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Application of Bio-fortification through Plant Breeding to Improve the Value of Staple Crops

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Abstract Mineral deficiency is one of the main global challenges to human health for people who live especially in developing world. It is known as 'Hidden hunger', results in poor growth and compromised psychomotor development of children, reduced immunity, fatigue, irritability, weakness, hair loss, wasting of muscles, sterility, morbidity and death. Iron and zinc mineral deficiency are the most common and widespread, afflicting more than half of the human population. Non-diversified cereal and plant based diets, which are poor in micronutrients, are the main reason for micronutrient deficiency in the populations. To alleviate this malnutrition problem, breeding strategies through use of bio-fortification is the best option to improve the quality of the plants through the addition of the desired minerals to food stuffs. Moreover, dietary diversification, supplementation, fortification and bio-fortification of crop plants are the main approaches to alleviate micronutrient malnutrition.

Keywords: *Bio-fortification, hidden hunger, mineral deficiency, plant breeding*

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1. Introduction

Plants are the source of many essential minerals nutrients. These mineral elements are vital for human beings for their survival and the continuity of life. Some plants are rich for some mineral and have deficiency to the other. No plant alone that contain all mineral elements in efficiently for human beings from in this planet. Deficiency of mineral is also known as 'Hidden hunger', results in poor growth and compromised psychomotor development of children, reduced immunity, fatigue, irritability, weakness, hair loss, wasting of muscles, sterility, morbidity and death (Stein, 2010). This is one of the main global challenges to human health for people who live especially in developing world. Of micro elements, iron and zinc mineral deficiency are the most common and widespread, afflicting more than half of the human population (White and Broadley, 2009). Due to its physico-chemical properties, iron takes part in most of the redox reactions in the body and also acts as a cofactor in numerous vital enzymatic reactions (Kim and Geurinot, 2007). Likewise, zinc is also an essential micronutrient for regulating gene expression and maintaining structural integrity of proteins (White and Broadley, 2009). It acts as a cofactor in more than 300 enzymatic reactions (King and Keen, 1999). Non-diversified cereal and plant based diets, which are poor in micronutrients, are the main reason for micronutrient deficiency in the populations of the developing world (GómezGalera *et al*., 2010). Moreover, anti-nutritional factors like phytic acid, fibres and tannins further reduce the bio-availability of these minerals from dietary intakes by preventing their absorption in the intestine (White and Broadley, 2006). Furthermore, processes like polishing, milling and pearling of cereals make them even poorer in micronutrients (Borg *et al*., 2009). Therefore, to alleviate this malnutrition problem, breeding strategies through use of bio-fortification is the best option to improve the quality of the cultivated plants. In this review, fortification means the addition of the desired minerals to food stuffs like iodine in salts, iron in flour, fluorine in toothpaste and zinc in flours. Moreover, dietary diversification, supplementation, fortification and bio-fortification of crop plants are the main approaches to alleviate micronutrient malnutrition (Stein, 2010). The application of these technologies have their own pros and cons, and a right mix of all the intervention approaches has to be employed to overcome the problem of hidden hunger (Gómez-Galera *et al*., 2010). Dietary diversification and modification suffers from difficulty in the change of dietary habits of people and high costs of diets with readily bio-available iron and zinc content (Zimmerman and Hurrel, 2007). The major drawback of these approaches is that these compounds have limited stability in the food stuffs (Allen, 2003).

Supplementation is another option to solve malnutrition problem that occur especially in the developing world. It encompasses the oral delivery of micronutrients in the forms of tables and syrups, has been used in chronic deficiencies. For instance, ferrous fumarate, ferrous sulphate and ferrous gluconate are the best absorbed forms of iron. Similarly, zinc can be supplied as zinc gluconate, zinc sulphate and zinc acetate. For instance, iron-fortified foods are susceptible to oxidation and also alter the taste of the food (Gómez-Galera *et al*., 2010). Similarly, folatefortified rice loses it while boiling owing to its increased solubility (Brinch-Pederson *et al*., 2007). Furthermore, the absorption of oral supplementation also depends on the type of food ingested. These approaches require recurring expenditure, robust distribution system and very careful implementation as overdose may also be harmful (Nestel *et al*., 2006). Cognizant to these facts, this review was designed to assess the application of bio-fortification technology through plant breeding to improve the quality crops.

2. Bio-fortification

Bio-fortification refers to increasing genetically the bio-available mineral content of food crops (Brinch-Pederson *et al*., 2007). Developing bio-fortified crops also improves their efficiency of growth in soils with depleted or unavailable mineral composition (Borg *et al*., 2009). Conventional breeding and genetic engineering techniques are the two approaches that may be used to bio-fortify the crops with minerals like iron and zinc (Tiwari *et al*., 2010). Cereals are the most important source of calories to humans. Rice, wheat and maize provide about 23%, 17% and 10%, respectively, of the calories acquired globally (Khush, 2003). To effectively target bio-fortification of cereals, five key steps can be targeted. These are (i) enhanced uptake from soil, (ii) increased transport of micronutrients to grains, (iii) increased sequestration of minerals to endosperm rather than husk and aleurone, (iv) reduction in anti-nutritional factors in grains and (v) increase in promoters of mineral bio-availability in grains.

2.1. Bio-fortification as Instrument to Combat Micronutrient Deficiencies

Micronutrient deficiencies are also referred to as "hidden hunger" since they are often not clinically visible, so that people might suffer from them without being aware. Iron, vitamin A, iodine and zinc deficiencies are among the world's most serious health risk factors and substantially contribute to the global burden of disease. It has been estimated that micronutrient deficiencies affect more than 2 billion people. They lead to low work productivity, permanent impairment of cognitive ability and increased rate of morbidity and mortality (WHO, 2005). The major cause of micronutrient malnutrition is a poor quality diet, mainly consisting of staple foods and lacking in animal products (Bouis, 2010). Therefore, a balanced diet would be the best way to prevent or counteract micronutrient malnutrition, but very often people have no access to the appropriate food (WHO, 2005).

Bio-fortification on staple foods could be a more sustainable strategy, also suitable for remote regions. Bio-fortified crops can potentially deliver iron, zinc and vitamin A to people with limited access to commercial markets (Mayer et al., 2008). The suitability of bio-fortification for the poor, who mainly eat staples that are not commercially processed and sold but rely on household produced crops, is the most noteworthy advantage (Tanumihardjo *et al*., 2008). Thus, bio-fortification has the potential to reduce the prevalence of micronutrient deficiencies and lower the number of people requiring interventions such as fortification and supplementation (Bouis and Welch, 2010). In contrast to dietary diversification, no behavioral changes are required from the consumers. However, the target crop has to be chosen carefully, following the dietary patterns of the consumers (Qaim *et al.,* 2007). The acceptance of the newly developed crop by the targeted population is a major issue for bio-fortification to be successful. To be accepted and cultivated by the farmers, the new variety must exhibit a high yield and resistance against disease and pests; in short be profitable. Characteristics of the newly developed plant such as yield, micronutrient concentration and disease and pests resistance should be stable over different environments and climatic zones. Moreover, the level of micronutrients must have the potential to significantly improve human health and ensure an adequate mineral bio-availability (Nestel *et al.*, 2006; Bouis and Welch, 2010).

Micronutrient enriched plants are more resistant to diseases, and their efficient uptake of minerals from soils might result in a higher yield since minerals are required for plant growth; this effect has particularly been observed in micronutrient depleted soil (Graham *et al*., 2001). Furthermore, bio-fortification might be a very cost effective approach. The major investments in bio-fortification occur during the development of the new varieties. It is estimated that the development of a micronutrient dense cultivar might cost only about $ 12 million (Khoshgoftarmanesh *et al*., 2010), whereas other interventions such as fortification and supplementation are more cost intensive. Once the bio-fortified plants are developed and grown by the farmers, seeds can be multiplied, reproduced and shared among the poor, with few additional costs occurring to maintain the high nutrient trait over time (Bouis, 2010). In contrast to other interventions, requiring larger funds on an annual basis, bio-fortification can provide benefits to the targeted populations over years without any noteworthy further investments.

3. Enhancing Mineral Deposition in Grains

The modern breeding practices have so far targeted improving the genetic potential of crops as the main objective due to which variability for other genetic traits got eroded. Modern day cultivars of all major crops have limited variability of mineral (Bouis, 2010). The Consultative Group on International Agricultural Research (CGIAR) through its HarvestPlus initiative has been exploring the genetic variability, heritability of mineral traits, stability over different environments, genetic studies and breeding strategies to enhance the mineral content in major edible crops such as wheat, rice, maize, beans and cassava (CIAT/IFPRI, 2002).

Crop wild relatives have been found to harbor sufficient variability for improvement in mineral content (White and Broadley, 2009) which could be used for improvement in modern day varieties. In rice, a fourfold difference was found in grain Fe and Zn content in some aromatic lines as compared to popular cultivars. In maize, Banziger and Long (2000) evaluated 1814 accessions in 13 trials over 6 years and reported that a range of 9.6–63.2 mg/kg of grain

Fe and 12.9–57.6 mg/kg of grain Zn. In beans, over 1000 genotypes of Centro Internacional de Agricultura Tropical (CIAT) core collections were screened and were found to have Fe content in ranges of 34–89 mg/kg and Zn in the range of 21–54 mg/kg (Beebe et al., 2000).

In wheat, many studies exploring variation in the grain iron and zinc content in the old and modern wheat cultivars, wild germplasm, and landraces have been done and wild relatives were found to contain three-fourfold higher grain iron and zinc content than the popular cultivars (Rawat *et al*., 2009). Wild relatives have been used to transfer genes for biotic and a biotic stress tolerance and yield and quality improvement in cultivated varieties, and likewise, these can also be used to transfer useful variability for grain iron and zinc content using conventional and modern breeding approaches (Chhuneja *et al*., 2008). Oury *et al*. (2006) studied GxE interactions in wheat cultivars for iron, zinc and magnesium concentrations and reported genotypes to have higher effect than environment.

4. The Impact Pathway of bio-fortification

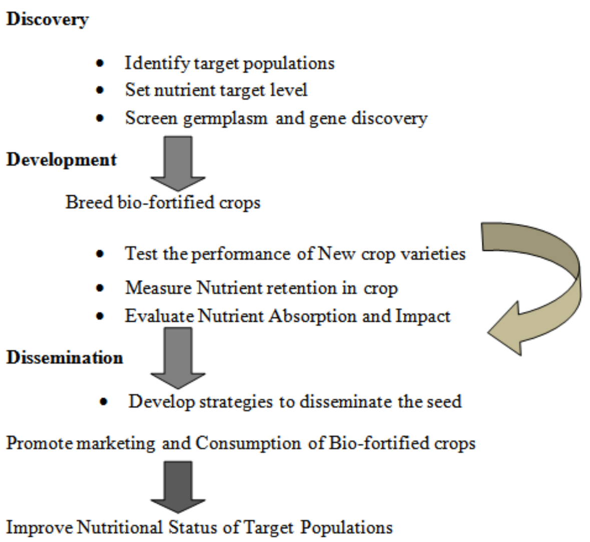

Figure 1. Simplified diagram of the pathway for bio-fortified crops (HarvestPlus, 2009)

For bio-fortification to succeed several factors have to be considered, starting with the identification of the targeted population and ending with the improvement of the nutritional status of this population. The "impact pathway for bio-fortified crops" as suggested by HarvestPlus is divided into the following three stages 1) discovery 2) development and 3) dissemination of the newly developed plant variety (Figure 1).

4.1. Discovery

The discovery stage starts with the identification of targeted populations, for which the bio-fortified crop should be developed. The targeted populations are not necessarily restricted to only one country and spillover effects to other countries or areas have to be taken into consideration. The selection should be done with regard to the prevalence of micronutrient deficiencies, the production and consumption of the targeted crop and the proportion and importance of self- or locally produced plants (Ortiz-Monasterio *et al.*, 2007). Fragmentary or missing data from national health surveys complicates the identification of populations effected by micronutrient deficiencies in many cases (Zapata-Caldas *et al.*, 2009). Further, to correctly assess the consumption of the targeted crop in a population, the availability of representative and reliable dietary intake data has to be assured (Hotz and McClafferty, 2007).

The appropriate target levels for micronutrients in the bio-fortified crop have to be set (Table 1). The setting of target levels should be done in co-operation of plant breeders and nutritionists (Ortiz-Monasterio *et al.*, 2007). Nutritionists estimate the micronutrient concentration, which is necessary to have an impact on the nutrition and health status of the targeted populations. For that, as mentioned above, the daily consumption of the crop has to be assessed, an exercise which has proven to be difficult since dietary intake data of many countries are often incomplete or inexistent. The retention of the nutrients following processing and cooking has to be taken into consideration as well as the bioavailability of the minerals after processing when eaten in a traditional diet (Hotz and McClafferty, 2007). Bioavailability of minerals is mainly influenced by the concentration of inhibitors and enhancers in the food (Hallberg, 1981), and their concentration in turn is strongly depending on food processing and dietary habits. It has been shown that soaking strongly effects the concentration of inhibitors in the food mainly due to their leaching into the water. However, if the water is not discarded but rather consumed, soaking has only minor effects (Luo *et al.*, 2009). An overview of the assumptions used to set micronutrient target levels are given in Table 1.

At the same time, plant breeders estimate possibilities in terms of breeding additional nutrients into the plant. This includes the identification of the genetic variability of the targeted crop by screening varieties which are able to accumulate high levels of the targeted minerals (Ortiz-Monasterio *et al*., 2007). During the screening process, lines have to be identified which accumulate and store a high proportion of the absorbed nutrients in their edible part and lines which have an increased nutrient uptake while maintaining the high proportion of nutrients in the edible part (Calderini and Ortiz- Monasterio, 2003). However genetic variability is limited and bioavailability of minerals in plant based diets often very low. Plant breeders should therefore not only focus on increasing the mineral concentration but also on increasing the mineral bioavailability from staple foods. Breeding for low/high concentrations of inhibitors/enhancers in combination with high mineral concentrations makes success of bio-fortification more likely (Nestel *et al*., 2006).

PA is the major cause of low mineral bioavailability from plant staples. Recently isolated low PA mutants (lpa) in wheat, rice, maize, barley (Larson *et al.*, 1998) and beans have the potential to alleviate bioavailability problems of micronutrients associated with PA. These mutants have normal phosphate levels, but reduced PA phosphate due to various mutations in the biosynthetic pathway of PA. However, the plants exhibit normal phosphate uptake and transport. So far lpa crops are in an early stage of development and most of them exhibit reduced yield and seed germination (Guttieri *et al*., 2006).

In addition, germplasm with greater abilities to cope with adverse climate or soil conditions should be selected. Additionally, for acceptance of the new variety by the farmers, plant breeders should focus on high yield and resistance against diseases (Ortiz-Monasterio *et al*., 2007). The screening of different varieties is basically done in a number of international research centers as e.g. the International Center for Tropical Agriculture (CIAT) or the International Rice Research Institute (IRRI), which are in turn supported by and linked to the Consultative Group on International Agricultural Research (CGIAR).

Table 1. Assumptions made to set micronutrient target levels for bio-fortified crops (Bouis and Welch, 2010)

Amount of nutrient	Criteria	Rice (Polished)	Wheat (Whole)	Pear millet (Whole)	Bean (Whole)	Maize (Whole)	Cassava (Freshwt.)	Sweet potato (Freshw.t)
Percapita consumption	Adult women	400	400	400	300	200	400	200
	Children 4-6yrs old	200	200	150	100	200	200	100
Fe	% of EAR to achieve				30			
	Ear non pregnant, non lactating women(µg/day)				1460			
	EAR children(4-6 years old)				500			
	Micro nutrient retention after processing (%)	90	90	90	90	90	90	90
	Bio-availability (µg/g)	10	5	5	5	5	10	10
	Baseline micro nutrient content (µg/g)	2	30	47	50	30	4	6
	Additional content required(µg/g)	11	22	30	44	22	11	22
	Final target content (µg/g)	13	52	77	94	52	15	28
	Final target content as dry weight (µg/g)	15	59	88	107	60	45	8
Zn	% of EAR to achieve				40			
	Ear non pregnant, non lactating women(µg/day)				1860			
	EAR children(4-6 years old)				830			
	Micro nutrient retention after processing (%)	90	90	90	90	90	90	90
	Bio-availability (µg/g)	25	25	25	25	25	25	25
	Baseline micro nutrient content (µg/g)	16	25	47	32	25	4	6
	Additional content required(µg/g)	8	8	11	17	8	8	17
	Final target content (µg/g)	24	33	58	49	33	12	23
	Final target content as dry weight (µg/g)	28	38	66	56	36	34	70
Pro-vitamin- A	% of EAR to achieve				50			
	Ear non pregnant, non lactating women(µg/day)				500			
	EAR children(4-6 years old)				275			
	Micro nutrient retention after processing (%)	50	50	50	50	50	50	50
	Bio-availability (µg/g)	12.1	12.1	12.1	12.1	12.1	12.1	12.1
	Baseline micro nutrient content (µg/g)	0	0	0	0	0	1	2
	Additional content required(µg/g)	15	15	20	30	15	15	30
	Final target content (µg/g)	15	15	20	30	15	16	32
	Final target content as dry weight (µg/g)	17	17	23	34	17	48	91

4.2. Development

The development stage mainly focuses on the development and testing of bio-fortified crops. An overview of crops currently undergoing the bio-fortification process is given in Table 2.The identification of promising lines by breeders is followed by mapping of genotypic differences. New varieties are developed by crossing promising lines and selecting those with favorable characteristics over many generations (Grusak and Cakmak, 2004). The performances of the newly developed bio-fortified varieties are then tested over different environments, to asses genetic and environment (GxE) interactions. It is suggested that the variability of minerals in the germplasm depends on the genotype, the environment and GxE interaction, but the impact of the various factors differs between the minerals and crops.

Once the desired variety is developed, the consumer acceptance in terms of taste, look and cooking quality is evaluated (Khoshgoftarmanesh *et al*., 2010). In a next step the performance of the new variety in terms of micronutrient retention is tested, followed by the investigation of micronutrient bioavailability in humans. If results from these preliminary tests are promising, the performance of the new variety is investigated in an efficacy trial in human subjects, which is usually implemented as a follow up study to an absorption study. Efficacy trials aim at examining whether an intervention produces the expected results under idealized conditions. This is why efficacy trials are very closely monitored, well-controlled and conducted by highly trained specialists (Hallfors *et al*., 2006). They require a rigorous research design including a specified and standardized treatment within standardized settings (Flay *et al*., 2005). Subjects often belong to a narrowly defined, homogenous group, who should be part of the targeted population. It has to be that the participants accept and comply with the treatment (Glasgow *et al*., 2003). To reduce the probability for bias, efficacy trials usually use a randomized controlled design.

Participants are randomly allocated to the intervention and control group to increase the likelihood of equal distribution of unknown factors. To further avoid bias, efficacy studies should ideally be blinded trials. The strict standardization of efficacy trials allows a direct attribution of observed effects to the intervention being studied (Glasgow *et al*., 2003).

If the outcome of the efficacy trial is positive, in a next step, the impact of the new variety on human health status is evaluated in an effectiveness trial. In this type of study the beneficial effects of the crop is tested under conditions simulating reality (Gartlehner *et al*., 2006). This is usually done among a broadly defined population which is representative for the targeted audience (Glasgow et al 2003). The food is prepared and eaten in traditional ways within the usual household environment (Khoshgoftarmanesh *et al.*, 2010). Standardization only takes places in terms of access and availability of the bio-fortified crop among the population. To be sure that a crop is ready for dissemination, an effectiveness trial should be implemented since the outcome might be different from the efficacy trial and hidden difficulties, such as lack of proper implementation or weak acceptance might be uncovered (Hallfors *et al*., 2006). It is debatable whether efficacy trials prior to effectiveness studies are necessary if the latter meet the standards of efficacy trials (Flay *et al.*, 2005).

Table 2. Crops currently undergoing bio-fortification process

Crop	Target nutrient	Nutrient range (µg/g)	Nutrient target level (µg/g)
Rice	Zinc	13-18	Polished rice
	Iron	6-24	
Wheat	Zinc	25-65	(Whole wheat)
	Iron	25-56	
Maize	β- Carotene	5-8.6	(Whole maize)
	Zinc	13-58	
	Iron	10-63	
Cassava	β- Carotene	0.1-20	(fresh wt.)
Beans	Iron	53-112	
	Zinc	20-55	
Sweet potato	β- Carotene	0-100	(fresh wt.)
Peirl millet	Iron	47	(whole peril millet)
	Zinc	47	

5. Conventional Plant Breeding Versus Genetic Engineering for Bio-Fortification

Conventional plant breeding and genetic engineering both involve changing the genotype of targeted crops with the aim of developing plants carrying genes that support the accumulation of bio-available minerals. The way of reaching this goal differs between the two approaches (Gomez-Galera *et al*., 2010). As already mentioned above, the main nutrients targeted for bio-fortification are beta-carotene, iron and zinc. Most work is currently done on traditional plant breeding techniques, exploiting the variability of mineral concentrations found in different germplasm (Qaim *et al*., 2007).

Not all crops have the genetic potential to meet desired micronutrient levels with traditional plant breeding, and therefore genetic engineering has to be applied to achieve sufficient improvements (Borg *et al*., 2009). It is suggested that genetic modification is an excellent approach to obtain high micronutrient concentrations (Bouis, 2010) and that genetically modified organisms (GMO) have the potential for increased agricultural productivity. A positive factor is the fast development and stable expression of GMO traits. To receive the desired new variety far fewer breeding generations are needed with genetic engineering compared to traditional plant breeding. Additionally, genetic engineering is more precise since single genes can be introduced in the targeted plants. But usually patented or patentable inventions are associated with the developed GMOs, making them inaccessible for researchers in developing countries and unaffordable for farmers (Pardey *et al.*, 2000).

About 70% of investments for the development of genetically modified plants come from the private sector, situated in developed countries, with hardly any focus on nutrition and the needs of developing populations (Fresco, 2003). Aside from numerous regulatory and political restrictions, transgenic plants often have to face social and ethical considerations causing a certain resistance to them (WHO, 2005). This resistance is often further intensified by existing health concerns (Seralini *et al*., 2007). Many genes and traits in GMOs are new and their use has not proven to be safe. The application of antibiotic resistance markers, which help recovering transformed cells, is discussed controversially in literature. It might be possible that transgenes survive human digestion and reach the colon where they are taken up by intestinal microflora, leading to a transfer of antibiotic resistance genes (Netherwood *et al*., 2004), but the risk is negligible and transfer has been shown not to occur at a detectable frequency (Demaneche *et al*., 2008).

Furthermore, it is assumed that GMOs might exhibit certain toxicity, allergenicity and carcinogenicity. Also GMOs might affect human health indirectly through negative impacts to the environment (invasion of natural habitats, reduction of biodiversity, horizontal gene transfer; Conner *et al (*2003), economic or social and ethical factors (WHO, 2005). However, the focus of bio-fortification initiatives is mainly on the development of new plant varieties by traditional plant breeding since the approach is regarded at present as the more appropriate strategy for developing countries.

6. Targeted Crops for Bio-Fortification

Welch *et al* (2005) found 7 times higher Fe and 4 times higher Zn values in grains from the durum wheat grown under hydroponic conditions in a nutrient solution than Ortiz-Monasterio *et al* (2007) who investigated the same cultivar grown in the field. Furthermore, especially in case of iron, it has to assured that the germplasm is not contaminated with iron from soil or dust (Hallberg and Bjornrasmussen, 1981).

6.1. Wheat

More than 3000 lines have been screened for Fe and Zn with concentrations for iron ranging from 25 µg to 56 µg per g wheat and for zinc ranging from 25 µg - 65 µg/ g wheat (Ortiz-Monasterio *et al*., 2007). They observed high

G x E interactions For zinc and iron and low G x E interactions for Mg and suggested that breeding for high concentrations of Fe and Zn might be difficult. Substantial impact of G x E interactions on mineral concentration was confirmed by a recently conducted study in India (Joshi *et al*., 2010), and it is suggested that genetic factors for zinc and iron concentration in the wheat plant are of minor importance.

Furthermore milling, which is accompanied by the removal of the seed coat and embryo, leads to a substantial decline in iron concentration of 40% (Borg *et al*., 2009). However, iron and zinc correlate positively in wheat (Zhang *et al.*, 2010) and highest concentrations (up to 85 µg /g) were detected in landraces as well as in wild and primitive relatives (Peleg *et al*., 2008). It is proposed that crossing wild wheat relatives with high yield cultivars might be the best strategy to increase the micronutrient concentration in wheat (Calderini and Ortiz- Monasterio, 2003).

The strategy of HarvestPlus is to obtain a new wheat variety by crossing high micronutrient wheat varieties with modern wheat (short stems and husk free). The newly developed wheat plant is expected to contain 40- 50% more iron and zinc than currently cultivated varieties (HarvestPlus, 2006). However, depending on wheat intake targeted levels might be lower than the levels recommended by WHO for wheat flour fortification. To improve iron status flour fortification levels should deliver about 6 mg additional iron in the form of ferrous sulfate. Bio-fortified wheat varieties would deliver only about 1 mg additional iron per 100 g wheat flour (40% losses due to milling).

6.2. Rice

The natural variation of iron in rice is quite low and milling and polishing usually results in a loss of up to 80% since iron is mainly stored in the aleurone layer and not in the endosperm (Brinch-Pedersen *et al.*, 2007). Iron and zinc concentrations in rice of different genotypes (n= 1138) were found to range between 6.3- 24.4 µg/ g and 13.5- 58.4 µg/ g, respectively, suggesting that there is at least some genetic potential to successfully breed high mineral rice.

Although Fe and Zn concentrations in rice are affected by G x E interactions, high levels can be more or less maintained over different environments (Graham *et al*., 1999). The first study investigating the effectiveness of a bio-fortified crop on micronutrient status of humans was done with bio-fortified rice, which was produced by traditional plant breeding. The influence of a high iron (9.8 µg/ g), high yield rice on iron status of Filipino women was tested in (Haas *et al*., 2005). No significant increase in body iron, serum ferrit in and hemoglobin was detected compared to the control group, after 9 month of feeding. Differences were only found in the non-anemic subgroup.

An important limitation of the study was that the bio=fortified rice only provided an additional amount of 2.8 µg iron per g rice, mainly due to losses during processing, and fortification was therefore far below the desired level. This finally led to a low iron intake from rice, which only accounted for less than 20% of total iron consumption, although the high iron rice used in the study almost contained the iron level recommended by HarvestPlus (14.5 µg /g).

Genetically modified rice, expressing soybean or *Phaseolus vulgaris* ferritin, showed an up to two fold increase in iron concentration in the grains (Vasconcelos *et al*., 2003), with accumulation also in the endosperm. However, the over expression of soybean ferritin in rice did not lead to a further iron increase in the seed, only to an exhaustion of iron reserves in the leaves (Qu *et al*., 2005). These results suggest that further iron enrichment is only feasible by increasing iron uptake or iron transport from the roots (Brinch-Pedersen *et al*., 2007). To concomitantly increase iron bioavailability, a phytase from *Aspergillus fumigatus* was introduced into rice grains, resulting in a 130-fold higher phytase activity than in non-transformed rice grains. Unfortunately, most phytases possess only limited thermo-tolerance and strongly loose activity during boiling, cooking and baking (Lucca *et al*., 2001). To further increase mineral bioavailability from rice, a gene encoding for myo-inositol synthase was suppressed, reducing PA concentration by 68% without any effects on seed weight, germination and plant growth.

Other workers introduced a nicotineamine synthase gene in rice grains to increase mineral concentration. Nicotineamine is a metal chelator which is also involved in metal assimilation and homeostasis and might be one of the limiting factors of mineral accumulation. Plants with higher concentrations in nicotineamine were found to have higher amounts of Fe and Zn in leaves and seeds (Lee *et al.*, 2009). Wirth *et al* (2009) developed transgenic high nicotineamine rice and observed 6 fold higher iron concentrations in the endosperm, when compared to the wild type.

Much research has been done on β- carotene enrichment of rice grains for over a decade. Rice, which normally contains beta-carotene only in the leaves, was genetically modified to produce carotenoids in the endosperm. Several generations of the so called "Golden Rice" have been developed up to 37 µg carotenoids per g rice (Paine *et al*., 2005).

6.3. Maize

Maize is one of the major targeted plants of genetic engineering and accounts together with cotton and soybean for 85% of globally planted transgenic varieties, the focus being on the development of insect resistant and herbicide tolerant maize (Fresco, 2003). In terms of bio-fortification a special effort was put into breeding maize with high concentrations of pro-vitamin A carotenoids (HarvestPlus, 2006). In the past, little focus was on high mineral maize mainly due to its low mineral concentrations and lack of genetic variability (Grusak and Cakmak, 2004). Researchers screened more than 1800 maize genotypes including improved germplasm and landraces grown in different locations and years. Iron and zinc concentrations ranged between 10 µg and 63 µg/ g and between 13 µg and 58 µg/ g, respectively, with the highest concentrations found in landraces. To investigate the environmental impact on mineral concentrations, the same germplasm were grown in six different locations. Average iron and zinc concentrations varied by 40% and 24%, respectively. According to the authors, environmental factors play a more significant role in terms of mineral concentration than genetic variation (Bänziger, 2000).

Another study investigated mineral concentrations in different maize varieties and the impact of environment, genetics and GxE interaction on mineral content. The genetic component accounted for 12% of variability, no impact of environment was detected and G x E interactions were highly significant (Oikeh et al., 2003). But results are inconsistent, and a recently conducted study found only small G x E interactions, indicating that environment has little impact (Simic *et al*., 2009). These workers concluded that there is enough genetic variability to increase iron and zinc concentration in maize by bio-fortification. However, Harvest Plus researchers discovered germplasm with iron and zinc concentrations of up to 45 µg/ g and 62 µg/ g, respectively. HarvestPlus states that 60 µg/g of iron and 55 µg/ g of zinc is sufficient to observe a significant impact on the mineral status of targeted populations (HarvestPlus, 2006).

6.4. Cassava

Cassava, an important crop in many developing countries, contains iron and zinc only in low concentrations. Thus, the focus of bio-fortification initiatives is exclusively on increasing beta-carotene concentration (Montagnac *et al*., 2009). The variation of carotenes in cassava is high and strongly depends on the root color. White varieties have by far the lowest concentration (1.3 µg/ g), whereas highest concentrations can be found in orange varieties (12.6 µg/ g). Iglesias *et al* (1997) analyzed 632 accessions from the CIAT germplasm collection of 5500 accessions. They detected germplasm with beta- carotene concentrations above 20 µg/ g, suggesting a high genetic variability that would make it possible to successfully bio-fortify cassava and meet the daily retinol requirements of adults. However, it also has to be taken into consideration that carotene concentration is effected by thermal processing.

6.5. Sweet Potato

The major aim of the bio-fortification programs is the replacement of white fleshed low pro-vitamin A sweet potato varieties with orange fleshed high pro-vitamin A plants. HarvestPlus has set the target level for sweet potatoes at 32 µg/ g (HarvestPlus, 2009), but varieties with concentrations up to 100 µg/ g already exist (Nestel *et al*., 2006). A study conducted in Durban, South Africa observed a significant improvement in vitamin A status of children, when fed with orange fleshed sweet potatoes. Workers provided the children with either orange fleshed potato with a beta carotene concentration of about 100 µg/ g in the cooked root or white fleshed potato without any beta- carotene over a period of 11 weeks (van Jaarsveld *et al*., 2005). Vitamin A liver stores were significantly increased in the treatment group compared to the control group. Furthermore it has been shown that the retention of beta- carotene from orange fleshed sweet potatoes when boiled is very high with about 80% of the initial concentration (van Jaarsveld *et al*., 2006).

7. Summary

Bio-fortification of crops is a feasible and most economical approach for overcoming 'hidden hunger'. Increasing the concentration of minerals in edible portions of cereals involves better uptake from soil and improved translocation to grains from leaves and finally enhanced sequestration to endosperm. Genetic diversity can be utilized to enhance micronutrient composition through conventional and modern breeding approaches. The most promising work plan to successfully alleviate micronutrient malnutrition will be to increase mineral content in the crops and simultaneously enhance their bioavailability by reducing anti-nutritional compounds and/or enhancing concentration of mineral absorption promoters. To effectively combat hidden hunger through bio-fortification, even after the development of bio-fortified varieties, it will be essential to address various socio-economical and sociopolitical challenges to popularize their cultivation by farmers and ultimately their consumption by the end users. A multi-tier coordinated strategy will play a pivotal role in overcoming hidden hunger.

References

[1] Allen, L. H. 2003. Interventions for micronutrient deficiency control in developing countries: past, present and future. J. Nutr. 133: 3877-3878.

[2] Bänziger, M., JL. 2000. The potential for increasing the iron and zinc density through plant breeding. *Food and Nutrition Bulletin* 2000, 21 (4): 397-400.

[3] Banziger, M., and J. Long. 2000. The potential for increasing the iron and zinc density of maize through plant-breeding. Food Nutr. Bull. 21: 397-400.

[4] Beebe, S., A. Gonzalez, and J. Rengifo. 2000. Research on trace minerals in the common bean. Food Nutr. Bull. 21: 387-391.

[5] Borg S, Brinch-Pedersen H, Tauris B, and Holm PB. 2009. Iron transport, deposition and bioavailability in the wheat and barley grain. *Plant and Soil* 325: 15-24.

[6] Bouis HE, Welch, RM. 2010. Bio-fortification: A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South. *Crop Science* 50: S20-S32.

[7] Bouis HE. 2003. Plant breeding: A new tool for fighting micronutrient malnutrition. *Journal of Nutrition*, 132 (3): 491S-494S.

[8] Brinch PH, Borg S, Tauris and B, Holm PB. 2007. Molecular genetics approaches to increasing mineral availability and vitamin content of cereals. *Journal of Cereal Science* 46: 308-326.

[9] Calderini DF, and Ortiz Monasterio I. 2003. Are synthetic hexaploids a means of increasing grain element concentrations in wheat? *Euphytica* 134: 169-178.

[10] Chhuneja, P., S. Kaur, R. K. Goel, M. Aghaee-Sarbarzeh, M. Prashar, and H. S. Dhaliwal. 2008. Transfer of leaf rust and stripe rustresistance from *Aegilops umbellulata* Zhuk. to bread wheat (*Triticum aestivum* L.). Genet. Res. Crop Evol. 55, 849-849.

[11] CIAT/IFPRI, 2002: Bio-fortified Crops for Improved Human Nutrition. A Challenge Program Proposal. Consultative Group on International Agricultural Research, Washington. Available at: http://www.cgiar.org/ bio-fortification.

[12] Conner AJ, Glare TR, and Nap JP. 2003. The release of genetically modified crops into the environment Part II. Overview of ecological risk assessment. *J. Plant* 33: 19-46.

[13] Demaneche S, Sanguin H, Pote J, Navarro E, Bernillon D, and Mavingui P. 2008. Anti bio-ticresistant soil bacteria in transigenic plant fields. *Proc Natl Acad Sci. U S A* 105: 3957-3962.

[14] Flay B, Biglan A, Boruch R, Castro F, Gottfredson D, and Kellam S., 2005. Standards of Evidence: Criteria for Efficacy, Effectiveness and Dissemination. *Prevention Science* 6: 20-26.

[15] Fresco L. 2003. Harnessing Genetic Resources and Making Use of Life Sciences, a New Contract for Sustainable Agriculture. In: Nations FaAOotU (ed). *Towards Sustainable Agriculture for*

Developing Countries: Options from Life Sciences and Biotechnologies: Brussels.

[16] Gartlehner G, Hansen RA, Nissman D, Lohr KN, and Carey TS. 2006. Criteria for distinguishing effectiveness from efficacy trials in systematic reviews. *AHRQ* 6-46.

[17] Glasgow RE, Lichtenstein E, and Marcus AC., 2003. Why don't we see more translation of health promotion research to practice? Rethinking the efficacy to effectiveness transition. *American J. Public Health,* 93: 1261-1267.

[18] Gomez-Galera S, Rojas E, Sudhakar D, Zhu CF, Pelacho AM and Capell T. 2010. Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic Res* 19: 165 180.

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

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

[21] Grusak M, and Cakmak I. 2004. Methods to improve the crop-delivery of minerals to humans and livestock. *International Workshop on Modelling Quality Traits and Their Genetic Variability for Wheat*; Jul 18-21; Clermont-Ferrand, FRANCE.

[22] Guttieri MJ, Peterson KM, and Souza EJ. 2006. Agronomic performance of low phytic acid wheat. *Crop Science,* 46: 2623-2629.

[23] Haas J, Beard J, Murray-Kolb L, del Mundo A, Felix A, and Gregorio G., 2005. Iron Bio-fortified rice improves the iron stores of non-anemic Filipino women. *Journal of Nutrition* 135: 2823-2830.

[24] Hallberg L and Bjornrasmussen, 1981. Phytates and the inhibitory effect of bran on iron-absorption in man. *American Journal of Clinical Nutrition*, 45 (5): 988-996.

[25] Hallfors D, Cho H, Sanchez V, Khatapoush S, Kim HM and Bauer D. 2006. Efficacy vs effectiveness trial results of an indicated "model" substance abuse program: Implications for public health. *Am J Public Health* 96: 2254-2259.

[26] HarvestPlus, 2006.. Bio-fortified Maize http://www.dfid.gov.uk/r4d/PDF/Outputs/Misc rop/maize. pdf: Houston.

[27] HarvestPlus, 2009. Provitamin A Sweet Potato for Uganda and Mozambique. Hotz C,

[28] Joshi AK, Crossa J, Arun B, Chand R, Trethowan R, and Vargas M. 2010. Genotype x Environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. *Field Crop Research,* 116: 268-277.

[29] Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, and Afyuni M., 2010. Micronutrient efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. *Agron Sustain Development,* 30: 83-107.

[30] Khush, G. S. 2003: Productivity improvements in rice. Nutr. Rev. 61, S114-S116.

[31] Kim, S. A. and M. L. Geurinot, 2007: Mining iron: iron uptake and transport in plants. FEB Letter 581: 2273-2280.

[32] King J. C., and C. L. Keen. 1999. Zinc. In: M. E. Shills, J. A. S. Olsem, M. Shike, and A. C.Ross (eds), Modern Nutrition in Health and Disease, 9th edn, 223-239. Williams and Wilkins, Baltimore.

[33] Larson SR, Young KA, Cook A, Blake TK, and Raboy V. 1998. Linkage mapping of two mutations that reduce phytic acid content of barley grain. *Theor Appl Genet* 97: 141- 146.

[34] Lee HC, Jenner AM, Low CS and Lee YK. 2009. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research Microbiology,* 157: 876-884.

[35] Lucca P, Hurrell R, and Potrykus I. 2001. Genetic engineering approaches to improve the bio-availability and the level of iron in rice grains. *Theor Appl Genet* 102: 392-397.

[36] Luo YW, Gu ZX, Han YB, and Chen ZG. 2009. The impact of processing on phytic acid, in vitro soluble iron and Phy/Fe molar ratio of faba bean (*Vicia faba* L.). *Journal of the Science of Food and Agriculture* 89: 861-866.

[37] Mayer JE, Pfeiffer WH, and Beyer P. 2008. Bio-fortified crops to alleviate micronutrient malnutrition. *Curr Opin Plant Biol*, 11 (2):166-170.

[38] Montagnac JA, Davis CR, and Tanumihardjo SA. 2009. Nutritional Value of Cassava for Use as a Staple Food and Recent Advances for Improvement. *Comprehensive Reviews in Food Science and Food Safety* 8: 181-194.

[39] Nestel P, Bouis HE, Meenakshi JV, and Pfeiffer W. 2006. Bio-fortification of staple food crops. *Journal of Nutrition* 136: 1064-1067.

[40] Netherwood T, Martin-Orue SM, O'Donnell AG, Gockling S, Graham J, and Mathers JC. 2004. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nat Biotechnol* 22: 204-209.

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

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

[43] Oury F. X., F. Leenhardt, F. Balfourier, and G.Charmet. 2006. Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. Eur. J. Agron. 25, 177-185.

[44] Paine JA, Shipton CA, Howells RM, Kennedy MJ, and Vernon G. 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat Biotechnol* 23: 482-487.

[45] Pardey PG, Wright BD, and Nottenburg C. 2000. Are Intellectual Property Rights Stifling Agricultural Bio-technology in Developing Countries? In: IFPRI (ed). *Annual Report*.

[46] Peleg Z, Saranga Y, Yazici A, Fahima T, Ozturk L, and Cakmak I. 2008. Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. *Plant and Soil* 306: 57-67.

[47] Qaim M, Stein AJ, and Meenakshi JV. 2007. Economics of bio-fortification. *Agr Econ-Blackwell* 37: 119-133.

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

[49] Rawat, N., V. K. Tiwari, P. Chhuneja, and H. S.Dhaliwal, 2009. Evaluation and utilization of *Aegilops a*nd wild *Triticum* species for enhancing iron and zinc content in wheat. Genet. Resour. Crop Evol. 56, 53-64.

[50] Seralini GE, Cellier D, and de Vendomois JS., 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Arch Environ Con Tox* 52: 596-602.

[51] Simic D, Sudar R, Ledencan T, Jambrovic A, Zdunic Z, and Brkic I. 2009. Genetic variation of bio-available iron and zinc in grain of a maize population. *Journal of Cereal Science* 50: 392-397.

[52] Stein A. J. 2010: Global impacts of human malnutrition. Plant Soil 335, 133-154.

[53] Tanumihardjo SA, Bouis H, Hotz C, Meenakshi JV, and McClafferty B., 2008. Bio-fortification of staple crops: An emerging strategy to combat hidden hunger. *Comprehensive Reviews in Food Science and Food Safety* 7: 329-334.

[54] Tiwari, V. K., N. Rawat, K. Neelam, S. Kumar, G. S. Randhawa, and H. S. Dhaliwal. 2010. Substitution of 2S and 7U chromosomes of Aegilops kotschyi in wheat enhances grain iron and zinc concentration. Theor. Appl. Genet. 121, 259-269.

[55] Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, and Benade AJS. 2005. beta-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *American Journal of Clinical Nutrition* 81: 1080-1087.

[56] Van Jaarsveld PJ, Marais DW, Harmse E, Nestel P, and Rodriguez-Amaya DB. 2006. Retention of beta-carotene in boiled, mashed orange-fleshed sweet potato. *Journal of Food Composition and Analysis* 19: 321-329.

[57] Vasconcelos M, Datta K, Oliva N, Torrizo L, and Krishnan S. 2003. Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Sci* 164: 371-378.

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

[59] White P. J., and MR. Broadley, 2009: Bio-fortification of crops with seven mineral elements often lacking in human diets iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182, 49- 84.

[60] WHO, 2005. Modern food biotechnology, human health and development: an evidence based study: Geneva.

[61] Wirth J, Poletti S, Aeschlimann B, Yakandawala N, Drosse B, and Osorio S. 2009. Rice endosperm iron bio-fortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnol J.* 7: 631-644.

[62] Zapata-Caldas E, Hyman G, Pachón H, Monserrate A, and Varela LV. 2009. Identifying candidate sites for crop bio-fortification in Latin America: case studies in Colombia, Nicaragua and Bolivia. *International Journal of Health Geographics.*

[63] Zhang Y, Song Q, Yan J, Tang J, Zhao R, Zhang Y., 2010. Mineral element concentrations in grains of Chinese wheat cultivars. *Euphytica* 174: 303-313.

[64] Zimmerman MB. and Hurrel RF. 2007. Nutritional iron deficiency. Lancet 370, 511-519.