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| Abstract | Micronutrients are essential minerals and vitamins required by humans in tiny amounts which play a vital role in human health and development. Over three billion people in the world are malnourished, particularly in the developing countries. Current food systems cannot provide sufficiently balanced micronutrients required to meet daily needs and to sustain the wellbeing of people in developing countries. Heavy and monotonous consumption of cereal-based foods which contain limited amounts of micronutrients is one of the major reasons for the significantly high prevalence of micronutrient deficiencies in many of the developing countries. The development of crops with enhanced micronutrient concentration is one of the most sustainable and cost-effective approaches to alleviate micronutrient malnutrition globally. In this chapter we focus on the research to improve mineral element concentration in crops through plant breeding strategies, especially in major cereal crops and a legume which are most widely cultivated and preferred in Africa and Asia. Biofortification is an appropriate strategy to increase the bioavailable concentrations of an element in edible portions of crop plants through traditional breeding practices or modern biotechnology to overcome the problem of micronutrient deficiencies. Therefore, conventional breeding with modern genetic engineering approaches are important for developing crop cultivars with enhanced micronutrient concentrations to improve human health. This chapter reports on biofortification research on rice, pearl millet, sorghum, maize, wheat and common bean. |


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| Keywords (separated by “ - ”) | Biofortification - Bioavailability - Micronutrient deficiency - Micro nutrients - Fe - Zn |

# Chapter 2
# Breeding Crop Plants for Improved Human Nutrition Through Biofortification: Progress and Prospects

**Prakash I. Gangashetty, Babu N. Motagi, Ramachandra Pavan, and Mallikarjun B. Roodagi**

**Abstract** Micronutrients are essential minerals and vitamins required by humans in tiny amounts which play a vital role in human health and development. Over three billion people in the world are malnourished, particularly in the developing countries. Current food systems cannot provide sufficiently balanced micronutrients required to meet daily needs and to sustain the wellbeing of people in developing countries. Heavy and monotonous consumption of cereal-based foods which contain limited amounts of micronutrients is one of the major reasons for the significantly high prevalence of micronutrient deficiencies in many of the developing countries. The development of crops with enhanced micronutrient concentration is one of the most sustainable and cost-effective approaches to alleviate micronutrient malnutrition globally. In this chapter we focus on the research to improve mineral element concentration in crops through plant breeding strategies, especially in major cereal crops and a legume which are most widely cultivated and preferred in Africa and Asia. Biofortification is an appropriate strategy to increase the bioavailable con-



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centrations of an element in edible portions of crop plants through traditional breeding practices or modern biotechnology to overcome the problem of micronutrient deficiencies. Therefore, conventional breeding with modern genetic engineering approaches are important for developing crop cultivars with enhanced micronutrient concentrations to improve human health. This chapter reports on biofortification research on rice, pearl millet, sorghum, maize, wheat and common bean.

**Keywords** Biofortification • Bioavailability • Micronutrient deficiency • Micro nutrients • Fe • Zn

## 2.1 Introduction

For good health, humans require at least 49 essential nutrients to meet their metabolic needs (Table 2.1).

Insufficient ingestion of even one of these essential nutrients will result in adverse metabolic disturbances leading to sickness, poor health, impaired development in children and high economic costs to society (Branca and Ferrari 2002; Golden 1991; Grantham-McGregor and Ani 1999; Ramakrishna et al. 1999). Micronutrient deficiency is the lack of essential vitamins and minerals required in small amounts by the body for proper growth and development. Micronutrients are not limited to



**Table 2.1** The 49 known essential nutrients for sustaining human life

| Water and energy | Protein (amino acids) | Lipids-fat (fatty acids) | Macro elements | Micro elements | Vitamins |
|---|---|---|---|---|---|
| Water | Histidine | Linoleic acid | Na | Fe | A |
| Carbohydrates | Isoleucine | Linolenic acid | K | Zn | D |
| | Leucine | | Ca | Cu | E |
| | Lysine | | Mg | Mn | K |
| | Methionine | | S | I | C |
| | Phenylalanine | | P | F | $B_1$ |
| | Threonine | | Cl | B | $B_2$ |
| | Tryptophan | | | Se | $B_3$ |
| | Valine | | | Mo | Niacin |
| | | | | Ni | $B_6$ |
| | | | | Cr | Folate |
| | | | | V | Biotin |
| | | | | Si | $B_{12}$ |
| | | | | As, Sn, Co (Cobalamin) | |

Source: Welch and Graham (2002)

vitamins A, B, C and D, but also include, calcium, folate, iodine, iron and zinc. Common micronutrient deficiencies among children and lactating women include iron, iodine, vitamin D, selenium, vitamin A, folate and zinc. The Food and Agricultural Organization, United Nations, and the World Health Organization (FAO/WHO 2000) reported the daily required amounts for some of the essential nutrients for adults, which are listed in Table 2.2. Agricultural products are the primary source of all these nutrients. If agricultural systems fail to provide enough products containing adequate quantities of all nutrients during all seasons, the result is a dysfunctional food system that cannot support healthy lives. Unfortunately, this

**Table 2.2** Recommended nutrient intakes for males and females between the ages of 25 and 50

| Nutrient | Assessment | Male | Female |
|---|---|---|---|
| Energy (kcal) | AEA | 2,900 | 2,200 |
| Protein (g) | AEA | 63 | 50 |
| Vitamin A(µg retinol equivalent) | RDA | 1,000 | 800 |
| Vitamin D (µg) | RDA | 5 | 5 |
| Vitamin E (mg) | RDA | 10 | 8 |
| Vitamin K (µg) | RDA | 80 | 65 |
| Riboflavin (mg) | RDA | 1.7 | 1.3 |
| Niacin (mg) | RDA | 19 | 15 |
| Thiamin (mg) | RDA | 1.5 | 1.1 |
| Pantothenic acid ($mgd^{-1}$) | ESADDI | 4–7 | 4–7 |
| Vitamin $B_6$ (mg) | RDA | 2 | 1.6 |
| Vitamin $B_{12}$ (µg) | RDA | 2 | 2 |
| Biotin ($\mu gd^{-1}$) | ESADDI | 30–100 | 30–100 |
| Folate (µg) | RDA | 200 | 180 |
| Vitamin C (mg) | RDA | 90 | 60 |
| Ca (mg) | RDA | 800 | 800 |
| P (mg) | RDA | 800 | 800 |
| Mg (mg) | RDA | 350 | 280 |
| Na (mg) | MR | 500 | 500 |
| K (mg) | MR | 2,000 | 2,000 |
| Cl (mg) | MR | 750 | 750 |
| Fe (mg) | RDA | 10 | 15 |
| Zn (mg) | RDA | 15 | 12 |
| Cu (mg) | ESADDIC | 1.5–3.0 | 1.5–3.0 |
| Se (µg) | RDA | 70 | 55 |
| I (µg) | RDA | 150 | 150 |
| Mn (µg) | ESADDI | 2–5 | 2–5 |
| Mo (µg) | ESADDI | 75–250 | 75–250 |
| Cr (µg) | ESADDI | 50–200 | 50–200 |
| F ((mg) | ESADDI | 1.5–4.0 | 1.5–4.0 |

Source: FAO/WHO (2000)

*AEA* Average Energy Allowance, *RDA* Recommended Dietary Allowances, *ESADDI* Estimated Safe and Adequate Daily Dietary Intakes, *MR* Minimum Requirement is the case for many agricultural systems in all developing countries (Graham et al. 2001; McGuire 1993; Schneeman 2001).

Micronutrient malnutrition has been designated as the most serious challenge to humanity (Bouis et al. 2011) because two-thirds of the world population is at risk of deficiency in one or more essential mineral elements (Stein 2010; White and Broadley 2009). The concern is more crucial in developing countries, especially among women, infants and children of resource-poor families. More than one-half of the total populations in developing countries are reported to be affected by micronutrient deficiency and therefore more susceptible to infections and impairment of physical and psycho-intellectual development (WHO 2005). The mineral elements most commonly lacking in human diets are iron (Fe) and zinc (Zn) (Stein 2010; White and Broadley 2009), whereas other essential minerals such as calcium (Ca), copper (Cu), magnesium (Mg), iodine (I) and vitamin A can be deficient in some human diets as well (Genc et al. 2005; White and Broadley 2005). These deficiencies are caused by customary diets that lack diversity (overly dependent on a single staple food), situations of food insecurity when populations do not have enough to eat (WHO 2002) as well as low intake of vegetables, fruits, and animal and fish products, which are rich sources of minerals. The widespread deficiencies of Fe and Zn in developing countries are mostly due to monotonous consumption of cereal-based foods with low concentrations and reduced bioavailability of Fe and Zn (Graham et al. 2001; Welch and Graham 1999). The recommended daily allowance (RDA) of both Fe and Zn is 12–15 mg for adults and 10 mg for children (FAO 2003; ICMR 2009). Both minerals have health and clinical significance as they affect growth and development and many physiological and neurophysiological functions (Sandstead 1994).

The causes of malnutrition among children and lactating women worldwide include:

(a) Inadequate maternal, prenatal and perinatal health care; poor prenatal diet,
(b) Premature infant birth; low or very low birth weight resulting in underdeveloped infants,
(c) Inadequate or no breastfeeding,
(d) Animal milk or milk products offered instead of fortified infant formula,
(e) Diluted or improperly prepared infant formula, which decreases the nutritional adequacy of the formula or introduces food safety risks,
(f) Premature introduction of solid foods to the infant diet,
(g) Insufficient amounts of food and/or lack of essential nutrient-rich foods,
(h) Insufficient feedings and/or inappropriate feeding practices in orphanages, particularly for children with special needs,
(i) Inadequate exposure to sunlight, which inhibits vitamin D production, a crucial vitamin that facilitates calcium absorption for bone growth,
(j) Cultural food practices introduced too early. For example, tea is often served with meals in many countries. Although tea has many health benefits, when consumed in large quantities as part of a nutrient-poor diet, naturally-occurring substances in tea may inhibit the absorption of important vitamins and minerals,
(k) Lack of fortified foods, beverages, and vitamin supplements due to high cost or unavailability,

(l) The stress of transitioning from birth mother to secondary care provider and then to the new family can disrupt a child's natural feeding cycle, resulting in nutritional issues (Adoption Nutrition- the go-to nutrition and feeding resource for adoptive and foster families www.adoptionnutrition.org/what-every-parent-needs-to know/contributing-factors-to-malnutrition).

Micronutrient malnutrition greatly increases mortality and morbidity rates, diminishes cognitive abilities of children and lowers their educational attainment, reduces labor productivity, stagnates national development efforts, contributes to continued high population growth rates and reduces the livelihood and quality of life for all those affected (Combs and Welch 1998; Welch and Graham 1999). In an attempt to reverse this scenario, research has been carried out to improve nutrient concentrations in edible crops by biofortification (Bouis et al. 2011; Mayer et al. 2008; Nestel et al. 2006; White and Broadley 2005). Biofortification can be achieved by combining breeding strategies with improved fertilization management (Bouis et al. 2011; Cakmak et al. 2010; Pfeiffer and McClafferty 2007; White and Broadley 2005). Biofortification of staple crops can be a sustainable and cost-effective approach to combat malnutrition (Bouis 1999; Meenakshi et al. 2010) especially of rural populations in remote, low-rainfall areas, with limited access to a diverse diet, commercially-fortified foods or supplements (Saltzman et al. 2013). Genetic variation of grain micronutrient densities in adapted genetic materials is the basic requirement for biofortification breeding programs, and thus needs to be assessed beforehand. Micronutrient-enriched crops can be obtained by conventional breeding or by biotechnological approaches (Brinch-Pedersen et al. 2007; Mayer et al. 2008). An understanding of the genetic basis of the accumulation of micronutrients in food grains and mapping of the quantitative trait loci (QTL) will provide the basis for devising plant-breeding strategies and to improve grain micronutrient content through marker-assisted selection (MAS). Developing micronutrient-enriched staple plant foods, either through traditional plant breeding methods or via molecular biological techniques, is a powerful intervention tool that targets the most vulnerable people (Bouis 2000; Combs Jr et al. 1996).

Studying the importance of malnutrition in developing and underdeveloped countries and also the availability of fortified crops in such countries is a major challenge for policymakers and researchers to provide the hungry world with nutrient rich foods. In many of the countries, agriculture is the main occupation and supplies food to the nation. Hence, biofortification of agriculturally-important crops like maize, rice, wheat, sorghum, pearl millet, manioc and common bean plays a major role in providing the essential micronutrients to this micronutrient deficient world.

This chapter mainly focuses on the genetic enhancement of crop plants for micronutrients with major focus on grain Fe and Zn in solving the problem of micronutrient deficiency through breeding major cereal crops like rice, wheat, pearl millet, sorghum, maize and common bean for improvement in grain yield associated with increased micronutrients. We discuss mainly the introduction and importance of micronutrients in human health. The consequences of deficiencies of micronutrients on human health with respect to Fe, Zn, iodine vitamin D, vitamin A, vitamin B, folate and selenium. We also discuss the genetic enhancement of crop plants for micronutrients, mainly in rice, sorghum, pearl millet, maize and common bean, for the current status of genetic variability for various micronutrients content along with their association with yield and yield components. Later we also discuss the genetic and environmental effect on grain micronutrient content and also on marker-assisted selection and transgenic approaches used for biofortification. The chapter concludes with a statement on biofortification as an improved tool for human health.

## 2.2 Consequences of Micronutrient Deficiencies on Human Health

The importance of some micronutrients and their consequences on human health are discussed under the following headings.

### *2.2.1 Iron (Fe)*

Iron is a micronutrient that is essential to the structure of every cell in the body, but particularly to red blood cells (hemoglobin), which transport oxygen in the blood to body tissues. In addition, iron is also a key component in proteins, in muscle tissue and is critical for the normal development of the central nervous system. Iron deficiency is the most common form of malnutrition worldwide. A lack of iron in the diet results in iron deficiency. The most commonly recognized condition associated with iron deficiency is anemia. Iron deficiency is a worldwide problem that is directly correlated with poverty and food insecurity. Approximately one-third of the world's population suffers from iron deficiency-induced anemia, 80 % of which are in developing countries (Boccio et al. 2003) (Fig. 2.1). In iron deficiency, the amount of iron stored for later use is reduced as indicated by a low serum ferritin level, but has no effect on the iron needed to meet the daily needs of an individual. If the body requires increased iron (due to a rapid growth spurt, for example), a person with inadequately stored iron has no reserves to use. When the body lacks sufficient iron to make adequate hemoglobin, red blood cells cannot transport sufficient oxygen to tissues throughout the body. This can cause iron-deficiency anemia, an advanced stage of iron deficiency. Iron is also critical for normal cardiac and skeletal muscle function and is a key component of enzymes involved in brain development. The major causes of iron deficiency are inadequate iron intake/availability from foods and blood loss or increased demand due to disease (e.g. malaria, HIV/AIDS) (Lemke 2005; Rosegrant et al. 2003; Skalicky et al. 2006).

The consequences of iron deficiency include increased mortality and morbidity rates, diminished cognitive abilities of children, and reduced labor productivity that in turn stagnate national development (Caballero 2002). Fe deficiency in pregnant women may cause irreversible damage to fetal brain development leading to irreversible damage to intellectual development in their children (Gordon 1997). The developed world

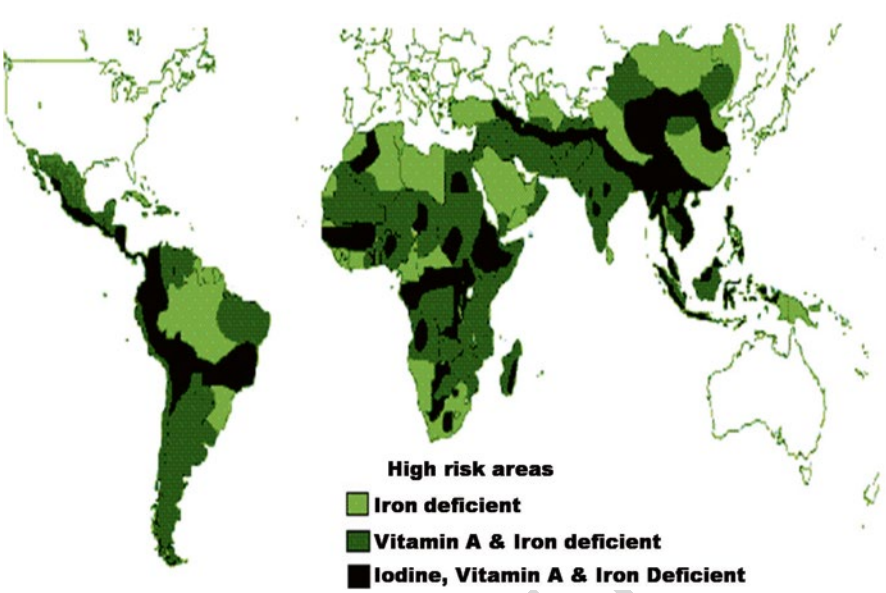


**Fig. 2.1** World map indicating the world population is affected from iron deficiency (Source: Sanghvi (1996))

has made tremendous progress in alleviating micronutrient deficiencies through dietary diversification, processed food fortification, improved public health care and supplementation. In developing countries, these strategies are often too expensive and difficult to sustain. Treatment for iron deficiency includes oral iron supplementation that can be used for both prevention and treatment of iron deficiency anemia. Oral iron supplements are usually best absorbed on an empty stomach. However, because iron can irritate a child's stomach, supplements may need to be taken with food. A source of vitamin C, like citrus juice, enhances iron absorption. It usually takes several months of iron supplementation to correct the deficiency; iron also is rich in foods such as meats, poultry and fish, fortified cereals and oatmeal, legumes (e.g. soybeans and lentils), leafy greens and seeds (e.g. sesame and pumpkin).

### *2.2.2 Zinc (Zn)*

Zinc is an essential mineral found in over 200 enzymes that are involved in a wide range of body functions. These zinc-containing enzymes play a role in immune function, wound healing, and making DNA and other proteins. Zinc supports normal growth and development during childhood and adolescence, and is required for a proper sense of taste and smell. Because zinc plays so many roles in the body, including brain development, a deficiency of zinc can impact multiple bodily functions and result in a wide variety of symptoms. Zinc deficiency alone is a major cause of child death in the world, and responsible for nearly 450,000 children deaths (4.4 % of the children deaths per year globally) under 5 years of age (Black et al. 2008). Deficiency of zinc in the human body will result in a number of cellular disturbances and impairments such as immune dysfunctions and high susceptibility to infectious diseases, retardation of mental development, altered reproductive biology, gastrointestinal problems and stunted growth of children, reduced growth and, sexual maturity and weakened immune defense system (Black et al. 2008). Zinc deficiency can also contribute to vitamin A deficiency, since lack of zinc impairs the synthesis of the retinol-binding protein. Low dietary zinc intake (in general) is the main cause of zinc deficiency. The risk of zinc deficiency is particularly high in populations which depend on diets with low levels of absorbable zinc and with no or only limited access to sources rich in bioavailable zinc such as meat. Zinc deficiency is a problem particularly in regions where the population consumes mainly cereals and where soils are low in phytoavailable zinc (Cakmak 2008). Kim et al. (1998) showed that marginal zinc deficiency lowers the lymphatic absorption of vitamin E ($\alpha$-tocopherol) in rats. Thus, intestinal absorption of vitamin E is reduced by low-zinc status. Zinc deficiency can be managed by supplements (zinc sulfate or zinc gluconate), increasing dietary intake, vitamin and mineral supplements to aid in zinc absorption (e.g. A, E, B6, magnesium, phosphorous and calcium). Foods high in zinc include meats and seafood, eggs, whole grains and oats, nuts and seeds, leafy greens, vegetables, herbs and yogurt.

### 2.2.3 Iodine (I)

Iodine is a nutrient essential for normal functioning of the thyroid gland, production of thyroid hormones and metabolism. Iodine deficiency is the world's most common, but preventable, deficiency and a cause of mental retardation. Iodine deficiency is common in areas where there is little iodine in the diet particularly in remote inland areas where no marine foods are eaten and in mountainous regions of the world where food is grown in iodine-poor soil. Iodine is typically found in small amounts in food and varies depending on environmental factors such as the soil concentration of iodine and the use of fertilizers. Prevention includes adding small amounts of iodine to table salt, a product known as iodized salt. Iodine compounds have also been added to other foodstuffs, such as bread (fortified), dairy products (e.g. cheese, cow milk and yogurt), soy milk, soy sauce and seafood. A meta-analysis found that iodine supplementation improves some maternal thyroid indices and may benefit aspects of cognitive function in school-age children, even in marginally iodine-deficient areas (Taylor et al. 2014). Iodine is not produced by the body, so it must be obtained through diet. Sufficient thyroid hormone is not produced without enough iodine. Iodine deficiency can lead to enlargement of the thyroid (goiter), hypothyroidism, and mental retardation in infants and children whose mothers were iodine deficient during pregnancy. Iodine deficiency resulting in goiter occurs in 187 million people globally as of 2010 (2.7 % of the population) (Vos et al. 2012). It resulted in 2,700 deaths in 2013 up from 2,100 deaths in 1990 (GBD 2013). Consuming foods high in iodine can help treat and prevent iodine deficiency (http://www.orphannutrition.org/understanding-malnutrition/micronutrient-malnutrition/#iodin).

### 2.2.4 Vitamin D

Vitamin D is a fat-soluble vitamin naturally produced in the body. It is essential to the absorption of calcium for proper bone development and function. Vitamin D is found in cod and cod liver oil, egg yolks, milk and butter, fortified cereals and salmon and shrimp. Hypovitaminosis D is a deficiency of vitamin D, which can lead to abnormalities in bone development and a condition in children called rickets, wherein, bones become soft and may bend, distort and/or fracture. It is one of the most common childhood diseases in many developing countries. Treatment of rickets involves vitamin D supplementation, increasing dietary intake of calcium, phosphates, and vitamin D, daily exposure to small amounts of sunlight (15 min/day for lighter-skinned children; longer for darker-skinned children), special braces to position the bones (severe cases), surgery (very severe skeletal deformities) (http://www.orphannutrition.org/understanding-malnutrition/identifying-malnutrition-in-orphans/#vitamin D).

Emerging evidence suggests that vitamin D plays a role in non-alcoholic fatty liver disease (NAFLD) pathogenesis (Eliades et al. 2013). NAFLD is one cause of a fatty liver, occurring when fat is deposited (steatosis) in the liver due to causes other than excessive alcohol use. NAFLD is the most common liver disorder in Western industrialized nations (Shaker et al. 2014).

### 2.2.5 Vitamin A

Vitamin A is a group of compounds that play a significant role in vision, bone development, immune support and normal bodily function. Retinol and beta-carotene are forms of pre-vitamin A which are converted to vitamin A in the body. Deficiency is a common problem in developing countries, but rarely seen in developed countries. In Africa, vitamin A deficiency (VAD) affects more than 30 million children, is a contributing factor to 10.8 million deaths overall and causes blindness in another 2.55 million annually. VAD is estimated to affect approximately one-third of children under the age of 5 around the world. It is estimated to claim the lives of 670,000 children under the age of 5 annually (WHO 1995–2005). Approximately 250,000–500,000 children in developing countries become blind each year owing to VAD, with the highest prevalence in Southeast Asia and Africa (Black et al. 2008). According to the World Health Organization, VAD is under control in the United States, but in developing countries is a significant concern. Nyctalopia (night blindness) is one of the first signs of VAD, later it can lead to xerophthalmia, keratomalacia and complete blindness since Vitamin A has a major role in phototransduction. As elucidated by Sommer et al. (1986), vitamin A deficiency leads to increased risk in children of developing respiratory and diarrheal infections, decreased growth rate, slow bone development and decreased likelihood of survival from serious illness. Treatment for vitamin A deficiency includes oral and injectable supplementation, food fortification and increasing consumption of vitamin A-rich foods from animals, fruits and vegetables.

### *2.2.6 Vitamin B12*

Vitamin B12 is a water-soluble vitamin that exists in several forms. Vitamin B12 is needed for proper red blood cell formation and the maintenance of healthy nerve cells. It is also essential in making DNA, the genetic material in cells. Vitamin B12 is found in fortified cereals and occurs naturally in foods coming from animals, including fish, meat poultry, eggs, milk and milk products. Vitamin B12 deficiency, also known as hypocobalaminemia, refers to low blood levels of vitamin B12 (Herrmann and Wolfgang 2011). Deficiency leads to a wide variety of signs and symptoms including a decreased ability to think and changes in personality such as depression, irritability, psychosis, abnormal sensations, changes in reflexes, poor muscle function, inflammation of the tongue, decreased taste, low red blood cells, reduced heart function and decreased fertility (Hunt et al. 2014). Without early treatment some of the changes may be permanent (Lachner et al. 2012). Increased requirements occur in HIV/AIDS and in those with rapid red blood cell breakdown (Hunt et al. 2014). Diagnosis is typically based on vitamin B12 blood levels below 120–180 picomol/L (normal level, 170–250 pg/mL) in adults. Once identified it is easily treated with supplementation by mouth or injection (Vidal et al. 2005), nasal sprays and increased consumption of animal products. Plants which provide vitamin B12 include vegetables and fortified cereal foods with meat, fish and eggs.

### *2.2.7 Folate ($C_{19}H_{19}N_7O_6$)*

Folate, also known as vitamin B9, is a water-soluble vitamin naturally occurring in foods. Folate is necessary for the production and maintenance of new cells and is especially important during periods of rapid cell division and growth, such as infancy and pregnancy. Both adults and children need folate to make normal red blood cells and prevent anemia. Folate is involved in adenosine, guanine and thymidine synthesis (part of DNA synthesis). Insufficient quantities cause the medicinal condition of folate deficiency anemia (Huethe et al. 2004). Initial symptoms of deficiency are loss of appetite and weight; additional signs are weakness, sore tongue, headache, heart palpitation, irritability and behavioral disorders. In adults, anemia (macrocytic, megaloblastic anemia) can be a sign of advanced folate deficiency (Haslam and Probert 1998). Folate occurs naturally in leafy greens (e.g. spinach and turnip greens), peas, beans, fruits and other vegetables. Folic acid (synthetic folate) is commonly added to enrich grain products such as cereals, rice, pasta, bread and flour. Inadequate dietary intake of folate can slow growth rate in infants and children. Folic acid is available in most multivitamins and in some foods. Supplementing the diet with vitamins and foods rich in folate or folic acid can help prevent and treat folate deficiency.

### *2.2.8 Selenium (Se)*

Selenium is a trace mineral needed in small amounts by the human body for good health. It is incorporated into proteins to make important antioxidant enzymes. These enzymes help prevent cellular damage from free radicals that can cause the development of chronic diseases such as cancer and heart disease. Selenium can be found in foods such as Brazil nuts, tuna, cod fish, beef, poultry, enriched pasta, rice, eggs, cottage cheese and oatmeal. In the USA, the Dietary Reference Intake for adults is 55 μg/day. In the UK it is 75 μg/day for adult males and 60 μg/day for adult females. The 55 μg/day recommendation is based on full expression of plasma glutathione peroxidase. Selenoprotein P (Papp et al. 2007) is a better indicator of selenium nutritional status and full expression of it would require more than 66 μg/day (Xia et al. 2005). Selenium deficiency is a result of inadequate selenium in the diet. Though rare, it can lead to three specific diseases: Keshan disease results in an enlarged heart and poor heart function in selenium-deficient children. Kashin-Beck disease results in osteoarthritis and weakened immune system in children (Moreno et al. 1998). Myxedematous endemic cretinism results in mental retardation in infants born to mothers deficient in both selenium and iodine. Selenium supplementation protects people from developing Keshan disease but cannot reverse heart muscle damage once it occurs. There is little evidence that improving selenium nutritional status prevents Kashin-Beck disease. It can occur in patients with severely compromised intestinal function, those undergoing total parenteral nutrition, those who have had gastrointestinal bypass surgery and also in individuals of advanced aged (e.g. over 90) (Ravaglia et al. 2000). Selenium is also necessary for the conversion of the thyroid hormone thyroxine (T4) into its more active counterpart, triiodothyronine and as such a deficiency can cause symptoms of hypothyroidism, including extreme fatigue, mental slowing, goiter, cretinism and recurrent miscarriage (http://www.atsdr.cdc.gov/toxprofiles/tp92-c3.pdf).



## 2.3 Genetic Enhancement of Crop Plants for Micronutrients

The success of any crop improvement program depends on the magnitude of genetic variability and the extent to which the desirable trait is heritable. The estimate of variability of yield and yield-contributing characters and their heritable components in the material is important in any crop breeding program. The presence of genetic variability in breeding material has been emphasized by Falconer

(1981), so as to exercise critical selection pressure. Information on the nature and magnitude of variation in the segregating population of a cross where selection is actually practiced will be more meaningful and is of immediate practical utility. Moreover, correlation studies provide information about the relative contribution of various component traits on grain yield per plant and help in effective identification and selection of superior plants. Since yield is polygenically controlled and highly influenced by environment, selection based on yield alone is not effective. Therefore, improvement in yield can be brought about by effecting indirect selection through yield attributes whose heritability is high and shows strong association with yield.

Genetic variability studies provide information about the extent of variation present in a population. The phenotypic variance measures the magnitude of variation arising out of difference in phenotypic values, while the genotypic variance measures the magnitude of variation due to differences in genotypic values. The absolute values of phenotypic and genotypic variances cannot be used for comparing the magnitude of variability for different characters since the mean and units of measurement of the characters may be different. Hence, the coefficients of variation expressed at the phenotypic and genotypic levels have been used to compare the variability observed among different characters. Although the genotypic coefficient of variation indicates the amount of genetic variability present in the character, the heritability estimates aid in determining the relative amount of heritable portion of variation. However, heritability values themselves provide no indication of the amount of genetic progress that would result from selecting the best individuals.

In recent years, the cognizance of genetic diversity and the evolutionary history of crop plants have yielded major advances in crop improvement. The measure of genetic divergence reveals the differences in gene frequencies. Mahalanobis's generalized distance estimated by the $D^2$ statistic (Rao 1952) is a unique tool for discriminating populations by considering a set of parameters together. In addition to estimation of variability, cognizance of the genetic diversity of the germplasm is necessary for effective choice of parents in hybridization. Knowledge of the amount of genetic variability present in a crop species with respect to yield and its attributes and their association, which reflects the nature and degree of relationship between any two measurable characters, is of great importance in achieving genetic improvement in that crop.

Biofortification breeding of crop plants focuses on improving grain Fe and Zn content. In a few studies researchers also have given importance to other micronutrients such as iodine and selenium. Genetic variability for micronutrient content in crop plants varies widely and micronutrient accumulation in grain also depends on agronomic practices, soil nutrient composition, environmental features and the variety or hybrid of each particular crop. In the following crops we discuss the genetic variability for grain Fe and Zn content, heritability, genes controlling the traits and so on, in individual crops with suggested breeding methods for biofortification programs.

### 2.3.1 *Rice (***Oryza sativa***)*

Rice is central to the lives of billions of people around the world. Possibly the oldest domesticated grain (~10,000 years), it is the staple food for 2.5 billion people (Anon 2004) and growing rice is the largest single use of land for food production, covering 9 % of the earth's arable land. Rice provides 21 % of global human per capita energy and 15 % of per capita protein (Anon 2002). Calories from rice are particularly important in Asia, especially among the poor, where it accounts for 50–80 % of daily caloric intake. As expected, Asia accounts for over 90 % of the world's production of rice, with China, India and Indonesia producing the most. Around 85 % of the rice that is produced in the world is used for direct human consumption (Anon 2002). Rice can also be found in cereals, snack foods, beverages, flour, oil, syrup and religious ceremonies to name a few other uses.

Rice belongs to the genus *Oryza* and has 2 cultivated and 22 wild species; the cultivated species are *O. sativa* and *O. glaberrima. Oryza sativa* is grown all over the world while *O. glaberrima* has been cultivated in West Africa for the past ~3,500 years (Anon 2002). Rice is grown under many different conditions and production systems worldwide, but most commonly in flooded fields. It is the only cereal crop that can grow for long periods of time in standing water (Anon 2004).

Rice is the world's most important food crop and a primary source of food for more than one-half the world's population. It is the predominant staple food crop for 15 countries in Asia and the Pacific, 10 in Latin America and the Caribbean, 7 in sub-Saharan Africa and 1 country in North Africa (FAO 1999). In developing countries, rice accounts for 715 kcal per capita per day, 27 % of dietary energy supply, 20 % of dietary protein and 3 % of dietary fat. Southeast Asian countries are heavily reliant upon rice. India accounts for nearly one-fourth (22 %) of the world's rice production, with China the leader. World rice production currently is around 597.8 million mt grown over 151 million ha with a productivity of 3.96 mt $ha^{-1}$. India has an area of 44 million ha under rice cultivation with an output of 99 million mt, which averages to a yield of around 2.10 mt $ha^{-1}$. Dietary intake surveys from China and India reveal an average adult intake of about 300 g of raw rice per day (FAO 1998). Technological advances during the last 40 years have led to an increase in rice production by 150 %. Rice production needs to increase even further to meet growing demand. Sustainable production will have to overcome a number of challenges including the decline in arable land, global water shortage and global climate change (Royal Society 2009).

Wide genetic variation exists for grain Fe and Zn content in rice germplasm accessions and this variation can be exploited in breeding programs to enhance Zn content in the grains (Graham et al. 1999; Welch and Graham 2004). A recent study by Gangashetty et al. (2013) screened germplasm accessions from the Western Ghats of Karnataka of non-basmati aromatic genotypes of rice for Fe and Zn content and found a range from 2–17.49 to 9.80–32.44 ppm, respectively. Anarudha et al. (2012) screened rice germplasm for Fe and Zn content and found Fe concentration ranged from 6.2 to 71.6 ppm and Zn from 26.2 to 67.3 ppm. Neelamraju et al. (2012) reported the Fe concentration in brown rice ranged from 6 ppm in Athira to 72 ppm in *Oryza nivara* and Zn concentration from 27 ppm in Jyothi to 67 ppm in *O. rufipogon*. Significant genetic variation was reported for Fe and Zn in *indica* and aromatic rice varieties (Brar et al. 2011). Another study showed wide variation for micronutrient levels recorded among 46 tested rice genotypes, which ranged from 4.82 to 22.69 µg/g for grain Fe and 13.95–41.73 µg/g for grain Zn content (Banerjee et al. 2010). Liu et al. (1995) reported Zn content in grains of rice ranged from 0.79 to 5.89 mg/100 g with an average of 3.34 mg/100 g in a study done among 57 rice varieties. Qui et al. (1995) reported a higher variability in mineral contents in some rice cultivars and the level of Fe content varied from 15.41 to 162.37 mg $kg^{-1}$ and Zn from 23.92 to 145.78 mg $kg^{-1}$.

### 2.3.2 *Pearl Millet [***Pennisetum glaucum** *(L.) R. Br.]*

Pearl millet is the staple cereal of what is undoubtedly the harshest of the world's major farming areas: the arid and semiarid regions stretching over 7,000 km from Senegal to Somalia. There, on the hot, dry, infertile sandy soils having low organic matter content, farmers produce some 50 % of the world's pearl millet grain. The agricultural research challenge is how to help farmers in this often drought-devastated zone, living on the edge of the world's largest desert, who have no access to irrigation, affordable mineral fertilizer, pesticides or other purchased inputs. The answer may lie in their age-old staple, pearl millet. Indeed, there is probably no better cereal to relieve the underlying threat of starvation in the Sahelian and northern Sudanian areas extending from Mauritania, Senegal and The Gambia in the west, to eastern and northeastern Kenya and the coastal lowlands of Yemen, Oman, and Iran. Millions of people entrust their daily lives to this single species and of all the inhabitants on the planet, they are among the poorest in economic terms and most in need of help. Yet, at the moment, pearl millet continues to suffer from neglect and misunderstanding, in part because the crop grows in some of the poorest countries and regions, and in some of the least hospitable habitats for humans and livestock. People have therefore unjustly stigmatized it as a poor crop, fit only for temporary support of poor people until something better is identified.

Pearl millet is the sixth most important of the world's cereals. Descended from a wild West African grass (also *Pennisetum glaucum*), it was domesticated more than 4,000 years ago, probably in what is now the heart of the Sahara Desert. In ancient times, it was dispersed from its homeland to East Africa and thence to India, reaching there more than 3,000 years ago. Both regions adopted it eagerly and it has become a much-favored staple food grain, feed and fodder crop. Today, pearl millet is sown on ~22 million ha in Africa and ~12 million ha in Asia, as well as more than 3 million ha in Latin America, much of it in Brazil where it serves as the best available mulch component of sustainable limited-tillage soybean production on acid soils in the Cerrado region. Global production of pearl millet grain probably exceeds 20 million mt annually, to which India contributes nearly one-half. At least 200 million people depend on pearl millet for at least several months each year and a large percentage of them depend upon it throughout the year.

Pearl millet's important characteristic is its concomitant ability to withstand heat, low soil fertility and low moisture availability (Gupta et al. 2015). Today, approximately 40 % of the world's pearl millet is grown in Africa and the rest mostly contributed by India. About 85 % of Africa's production is in the West African countries, including Nigeria (5 M ha), Niger (7 M ha), Burkina Faso (1.5 M ha), Chad (3 M ha), Mali (1.5 M ha) and Senegal (1 M ha). Sudan (2 M ha), Tanzania (0.2 M ha), Eritrea, Namibia and Uganda (0.1 M ha each) are other producing countries in Africa. In these regions, pearl millet is a staple food of more than 90 million people. Pearl millet is a highly nutritious cereal with high levels of metabolizable energy and protein, and a more balanced amino acid profile (Andrews and Kumar 1992). Pearl millet grains from crops grown with 20–40 kg $ha^{-1}$ of applied nitrogen have 10–11 % protein, comparable to the protein found in wheat cultivars. Processing technologies for preparing various types of alternative and health food products have been developed. These products have been shown to have lower glycemic index levels than similar products produced from wheat (Sehgal et al. 2004), thus increasing the food value of pearl millet for those prone to diabetes. Pearl millet grains lack gluten, unlike most of the major cereals, thus enhancing its health value for those allergic to gluten (Dahlberg et al. 2004).

Pearl millet is less prone to aflatoxin contamination than sorghum and maize. Collins et al. (1997) reported that eggs produced by chickens fed pearl millet-based diets have lower levels of low-density lipoprotein, thus making possible the production of *designer* eggs for those with high cholesterol. These findings suggest that pearl millet can play an important role not only in contributing to the nutritional security of the poor in the pearl millet growing areas of India and Sub-Saharan Africa, but could also have potential health value for the affluent.

Pearl millet has both natural relatively high concentrations of Fe and Zn with demonstrated potential to increase these levels further with plant breeding. Several reports indicate the existence of large variability for grain Fe and Zn in various types of genetic materials of pearl millet. For example, a recent study showed for all tested minerals a moderate to high range in mineral density among the West and Central Africa (WCA) pearl millet accessions studied (Burger et al. 2014). The study focused on the grain density of several minerals in 225 Sudanese pearl millet accessions evaluated in Sudan also found wider density ranges for all 8 minerals (Bashir et al. 2014). A study conducted with a limited number of 27 genotypes at ICRISAT showed high levels and large variability of both Fe (40–580 ppm) and Zn (10–66 ppm) in pearl millet grains (Jambunathan and Subramanian 1988). Other studies on grain Zn and Fe densities in pearl millet material, based on means of two environments reported from India, ranged around 30–80 mg $kg^{-1}$ Fe and 20–70 mg $kg^{-1}$ Zn (Govindaraj et al. 2013; Velu et al. 2007). Parthasarathy Rao et al. (2006) reported that in the major pearl millet growing states of India, pearl millet accounts for the largest share of Fe and Zn intake by the population, and it is also the cheapest source of these micronutrients as compared to other cereals and even vegetables. Pearl millet is a significant source of these micronutrients both in India and Sub-Saharan Africa.

### 2.3.3 *Maize (*Zea mays *L.)*

Maize is a major component of the daily diet of many of the neediest people of the world, and was selected as a target crop by the HarvestPlus Biofortification Program (Nestel et al. 2006). Maize is a major cereal crop widely consumed in developing countries, which have a high incidence of iron deficiency anemia. The major cause of Fe deficiency in these countries is inadequate intake of bioavailable Fe, where poverty is a major factor. Therefore, biofortification of maize by increasing Fe concentration and/or bioavailability has great potential to alleviate this deficiency. Maize is also a model system for genomic research and thus allows the opportunity for gene discovery. The development of an efficient breeding program to increase mineral concentrations in maize depends on the presence of genetic variability in this species. A study evaluating the kernel Fe and Zn of 67 diverse maize genotypes grown during 2006–2008 indicated significant variation for both micronutrients. Kernel Fe concentration in 2006 varied from 20.38 to 43.79 mg/kg, whereas the same ranged from 23.23–54.29 to 29.22–49.24 mg/kg, in 2007 and 2008, respectively. Kernel Zn varied from 15.06–29.88, 7.01–22.01 to 13.64–26.54 mg/kg, in 2006, 2007 and 2008, respectively (Agrawal et al. 2012). Queiroz et al. (2011) reported significant variability in the contents of Zn (17.5–42 $mgkg^{-1}$) and Fe (12.2–36.7 $mgkg^{-1}$) in 22 tropical maize inbred lines with different genetic backgrounds. Significant differences in the Fe and Zn concentrations in maize have been reported in many genotypes in trials conducted in Mexico and Zimbabwe by Banziger and Long (2000) and in Nigeria by Menkir (2008). Fe and Zn concentrations of more than 1,000 CIMMYT improved maize genotypes and 400 *core accessions* (landraces) from different environments were analyzed and little variation of Fe levels in grain (average 2,075 mg/g) and moderate variation for Zn concentration in grain (mostly 15–35 mg/g) were reported (Banziger and Long 2000; Long et al. 2004). Hence maize also serves as a major food source for Fe and Zn in many parts of the world.

### 2.3.4 *Sorghum [*Sorghum bicolor *(L.) Moench]*

Sorghum is an affordable staple food for more than 400 million people in Africa and some parts of Asia, many of whom live in the drier, more vulnerable agricultural areas. However, sorghum is deficient in most essential nutrients, and it is difficult to digest when cooked. If enhanced with key nutrients it could benefit key targeted populations who suffer from micronutrient deficiency. Sorghum is a crop with many advantages; it grows quickly and can tolerate much more heat and drought than most other crops. Sorghum also is gluten free and can be a good substitute for wheat in baked goods and other products. In Africa, sorghum is used to make bread and nutritious porridge, and can even be popped like corn. Sorghum is an important crop in Africa, with 23.4 million mt produced in 2012. While world production of sorghum appears to be level, production is slowly increasing in Africa.

In an attempt to create a sorghum database for grain Fe and Zn content at ICRISAT, Kumar et al. (2012) evaluated the ICRISAT germplasm core collection, improved varieties and partner institution selected varieties. In this study the range for Fe was 8–192 mg $kg^{-1}$ and 14–91 mg $kg^{-1}$ for Zn in the landraces. In a more recent study Kumar et al. (2013) at ICRISAT-India studied three particularly-derived diallel crosses for combining grain Fe and Zn content and also studied the heterosis for grain Fe and Zn content. Results indicated a large exploitable genetic variability available in sorghum germplasm and also observed the heterosis for grain Fe and Zn content without affecting the yield. This study indicated that the expression of grain Zn concentrations in sorghum is governed predominantly by additive gene effects, suggesting the high effectiveness of progeny selection in pedigree selection or population breeding to develop lines with increased levels of grain Zn concentrations, while the grain Fe concentration is governed predominantly by non-additive gene effects in combination with additive gene effects, suggesting scope for heterosis breeding in addition to progeny selection to develop lines with increased levels of grain Fe concentrations. The performance of the crosses can be predicted based on general combining ability (GCA) for grain Zn but information on both GCA and specific combining ability (SCA) is required for Fe. There is scope for exploitation of heterosis to improve grain Fe content. Some of the crosses developed in the study significantly outperformed parents for Fe and Zn concentration with no yield loss, indicating that it is possible to develop high grain Fe and Zn cultivars in high-yielding backgrounds. Nguni et al. (2011) evaluated sorghum genotypes of improved and farmers varieties from southern Africa for grain Fe and Zn; analysis ranged from 2.74–8.18 mg/100 g to 2.03–5.53 mg/100, respectively. The availability of wide genetic variability for grain Fe and Zn content in sorghum will help breeders select superior genotypes with high yield while improving micronutrients content.



### *2.3.5 Phaseolus Bean (***Phaseolus vulgaris** *L.)*

The common bean is the most important economic variety of the genus *Phaseolus* and is grown throughout the world. It requires much warmth and sun; cool weather and wind hamper growth. The crops prefers moderately-heavy or light soils are preferred. It is the most important legume worldwide for direct human consumption. The crop is consumed principally for its dry (mature) beans, shell beans (seeds at physiological maturity) and green pods. When consumed as seed, beans constitute an important source of dietary protein (22 % of seed weight) that complements cereals for over one-half billion people, mainly in Latin America. The largest producers of dry beans are Brazil, Mexico, China and the USA. Annual production of green beans is around 4.5 million mt, with the largest production taking place around the Mediterranean and in the USA. The common bean was used to derive important principles in genetics.

The degree of genetic variability present in Fe and Zn concentrations in common beans seeds was observed by researchers at the International Center for Tropical Agriculture (CIAT). A core collection of over 1,000 accessions of common beans were evaluated (Beebe et al. 2000), and showed a range in Fe concentrations from 34 to 89 $\mu gg^{-1}$ Fe (average 55 $\mu gg^{-1}$ Fe) while the Zinc concentrations in these same accessions ranged from 21 to 54 $\mu gg^{-1}$ Zn (average 35 $\mu gg^{-1}$Zn) (Graham et al. 1999). Recently, some common bean accessions from Peru were found to contain high levels of Fe averaging over 100 $\mu gg^{-1}$Fe. The results showed that there is sufficient genetic variability available to increase significantly Fe (~80 %) and Zn (~50 %) concentrations in common beans.

### *2.3.6 Breeding Strategies*

A common breeding strategy can be used to enhance micronutrient content in crop plants based on their pollination systems (Fig. 2.2). Applied breeding programs begin with introduction of material developed elsewhere for improved micronutrient content. Advanced breeding lines, released varieties and hybrids also can be used as base material for developing new elite lines with trait breeding for micronutrients. Availability of genetic variability in the population can be used at the beginning to harness the genetic variability for developing new breeding lines. If the available genetic variability is not sufficient to develop the breeding lines, then it can be created by hybridization, mutation and polyploidy breeding approaches. A



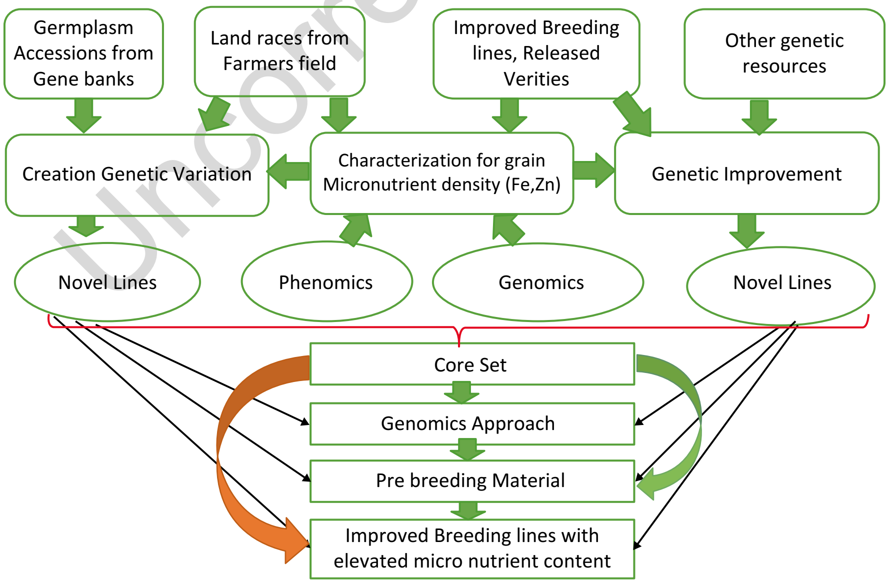


**Fig. 2.2** Breeding strategy for micronutrient enhancement in crop plants

core set of genetic germplasm will be developed by evaluating complete genetic material and breeding lines available in particular crop. At present molecular marker-assisted breeding is gaining importance in fast-track breeding for developing genetic material. The combination of molecular breeding and conventional breeding will be of great help in developing the genetic material and elite breeding lines in the shortest time available. Based on the pollination systems in crop plants, breeding methods can be applied. Breeding methods used in rice, sorghum and beans include mass selection, pedigree selection, single seed descent method of selection, back-cross breeding, mutation breeding and marker-assisted selection. The breeding methods commonly followed in maize and pearl millet include population improvement approaches, mass selection and marker-assisted selection. If a crop is often cross-pollinated, like sorghum, either of the selection methods used for self-pollinated and cross-pollinated selection methods can be practiced depending upon the breeding objectives.

## 2.4 Effect of Genetics and Environment on Grain Micronutrient Content

Genotype by environment (G × E) interaction is the differential response of crop genotypes to changing environmental conditions. Such interactions complicate testing and selection in breeding programs and result in reduced overall genetic gains of desired traits (Shafii and Price 1998). Understanding the G × E interaction therefore allows the making of informed choices regarding which locations and input systems to use in the breeding efforts. Burger et al. (2014) reported significant G × E interaction effects for grain Fe and Zn densities in WCA pearl millet, showing the importance of multi-environmental evaluation to identify genotypes stable across environments. Studies on pearl millet in general have shown a significant G × E interaction effect for grain Fe and Zn densities as well (Govindaraj et al. 2013; Gupta et al. 2009; Velu et al. 2011), indicating the general importance of basing biofortification breeding programs on multiple environment testing.

Environment, genotype and G × E interaction significantly affected Fe concentration in rice grains (Anuradha et al. 2012; Suwarto and Nasrullah 2011). The pH, organic matter content and Fe/Zn levels of native soils showed significant effects on grain Fe and Zn content in rice (Chandel et al. 2011). Comparative analysis of grain nutrient contents (Fe and Zn) of genotypes grown in three locations showed significant differences, thus indicating a strong influence of native soil properties on Fe and Zn levels in grain (Banerjee et al. 2010). Several studies carried out in The Philippines, Bangladesh, Korea and Vietnam have reported a significant G × E interaction effect on grain nutritive-value related traits in rice, including factors, such as, wet and dry season, inherent soil properties like saline, acidic or neutral soils, nitrogen supply and period of flooding during crop growth (Graham et al. 2005; Gregorio et al. 2000).

In wheat, significant G × E interactions on grain nutrients were reported, demonstrating the importance of environmental effects on Fe and Zn concentrations (Badakhshan et al. 2013). Several studies reported significant G × E interactions for grain nutrient concentrations such as Fe and Zn for bread wheat varieties (Morgounov et al. 2007; Oury et al. 2006; Wang et al. 2010) as well as for their wild and cultivated relatives (Chatzav et al. 2010; Gomez-Becerra et al. 2010a, b; Peleg et al. 2008).

In maize, Queiroz et al. (2010) showed that there were highly significant effects of maize genotypes in mineral content, but the location effect was not significant in terms of the concentration of any kernel minerals, except Zn, in the majority of the trials. The mineral concentrations in maize grains can be affected by soil type and fertility, soil moisture, environmental factors, crop genotype and interactions among nutrients (Feila et al. 2005). Oikeh et al. (2003) reported that the effects of G × E were significant ($P < 0.05$) for grain Fe and Zn and was about double the contribution of the genotype (G) for grain Fe and Zn. However, G × E interaction can greatly influence genotypic performance across different crop-growing scenarios.

In common bean, results also indicate that the traits responsible for genetic improvements in Fe and Zn concentrations are stable across environments. Significant location and location × genotype effects indicate that environments have an influence on the concentrations of Fe and Zn in bean seeds. However, high-Fe and high-Zn accumulating genotypes will accumulate more nutrients when compared to low-Fe and low-Zn accumulating genotypes which were grown simultaneously at the same location, which once again shows that the environmental effect was absent and variation is purely due to the genotype. Interestingly, a very highly significant positive correlation of 0.52 between the concentrations of Fe and Zn across different genotypes were observed by CIAT researchers.

## 2.5 Genetic Association of Grain and Grain Yield in Micronutrient Concentration

Iron, zinc and copper are essential micronutrients for plants as well as humans (Asad and Rafique 2000; Hao et al. 2007). A deficiency of one of these nutrients can greatly reduce plant yield and even cause plant death. The correlation coefficients between Fe and Zn concentration and grain yield in cereal grain reported by earlier researchers are presented in Table 2.3. A recent study on micronutrient density in pearl millet showed no significant correlation between grain yield and Zn and Fe densities (Burger 2014). Govindaraj et al. (2009) studied correlations between agro-morphological traits and densities of four minerals (P, Ca, Zn and Fe) in pearl millet, where no association with grain yields was observed for any of the four. However, studies on pearl millet, reported significant negative to no correlations between Zn (Fe) density and grain yield (Gupta et al. 2009; Rai et al.

**Table 2.3** Correlation coefficients between Fe and Zn concentrations and grain yield in cereal grains

| Crop | Correlation coefficient (r) | References |
|---|---|---|
| Grain Fe and grain yield | | |
| Bean | 0.34* | Gelin et al. (2007) |
| Maize | −0.26* | Chakraborti et al. (2009) |
| Pearl millet | −0.02$^{ns}$ | Gupta et al. (2009) |
| Sorghum | −0.32* | Reddy et al. (2005) |
| | −0.36* | Ashok Kumar et al. (2009) |
| Wheat | −0.39** | Vogel (1989) |
| | −0.41* | Morgounov et al. (2007) |
| | −0.19$^{ns}$ | Ficco et al. (2009) and Zhao et al. (2009) |
| | −0.51$^{ns}$ | Oury et al. (2006) |
| Grain Zn and grain yield | | |
| Bean | 0.21* | Gelin et al. (2007) |
| Maize | 0.18$^{ns}$ | Chakraborti et al. (2009) |
| Pearl millet | −0.1$^{ns}$ | Gupta et al. (2009) |
| Sorghum | −0.54** | Reddy et al. (2005) |
| | −0.46** | Ashok Kumar et al. (2009) |
| Wheat | −0.64** | Morgounov et al. (2007) |
| | −0.57 to −0.61** | McDonald et al. (2008) |
| | −0.41** | Ficco et al. (2009) |
| | −0.64** | Morgounov et al. (2007) |
| | | Oury et al. (2006) |
| | −0.439** | Zhao et al. (2009) |

*, ** = Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; *ns* non-significant



2012; Velu et al. 2008). A negative correlation was observed between the concentrations of Fe and Zn in grain and grain yield were reported in many studies in wheat, although the strength of these relationships was influenced greatly by the environment (White and Broadley 2009). There were obviously significant negative correlations between yield and Zn concentration with the correlation coefficients ranging from −0.67 to −0.41, while there was no significant correlation for Fe (Morgounov et al. 2007; Oury et al. 2006). In maize and sorghum, grain yield was found negatively associated with grain Fe (r = −0.26) and (r = −0.32 to −0.36), respectively. A low but positive correlation (r = 0.21) between grain yield and Zn and Fe have been reported in common bean. Grain yield and grain Zn were negatively associated in sorghum (r = −0.46 to −0.54). However, Anand et al. (2012) reported negative correlation between grain yield and mineral contents in rice. Grain Zn concentration showed negative correlation with grain yield per plant (r = −0.27) in recombinant inbred lines (RILs) of rice.

## 2.6 Heritability Estimates of Grain Iron and Zinc Concentrations

The inheritance of nutritional traits appears to be mostly quantitative, influenced by the environment, but more specific to source genotypes (Blair et al. 2009; Cichy et al. 2005, 2009). To determine whether Fe and Zn concentration in a particular crop can be improved by traditional breeding methods, it must be determined to what extent these traits are heritable. Heritability estimates are limited to experimental material and setup, and may differ widely in the same crop and for the same trait (Garcia-Oliveira et al. 2009). Heritability is a measure of genetic differences among individuals in a population, not simply of whether or not a trait is inherited (Gomez-Becerra et al. 2010b). Heritability of Fe and Zn in the cited study was estimated by some researchers previously. Recently Govindaraj et al. (2011, 2013) and Bashir et al. (2013) reported high heritability estimates in pearl millet and suggested the predominance of additive gene effects in the inheritance of the nutritional traits. Both high heritability for grain Fe (65–71.2 %) and Zn (65–80 %) (Gupta et al. 2009) and heritability for grain Fe (80 %) and Zn (77 %) (Velu et al. 2007) have been reported in pearl millet, indicating that a substantial portion of the total variation for Fe/Zn is due to genetic effects. In wheat, estimates of broad-sense heritability (h2B) ranged from 90.62 % for Fe in 2010, to 90.90 % for Zn in 2011 (Badakhshan et al. 2013). Rawat et al. (2009) reported high heritability for grain Fe (0.98) and Zn (0.96) in wheat genotypes. Khodadadi et al. (2014) reported that the heritability of grain Fe and Zn in wheat was 0.74 and 0.61 in 2009 and 0.85 and 0.92 in 2010, respectively. Garcia-Oliveira et al. (2009) reported medium to high heritability for Fe and Zn, with estimates of 72.8 % and 40.6 %, respectively, in a set of recombinant inbred lines of rice. Chakraborti et al. (2010) reported high heritability for grain Fe (78 and 73 %) and grain Zn (71 and 76 %) in maize. Both moderate heritability (54 %) and high heritability (78–82 %) were reported for grain Zn in common bean (Cichy et al. 2005). Thus, heritability estimates are useful for the biofortification of high-yielding crop varieties.



## 2.7 Molecular Marker-Assisted Breeding for Genetic Improvement of Grain Fe and Zn Content in Crop Plants

The rapid development of DNA marker technology provides great opportunities to enhance nutritive values of traditionally-cultivated crops and grains. Molecular markers augment conventional plant breeding for efficient and precise identification or selection of a trait of interest linked to them. During the last few decades, molecular markers have been widely used in plant biotechnology and genetic studies. They are used in the assessment of genetic variability and characterization of germplasm; estimation of genetic distance between populations, inbred and breeding material; genetic mapping; detection of monogenic and quantitative trait loci (QTLs); marker-assisted selection; increase in the speed and quality of backcrossing to introgress desirable traits from closely related varieties to elite germplasm and identification of sequences of useful candidate genes, etc. (Farooq and Azam 2002; Murtaza et al. 2005; Rana and Bhat 2005). Recent developments in quantitative genetics of molecular markers allow construction of linkage maps to determine the map position and effect of different loci/genes of metric characters i.e. QTLs. In QTL analysis, scientists attempt to identify associations between quantitative traits and marker alleles within a segregating population (Lander and Bostein 1989; Weller et al. 1990) to identify the genomic locations of loci contributing to complex traits, the contribution of each and the interaction between loci. QTL analysis provides a powerful approach to identify the genes underlying the natural variation for Fe and Zn concentrations (Ghandilyan et al. 2006). Molecular markers have been used to identify the genetic regions involved in grain Zn content in plants. Subsequently, there have been thousands of QTL studies carried out in different plant species.

In a study of wheat, nine additive and four epistatic QTLs were identified, among which six and four, respectively, were effective at the two environments (Xu et al. 2012). Peleg et al. (2009) found 11 QTLs on chromosomes 2A, 5A, 6B, 7A and 7B for Fe and 6 QTLs on chromosomes 2A, 2B, 3A, 4B, 5A, 6A, 6B, 7A and 7B for Zn. Shi et al. (2008) identified 4 QTLs for grain Zn concentration (mg/kg) on wheat chromosomes 4 and 5 contributing 11.9 % and 10.9 %, respectively, to the variance whereas for grain Zn content (μg/seed) seven major QTLs were found on chromosomes 2 and 7 in a double haploid wheat population. Genc et al. (2009) also reported major QTLs for grain Zn concentration on chromosomes 4 and 7 in wheat. A total of five significant QTLs controlling grain Zn and Fe content were detected in a maize $F_{2:3}$ mapping population (Jin et al. 2013). Lungaho et al. (2011) reported three modest QTLs for grain Fe concentration (FeGC) and ten QTLs for grain Fe bioavailability (FeGB) from an intermated B736Mo17 (IBM) recombinant inbred (RI) population of maize.

Identifying QTLs for Fe and Zn in rice grains, 14 QTLs were detected and QTLs for Fe were co-located with QTLs for Zn on chromosomes 7 and 12 (Anuradha et al. 2012). A total of seven QTLs for Fe and six for Zn were identified each explaining >30 % phenotypic variance in rice accessions (Neelamraju et al. 2012). Garcia-Oliveira et al. (2009) reported two QTLs for Fe on chromosomes 2 and 9 and three QTLs for Zn on chromosomes 5, 8 and 12. Three QTLs for Fe on chromosomes 2, 8 and 12, while two QTLs for Zn on chromosomes 1 and 12 and a common QTL for Fe and Zn accounted for a 13–14 % variation, as identified by Stangoulis et al. (2006). In common bean, a total of 26 QTLs were identified in an inter-gene pool mapping population for the mineral × trial × method combinations of which one-half were for Fe concentration and one-half for Zn concentration (Blair et al. 2009). Cichy et al. (2009) reported 11 QTLs on 6 linkage groups (LGs) accounting for 8–36 % variation for Fe and 11 QTL on 4 LGs accounting for 9–39 % variation in Zn.

However, marker-assisted selection is useful in improving the efficiency of selection early in the breeding cycle by helping to improve characters with low heritability. Thus, identifying the target QTL genes will help achieve biofortification with greater precision and accuracy.

## 2.8 Transgenic Approaches for Micronutrient Improvement

Transgenic approaches are advantageous when a micronutrient does not naturally exist in a crop (e.g. provitamin A in rice) or when sufficient amounts of bioavailable micronutrients cannot be effectively bred into the crop. However, once a transgenic line is obtained, several years of conventional breeding are needed to ensure that the transgenes are stably inherited and to incorporate the transgenic line into varieties that farmers prefer. While transgenic breeding can sometimes offer micronutrient gains beyond those available to conventional breeders, many countries lack the legal framework to allow release and commercialization of these varieties. To attain higher levels of provitamin A, Zn and Fe content in crops where genetic variation for these traits has not been identified, HarvestPlus, its partners, and other organizations have explored transgenic approaches, discussed below in detail.

### *2.8.1 Golden Rice*

Golden Rice is a variety of *Oryza sativa* produced through genetic engineering to biosynthesize beta-carotene, a precursor of vitamin A, in the edible parts of rice (Ye et al. 2000). It was first developed at the Swiss Federal Institute of Technology and the University of Freiburg, Germany. Golden Rice was created by transforming rice with only two beta-carotene biosynthesis genes: psy (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*) and crtI (carotene desaturase) from the soil bacterium *Erwinia uredovora* (Fig. 2.3).

In 2005, a research team at the Syngenta biotechnology company produced a variety of Golden Rice called Golden Rice 2. It combined the phytoene synthase gene from maize with crt1 from the original Golden Rice. Golden Rice 2 produces 23 times more carotenoids than the original Golden Rice (up to 37 μg/g), and preferentially accumulates beta-carotene (up to 31 μg/g of the 37 μg/g of carotenoids) (Paine et al. 2005). To receive the Recommended Dietary Allowance (RDA), it is estimated that 144 g of the highest-yielding strain would have to be eaten. Bioavailability of the carotene from Golden Rice has been confirmed and found to be an effective source of Vitamin A for humans (Datta et al. 2007; Tang et al. 2009). Bioavailability testing has confirmed that Golden Rice is an effective source of vitamin A in humans, with an estimated conversion rate of beta-carotene to retinol of 3.8:1 and 2:1 (Tang et al. 2009, 2012).

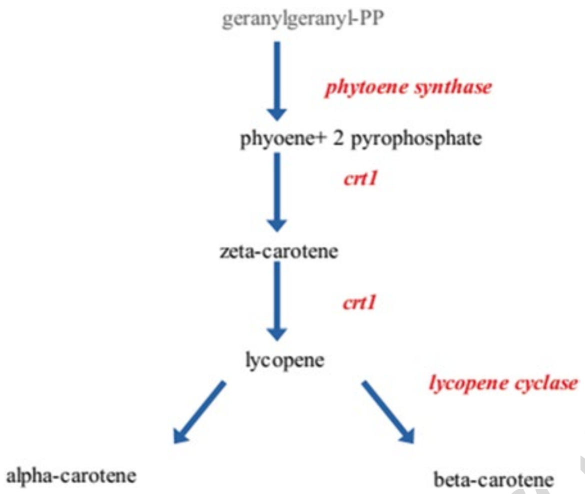


**Fig. 2.3** A simplified overview of the carotenoid biosynthesis pathway in Golden Rice. The enzymes expressed in the endosperm of Golden Rice, shown in red, catalyze the biosynthesis of beta-carotene from geranylgeranyl diphosphate. Beta-carotene is assumed to be converted to retinal and subsequently retinol (vitamin A) in the animal gut (Source: http://en.wikipedia.org/wiki/Golden_rice)



### *2.8.2 Iron-Rich Rice*

Iron deficiency is considered one of the world's most widespread micronutrient deficiencies. Despite the fact that whole grains, vegetables and fruits contain Fe, absorption of the micronutrient is poor from these food sources because it is bonded with phytic acid. Since rice is a staple food for over three billion people, improving its Fe content (normal availability of Fe 0.2–2.8 mg/100 g rice) could help resolve the problem of Fe deficiency especially in developing countries. Researchers have incorporated pAGt lFe containing the gene for the ferritin protein from *Phaseolus vulgaris* and pAGt 1Me with metallothirnein-like protein followed by agrobacterium-mediated transformation, which increased the Fe content in the rice endosperm twofold (Lucca et al. 2002). To address the bioavailability problem, Lucca et al. (2002) integrated the gene from *Aspergillus fumigatus* encoding a thermotolerant phytase protein and the gene for endogenous cysteine-rich metallothionein-like protein. Cysteine helps increase Fe uptake during digestion. The concerted effect of these genes resulted in a sevenfold increase in cysteine level and a 130-fold increase in phytase level. Masuda et al. (2013) recently reported seven transgenic approaches to increase the Fe concentration of rice seeds (Tables 2.4 and 2.5) and also proposed some additional prospective target genes for the Fe biofortification of rice.

**Table 2.4** Approaches of Fe biofortification of rice: single transgenic approaches

| Approach cultivation | Introduced genes | Rice cultivar | Cultivation condition | Fold increase in Fe concentration compared to non-transgenic rice[a] | References |
|---|---|---|---|---|---|
| Approach 1: enhancement of Fe storage in rice seeds by ferritin | OsGluB1pro-SoyferH1 | Japonica cv. Kitaake | Soil cultivation in greenhouse | 2 fold (polished seeds) | Goto et al. (1999) |
| | OsGluB1 pro-SoyferH1[b] | Japonica cv. Kitaake | Soil cultivation in greenhouse | 3 fold (brown seeds) | Qu et al. (2005) |
| | OsGlb1 pro-SoyferH1b | | | 1.5 fold (brown seeds) | |
| | OsGluB1 pro-SoyferH1 | Japonica cv. Taipei 309 | Soil cultivation in greenhouse | 2.2 fold (brown seeds) | Lucca et al. (2002) |
| | OsGluB1 pro-SoyferH1 | Indica cv. IR68144 | Soil cultivation in greenhouse | 3.7 fold (polished seeds) | Vasconcelos et al. (2003) |
| | OsGluA2 pro-OsFer2 | Indica cv. Pusa-Sugandh II (aromatic rice) | Soil cultivation in greenhouse | 2.1 fold (polished seeds) | Paul et al. (2012) |
| Approach 2: enhancement of Fe translocation by overexpression of NAS | OsActin1 pro-HvNAS1c | Japonica cv. Tsukinohikari | Soil cultivation in greenhouse | 2 fold (polished seeds) | Masuda et al. (2009) |
| | 35S pro-HvNAS1c | | | | |
| | Activation tag line of OsNAS3 | Japonica cv. Dongjin | Soil culture in greenhouse | 3 fold (polished seeds) | Lee et al. (2009c) |
| | 35S pro-OsNAS1, 2, 3 | Japonica cv. Nipponbare | Soil cultivation in greenhouse | 4 fold (polished seeds) | Johnson et al. (2011) |
| Approach 3: enhancement of Fe transportation by Fe transporter | OsSUT1 pro-OsYSL2 | Japonica cv. Tsukinohikari | Soil cultivation in greenhouse | 4 fold (polished seeds) | Ishimaru et al. (2010) |

(continued)

**Table 2.4** (continued)

| Approach cultivation | Introduced genes | Rice cultivar | Cultivation condition | Fold increase in Fe concentration compared to non-transgenic rice[a] | References |
|---|---|---|---|---|---|
| Approach 4: enhancement of Fe uptake and translocation by IDS3 gene | Barley IDS3 genome fragment | Japonica cv. Tsukinohikari | Andosol soil in paddy field | 1.4 fold (polished seeds) | Masuda et al. (2008) |
| | | | | 1.3 fold (brown seeds) | |
| | | | Calcareous soil in paddy field | 1.3 fold (brown seeds) | Suzuki et al. (2008) |
| Approach 5: overexpression of Fe transporter | Ubiquitin pro-OsIRT1 | Japonica cv. Dongjin | Paddy field | 1.7fold (leaves) | Lee et al. (2009a) |
| | | | | 1.1 fold (brown seeds) | |
| | OsActin1 pro-OsYSL15 | Japonica cv. Dongjin | Paddy field | 1.3 fold (brown seeds) | Lee et al. (2009b) |
| Approach 6: overexpression of transcription factor | 35S pro-OsIRO2 | Japonica cv. Tsukinohikari | Calcareous soil in greenhouse | 3 fold (brown seeds) | Ogo et al. (2011) |
| Approach 7: knockdown of OsVITs genes | OsVIT1 or OsVIT2 T-DNA insertion mutant lines | Japonica cv. Zhonghua11 | Hydroponic culture | 1.4 fold (brown seeds) | Zhang et al. (2012) |
| | | Japonica cv. Dongjin | Paddy field | 1.4 fold (brown seeds) | |
| | OsVIT2 T-DNA insertion mutant line | Japonica cv. Dongjin | Soil cultivation in greenhouse | 1.3 fold (brown seeds) | Bashir et al. (2013) |
| | | | | 1.8 fold (polished seeds) | |

Source: Masuda et al. (2013)

[a]The tissue name in parentheses is the rice tissue where Fe concentration was increased

[b]They introduced these two genes into same transgenic lines

[c]These two genes were introduced separately into rice and they analyzed these two types of transgenic lines

**Table 2.5** Approaches of Fe biofortification of rice: multi-transgenic approaches

| Approach cultivation | Introduced genes[a] | Rice cultivar | Cultivation condition | Fold increase in Fe concentration compared to non-transgenic rice[b] | References |
|---|---|---|---|---|---|
| Combination of approaches 1 and 2 | OsGlb pro-Pvferritin | Japonica cv. Taipei 309 | Hydroponic culture | 6 fold (polished seeds) | Wirth et al. (2009) |
| | 35S pro-AtNAS1 | | | | |
| | OsGlb pro-Afphytase | | | | |
| Combination of approaches 1, 2 and 3 | OsGluB1 pro-SoyferH2 | Japonica cv. Tsukinohikari | Soil cultivation in greenhouse | 6 fold (polished seeds) | Masuda et al. (2012) |
| | OsGlb1 pro-SoyferH2 | | | | |
| | OsActin1 pro-HvNAS1 | | Paddy field | 4.4 fold (polished seeds) | |
| | OsSUT1 pro-OsYSL2 | | | | |
| | OsGlb1 pro-OsYSL2 | | | | |
| | OsGluB1pro-SoyferH2 | Tropical Japonica cv. Paw San Yin (Myanmar high quality rice) | Soil cultivation in greenhouse | 3.4 fold (polished seeds) | Aung et al. (2013) |
| | OsGlb1 pro-SoyferH2 | | | | |
| | OsActin1pro-HvNAS1 | | | | |
| | OsSUT1 pro-OsYSL2 | | | | |
| | OsGlb1 pro-OsYSL2 | | | | |
| Combination of approaches 1 and 4 | OsGluB1 pro-SoyferH2 | Japonica cv. Tsukinohikari | Normal soil in greenhouse | 4 fold (polished seeds) | Masuda et al. (2013) |
| | OsGlb1 pro-SoyferH2 | | | | |
| | HvNAS1, HvNAAT-A,-B and IDS3 genome fragments | | Calcareous soil in greenhouse | 2.5 fold (polished seeds) | |

Source: Masuda et al. (2013)

[a]These gene expression cassettes were introduced concomitantly

[b]The tissue name in parentheses is the rice tissue where Fe concentration was increased

## 2.9 Micronutrient Bioavailability

The total amount of a micronutrient from a plant source does not represent the actual micronutrient content of the food that is utilizable by the consumer. The bioavailability of micronutrients must be determined independently using methodologies especially developed for such purposes. In human nutrition terms, bioavailability is commonly defined as the amount of a nutrients in a meal that is absorbable and utilizable for metabolic processes in the body (Welch and Graham 2004). Determining the bioavailability of micronutrients to humans in plant foods is fraught with difficulty (Fig. 2.4). Ultimately to determine the bioavailability of a particular micronutrient a number of factors interact in the body of an individual eating a mixed diet within a given environment. Because of this complexity, the data obtained using various bioavailability model systems are always ambiguous (House 1999; Van Campen and Glahn 1999).

Not all ingested minerals are completely absorbed and utilized by humans or livestock (Grusak and Cakmak 2004); moreover, only a small portion of accumulated minerals in edible parts is bioavailable leading to certain groups of people who are vegetarians being at risk of deficiencies of Fe, Zn and other trace elements. Thus, determining the bioavailability of Fe and Zn in genetically-enhanced new lines is an important aspect of a crop biofortification program. The levels of bioavailable Fe and Zn in staple food crop grains are as low as 5 % and 25 %, respectively (Bouis and Welch 2010). Researchers should therefore consider the bioavailability of micronutrients and their concentration while conducting breeding experiments.

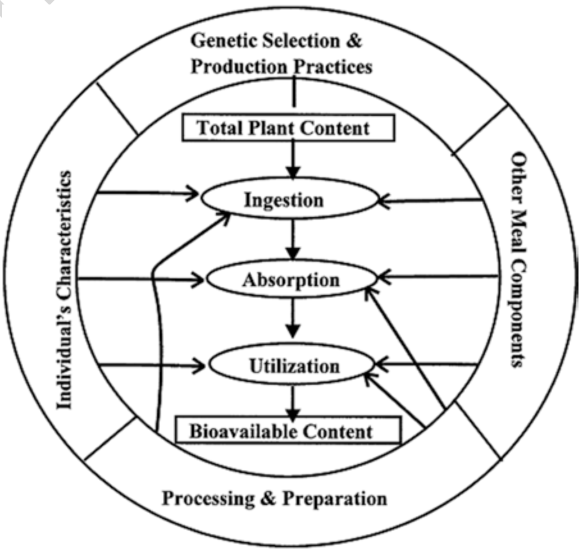


**Fig. 2.4** The complexities of bioavailability in human nutrition (Source: Graham et al. 2001)

Only data from feeding trials in micronutrient-deficient test populations under free-living conditions can delineate the efficacy of using micronutrient-enriched varieties of plant foods as an intervention tool. Unfortunately, it is impractical to test the bioavailability of selected micronutrients in numerous genotypes of staple plant foods that can be generated in plant breeding programs (Graham and Welch 1996). Therefore, to screen large numbers of promising lines of micronutrient-enriched genotypes identified through a breeding program one must use a bioavailability model before advancing them within these programs.

### *2.9.1 Bioavailability Models*

Various bioavailability models have been developed to determine the micronutrients in human plant foods (House 1999; Van Campen and Glahn 1999). Among these in wide use are in vitro models such as cultured human intestinal cells (i.e. Caco-2 cell model), animal models (e.g. rats, pigs and poultry) and small-scale human clinical trials (Underwood and Smitasiri 1999). The rat and poultry models are easy to execute and relatively cost effective, but the results obtained are limited in their acceptance by the nutrition community (Greger 1992). In vitro cultured human intestinal cell models such as the Caco-2 cell model are rapid, inexpensive and can be used to screen large numbers of genotypes for bioavailable Fe (Van Campen and Glahn 1999). However, the Caco-2 cell model needs further development before adopting it to determine the bioavailability of Zn and provitamin A carotenoids in staple plant foods. The pig animal model is a currently and widely accepted, as it is the most accurate of the animal models available to study the bioavailability of Fe, Zn and provitamin A carotenoids in plant foods (Miller and Ullrey 1987). Current breeding efforts to screen large numbers of promising genotypes rich in micronutrients of staple foods crops (rice, maize, pearl millet, sorghum, wheat, beans and manioc) at several CGIAR Centers (IRRI, CIMMYT, ICRISAT, CIAT and IITA) for bioavailable Fe, rely on an in vitro Caco-2 cell model.

Bioavailability of Fe and Zn is known to be influenced by various dietary components, which include both absorption inhibitors and enhancers. Among the inhibitors, phytic acid (PA), tannins, dietary fiber and calcium are the most potent, while organic acids are known to promote Fe absorption (Gibson 1994; Hambidge et al. 2010; Sandberg 2002; Elad et al. 2015). Phytate, a complex of phytic acid and mineral elements, decreases the bioavailable concentration of nutrient elements and thus leads to health problems, such as Fe and Zn deficiency, in populations with diets based mainly on cereals and legumes (Liu et al. 2006). These compounds are normal plant metabolites and only small changes in their concentration may have significant effects on the bioavailability of micronutrients.

Several studies have demonstrated the negative effect of phytate on Zn and Fe absorption, causing nutritional deficiencies both in humans and livestock (Lonnerdal 2000). A study of pearl millet showed that Fe was chelated by phytates and insoluble fibers, whereas Zn was almost exclusively chelated by phytates. A recent study on high Fe pearl millet by Tako et al. (2015) showed that higher-Fe pearl millet provides more absorbable Fe that is limited by increased polyphenolic content. Similarly, in the case of higher fiber and tannin contents, the chelating effect of these compounds was higher than that of phytates (Lestienne et al. 2005). Results of pilot studies among maize consumers in the USA and Guatemala showed that genetically-selected low phytic acid plants have the potential to be used as primary or complementary strategies in the prevention of human Zn deficiency (Hambidge et al. 2004). Studies in animals have shown the positive effect of diets containing low phytate maize to improve the use of minerals (Li et al. 2000; Veum et al. 2001). Therefore, food crop breeding strategies for higher levels of nutrients and low levels of anti-nutritional substances, such as phytic acid, are desirable (Ghandilyan et al. 2006). Thus, the inhibitory effect of phytate should be taken into account when assessing Fe and Zn deficiencies.

Recent technological advancements have improved the accuracy and precision of methods used in the study of bioavailability and absorption of trace elements. Currently two models are used to evaluate mineral bioavailability in foods and diets, each giving a great variability of results: in vivo and in vitro models (Vitali et al. 2007; Welch and Graham 2002). In vivo investigations generally include work with rats or clinical studies with humans. In vitro methods involve determining the soluble and/or dialyzable fraction of the mineral and are important as screening techniques (Fairweather-Tait et al. 1995). Due to the phytic acid influence on mineral absorption, researchers have also used the molar ratio of phytic acid/mineral as a simpler and less costly method to estimate the Fe and Zn bioavailability in food (Abebe et al. 2007; Lestienne et al. 2005). In vivo and in vitro studies on the availability of Fe in a nutritional formulation indicated low Fe availability and absorption in humans (Bueno et al. 2013).



## 2.10 Biofortification: A Tool for Improved Human Health

Breeding staple cereal crops richer in minerals is a low-cost, sustainable strategy to ameliorate micronutrient malnutrition for people living in developing countries who cannot afford to include sufficient amounts of pulses, fruits, vegetables, fish and animal products, rich or enriched with micronutrients in their diet (Cakmak 2008; Martinez et al. 2010). A combination of strategies involving food fortification, pharmaceutical supplementation and dietary diversification has been suggested to combat micronutrient malnutrition (Stein et al. 2005). However, neither of strategy has been universally successful in developing countries, largely due to lack of safe delivery systems, stable government policies, appropriate infrastructure and continued adequate investment (Bouis 2003; Timmer 2003). Thus, biofortification has been proposed as an alternative solution to micronutrient malnutrition (Bouis 2003). Biofortification is a new approach to combat micronutrient deficiencies, by increasing the concentration and/or bioavailability of essential elements in the edible part of the plant by traditional plant breeding or genetic engineering (White and Broadley 2005). By definition, the focus of plant breeders and biofortification initiatives is on breeding crops with a high density and increased bioavailability of nutrients. HarvestPlus (www.harvestplus.org) is a major international consortium created to develop new plant genotypes with high concentrations of micronutrients by applying classical and modern breeding tools (i.e. genetic biofortification). Although plant breeding is the most sustainable solution to the problem, developing new micronutrient-rich plant genotypes is a protracted process and its effectiveness can be limited by the low amount of readily-available pools of soluble micronutrients in soils (Cakmak 2008). Application of fertilizers containing Zn and Fe (i.e. agronomic biofortification) is a short-term solution and represents a complementary approach to breeding. Biofortified crops, once developed, adapted and released for cultivation, will continue to be grown and consumed yearly, thus contributing significantly to overcoming malnutrition (Graham et al. 2007; Stein et al. 2005, 2010; White and Broadley 2009). Recent studies report clear increases in Fe and Zn absorption when biofortified pearl millet grain of Indian origin is consumed by young women or children (Cercamondi et al. 2013; Kodkany et al. 2013). Another study showed strong positive correlation (r = 0.73) between Zn and Fe, showing that the simultaneous selection for high Zn and Fe densities could be very efficient (Burger et al. 2014; Kanatti et al. 2014). Several studies reported a high correlation between Zn and Fe in pearl [AU25] millet (Bashir et al. 2013; Govindaraj et al. 2009; Velu et al. 2007) and in wheat (Gomez et al. 2010a, b; Velu et al. 2012). In wheat, Fe and Zn correlate positively and the highest concentrations (up to 85 µg/g) were detected in landraces as well as in wild and primitive relatives (Ortiz-Monasterio et al. 2007; Peleg et al. 2009). In India, application of Zn-coated urea fertilizer significantly improved both grain yield and grain Zn concentrations (Shivay et al. 2008).

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 enhanced accumulation of bioavailable minerals. The means of achieving this goal differ between the two approaches (Gomez-Galera et al. 2010). The main nutrients targeted for biofortification are beta carotene, Fe and Zn. Most current research is being 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 2007) and that genetically-modified organisms (GMOs) have the potential for increased agricultural productivity.

Another genetic engineering approach to increasing the bioavailability of Fe in diets is the elimination of phytate. This sugar-like molecule binds a high proportion of dietary Fe, so that the human body is unable to absorb it. Lucca et al. (2001) introduced a fungal gene for the enzyme phytase, which breaks down phytate synthesis, thus improving the bioavailability of Fe in rice diets. Wei et al. (2012) reported that foliar Zn fertilization reduced the phytic acid content and increased the accumulation of bioavailable Zn in polished rice. In maize, overexpression of *Aspergillus niger* phytase gene (phyA2) in seeds using a construct driven by the maize embryo-specific globulin-1 promoter resulted in about 5,000 % increase in phytase activity and 30 % decrease in seed phytate concentration. On the other hand, a very novel and interesting approach has been used in maize and soybean to silence the genes involved in the biosynthesis of phytic acid (PA) (Shi et al. 2008). It was found that maize lpa1 mutants are defective in a MRP ATP-binding cassette (ABC) transporter that is more highly expressed in embryos, but also in immature endosperm, germinating seeds and vegetative tissues. The expression of this transporter was silenced in an embryo-specific manner. The concentration of PA in seeds of transgenic maize was found to be reduced by up to 87 % depending upon the transgenic line, and the transgenic plants were not adversely affected in grain yield or seed germination in contrast to the lpa mutants. Similarly, silencing of MRP (expansion) transporter in sorghum decreased the PA concentration in seeds by 80–86 %, and a consequent increase in Fe and Zn absorption was observed when analyzed in Caco-2 cell lines (Kruger et al. 2013). These remarkable findings indicate the possibility of producing GMO cereals with low PA and without affecting agronomic performance by silencing the expression of transporters involved in the biosynthesis of PA.

## 2.11 Conclusion and Prospects

Biofortification is a method of breeding crops to increase their nutritional value. This can be done either through conventional selective breeding or through genetic engineering. Biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as they are growing, rather than having nutrients added to processed foods. This is an improvement over ordinary fortification when it comes to providing nutrients for the rural poor, who rarely have access to commercially-fortified foods. As such, biofortification is seen as a future strategy to deal with deficiencies of micronutrients in the developing world. In the case of Fe, WHO estimated that biofortification could help cure the two billion people suffering from iron deficiency-induced anemia.

There is very compelling global human health and nutritional evidence to convince plant breeders that micronutrient density traits should be primary objectives in their work, and targeted to the developing world. Therefore, biofortification is of great importance in enriching seeds with mineral micronutrient. Both plant breeding and genetic modification offer good opportunities to increase the micronutrient contents of edible parts of major crops. Anti-nutrient factors should be minimized to maximize micronutrient bioavailability. Understanding the genetic basis for breeding crop cultivars with higher grain micronutrient concentration is required. Emerging cost-effective genomics tools should be used to accelerate the breeding process and product development targeting these micronutrients. After development of new breeding lines and varieties, dissemination of biofortified breeding lines and hybrid parents to and their utilization by user-research organizations in the public and private sector on a continuing basis will make biofortified cultivar development a routine matter and significantly contribute to improved human nutrition.

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# Author Queries

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| Queries | Details Required | Author's Response |
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| AU1 | WHO (2002, 2005), Welch and Graham (1999, 2004), FAO (2003), Combs and Welch (1998), Mayer et al. (2008), Brinch-Pedersen et al. (2007), Combs Jr et al. (1996), Boccio et al. (2003), Lemke (2005), Rosegrant et al. (2003), Caballero (2002), Gordon (1997), Herrmann and Wolfgang (2011), Huethe et al. (2004), Long et al. (2004), Kumar et al. (2012, 2013), Nguni et al. (2011), Suwarto and Nasrullah (2011), Chandel et al. (2011), Graham et al. (2005), Wang et al. (2010), Peleg et al. (2008), Queiroz et al. (2010), Burger (2014), Govindaraj et al. (2009), Garcia-Oliveira et al. (2009), Govindaraj et al. (2011), Grusak and Cakmak (2004), Greger (1992), Gibson (1994), Sandberg (2002), Graham et al. (2007), Stein et al. (2010), Bouis (2007), Gelin et al. (2007), Chakraborti et al. (2009), Ashok Kumar et al. (2009), Vogel (1989), Ficco et al. (2009); Zhao et al. (2009), McDonald et al. (2008), Lee et al. (2009c) are not provided in the reference list. Please provide. | |
| AU2 | Please fix "a" for "b" for Gomez-Becerra et al. (2010). | |
| AU3 | Please provide in-text citation for Ahmed et al. (1998), Ashok et al. (2010, 2013), Del et al. (2003), Dickson et al. (2011), FAO/WHO (2002, 2003, 2005, Gibson et al. (1994), Herrmann and Obeid (2011), Huether and McCance (2004), Lee and An (2009), Long et al. (2008), Penelope et al. (2006), Philip et al. (2009), Rosegrant et al. (2002), Ross and Welch (2004), Sandberg and Andlid (2002), Suwarto (2011), Welch (1999). | |
| AU4 | Please confirm the inserted publisher name and location for ICMR (2009). | |
| AU5 | Please confirm the inserted volume, issue number and page range for Penelope et al. (2006). | |