and alleviating malnutrition requires further research in the laboratory,

as well as community-based trials. Research should consider the impacts of biofortified crops for larger groups of different gender and age and for long-term consumption (Bouis and Saltzman 2017). Furthermore, additional research is required to assess the nutrients bioavailability of various genotypes of biofortified crops, in particular genetic engineered crops, using different in-vitro and/or in-vivo tests. Additionally, trials need to be carried out to evaluate the agricultural, environmental, and socioeconomic impacts, and these trials must include the communities, the stakeholders, and the policy makers as well (King 2002).

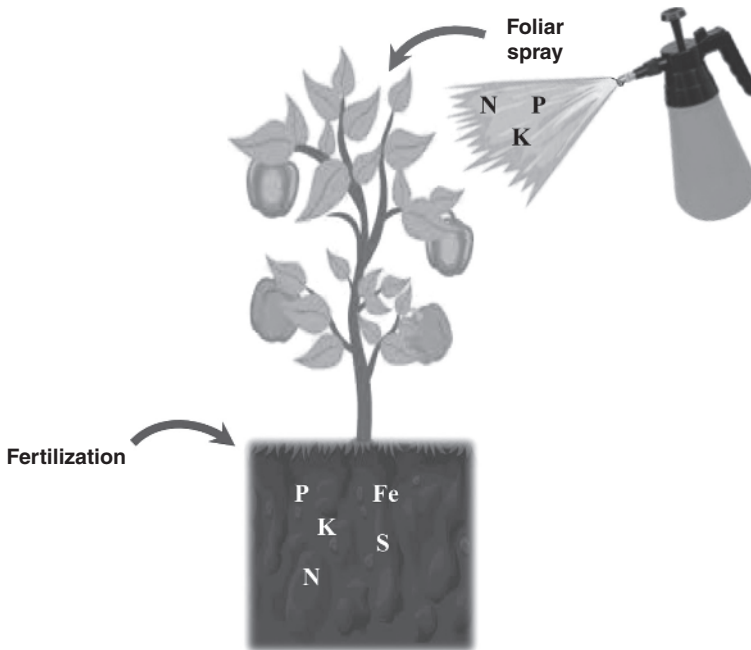
Biofortification of food plants also causes potential alterations of the plants metabolism, and these alterations should be thoroughly assessed beyond the few studies that have already analyzed these alterations. The alteration of these different metabolic pathways might affect growth, development, and productivity of these plants, and it is imperative to determine to what extent these alterations can be minimized or even avoided. However, recent development in omics, particularly metabolomics and related techniques, is significantly contributing to deciphering the potential alterations in plants caused by biofortification (Hall et al. 2008).

### 1.3 Cultural Practices and Plants Biofortification

Agricultural practices are more likely the simplest and readily most accessible to farmers to overcome the problem of nutrients deficiency of edible plants, and these agronomic-based strategies might be interesting alternative solutions. Indeed, agronomic-based strategies have shown be efficient in improving nutrient contents by many folds. Although the application methods differ, soil fertilization and/or foliar application of fertilizers at different stages of plant growth have shown to increase the nutrients levels of many crops, and fertilization as agronomic strategy for plants biofortification is considered to be a good and effective way (Mao et al. 2014; Zhao and Shewry 2011) (Figure 1.2) (Table 1.1). Another strategy consisting of intercropping between dicots and gramineous species to increase mineral contents of crops (Zuo and Zhang 2009).

Worldwide, iron (Fe) is the most prevalent deficient nutrient. Different attempts to increase iron contents of food crops have been conducted, and the results have been encouraging. The application of iron fertilizers, either inorganic or chelated forms, did not show any Fe increase in cereal. However, improved nitrogen nutrition of plants increased Fe content (Aciksoz et al. 2011; Cakmak 2012; Cakmak et al. 2010), and this might be due to the contrasting abilities of cereals to acquire Fe because the phyto-siderophores chemistry is species-specific and determines iron absorption by plants (Bashir et al. 2006). Similarly, foliar application of Fe-fertilizers had shown no or little positive effect on grain-Fe content (Aciksoz et al. 2011); however, when urea (Aciksoz et al. 2011) or boron (Jin et al. 2008) were incorporated to foliar fertilizers, grain Fe concentration was increased by threefold. Hence, nitrogen plant status deserves special attention in Fe-crops biofortification (for further details, see Chapter 6).

Zinc, which is often associated to iron, is the most ubiquitous micronutrient deficiency issue in plants, and this deficiency is to a large extent caused by soils factors



**Figure 1.2** Foliar application of mineral solution for corps biofortification.

including Zn deficiency (Alloway 2009; Cakmak 2008). Many plants, particularly cereals, might be fortified with Zn using agronomic approaches. Trials showed that foliar application of  $\text{ZnSO}_4$  to wheat crop increased grain Zn concentration by threefold (Cakmak 2008). However, trials on Zn fertilization did not increase the concentration of Zn in rice grain (Wissuwa et al. 2008). Other studies showed that the application of inorganic Zn salts ameliorates crops Zn deficiency (Takkur and Walker 1993), while when high Zn concentrations in grains are desired, studies showed that soil fertilization combined with foliar application is the most effective method of Zn application (Yilmaz et al. 1997).

These discrepancies between the different studies on Zn fertilization and Zn biofortification of plants might be explained by the zinc chemistry and its behaviour in soils, its concentration, soil pH, calcite and inorganic matter, and the concentration of other minerals such as Na, Ca, Mg (Alloway 2009) (for details, see Chapter 10).

By adding a small amount of selenium (Se) to fertilizers, its concentration was increased in many plants in Finland, which was the first country to adopt this agronomic approach (Hartikainen 2005), and a similar strategy was adopted in the UK, where Se concentration in crops was increased by about tenfold (Broadley et al. 2010). Addition to iodine (I) to fertilizers also showed that plant can be fortified with higher concentration of iodine. Trials iodine fertigation (iodination of irrigation water) increased significantly iodine intake through foods intake (Cao et al. 1994; DeLong et al. 1997).

**Table 1.1** Studies of crops biofortification using agronomic approaches.

Class	Crops	Nutrients	References	
Cereals	Rice	Iron	Fang et al. (2008), He et al. (2013), Wei et al. (2012a), Yuan et al. (2013)	
		Zinc	Boonchuay et al. (2013), Fang et al. (2008), Guo et al. (2016), Jiang et al. (2008), Mabesa et al. (2013), Ram et al. (2016), Shivay et al. (2008, 2015), Wei et al. (2012b)	
		Selenium	Chen et al. (2002), Fang et al. (2008), Giacosa et al. (2014), Liu and Gu (2009), Premarathna et al. (2012), Ros et al. (2016), Xu and Hu (2004)	
	Wheat	Iron	Aciksoz et al. (2011)	
		Zinc	Cakmak and Kutman (2018), Cakmak et al. (2010), Yang et al. (2011)	
		Selenium	Aro et al. (1995)	
	Maize	Zinc	Alvarez and Rico (2003), Fahad et al. (2015), Lopez-Valdivia et al. (2002), Wang et al. (2012), Zhang et al. (2013)	
	Legumes	Soybean	Selenium	Yang et al. (2003)
		Chickpea	Zinc	Shivay et al. (2015)
Selenium			Poblaciones et al. (2014)	
Pea		Iron	Poblaciones and Rengel (2016)	
Common Bean		Zinc	Ibrahim and Ramadan (2015), Ram et al. (2016)	
Vegetables	Potato	Iron	Márquez-Quiroz et al. (2015)	
		Zinc	White et al. (2012b)	
	Sweet Potato	Selenium	Cuderman et al. (2008), Poggi et al. (2000)	
		$\beta$ -carotene	Laurie et al. (2012)	
	Carrot	Iodine	Smolen et al. (2016)	
		Selenium	Smolen et al. (2014)	
	Lettuce	Iodine	Smolen et al. (2014)	
		Selenium	Carvalho et al. (2003), Smolen et al. (2014)	
Fruits	Tomato	Iodine	Landini et al. (2011)	

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## 1.4 Conventional Breeding and Crops Biofortification

For a long time, plant breeding was limited in crossing individuals of interesting and targeted traits and thereafter selecting the interesting varieties. This approach changed with the discovery of the genetic and the sexual propagation of plants. The twentieth century has seen the introduction of hybrid technology and boosted breeding of many crops (Poletti and Sautter 2005). Vitamins and micronutrients biofortification of crops through breeding

has been considered for decades (Graham et al. 1998, 1999). This strategy is based on two different approaches:

- 1) Explore the genetic diversity of the existing species by identifying the parental genotypes potentially interesting for crosses.
- 2) Identify the existing varieties or germplasms (Welch and Graham 2004).

However, and before contemplating this strategy, some criteria should be considered. Among these breeding criteria, the first to consider is the productivity or the yield of the bred crop (Bouis 1996; Graham et al. 1998), the biofortification level, the stability of the enriched mineral or vitamin across cropping, the varying cropping and environmental conditions, and the nutritional bioavailability of the nutrient (Ortiz-Monasterio et al. 2007; Welch and Graham 2004).

Many attempts have been successful in increasing vitamins and minerals by conventional breeding, including increasing the concentration of  $\beta$ -carotene and carotenoids (Champagne et al. 2013; Pixley et al. 2013; Suwarno et al. 2014), iron and zinc (Pixley et al. 2011; Velu et al. 2007), as well as other minerals such as selenium (Graham et al. 1999, 2005). However, crops biofortification through breeding has shown some limits and constraints. One example is the inverse correlation noted between the increase of iron and zinc content of crops and the yield due to a dilution effect caused by enhanced starch content (Table 1.2) (Bänzinger and Long 2000).

Indeed, crop breeding for biofortification has targeted widely used staple food like maize, cassava, sweet potato, banana, and some legumes. Vitamin A-rich orange sweet potato is likely the most successful development in biofortification resulting from crop breeding. Although many food crops bred, especially fruits, are providing sufficient levels of nutrients to targeted populations, a greater emphasis is being laid on transgenic research. However, breeding is much accepted by the greater consumers compared to transgenic crops.

Unfortunately, crops biofortification through conventional breeding have known limited success in general, because this approach requires years to achieve significant enhancement in adapted varieties. The absence of key vitamins and minerals in many crops reflects the fact that the corresponding metabolic pathways are absent, truncated, or inhibited in the targeted species. Therefore, it is obvious that in order to enhance the biosynthesis vitamins pathways and minerals accumulation, genes encoding key enzymes of vitamins biosynthesis and minerals accumulation should be introduced using transgenic methods (Christou and Twyman 2004; Khan et al. 2013; Zhu et al. 2007).

## 1.5 Molecular Engineering and Crops Biofortification

During the last few decades, molecular engineering has entered an exciting phase of rapid development and discovery. More generally, genomics tools have been and are still being developed and applied to improve plant crops for human benefit. Most of the edible crops are now subject of in-depth molecular engineering investigations driven by a need for support of biofortification programmes to enhance nutritional quality of food-plants. Indeed, biofortification of crops using genetic techniques focuses on genes with

**Table 1.2** Studies of crops biofortification using conventional breeding approaches.

Class	Crops	Nutrients	References
Cereals	Rice	Iron	Sperotto et al. (2012)
		Zinc	Gregorio et al. (2000)
	Wheat	Iron	Cakmak et al. (1999, 2004), Monasterio and Graham (2000), Welch et al. (2005)
		Zinc	Velu et al. (2014)
	Orange Maize	Vitamin A, Provitamin A and Carotenoids	Maqbool et al. (2018), Palmer et al. (2016), Pixley et al. (2013)
		Vitamin E	Goffman and Böhme (2001), Muzhingi et al. (2016)
	Sorghum	Iron	Reddy et al. (2005)
		Zinc	Reddy et al. (2005)
		$\beta$ -carotene	Reddy et al. (2005)
	Millet	Iron	Rai et al. (2012), Velu et al. (2007)
Zinc		Rai et al. (2012), Velu et al. (2007)	
Legumes	Lentils	Iron	Kumar et al. (2016)
		Zinc	Kumar et al. (2016)
	Cow pea	Iron	Santos and Boiteux (2013)
		Zinc	Santos and Boiteux (2013)
	Bean	Iron	Beebe et al. (2000), Gelin et al. (2007), Hoppler et al. (2014)
		Zinc	Beebe et al. (2000), Blair et al. (2009), Gelin et al. (2007)
Vegetables	Potato	Iron	Brown et al. (2010), Burgos et al. (2007), Haynes et al. (2012)
		Zinc	Brown et al. (2010), Burgos et al. (2007), Haynes et al. (2012)
		Copper	Haynes et al. (2012)
		Manganese	Haynes et al. (2012)
	Sweet Potato	See Chapter 4	
	Cauliflower	$\beta$ -carotene	Muthukumar (2016)
	Cassava	$\beta$ -carotene	Chavez et al. (2005), Maziya- Dixon et al. (2000), Peninah et al. (2014)
		Carotene	
		Iron	Chavez et al. (2005), Maziya- Dixon et al. (2000)

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different expression patterns targeting for development into breeding new varieties of crops with greatest levels of nutrients. Moreover, the efficiency in genetic techniques has been enhanced through the use of diagnostic DNA-based markers to develop biofortified crops and the approach of using modern technologies might be viewed as one of the key decisions that breeders make concerning the objectives of biofortification programmes to achieve this goal. The genetic transformation of food-plants is considered a faster method to achieve the nutritional improvement of crops, and the transgenic approaches might be a good and more realistic alternative, particularly where breeding approaches have not been successful or to overcome its limitation (Brinch-Pedersen et al. 2006; Kumari et al. 2014; Zhu et al. 2007). Consequently, the number and genetically modified and biofortified crops with different vitamins and minerals is much larger compared to the number of biofortified crops using agronomic or conventional breeding. Furthermore, spectacular advances in genes and genotyping had led at dissecting in depth the architecture of important traits providing the most advanced genomic platforms and analysis methods available for food-plants. These techniques combined to new advances in genomic research have led to unprecedented access into the structure and function of crop genomes.

From a genetic engineering perspective, different transgenic crops have been developed because of the identification of the respective genes encoding the biosynthesis of vitamins and minerals accumulation in plants (Table 1.3) (White et al. 2012a,b; Zhang et al. 2009). For example, many cereals have been successfully biofortified, such as  $\beta$ -carotene, vitamin B<sub>9</sub> (folate), iron and zinc biofortified rice, high provitamin A and iron content wheat, provitamin A, vitamin E, ascorbic acid and iron biofortified maize, high zinc content barley, and  $\beta$ -carotene biofortified sorghum. Furthermore, many other legumes, fruits, and vegetables have been biofortified with minerals and vitamins using transgenic approaches (Table 1.4).

Nevertheless, crops biofortification using transgenic approaches is constrained by two limitations. The first limitation is the knowledge gap in the genes' functions and their interaction with the environment, and without this knowledge the transgenic transformation of plants remains still limited to some nutrients or vitamins and to some species as well. The second and perhaps more constraining limitation is the regulatory issues restricting the development and commercialization of transgenic biofortified crops (Johnson et al. 2007; Powell 2007; Ramessar et al. 2009). Moreover, in order to select appropriate crops for minerals and vitamins biofortification using transgenic transformation, two criteria are essential. First, the selected crop for transformation should be of large consumption and economically interesting. Second, the accumulation of the targeted nutrient should not limit the accumulation of another nutrient nor the physiology and development of the crop.

## 1.6 Conclusions and Future Perspective

Conclusively, biofortification of edible crops for food is considered the most useful and efficient approach to supplement diets and alleviate malnutrition. Agricultural research, both fundamental and applied, is aimed at developing biofortified crops. The relationship between diet and health has long been demonstrated and numerous scientific studies indicate that food components affect our body and health by influencing the physiological

**Table 1.3** Studies in which chromosomal loci (QTL) have been identified in crop plants that affect the concentrations of vitamins and essential mineral elements most commonly lacking in human diets.

Crop species	Tissue	Elements	References
Rice ( <i>Oryza sativa</i> )	Grain	Fe, Zn	Garcia-Oliveira et al. (2009), Gregorio et al. (2000), Lu et al. (2008), Norton et al. (2010), Stangoulis et al. (2007), Zhang et al. (2011)
		Provitamin A	Paine et al. (2005)
Oilseed rape ( <i>Brassica napus</i> )	Seed	Fe, Zn	Ding et al. (2010)
Potato ( <i>Solanum tuberosum</i> )	Tuber	Fe, Zn	Subramanian (2012)
		Carotenoids	Ducreux et al. (2005), Diretto et al. (2007)
Sweetpotato	Tuber	$\beta$ -carotene	Cervantes-Flores (2006)
Cassava	Root	Carotenoids	Welch et al. (2010)
Cauliflower ( <i>Brassica oleracea</i> )	Leaf	Zn	Broadley et al. (2010)
		Carotenoids	Crisp et al. (1975), Dickson et al. (1988), Li et al. (2001)
Canola ( <i>Brassica napus</i> )	Seed	Carotenoids	Fujisawa et al. (2009), Ravanello et al. (2003)
<i>Brassica rapa</i>	Leaf	Fe, Zn	Wu et al. (2008)
Wheat ( <i>Triticum</i> spp.)	Grain	Fe, Zn	Distelfeld et al. (2007), Genc et al. (2009), Peleg et al. (2009), Shi et al. (2008)
Barley ( <i>Hordeum vulgare</i> )	Grain	Zn	Lonergan et al. (2009), Sadeghzadeh et al. (2009), Zeng et al. (2016)
Maize ( <i>Zea mays</i> )	Grain	Fe, Zn	Jin et al. (2013), Lung'aho et al. (2011), Simić et al. (2012)
		Carotenoid Provitamin A	Burt et al. (2011), Chandler et al. (2013), Harjes et al. (2008)
Bean ( <i>Phaseolus vulgaris</i> )	Seed	Fe, Zn	Beebe et al. (2000), Blair et al. (2009, 2010, 2011), Cichy et al. (2005, 2009), Gelin et al. (2007), Guzman-Maldonado et al. (2003)

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processes. Thus, biofortified crops are of increasing interest in the prevention of malnutrition and its related diseases.

For years, it was thought that plant-based foods simply provide energy and essential nutrients, but medical and nutrition sciences have demonstrated that many other micronutrients are required although some are at low levels. Food crops are known to have different nutritional profiles and the dietary insufficiency of one or more micronutrients, and the deficiency of one or more nutrient remains a major concern in populations that have an unbalanced diet, leading to nutritional deficiencies and metabolic diseases and disorders. Furthermore, most of the plant-based and staple crops consumed in many regions do not contain many essential nutrients or their levels are not sufficient to meet minimum daily intake.

**Table 1.4** Studies of crops biofortification using molecular engineering approaches.

Class	Crops	Nutrients	References
Cereals	Rice	$\beta$ -carotene	Beyer et al. (2002), Burkhardt et al. (1997), Datta et al. (2003), Paine et al. (2005), Ye et al. (2000)
		Vitamin B9	Blancquaert et al. (2015), Storozhenko et al. (2007)
		Iron	Goto et al. (1999), Lee and An (2009), Lee et al. (2012), Lucca et al. (2002), Masuda et al. (2012, 2013), Paul et al. (2012), Takahashi et al. (2001, 2016), Vasconcelos et al. (2003), Wirth et al. (2009), Zheng et al. (2010)
	Wheat	Zinc	Lee and An (2009), Masuda et al. (2008)
		Iron	Borg et al. (2012), Sui et al. (2012)
	Barley	Provitamin A Carotenoids	Cong et al. (2009), Wang et al. (2014)
		Zinc	Ramesh et al. (2004)
	Maize	Provitamin A Carotenoids	Aluru et al. (2008), Decourcelle et al. (2015), Zhu et al. (2007)
		Vitamin E	Cahoon et al. (2003)
		Vitamin C	Chen et al. (2003), Levine et al. (1995)
Legumes	Sorghum	Provitamin A	Lipkie et al. (2013)
	Soybean	$\beta$ -carotene	Kim et al. (2012), Pierce et al. (2015), Schmidt et al. (2015)
		Vitamin E	Van Eenennaam et al. (2003)
Vegetables	Lentils	Manganese	Ates et al. (2018)
		$\beta$ -carotene Zeaxanthin	Diretto et al. (2006), Ducreux et al. (2005), Lopez et al. (2008), Romer et al. (2002), Song et al. (2016), Van Eck et al. (2007)
	Potato	Zinc	Burgos et al. (2007), Brown et al. (2010), Haynes et al. (2012)
		Copper	Haynes et al. (2012)
		Manganese	Haynes et al. (2012)
	Sweet Potato	See Chapter 4	
	Cauliflower	$\beta$ -carotene	Lu et al. (2006)
	Lettuce	Iron	Goto et al. (2000)
	Carrot	Calcium	Morris et al. (2008), Park et al. (2004)
	Cassava	$\beta$ -carotene Provitamin A	Telengech et al. (2015), Welch et al. (2010)
Fruits		Banana	$\beta$ -carotene
	Vitamin A Provitamin A		Davey et al. (2008) Paul et al. (2017)
	Tomato	Folate, $\beta$ -carotene Lycopene Provitamin A Carotenoid	Apel and Bock (2009), Davuluri et al. (2005), Dharmapuri et al. (2002), Enfissi et al. (2005), Fraser et al. (2007), Huang et al. (2013), Rosati et al. (2000), Wurbs et al. (2007)
Oilseeds	Linseed	Carotenoids	Fujisawa et al. (2008)
	Canola	$\beta$ -carotene	Fujisawa et al. (2009), Ravanello et al. (2003), Shewmaker et al. (1999), Wei et al. (2009), Yu et al. (2008)

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Malnutrition and deficiencies or hidden hunger have been significantly alleviated in many regions of the world, particularly in the developing and poor countries as results of programs that aim to fortify food crops. Nevertheless, much remains to be done regarding biofortification of staple food crops in the poor countries because these approaches have not always been successful due of the limited agricultural resources.

Demand for biofortified food will likely increase in the future due to numerous issues faced by the growing population, including the possible effects of climate change on food production and food quality. Therefore, it will be even more important to educate consumers on the benefits of biofortified foods.

Nowadays, the real challenge is no longer the science of biofortification. We know it works and have showed its efficiency in dealing with nutritional deficiencies; but the real challenge is to make biofortified crops common plant-foods for populations at risk, and to do this, rather than modifying their diet, it is more efficient to provide biofortified staple crops. Farmers, stakeholders and policy-makers should thus scale up biofortified crops to reach millions of households through institutional, regulatory, and financial policies.

## Summary

Large number of nutrients are not only required and crucial for the growth and development of our organisms, but they also help in preventing many diseases and disorders. However, often the daily diet is not balanced and does not provide the required amount of these nutrients. To supply adequate nutrients, biofortification of edible plants is becoming one the most efficient strategies to improve daily diet and overcome malnutrition. For the last few decades, crops biofortification technology using different approaches has been identified as a priority initiative and has shown great potential for mitigating malnutrition and enhancing nutrient bioavailability. To improve the nutritional quality of food crops, agronomic, conventional breeding, and molecular engineering approaches have been used. Technically, each approach has its benefits, disadvantages, and constraints. However, to be viable, each approach should be economically feasible, less time consuming, and readily apparent to the consumers.

## References

- Aciksoz, S.B., Yazici, A., Ozturk, L., and Cakmak, I. (2011). Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. *Plant and Soil* 349: 215–225.
- Alloway, B.J. (2009). Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health* 31: 537–548.
- Aluru, M., Xu, Y., Guo, R. et al. (2008). Generation of transgenic maize with enhanced provitamin A content. *Journal of Experimental Botany* 59: 3551–3562.
- Alvarez, J.M. and Rico, M.I. (2003). Effects of zinc complexes on the distribution of zinc in calcareous soil and zinc uptake by maize. *Journal of Agricultural and Food Chemistry* 51: 5760–5767.

- Apel, W. and Bock, R. (2009). Enhancement of carotenoid biosynthesis in transplastomic tomatoes by induced lycopene-to-provitamin A conversion. *Plant Physiology* 151: 59–66.
- Aro, A., Alfthan, G., and Varo, P. (1995). Effects of supplementation of fertilizers on human selenium status in Finland. *The Analyst* 120: 841–843.
- Ates, D., Aldemir, S., Yagmur, B. et al. (2018). QTL mapping of genome regions controlling manganese uptake in lentil seed. *Genes, Genomes, Genetics* 8: 1409–1416.
- Bänzinger, M. and Long, J. (2000). The potential for increasing the iron and zinc density of maize through plant breeding. *Food and Nutrition Bulletin* 21: 397–400.
- Bashir, K., Inoue, H., Nagasaka, S. et al. (2006). Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. *Journal of Biological Chemistry* 281: 32395–32402.
- Beebe, S., Gonzalez, A.V., and Rengifo, J. (2000). Research on trace minerals in the common bean. *Food and Nutrition Bulletin* 21: 387–391.
- Beyer, P., Al-Babili, S., Ye, X. et al. (2002). Golden rice: introducing the  $\beta$ -carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *Journal of Nutrition* 132: 506S–510S.
- Blair, M., Astudillo, C., Grusak, M.A. et al. (2009). Inheritance of seed iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding* 23: 197–207.
- Blair, M.W., Astudillo, C., Rengifo, J. et al. (2011). QTL analyses for seed iron and zinc concentrations in an intra-genepool population of Andean common beans (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 122: 511–521.
- Blair, M.W., Medina, J.I., Astudillo, C. et al. (2010). QTL for seed iron and zinc concentration and content in a Mesoamerican common bean (*Phaseolus vulgaris* L.) population. *Theoretical and Applied Genetics* 121: 1059–1070.
- Blancaquert, D., Van daele, J., Strobbe, S. et al. (2015). Improving folate (vitamin B9) stability in biofortified rice through metabolic engineering. *Nature Biotechnology* 33: 1076–1078.
- Boonchuay, P., Cakmak, I., Rerkasem, B., and Prom-U-Thai, C. (2013). Effect of different foliar zinc application at different growth stages on seed zinc concentration and its impact on seedling vigor in rice. *Soil Science and Plant Nutrition* 59: 180–188.
- Borg, S., Brinch-Pedersen, H., Tauris, B. et al. (2012). Wheat ferritins: improving the iron content of the wheat grain. *Journal of Cereal Science* 56: 204–213.
- Bouis, H. (1996). Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition Reviews* 54: 131–137.
- Bouis, H.E., Meenakshi, J.V., and Pfeiffer, W. (2006). Biofortification of staple food crops. *The Journal of Nutrition* 136: 1064–1067.
- Bouis, H.E. and Saltzman, A. (2017). Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security* 12: 49–58.
- Bouis, H.E. and Welch, R.M. (2010). Biofortification – a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science* 50: S20–S32.
- Branca, F. and Ferrari, M. (2002). Impact of micronutrient deficiencies on growth: the stunting syndrome. *Annals of Nutrition and Metabolism* 46 (S1): 8–17.
- Brinch-Pedersen, H., Hatzack, F., and Stoger, E. (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, M.R., Hammond, J.P., King, G.J. 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, M.R., Hammond, J.P., and King, G.K. (2009). Biofortifying brassica with calcium (Ca) and magnesium (Mg). *Proceedings of the 16<sup>th</sup> International Plant Nutrition Colloquium*, Paper 1256 (23 March 2019). <https://escholarship.org/content/qt9936g2vv/qt9936g2vv.pdf>.
- Broadley, M.R., Lochlainn, S.O., Hammond, J.P. et al. (2010). Shoot zinc (Zn) concentration varies widely within Brassica oleracea L. and is affected by soil Zn and phosphorus (P) levels. *Journal of Horticultural Science and Biotechnology* 85: 375–380.
- Brodeur, J.M., Laurin, D., Vallee, R., and Lachapelle, D. (1993). Nutrient intake and gastrointestinal disorders related to masticatory performance in the edentulous elderly. *The Journal of Prosthetic Dentistry* 70: 468–473.
- Brown, C.R., Haynes, K.G., Moore, M. et al. (2010). Stability and broad-sense heritability of mineral content in potato: iron. *American Journal of Potato Research* 87: 390–396.
- Burgos, G., Amoros, W., Morote, M. et al. (2007). Fe and Zn concentration of native Andean potato cultivars from a human nutrition perspective. *Journal of Food Science and Agriculture* 87: 668–675.
- Burkhardt, P.K., Beyers, P., Wuenn, J. et al. (1997). Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *The Plant Journal* 11: 1071–1078.
- Burt, A.J., Grainger, C.M., Smid, M.P. et al. (2011). Allele mining of exotic maize germplasm to enhance macular carotenoids. *Crop Science* 51: 991–1004.
- Cahoon, E.B., Hall, S.E., Ripp, K.G. et al. (2003). Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nature Biotechnology* 21: 1082–1087.
- Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and Soil* 302: 1–17.
- Cakmak, I. (2012). Biofortification of cereals with zinc and iron through fertilization strategy. *19th World Congress of Soil Science, Soil Solutions for a Changing World* 5: 1–6.
- Cakmak, I., Kalaycı, M., Ekiz, H. et al. (1999). Zinc deficiency as a practical problem in plant and human nutrition in Turkey: a NATO- science for stability project. *Field Crops Research* 60: 175–188.
- Cakmak, I. and Kutman, U.B. (2018). Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science* 69: 172–180.
- Cakmak, I., Pfeiffer, W.H., and McClafferty, B. (2010). Biofortification of durum wheat with zinc and iron. *Cereal Chemistry* 87: 10–20.
- Cakmak, I., Torun, A., Millet, E. et al. (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.
- Cao, X.Y., Jiang, X.M., Kareem, A. et al. (1994). Iodination of irrigation water as a method of supplying iodine to a severely iodine-deficient population in Xinjiang, China. *The Lancet* 344: 107–110.
- Carvalho, K.M., Gallardo-Williams, M.T., Benson, R.F., and Martin, D.F. (2003). Effects of selenium supplementation on four agricultural crops. *Journal of Agricultural and Food Chemistry* 51: 704–709.

- Cervantes-Flores, J.C. (2006). Development of a genetic linkage map and QTL analysis in sweet potato. *Molecular Breeding* 21: 511–532.
- Champagne, A., Legendre, L., and Lebot, V. (2013). Biofortification of taro (*Colocasia esculenta*) through breeding for increased contents in carotenoids and anthocyanins. *Euphytica* 194: 125–136.
- Chandler, K., Lipka, A.E., Owens, B.F. et al. (2013). Genetic analysis of visually scored orange kernel color in maize. *Crop Science* 53: 189–200.
- Chavez, A.L., Sanchez, T., Jaramillo, G. et al. (2005). Variation of quality traits in cassava roots evaluated in landraces and improved clones. *Euphytica* 143: 125–133.
- Chen, L., Yang, F., Xu, J. et al. (2002). Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agricultural and Food Chemistry* 50: 5128–5130.
- Chen, Z., Young, T.E., Ling, J. et al. (2003). Increasing vitamin C content of plants through enhanced ascorbate recycling. *Proceedings of the National Academy of Sciences of the United States of America* 100: 3525–3530.
- Christou, P. and Twyman, R.M. (2004). The potential of genetically enhanced plants to address food insecurity. *Nutrition Research Reviews* 17: 23–42.
- Cichy, K.A., Caldas, G.V., Snapp, S.S., and Blair, M.W. (2009). QTL analysis of seed iron, zinc, and phosphorus levels in an Andean bean population. *Crop Science* 49: 1742–1750.
- Cichy, K.A., Forster, S., Grafton, K.F., and Hosfield, G.L. (2005). Inheritance of seed zinc accumulation in navy bean. *Crop Science* 45: 864–870.
- Cong, L., Wang, C., Chen, L. et al. (2009). Expression of phytoene synthase1 and carotene desaturase crtI genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry* 57: 8652–8660.
- Crisp, P., Walkey, D.G.A., Bellman, E., and Roberts, E.A. (1975). A mutation affecting curd colour in cauliflower (*Brassica oleracea* L. var. botrytis DC). *Euphytica* 24: 173–176.
- Cuderman, P., Kreft, I., Germ, M. et al. (2008). Selenium species in selenium-enriched and drought-exposed potatoes. *Journal of Agricultural and Food Chemistry* 56: 9114–9120.
- Datta, K., Baisakh, N., Oliva, N. et al. (2003). Bioengineered ‘golden’ Indica rice cultivars with beta-carotene metabolism in the endo- sperm with hygromycin and mannose selection systems. *Plant Biotechnology Journal* 1: 81–90.
- Davey, M.W., Garming, H., Ekese, B. et al. (2008). Exploiting banana biodiversity to reduce vitamin A deficiency-related illness: a fast and cost-effective strategy. *Proceedings of the tropical fruits in human nutrition and health conference*, Queensland Primary Industries and Fisheries. Australia (17 March 2019). [http://era.daf.qld.gov.au/id/eprint/1553/1/4549\\_Tropical\\_fruit\\_conference\\_proceedings\\_v2.pdf#page=167](http://era.daf.qld.gov.au/id/eprint/1553/1/4549_Tropical_fruit_conference_proceedings_v2.pdf#page=167).
- Davuluri, G.R., Van Tuinen, A., Fraser, P.D. et al. (2005). Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. *Nature Biotechnology* 23: 890–895.
- Decourcelle, M., Perez-Fons, L., Baulande, S. et al. (2015). Combined transcript, proteome, and metabolite analysis of transgenic maize seeds engineered for enhanced carotenoid synthesis reveals pleiotropic effects in core metabolism. *Journal of Experimental Botany* 66: 3141–3150.
- DeLong, G.R., Leslie, P.W., Wang, S.-H. et al. (1997). Effect on infant mortality of iodination of irrigation water in a severely iodine-deficient area of China. *The Lancet* 350: 771–773.

- Dharmapuri, S., Rosati, C., Pallara, P. et al. (2002). Metabolic engineering of xanthophyll content in tomato fruits. *FEBS Letters* 519: 30–34.
- Dickson, M.H., Lee, C.Y., and Blamble, A.E. (1988). Orange-curd high carotene cauliflower inbreds. *HortScience* 23: 778–779.
- Ding, G., Yang, M., Hu, Y. et al. (2010). Quantitative trait loci affecting seed mineral concentrations in *Brassica napus* grown with contrasting phosphorus supplies. *Annals of Botany* 105: 1221–1234.
- Diretto, G., Al-Babili, S., Tavazza, R. et al. (2007). Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini- pathway. *PLoS One* 2: e350.
- Diretto, G., Tavazza, R., Welsch, R. et al. (2006). Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. *BMC Plant Biology* 6: 13.
- Distelfeld, A., Cakmak, I., Peleg, Z. et al. (2007). Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Physiologia Plantarum* 129: 635–643.
- Ducreux, L.J.M., Morris, W.L., Hedley, P.E. et al. (2005). Metabolic engineering of high carotenoid potato tubers containing enhanced levels of  $\beta$ -carotene and lutein. *Journal of Experimental Botany* 56: 81–89.
- Enfissi, E., Fraser, P.D., Lois, L.M. et al. (2005). Metabolic engineering of the mevalonate and nonmevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. *Plant Biotechnology Journal* 3: 17–27.
- Fahad, S., Hussain, S., Saud, S. et al. (2015). Grain cadmium and zinc concentrations in maize influenced by genotypic variations and zinc fertilization. *CLEAN – Soil, Air, Water* 43 (10): 1433–1440.
- Fang, Y., Wang, L., Xin, Z. et al. (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* 6: 2079–2084.
- Finkelstein, J.L., Mehta, S., Udipi, S.A. et al. (2015). A randomized trial of iron-biofortified pearl millet in school children in India. *The Journal of Nutrition* 145: 1576–1581.
- Fraser, P.D., Enfissi, E.M., Halket, J.M. et al. (2007). Manipulation of phytoene levels in tomato fruit: effects on isoprenoids, plastids, and intermediary metabolism. *The Plant Cell* 19: 3194–3211.
- Fujisawa, M., Takita, E., Harada, H. et al. (2009). Pathway engineering of *Brassica napus* seeds using multiple key enzyme genes involved in ketocarotenoid formation. *Journal of Experimental Botany* 60: 1319–1332.
- Fujisawa, M., Watanabe, M., Choi, S.K. et al. (2008). Enrichment of carotenoids in flaxseed (*Linum sitatissimum*) by metabolic engineering with introduction of bacterial phytoene synthase gene crtB. *Journal of Bioscience and Bioengineering* 105: 636–641.
- Garcia-Oliveira, A.L., Tan, L., Fu, Y., and Sun, C. (2009). Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology* 51: 84–92.
- Garg, M., Sharma, N., Sharma, S. et al. (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition* 5: 1–33.
- Gelin, J.R., Forster, S., Grafton, K.F. et al. (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., Verbyla, A.P., Torun, A.A., and Cikmak, I. (2009). Quantitative trait loci analysis of zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. *Plant and Soil* 314: 49–66.
- Giacosa, A., Faliva, M.A., Perna, S. et al. (2014). Selenium fortification of an Italian rice cultivar via foliar fertilization with sodium selenate and its effects on human serum selenium levels and on erythrocyte glutathione peroxidase activity. *Nutrients* 6: 1251–1261.
- Global Panel (2015). Biofortification: An agricultural investment for nutrition. *Policy Brief No. 1*. Global Panel on Agriculture and Food Systems for Nutrition. London, UK.
- Goffman, F.D. and Böhme, T. (2001). Relationship between fatty acid profile and vitamin E content in maize hybrids (*Zea mays* L.). *Journal of Agricultural and Food Chemistry* 49: 4990–4994.
- Goto, F., Yoshihara, T., and 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. et al. (1999). Iron fortification of rice seed by the soybean ferritin gene. *Nature Biotechnology* 17: 282–286.
- Graham, L., Ortiz-Monasterio, I., Stangoulis, J., and Graham, R. (2005). Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? *Plant and Soil* 269: 369–380.
- Graham, R.D., Senadhira, D., Beebe, S.E., and Iglesias, C. (1998). A strategy for breeding staple-food crops with high micronutrient density. *Soil Science and Plant Nutrition* 43: 1153–1157.
- Graham, R.D., Senadhira, D., Beebe, S.E. et al. (1999). Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Research* 60: 57–80.
- Gregorio, G.B., Senadhira, D., Htut, H., and Graham, R.D. (2000). Breeding for trace mineral density in rice. *Food and Nutrition Bulletin* 21: 382–386.
- Guo, J.X., Feng, X.M., Hu, X.Y., and Tian, G.L. (2016). Effects of soil zinc availability, nitrogen fertilizer rate and zinc fertilizer application method on zinc biofortification of rice. *Journal of Agricultural Science* 154: 584–597.
- Guzman-Maldonado, S.H., Martinez, O., Acosta-Gallegos, J.A. et al. (2003). Putative quantitative trait loci for physical and chemical components of common bean. *Crop Science* 43: 1029–1035.
- Haas, J.D., Luna, S.V., Lung'aho, M.G. et al. (2016). Consuming iron biofortified beans increases iron status in Rwandan women after 128 days in a randomized controlled feeding trial. *The Journal of Nutrition* 146: 1586–1592.
- Hall, R.D., Brouwer, I.D., and Fitzgerald, M.A. (2008). Plant metabolomics and its potential application for human nutrition. *Plant Physiology* 132: 162–175.
- Harjes, C.E., Rocheford, T.R., Bai, L. et al. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* 319: 330–333.
- 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.
- Haynes, K.G., Yencho, G.C., Clough, M.E. et al. (2012). Genetic variation for potato tuber micronutrient content and implications for biofortification of potatoes to reduce micronutrient malnutrition. *American Journal of Potato Research* 89: 192–198.
- He, W., Shohag, M.J., Wei, Y. et al. (2013). Iron concentration, bioavailability, and nutritional quality of polished rice affected by different forms of foliar iron fertilizer. *Food Chemistry* 141: 4122–4126.

- Hoppler, M., Egli, I., Petry, N. et al. (2014). Iron speciation in beans (*Phaseolus vulgaris*) biofortified by common breeding. *Journal of Food Science* 79: C1629–C1634.
- Huang, J.C., Zhong, Y.J., Liu, J. et al. (2013). Metabolic engineering of tomato for high-yield production of astaxanthin. *Metabolic Engineering* 17: 59–67.
- Ibrahim, E.A. and Ramadan, W.A. (2015). Effect of zinc foliar spray alone and combined with humic acid or/and chitosan on growth, nutrient elements content and yield of dry bean (*Phaseolus vulgaris* L.) plants sown at different dates. *Scientia Horticulturae* 184: 101–115.
- Jiang, W., Struik, P.C., Keulen, H.V. et al. (2008). Does increased zinc uptake enhance grain zinc mass concentration in rice? *Annals of Applied Biology* 153: 135–147.
- Jin, T., Zhou, J., Chen, J. et al. (2013). The genetic architecture of zinc and iron content in maize grains as revealed by QTL mapping and meta-analysis. *Breeding Science* 63: 317–324.
- Jin, Z., Minyan, W., Lianghuan, W. et al. (2008). Impacts of combination of foliar iron and boron application on iron biofortification and nutritional quality of rice grain. *Journal of Plant Nutrition* 31: 1599–1611.
- Johns, T. and Eyzaguirre, E.B. (2007). Biofortification, biodiversity and diet: a search for complementary applications against poverty and malnutrition. *Food Policy* 32: 1–24.
- Johnson, K.L., Raybould, A.F., Hudson, M.D., and Poppy, G.M. (2007). How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends in Plant Science* 12: 1–5.
- Jones, K.M. and de Brauw, A. (2015). Using agriculture to improve child health: promoting orange sweet potatoes reduces diarrhea. *World Development* 74: 15–24.
- Khan, S., Ghanghro, A.B., Memon, A.N. et al. (2013). Quantitative analysis of wheat proteins in different varieties grown in Sindh, Pakistan. *International Journal of Agriculture and Crop Sciences* 5: 1836–1839.
- Kim, M.J., Kim, J.K., Kim, H.J. et al. (2012). Genetic modification of the soybean to enhance the  $\beta$ -carotene content through seed-specific expression. *PLoS One* 7: e48287.
- King, J.C. (2002). Evaluating the impact of plant biofortification on human nutrition. *The Journal of Nutrition* 132: 511S–513S.
- Kodkany, B.S., Bellad, R.M., Mahantshetti, N.S. et al. (2013). Biofortification of pearl millet with iron and zinc in a randomized controlled trial increases absorption of these minerals above physiologic requirements in young children. *The Journal of Nutrition* 143: 1489–1493.
- Kumar, J., Gupta, D.S., Kumar, S. et al. (2016). Current knowledge on genetic biofortification in lentil. *Journal of Agricultural and Food Chemistry* 64: 6383–6396.
- Kumari, V.V., Hoekenga, O., Salini, K., and Sarath-Chandran, M.A. (2014). Biofortification of food crops in India: an agricultural perspective. *Asian Biotechnology and Development Review* 16: 21–41.
- Landini, M., Gonzali, S., and Perata, P. (2011). Iodine biofortification in tomato. *Journal of Plant Nutrition and Soil Science* 174: 480–486.
- Laurie, S.M., Faber, M., Van Jaarsveld, P.J. et al. (2012).  $\beta$ -Carotene yield and productivity of orange-fleshed sweet potato (*Ipomoea batatas* L. Lam.) as influenced by irrigation and fertilizer application treatments. *Scientia Horticulturae* 142: 180–184.
- Lee, S. and An, G. (2009). Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. *Plant, Cell & Environment* 32: 408–416.
- Lee, S., Kim, Y.S., Jeon, U.S. et al. (2012). Activation of rice nicotinamine synthase 2 (OsNAS2) enhances iron availability for biofortification. *Molecular Cell* 33: 269–275.

- Levine, M., Dhariwal, K.R., Welch, R.W. et al. (1995). Determination of optimal vitamin C requirements in humans. *American Journal of Clinical Nutrition* 62: 1347S–13456S.
- Li, L., Paolillo, D.J., Parthasarathy, M.V. et al. (2001). Novel gene mutation that confers abnormal patterns of beta-carotene accumulation in cauliflower (*Brassica oleracea* var. botrytis). *The Plant Journal* 26: 59–67.
- Lipkie, T.E., De Moura, F.F., Zhao, Z.-Y. et al. (2013). Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. *Journal of Agricultural and Food Chemistry* 61: 5764–5771.
- Liu, K. and Gu, Z. (2009). Selenium accumulation in different brown rice cultivars and its distribution in fractions. *Journal of Agricultural and Food Chemistry* 57: 695–700.
- Lividini, K. and Fiedler, J.L. (2015). Assessing the promise of biofortification: a case study of high provitamin A maize in Zambia. *Food Policy* 54: 65–77.
- Lonergan, P.F., Pallotta, M.A., Lorimer, M. et al. (2009). Multiple genetic loci for zinc uptake and distribution in barley (*Hordeum vulgare*). *New Phytologist* 184: 168–179.
- Lopez, A.B., Van Eck, J., Conlin, B.J. et al. (2008). Effect of the cauliflower or transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. *Journal of Experimental Botany* 59: 213–223.
- Lopez-Valdivia, L.M., Fernandez, M.D., Obrador, A., and Alvarez, J.M. (2002). Zinc transformations in acidic soil and zinc efficiency on maize by adding six organic zinc complexes. *Journal of Agricultural and Food Chemistry* 50: 1455–1460.
- Low, J.W., Mwangi, R.O.M., Andrade, M. et al. (2017). Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. *Global Food Security* 14 (2): 3–30.
- Lu, K., Li, L., Zheng, X. et al. (2008). Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. *Journal of Genetics* 87: 305–310.
- Lu, S., Van Eck, J., Zhou, X. et al. (2006). The cauliflower or gene encodes a DNA-J cysteine-rich domain-containing protein that mediates high levels of  $\beta$ -carotene accumulation. *The Plant Cell* 18: 3594–3605.
- Lucca, P., Hurrell, R., and Potrykus, I. (2002). Fighting iron deficiency anemia with iron-rich rice. *Journal of the American College of Nutrition* 21: 184S–190S.
- Lung'aho, M.G., Mwaniki, A.M., Szalma, S.J. et al. (2011). Genetic and physiological analysis of iron biofortification in maize kernels. *PLoS One* 6: e20429.
- Mabesa, R.L., Impa, S.M., Grewal, D., and Beebouts, S.E.J. (2013). Contrasting grain-Zn response of biofortification rice (*Oryza sativa* L.) breeding lines to foliar Zn application. *Field Crops Research* 149: 223–233.
- Mao, H., Wang, J., Wang, Z. et al. (2014). Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China. *Journal of Soil Science and Plant Nutrition* 14: 459–470.
- Maqbool, M.A., Aslam, M., Beshir, A., and Khan, M.S. (2018). Breeding for provitamin A biofortification of maize (*Zea mays* L.). *Plant Breeding* 137: 451–469.
- Márquez-Quiroz, C., De-la-Cruz-Lázaro, E., Osorio-Osorio, R., and Sánchez-Chávez, E. (2015). Biofortification of cowpea beans with iron: iron's influence on mineral content and yield. *Journal of Soil Science and Plant Nutrition* 15: 839–847.
- Masuda, H., Ishimaru, Y., Aung, M.S. et al. (2012). Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Scientific Reports* 2: 534.

- Masuda, H., Kobayashi, T., Ishimaru, Y. et al. (2013). Iron-biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. *Frontiers in Plant Science* 4: 132.
- Masuda, H., Suzuki, M., Morikawa, K.C. et al. (2008). Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. *Rice* 1: 100–108.
- Maziya-Dixon, B., Kling, J.G., Menkir, A., and Dixon, A. (2000). Genetic variation in total carotene, iron, and zinc contents of maize and cassava genotypes. *Food and Nutrition Bulletin* 21: 419–422.
- Monasterio, I. and Graham, R.D. (2000). Breeding for trace minerals in wheat. *Food and Nutrition Bulletin* 21: 392–396.
- Morris, J., Hawthorne, K.M., Hotze, T. et al. (2008). Nutritional impact of elevated calcium transporter activity in carrots. *Proceedings of the National Academy of Sciences of the United States of America* 105: 1431–1435.
- Muthukumar, P. (2016). Genetics of yield and its components and marker assisted introgression of “Or” gene for enhancing  $\beta$ -carotene in cauliflower. PhD thesis. Division of Vegetable Science, Indian Agricultural Research Institute.
- Muzhingi, T., Palacios, N., Miranda, A. et al. (2016). Genetic variation of carotenoids, vitamin E and phenolic compounds in biofortified maize. *Journal of the Science of Food and Agriculture* 97: 793–801.
- Nestel, P., Bouis, H.E., Meenakshi, J.V., and Pfeiffer, W.H. (2006). Biofortification of staple food crops. *Journal of Nutrition* 136: 1064–1067.
- Norton, G.J., Deacon, C.M., Xiong, L. et al. (2010). Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant and Soil* 329: 139–153.
- Ortiz-Monasterio, J.I., Palacios-Rojas, N., Meng, E. et al. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science* 46: 293–307.
- Paine, J.A., Shipton, C.A., Chaggar, S. et al. (2005). Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nature Biotechnology* 23: 482–487.
- Palmer, A.C., Healy, K., Barffour, M.A. et al. (2016). Provitamin A carotenoid-biofortified maize consumption increases pupillary responsiveness among Zambian children in a randomized controlled trial. *Journal of Nutrition* 146: 2551–2558.
- Park, S., Kim, C.-K., Pike, L.M. et al. (2004). Increased calcium in carrots by expression of an Arabidopsis H<sup>+</sup>/Ca<sup>2+</sup> transporter. *Molecular Breeding* 14: 275–282.
- Paul, J.Y., Khanna, H., and Kleidon, J. (2017). Golden bananas in the field: elevated fruit provitamin A from the expression of a single banana transgene. *Plant Biotechnology Journal* 15: 520–532.
- Paul, S., Ali, N., Gayen, D. et al. (2012). Molecular breeding of Osfer2 gene to increase iron nutrition in rice grain. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain* 3 (4): 310–316.
- Peleg, Z., Cakmak, I., Ozturk, L. et al. (2009). Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat  $\times$  wild emmer wheat RIL population. *Theoretical and Applied Genetics* 119: 353–369.

- Peninah, N., Richard, E., and Josep, K. (2014). Combining ability for beta-carotene and important quantitative traits in a cassava fl population. *Journal of Plant Breeding and Crop Science* 6: 24–30.
- Pierce, E.C., LaFayette, P.R., Ortega, M.A. et al. (2015). Ketocarotenoid production in soybean seeds through metabolic engineering. *PLoS One* 10: e0138196.
- Pixley, K., Rojas, N.P., Babu, R. et al. (2013). Biofortification of maize with Provitamin A carotenoids. In: *Carotenoids and Human Health: Nutrition and Health* (ed. S. Tanumihardjo), 271–292. Totowa, NJ: Humana Press.
- Pixley, K.V., Palacios-Rojas, N., and Glahn, R.P. (2011). The usefulness of iron bioavailability as a target trait for breeding maize (*Zea mays* L.) with enhanced nutritional value. *Field Crops Research* 123: 153–160.
- Poblaciones, M.J. and Rengel, Z. (2016). Soil and foliar zinc biofortification in field pea (*Pisum sativum* L.). Grain accumulation and bioavailability in raw and cooked grains. *Food Chemistry* 212: 427–433.
- Poblaciones, M.J., Rodrigo, S., Santamaria, O. et al. (2014). Selenium accumulation and speciation in biofortified chickpea (*Cicer arietinum* L.) under Mediterranean conditions. *Journal of the Science of Food and Agriculture* 94: 1101–1106.
- Poggi, V., Arcioni, A., Filippini, P., and Pifferi, P.G. (2000). Foliar application of selenite and selenate to potato (*Solanum tuberosum*): effect of a ligand agent on selenium content of tubers. *Journal of Agricultural and Food Chemistry* 48: 4749–4751.
- Poletti, S. and Sautter, C. (2005). Biofortification of the crops with micronutrients using plant breeding and/or transgenic strategies. *Minerva Biotechnologica Torino* 17: 1–11.
- Powell, K. (2007). Functional foods from biotech – an unappetizing prospect? *Nature Biotechnology* 25: 525–531.
- Premarathna, L., McLaughlin, M.J., Kirby, J.K. et al. (2012). Selenate-enriched urea granules are a highly effective fertilizer for selenium biofortification of paddy rice grain. *Journal of Agricultural and Food Chemistry* 60: 6037–6044.
- Prendiville, J.S. and Manfredi, L.N. (1992). Skin signs of nutritional disorders. *Seminars in Dermatology* 11: 88–97.
- Rai, K.N., Govindraj, M., and Rao, A.S. (2012). Genetic enhancement of grain iron and zinc content in pearl millet. *Crops Food* 4: 119–125.
- Ram, H., Rashid, A., Zhang, W. et al. (2016). Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. *Plant and Soil* 1: 389–401.
- Ramesh, S.A., Choimes, S., and Schachtman, D.P. (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.
- Ramessar, K., Capell, T., Twyman, R.M. et al. (2009). Calling the tunes on transgenic crops: the case for regulatory harmony. *Molecular Breeding* 23: 99–112.
- Ravanello, M.P., Ke, D., Alvarez, J. et al. (2003). Coordinate expression of multiple bacterial carotenoid genes in canola leading to altered carotenoid production. *Metabolic Engineering* 5: 255–263.
- Reddy, B.V.S., Ramesh, S., and Longvah, T. (2005). Prospects of breeding for micronutrients and  $\beta$ -carotene-dense sorghums. *International Sorghum and Millets Newsletter* 46: 10–14.

- Romer, S., Lubeck, J., Kauder, F. et al. (2002). Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. *Metabolic Engineering* 4: 263–272.
- Ros, G.H., VanRotterdam, A.M.D., Bussink, D.W., and Bindraban, P.S. (2016). Selenium fertilization strategies for bio-fortification of food: an agro-ecosystem approach. *Plant and Soil* 404: 99–112.
- Rosado, J.L., Hambidge, K.M., Miller, L.V. et al. (2009). The quantity of zinc absorbed from wheat in adult women is enhanced by biofortification. *Journal of Nutrition* 139: 1920–1925.
- Rosati, C., Aquilani, R., Dharmapuri, S. et al. (2000). Metabolic engineering of  $\beta$ -carotene and lycopene content in tomato fruit. *The Plant Journal* 24: 413–419.
- Sadeghzadeh, B., Rengel, Z., Li, C., and Yang, H. (2009). Molecular marker linked to a chromosome region regulating seed Zn accumulation in barley. *Molecular Breeding* 25: 167–177.
- Santos, C.A.F. and Boiteux, L.S. (2013). Breeding biofortified cowpea lines for semi-arid tropical areas by combining higher seed protein and mineral levels. *Genetics and Molecular Research* 12: 6782–6789.
- Schmidt, M.A., Parrott, W.A., Hildebrand, D.F. et al. (2015). Transgenic soya bean seeds accumulating  $\beta$ -carotene exhibit the collateral enhancements of oleate and protein content traits. *Plant Biotechnology Journal* 13: 590–600.
- Shewmaker, C.K., Sheehu, J.A., Daley, M. et al. (1999). Seed-specific overexpression of phytoene synthase: increase in carotenoids and metabolic effects. *The Plant Journal* 20: 401–412.
- Shi, R., Li, H., Tong, Y. et al. (2008). Identification of quantitative trait locus of zinc and phosphorus density in wheat (*Triticum aestivum* L.) grain. *Plant and Soil* 306: 95–104.
- Shivay, Y.S., Kumar, D., Prasad, R., and Ahlawat, I.P.S. (2008). Relative yield and zinc uptake by rice from zinc sulphate and zinc oxide coatings onto urea. *Nutrient Cycling in Agroecosystems* 80: 181–188.
- Shivay, Y.S., Prasad, R., and Pal, M. (2015). Effects of source and method of zinc application on yield, zinc biofortification of grain, and Zn uptake and use efficiency in chickpea (*Cicer arietinum* L.). *Communications in Soil Science and Plant Analysis* 46: 2191–2200.
- Simić, D., Mladenović Drinić, S., Zdunić, Z. et al. (2012). Quantitative trait loci for biofortification traits in maize grain. *Journal of Heredity* 103: 47–54.
- Smolen, S., Kowalska, L., and Sady, W. (2014). Assessment of biofortification with iodine and selenium of lettuce cultivated in the NFT hydroponic system. *Scientia Horticulturae* 166: 9–16.
- Smolen, S., Skoczylas, L., Ledwozyw-Smolen, L. et al. (2016). Biofortification of carrot (*Daucus carota* L.) with iodine and selenium in a field experiment. *Frontiers in Plant Science* 7: 730.
- Song, X.Y., Zhu, W.J., Tang, R.M. et al. (2016). Over-expression of StLCYb increases  $\beta$ -carotene accumulation in potato tubers. *Plant Biotechnology Reports* 10: 95–104.
- Sperotto, R.A., Ricachenevsky, F.K., de Abreu Waldow, V., and Fett, J.P. (2012). Iron biofortification in rice: It's a long way to the top. *Plant Science* 190: 24–39.
- Stangoulis, J.C.R., Huynh, B.L., Welch, R.M. et al. (2007). Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* 154: 289–294.
- Storozhenko, S., De Brouwer, V., Volckaert, M. et al. (2007). Folate fortification of rice by metabolic engineering. *Nature Biotechnology* 25: 1277–1279.
- Subramanian, N.K. (2012). Genetics of mineral accumulation in potato tubers. PhD thesis. University of Nottingham.

- Sui, X., Yan, Z., and Shubin, W. (2012). Improvement Fe content of wheat (*Triticum aestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. *Journal of Agricultural Biotechnology* 20: 766–773.
- Suwarno, W.B., Pixley, K.V., Palacios-Rojas, N. et al. (2014). Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Science* 54: 14–24.
- Takahashi, M., Nakanishi, H., Kawasaki, S. et al. (2001). Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nature Biotechnology* 19: 466–469.
- Takkar, P.N. and Walker, C.D. (1993). The distribution and correction of zinc deficiency. In: *Zinc in Soils and Plants: Developments in Plant and Soil Sciences* (ed. A.D. Robson), 151–165. Dordrecht: Springer.
- Telengech, P.K., Maling'a, J.N., Nyende, A.B. et al. (2015). Gene expression of beta carotene genes in transgenic biofortified cassava. *3 Biotech* 5: 465–472.
- Trijatmiko, K., Duenas, C., Tsakirpaloglou, N. et al. (2016). Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Scientific Reports* 6: 19792.
- Van Eck, J., Conlin, B., Garvin, D.F. et al. (2007). Enhancing beta-carotene content in potato by RNAi-mediated silencing of the beta-carotene hydroxylase gene. *American Journal of Potato Research* 84: 331–342.
- Van Eenennaam, A.L., Lincoln, K., Durrett, T.P. et al. (2003). Engineering vitamin E content: from Arabidopsis mutant to soy oil. *Plant Cell* 15: 3007–3019.
- Vasconcelos, M., Datta, K., Oliva, N. et al. (2003). Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Science* 164: 371–378.
- Velu, G., Ortiz-Monasterio, I., Cakmak, I. et al. (2014). Biofortification strategies to increase grain zinc and iron concentrations in wheat. *Journal of Cereal Science* 59: 365–372.
- Velu, G., Rai, K., N., Muralidharan, V. et al. (2007). Prospects of breeding biofortified pearl millet with high grain iron and zinc content. *Plant Breeding* 126: 182–185.
- Waltz, E. (2014). Vitamin A super banana in human trials. *Nature Biotechnology* 32: 857.
- Wang, C., Zeng, J., Li, Y. et al. (2014). Enrichment of provitamin A content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI. *Journal of Experimental Botany* 65: 2545–2556.
- Wang, J., Mao, H., Zhao, H. et al. (2012). Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in loess plateau, China. *Field Crops Research* 135: 89–96.
- Wei, S., Li, X., Gruber, M.Y. et al. (2009). RNAi-mediated suppression of DET1 alters the levels of carotenoids and sinapate esters in seeds of *Brassica napus*. *Journal of Agricultural and Food Chemistry* 57: 5326–5333.
- Wei, Y., Shohag, M.J., and Yang, X. (2012b). Biofortification and bioavailability of rice grain zinc as affected by different forms of foliar zinc fertilization. *PLoS One* 7: e45428.
- Wei, Y., Shohag, M.J., Yang, X., and Yibin, Z. (2012a). Effects of foliar iron application on iron concentration in polished rice grain and its bioavailability. *Journal of Agricultural and Food Chemistry* 60: 11433–11439.
- Welch, R., Arango, J., Bar, C. et al. (2010). Provitamin A accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *The Plant Cell* 22: 3348–3356.

- Welch, R.M. (2005). Biotechnology, biofortification, and global health. *Food and Nutrition Bulletin* 26: 419–421.
- Welch, R.M. and Graham, R.D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* 55: 353–364.
- Welch, R.M., House, R.A., Ortiz-Monasterio, I., and 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.
- White, P.J., Broadley, M.R., and Greg, P.J. (2012a). Managing the nutrition of plants and people. *Applied and Environmental Soil Science* 2012: 104826.
- White, P.J., Broadley, M.R., Hammond, J.P. et al. (2012b). Bio-fortification of potato tubers using foliar zinc-fertiliser. *Journal of Horticultural Science and Biotechnology* 87: 123–129.
- WHO (2009). Global prevalence of vitamin A deficiency in populations at risk 1995–2005. *WHO Global Database on Vitamin A Deficiency*. World Health Organization (22 January 2019). [http://apps.who.int/iris/bitstream/handle/10665/44110/9789241598019\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/handle/10665/44110/9789241598019_eng.pdf?ua=1).
- Wirth, J., Poletti, S., Aeschlimann, B. et al. (2009). Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnology Journal* 7: 631–644.
- Wissuwa, M., Ismail, A.M., and Graham, R.D. (2008). Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. *Plant and Soil* 306: 37–48.
- Wu, J., Yuan, Y., Zhang, X. et al. (2008). Mapping QTLs 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., Rup, S., and Bock, R. (2007). Contained metabolic engineering in tomatoes by expression of carotenoid biosynthesis genes from the plastid genome. *The Plant Journal* 49: 276–288.
- Xu, J. and Hu, Q. (2004). Effect of foliar application of selenium on the antioxidant activity of aqueous and ethanolic extracts of selenium-enriched rice. *Journal of Agricultural and Food Chemistry* 52: 1759–1763.
- Yang, F., Chen, L., Hu, Q., and 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.W., Tian, X.H., Lu, X.C. et al. (2011). Impacts of phosphorus and zinc levels on phosphorus and zinc nutrition and phytic acid concentration in wheat (*Triticum aestivum* L.). *Journal of the Science of Food and Agriculture* 91: 2322–2388.
- Ye, X., Al-Babili, S., Klott, A. et al. (2000). Engineering the provitamin A ( $\beta$ -carotene) biosynthetic pathway into (carotenoids-free) rice endosperm. *Science* 287: 303–305.
- Yilmaz, A., Ekiz, H., Torun, B. et al. (1997). Effect of different zinc application methods on grain yield and zinc concentration in wheat cultivars grown on zinc-deficient calcareous soils. *Journal of Plant Nutrition* 20: 461–471.
- Yu, B., Lydiate, D.J., Young, L.W. et al. (2008). Enhancing the carotenoid content of *Brassica napus* seeds by downregulating lycopene epsilon cyclase. *Transgenic Research* 17: 573–585.

- Yuan, L., Wu, L., Yang, C., and Quin, L.V. (2013). Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *Journal of the Science of Food and Agriculture* 93: 254–261.
- Zeng, Y.W., Du, J., Yang, X.M. et al. (2016). Identification of quantitative trait loci for mineral elements in grains and grass powder of barley. *Genetics and Molecular Research* 15: gmr15049103.
- Zhang, B., Chen, P., Shi, A. et al. (2009). Putative quantitative trait loci associated with calcium content in soybean seed. *Journal of Heredity* 100: 263–269.
- Zhang, X., Zhang, G., Guo, L., and Wang, H. (2011). Identification of quantitative trait loci for Cd and Zn concentrations of brown rice grown in Cd-polluted soils. *Euphytica* 180: 173–179.
- Zhang, Y.Q., Pang, L.L., Yan, P. et al. (2013). Zinc fertilizer placement affects zinc content in maize plant. *Plant and Soil* 372: 81–92.
- Zhao, F.-J. and Shewry, P.R. (2011). Recent developments in modifying crops and agronomic practice to improve human health. *Food Policy* 36: S94–S101.
- Zheng, L., Cheng, Z., Ai, C. et al. (2010). Nicotianamine, a novel enhancer of rice iron bioavailability to humans. *PLoS One* 5: e10190.
- Zhu, C., Naqvi, S., Gomez-Galera, S. et al. (2007). Transgenic strategies for the nutritional enhancement of plants. *Trends in Plant Science* 12: 548–555.
- Zuo, Y. and Zhang, F. (2009). ‘Iron and zinc biofortification strategies in dicot plants by intercropping with gramineous species’, a review. *Agronomy for Sustainable Development* 29: 63–71.

