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REVIEW

Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains

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Abstract More than half of the world populations are affected by micronutrient malnutrition and one third of world's population suffers from anemia and zinc deficiency, particularly in developing countries. Iron and zinc deficiencies are the major health problems worldwide. Phytic acid is the major storage form of phosphorous in cereals, legumes, oil seeds and nuts. Phytic acid is known as a food inhibitor which chelates micronutrient and prevents it to be bioavailabe for monogastric animals, including humans, because they lack enzyme phytase in their digestive tract. Several methods have been developed to reduce the phytic acid content in food and improve the nutritional value of cereal which becomes poor due to such antinutrient. These include genetic improvement as well as several pre-treatment methods such as fermentation, soaking, germination and enzymatic treatment of grains with phytase enzyme. Biofortification of staple crops using modern biotechnological techniques can potentially help in alleviating malnutrition in developing countries.

Keywords Phytic acid · Phytase · Dephytinization · Micronutrients · Monogastric animals

Introduction

Micronutrient malnutrition affects more than half of the world population, particularly in developing countries. Iron, zinc and vitamin A deficiencies are the most serious health constraints worldwide (Jorge et al. 2008). In developing countries plants are the major source of food. In unrefined cereal and legumes foods the low bioavailability of Fe, Zn causes metabolic disorder related to these nutritional factors. So improving the nutritional value of such type of food will improve the nutritional status of entire population. Mineral, phosphorous and phytate content is much higher in the bran than the whole grain (Iskander and Morad 1986; Guttieri et al. 2004; Steiner et al. 2007).

Food fortification programs depend on widely distributed, industrially processed food items usually not affordable for half of the world's population (Jorge et al. 2008). More than one third of the world's population suffers from anemia, half of it caused by iron deficiency. Iron deficiency adversely affects cognitive development, resistance to infection, work capacity, productivity and pregnancy. Zinc is involved in cellular growth and differentiation. While mild to moderate zinc deficiency is common throughout the world (Sandsted 1995) one third of world's population at high risk live in low income countries. Zinc deficiency causes impaired growth, immune dysfunction, increased morbidity and mortality, adverse pregnancy outcomes and abnormal neurobehavioral development. The in vitro bioaccessibility of minerals varied significantly, depending on the mineral and the type of the food matrix. In general the best sources of bioaccessible Fe and Zn were found to be pulses and nuts (Joanna and Zbigniew 2011). The limited bioavailability of cereals mineral content due to relatively low mineral levels and the presence of phytic acid and other antinutritional factors that reduce their bioavailability to 5–15 % offers challenges in nutrition point of view (Das et al. 2011). This study focuses on sources of phytic acid in food and different strategies to reduce such phytic acid food inhibitor in major food grain to improve nutritional quality of food.

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Phytic acid and its sources in foods

The Phytic acid is myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate. Phytic acid is the major storage form of phosphorous comprising 1–5 % by weight in cereals, legumes, oil seeds and nuts (Vats and Banerjee 2004). It represents 50–85 % of total phosphorous in plants (Reddy et al. 1982). Phytate rapidly accumulates in seeds during the ripening period. It is stored in leguminous seeds and oil seeds in the globoid crystal within the protein bodies (Erdman 1979). In cereal grains, rice and wheat, it is found in bran fraction such as aleurone layer and pericarp, in corn it is seen in endosperm (O'Dell et al. 1972). Monogastric animals including poultry and humans are unable to metabolize phytic acid due to the lack of sufficient level of phytate degrading enzymes activity in their digestive tract (Wodzinski and Ullah 1996; Schroder et al. 1996; Maenz and Classen 1998; Boling et al. 2000; Singh et al. 2011) and it is largely excreted in their manure. Hence food products have to supplement with inorganic phosphate to meet the phosphorous requirement (Reddy et al. 1982; Vats and Banerjee 2004). About 70 % of total P in feed is released in excreta due to inefficient uptake of phosphorous by monogastric animals (Milko et al. 2008). Hence the presence of phytic acid in animal feed for chickens and pigs are undesirable. Such high levels of phytate and inorganic phosphate through leaching or surface run-off, can lead to the eutrophication of surface water and algal blooms (Boesch et al. 2001; Turner and Haygarth 2000; Milko et al. 2008), hypoxia, death of fish and aquatic animals and production of nitrous oxide, a potent green house gas (Mallin 2000; Naqvi et al. 2000). The projected growth of livestock industry is expected to accelerate such environmental problems on a global scale**.** Supplementation of animal feeds with phytase improves the phosphorus bioavailability and reduces the amount of phosphorus excreted (Yano et al. 1999). It reduces phosphorus excretion by 30 % to 50 % (Lie and Porres 2003; Haefner et al. 2005; Greiner and Carlsson 2006; Selle and Ravindran 2007). Thus, for both industrial and ecological reason phytase and phytase producing microbes have attracted significant interest. Furthermore, phytic acid acts as antinutritive agent by blocking the absorption of minerals such as Fe, Zn, and Ca. The binding results in insoluble salt with poor bioavailability of minerals (Zhou and Erdman 1995; Urbano et al. 2000; Feil 2001).

Sources of phytic acid in food are cereals, legumes, oilseeds and nuts which are important for human nutrition (Table 1). It represents approximately 40 % and 60 % of total calorie intake for human in developed and developing countries respectively (Schlemmer et al. 2009). Cereals are rich in phytate and cereal food products show higher phytic acid content (Wise 1983). The phytic acid concentration reported in wheat germs and wheat bran are 1.1–3.9 % and 2.0–5.3 % respectively (Kasim and Edwards 1998). In rice bran, the phytic acid content is present upto 8.7 % (Lehrfeld 1994). In the semi refined pearl millet flour phytic acid content is significantly ($P<0.05$) lower while, bran rich fraction retained significant ($P<0.05$) amounts (Suma and Urooj 2011). In legume seeds, phytate is located in protein bodies of endosperm. Phytic acid content in whole seed ranged from 0.2 to 2.9 % and is higher (greater than 3.7 %) in cotyledons (Ravindran et al. 1995; Harland and Prosky 1979; Lestienne et al. 2005). The wild type legume seeds contain 0.98–3.14 g/100 g DM of phytic acid. Phytic acid content is drastically reduced during soaking plus cooking (Vellingiri and Hans 2010).

The phytic acid content varies from approx. 1.0–5.4 % (dw) in oilseeds such as soybeans, sesame seeds, sunflower kernels, linseeds and rape seeds (Lolas et al. 1976). A maximum phytic acid content of 10.7 % is reported in soy concentrates (Lehrfeld 1994). The next group of phytate rich food is nuts such as walnuts, almond, cashew nuts etc. in

Table 1 Content of phytic acid in major cereals, legumes, oilseeds and nuts (Schlemmer et al. 2009)

Name	Phytic acid g/100 g(dw)	References
Cereals		
Maize germ	6.39	Kasim and Edwards 1998
Wheat bran	2.1–7.3	Harland and Oberleas 1986; Wise 1983
Wheat germ	1.14–3.91	Wise 1983
Rice bran	2.56–8.7	Kasim and Edwards 1998; Lehrfeld 1994
Barley	0.38–1.16	Kasim and Edwards 1998
Sorghum	0.57–3.35	Kasim and Edwards 1998
Oat	0.42–1.16	Harland and Oberleas 1986
Rye	0.54–1.46	Harland and Prosky 1979
Millet	0.18–1.67	Lestienne et al. 2005
Legumes		
Kidney beans	0.61–2.38	Lehrfeld 1994
Peas	0.22–1.22	Ravindran et al. 1994
Chickpeas	0.28–1.60	Ravindran et al. 1994
Lentils	0.27–1.51	Ravindran et al. 1994
Oilseeds		
Soybeans	1.0–2.22	Lolas et al. 1976
Linseed	2.15–3.69	Wise 1983
Sesame seed	1.44–5.36	Harland and Oberleas 1986
Sunflower meal	3.9–4.3	Kasim and Edwards 1998
Nuts		
Peanuts	0.17–4.47	Venktachalam and Sathe 2006
Almonds	0.35–9.42	Harland and Oberleas 1986
Walnuts	0.20–6.69	Chen 2004
Cashew nuts	0.19–4.98	Chen 2004

which phytic acid content ranged from approx.0.1–9.4 % (Chen 2004; Venktachalam and Sathe 2006; Schlemmer et al. 2009). Zhang and Bai (2011) extracted phytic acid from rice bran and its content was 2.15 %.

Dephytinization and nutrition

Phytic acid binds to minerals and makes them unavailable due to its chelating property. It has been reported that phytic acid inhibits absorption of iron, zinc calcium, magnesium and manganese (Hallberg et al. 1989; Reddy et al. 1996; Bohn et al. 2004; Phillippy 2006). Removal of phytic acid increases bioavailability of many cations and thus nutritional value of meal. There are several methods which are developed for removal of phytic acid from grains (Nout 1993).

Milling and soaking Milling is the most commonly used method to remove phytic acid from grains. This technique removes the phytic acid but also has major disadvantages as it also removes major parts of minerals and dietary fibers. Soaking is widely applied and most important method in germination and fermentation process of cereals. Soaking of cereal such as pearl millet with endogenous or exogenous phytase increased in vitro solubility of iron and zinc by 2–23 % (Lestienne et al. 2005). Soaking of sorghum flour for 24 h at room temperature reduces phytic acid level by 16–21 % (Mahgoub and Elhag 1998). Together soaking and cooking has shown much more effective to reduce phytic acid than only soaking for a short duration (Vidal-Valverde et al. 1994). In case of grains and beans soaking to be quite effective for reduction of phytic acid as well as consequent increase in mineral bioavailability (Perlas and Gibson 2002; Coulibaly et al. 2011). This method involves the complete submergence of grains in water for certain amount of time period which results in the activation of endogenous phytases. Soaking at temperature between 45 and 65 °C and pH value between 5 and 6 a considerable percentage of phytate was hydrolysed (Greiner and Konietzny 2006). These phytases are present in grains so by activation of these enzymes it has been reported that significant amount of phytic acid content in grains have been removed. This treatment also has certain disadvantages as during this treatment there occurs loss of minerals and water extractable proteins. As soaking time increased from 2 to 12 h phytic acid content in chick pea decreased by 47.4 to 55.71 % (Ertas and Turker 2012).

Fermentation Fermentation is a metabolic process in which carbohydrates are oxidized to release energy in absence of external electron acceptor. Fermentation of food grains improves bioavailability of minerals. Phytic acid is present in cereals in the form of complexes with metal cations viz. iron, zinc, calcium and proteins. The enzymatic degradation of phytic acid requires an optimum pH which can be provided by natural fermentation. Such a degradation of phytic acid can increase the amount of soluble iron, zinc and calcium a number of folds (Haard et al. 1989). It have been reported that fermentation of millet grain for 12 and 24 h could reduce the food inhibitors, phytic acid and tannins (Coulibaly et al. 2011). Natural fermentation can achieve a large reduction in phytic acid in rice flour by the action of microbial as well as grain phytases. Phytases reduces the hexa form of phytic acid (IP6, myo-inositol 1,2,3,4,5,6-hexakisphosphate) into lower forms, such as IP5,IP4,IP3, IP2,IP1 and myo-inositol (Ragon et al. 2008). The lower forms of phytic acid have a lower binding capacity for metals like iron and zinc (Agte et al. 1997). There are 88.3 % reduction in phytate content was recorded when germinated pearl millet sprouts were fermented with mix pure cultures of *Saccharomyces diasticus, S. cerevisiae, Lacto-bacillus brevis* and *L. fermentum* at 30 °C for 72 h (Kaur et al. 2011).

Germination This method reduces phytic acid content by up to 40 % (Masud et al. 2007). In non-germinated cereal and legume grains a little endogenous activity is found but during germination a marked increase in phytate degrading activity was observed (Greiner and Konietzny 2006). It is reported that malting of millet reduces 23.9 % phytic acid after 72 h and 45.3 % after 96 h (Makokha et al. 2002; Coulibaly et al. 2011). The greatest reduction of phytic acid phosphorus has been found in rye while smallest decrease was found in maize (Poiana et al. 2009). Marshall et al. (2011) screened cereal grains for phytic acid content and found that germination for 10 days resulted in a significant reduction ($P<0.05$) in the phytate contents of all cereal grains screened. Autoclave and Microwave treatments decreased phytic acid content as they also increased total mineral content and HCl-extractability of minerals in whole wheat bread (Mustafa and Adem 2011).

Phytases and its classification

The hydrolysis of phytate to orthophosphate and lower substituted inositol phosphates is achieved enzymatically with phytase. This is the most beneficial method for reducing phytic acid content in grains as it can remove maximum amount of phytic acid without reducing mineral content of grains.

Phytases are myo-inositol hexakisphosphate phosphohydrolase that catalyses the hydrolysis of phytic acid to inorganic phosphate and myo inositol phosphate derivative (Mullaney et al. 2002). It is an acid phosphohydrolase that

hydrolyzes phosphomonoester bonds from phytate thereby liberating inorganic phosphate. Phytases can be used as additives in many food products which strengthen the interest of isolation of new and efficient phytase producing microbes, obtaining efficient phytases that able to release adequate food phosphate in digestive tract and selecting thermostable phytases which are stable during processing with lowest production cost (Lei and Stahl 2001; Greiner and Konietzny 2006).

Phytases have been classified as 3-phytases (EC 3.1.3.8), and 6-phytases (EC 3.1.3.26) based on the position of first phosphate hydrolyzed. The 3-phytases initiates dephosphorylation of phytic acid at the 3 position of phytic acid and 6-phytases at position 6. The 3-phytases are the largest group of phytases which are generally found in bacteria and fungi. The 6-phytases acts basically on the carbon atom next to C5 of the inositol ring. Plant phytases acts preferentially at the C6 carbon and are 6-phytase. Phytases can be categorized into acid phytases and alkaline phytases on the basis of pH optimum (Milko et al. 2008). On the basis of catalytic property phytases have also been classified as Histidine acid phosphatase (HAP), b-Propeller phytase (BPP), cysteine phosphatase (CP) and purple acid phosphatase(PAP) (Vats and Banerjee 2004; Mullaney and Ullah, 2003; Singh et al. 2011). The 3-phytases are structurally homologous with beta-propeller phosphates and histidine acid phosphatases. It has been suggested that end product by action of 3-phytases on phytic acid is Ins (2, 4, 6) P3. Most bacterial, fungal and plant phytases belong to the HAPs. HAPs can initiate hydrolysis of phytic acid on either the C3 or the C6 position of the inositol ring and produce *myo*-inositol monophosphate as the final product.

Sources of phytase

Phytases are commonly found in nature and can be obtained from a number of sources including plant, animal and microorganisms (Konietzny and Greiner 2002; Vohra and Satyanarayana 2003; Milko et al. 2008). Phytase occurs very frequently in the plant kingdom. Its activity was detected in many plants species such as wheat, rye, barley, pea, bean, soybean, maize, rice, lettuce, spinach, grass, lily pollen, etc. Generally, it is assumed that during seed germination phytate, after decomposition by phytase, is utilized in the form of phosphate and inositol (Asada et al. 1969). The first report on animal phytase in calf liver and blood was given by McCollum and Hart (1908). However, phytase was reported in the blood of lower vertebrates, birds, reptiles, fishes, batrachians, sea turtle (Rapoport et al. 1941).

The major problem in production of plant phytases is that a cost-effective and efficient production of the enzymes is yet to be developed. The higher pH and thermal stability of microbial phytases compared to plant phytases have made the microbial phytases more investigated for industrial purposes (Bohn et al. 2008). The phytase production from plant is not economical since preliminary treatment is necessary and production procedure becomes time consuming, troublesome and expensive. So, the production of phytase from microbial origin is of greater potential in development. Various strains of microbial origin for phytase production were discovered which are highly responsible for the production of the phytase. Several screening programs have been carried out aiming at the isolation of different groups of bacteria, yeast and fungi having extra cellular phytase activity. Singh et al. (2013) isolated phytase producing bacteria from different soil sample and screened using phytase screening medium (PSM). Bacterium isolated from poultry farm soil (*Bacillus sp*.) shows 39 mm clear zone on PSM and having better phytase activity.

Over 200 fungal isolates belonging to genera *Aspergillus, Mucor, Penicillium* and *Rhizopus* have been tested for Phytase production. All isolates produces active extra cellular phytase. *A. niger* was identified as the most active fungal phytase producer. A survey of fungi for production of extra cellular phytase has been reported (Shieh and Ware 1968). More than 58 strains of fungi exhibited the ability of hydrolyzing phytate when grown in rape seed meal. Of them, most efficient producer of active phytase was *A. ficuum.* Extra cellular phytase has also been found in other *Aspergillus* species such as *A. oryzae, A. amstelodami, A. candidus, A. flavus* and *A. repen* (Hawson and Davis 1983). *Bacillus, Klebsiella, E.coli, Pseudomonas* sp. are some examples of phytase producing bacteria (Greiner and Carlsson 2006*)*. There are few studies on the phytase of yeast such as *Saccharomyces cerevisiae* and *Schwanniomyces castellii*.

Properties of phytases

Properties of enzymes are important in determining their potential use in industrial application. The different molecular forms of phytases obtained from different sources exhibited differences in properties such as thermostability, optimum pH etc. Temperature optimum for phytases ranged from 25 to 80 °C. *Thermomyces lanuginosus,* a thermophilic fungus, has optimum phytase activity at 65 °C. Mesophilic fungal sps. *A. fumigatus* and *A. niger* NRRL 3135 have optimum activity at 37 °C and 55 °C respectively. Temperature optimum of phytase produced by *Thermoascus auranticus* is 55 °C. When temperature increases to 70 °C its 80 % activity still remained. A highly thermostable phytase from *A. fumigatus* can withstand temperature upto 100 °C over a period of 20 min with loss of only 10 % of initial enzyme activity, was reported by Pasamonts et al. (1997). Bacterial phytase from *B. subtilis war. Notto* remains active at temperature 60 °C (Shimizu

1992). Phytases active within pH range 4.5–6.0 and stability decrease dramatically when pH value is less than 3.0 and greater than 7.5. pH optimum for fungal origin phytases is between 4.5 to 5.5 and 6.5 to 7.5 for bacterial origin. *A. niger* NRRL3135 produces two different types of phytase-Phy A and Phy B. Phy A has optimum pH of 5.5 whereas optimum pH for Phy B is 2.0. Plant seeds phytases have been described to have usually pH optimum between 4.0 and 5.6. Recently, alkaline phytases having a pH optimum at eight were extracted by a non-ionic detergent from legume seeds (Scott 1991). Another alkaline phytase with a pH optimum at 8 was found in mature lily pollen (Hara et al. 1985; Scott and Loewus 1986). Phytases show broad substrate specificity with highest affinity for phytic acid. Phytases are high molecular weight protein ranging from 40 to 500 kDa. *A. ficuum* phytase contained 594 amino acids. The phytase gene (phy) of *A. niger* is cloned and characterized whose translated product resulted in peptide sequence containing 10 potential glycosylation sites.

Enzyme assay

Phytase activity has been detected by several assay procedures developed by Fiske and Subbarao (1925), Ames (1966), Harland and Harland (1980), Heinonen and Lahti (1981). The most common method to detect phytase activity is by measuring the phosphate liberated by action of enzyme. The hydrolyzed inorganic phosphate is measured by the method based on colorimetric measurement of phosphomolybdate. Assay developed by Harland and Harland (1980) is most commonly employed.

Genetic modification of phytase source

Genetic modification technique can be used efficiently to reduce phytic acid content in cereals by cloning the genes of phytase enzyme and by creating the transgenic plant with modified genome encoding for phytase enzyme. In this scenario transgenic rice has been developed to overexpress genes encoding for phytase from *Aspergillus fumigatus*, ferritin from *Phaseolus vulgaris* and a cysteine-rich metallothionein-like protein to improve rice iron bioavailability to humans. The plant has been crossed with a recently developed β-carotene producing rice line (Lucca et al. 2001; Lie and Porres 2003). Genetic modification of crop plants for production of heterologous phytase reduces phosphate load on agricultural ecosystems as well as improving phosphate bioavalability (Brinch-Pedersen et al. 2002; Vats and Banerjee 2004). After expression of *A. niger* NRRL3135 phyA gene in soybean it was found that recombinant phytase exhibits similar temperature and pH optima as of native enzyme except the molecular weight of enzyme (Vats and Banerjee 2004). It has been reported that biofarming of phytase is cost effective approach for phytase production. Strain improvement studies of *A. niger* NRRL 3135 by UV radiation, has been showed that phytase catalytic mutant producing 3.3-fold higher phytase (phyA) than the wild type strain (Chelius and Wodzinski 1994; Vats and Banerjee 2004).

Protein modification

An 'ideal phytase' that has the desirable characteristics for application in animal feed industry should be active in the stomach, catalytically efficient, thermostable during animal feed processing and storage, proteolysis-resistant and cheap (Lei and Stahl 2001). It should be easily processed by the feed manufacturer for its suitability as an animal feed additive (Singh et al. 2011). Genetic manipulation techniques such as site directed mutagenesis could be employed for further amelioration of the properties. There is a need to improve phytase enzyme structure by genetic manipulations in coding sequence of genes (Kostrewa et al. 1997; Lim et al. 2000). But before designing an ideal phytase and its genetic manipulation, its structure is important. The specific activity of the heat-stable *A. fumigatus* phytase (Tomschy et al. 2000), pH stabilility of *A. niger* PhyA phytase (Mullaney et al. 2002) and thermostability of *E. coli* AppA phytase has been improved using this approach (Rodriguez et al. 2000; Lie and Porres 2003).

Application of phytase

There are many areas where phytase can be used such as phytate elimination in feed and food industries, in fighting phosphorus pollution or environmental protection, plant growth promotion and the preparation of special myo-inositol phosphates as tools for biochemical investigation (Idriss et al. 2002; Greiner and Carlsson 2006; Singh et al. 2011). Phytase, releasing phosphate from phytate, reduces the need of feed supplementation by phosphorus. There are two basic ways how to use phytase in feeds. The first possibility is replacement of inorganic phosphorus supplementation with phytase. However, the pH, temperature etc. condition in the animal stomach or intestine are not optimal, the second method of phytase use, feed pretreatment, becomes more attractive. Canola meal used as a feedstuff for livestock and fowl was successfully dephytinitated by *A. niger* NRRL 3135 in a solid-state fermentation (Nair and Duvnjak 1990; Ebune et al. 1995) aiming to increase phytate phosphorus bioavailability and the nutritive value of the feed. Segueilha et al. (1993) removed phytic acid in

wheat bran using phytase from the yeast *S. castelii*. Increased dietary consumption of cereal fibers, legumes and soy protein isolates results in an increased intake of phytate. Some food processing methods such as cooking, germination, hydrothermal treatment, fermentation and soaking are shown to reduce or remove considerable amounts of phytate in legumes (Nout and Rambouts 1990; Rehms and Barz 1995). Phytase is incorporated into commercial poultry, swine, and fish diets to improve the availability of phosphorus, minerals, amino acids, and energy. The phytate molecule and thus the nutrients bound to it cannot be absorbed in the digestive tract without enzymatic degradation by phytases. It is well known for many years that degradation of phytic acid during bread making effect mineral bioavailability (Mollgaard 1946). Therefore, several bread making procedures designed to decrease the phytate content have been reported. These include the addition of commercial phytase from wheat to whole wheat flour (Knorr et al. 1981) and the activation of the naturally occurring phytase by soaking and malting the grain. Feed treatment with phytase increases the bioavailability of inorganic phosphorus thus improving the nutritional value of food and help in fighting phosphorus pollution. Pollution caused by excess of phosphorus accumulation in soil and water can be decreased by phytases (Nahm 2002). From *Bacillus subtilis,* a beta-propeller phytase was constitutively expressed in tobacco and *Arabidopsis*. Phytase activities in leaf and root extracts in transgenic tobacco, were 7 to 9-fold higher than those in wild-type and 4 to 6-fold higher extracellular phytase activity had been recorded in transgenic plants (Lung et al. 2005; Singh et al. 2011).

Screening of micronutrient dense germplasm

Minerals and vitamins in food staples eaten widely by the poor may be increased either through conventional plant breeding or through use of transgenic technique, a process known as biofortification. Genetic variation needed for germplasm collection is a new technique such as TILLING (Till et al. 2007) proved to be very beneficial in achieving the required goal of creation of genetically variant population. The transgenic approaches can supplement ongoing breeding efforts and provide the urgently needed biofortified crop to feed the world population with nutritious food. These include recent work on tomato, which increased folate accumulation 15 fold by targeting a highly compartmentalized pathway (Diaz et al. 2007). Also iron content in rice grain was doubled by over expression of bean ferritin (Lucca et al. 2001).

A small content of Fe and Zn present in wheat (21–32 mg kg−1 and 15–22 mg kg−1) respectively (Rawat et al. 2009) and a very small portion of existing amount is retained during processing, hence low bioavailable due to presence of phytic acid food inhibitors. Using genetic biofortification approach to reduce the amount of Zn deficiency is cost-effective, easily applicable and affordable in the target populations. For a breeding program to develop new genotypes with high Zn concentration first requires existence of useful genetic variation for Zn accumulation in grain. Compared to cultivated wheat, wild and primitive wheat is a better genetic resource for high Zn concentrations. Among wild wheat tested so far, the collections of wild emmer wheat, *Triticum turgidum* ssp. dicoccoides, showed prominent genetic variation and the highest concentrations of Zn ranging from 14 to 190 mg Zinc kg −1. New wild emmer wheat accession have been identified recently showing very high concentrations of Zn up to 139 mg kg −1, Iron up to 88 mg kg −1 and protein up to 380 g kg −1 in seed . It has also high tolerance to drought stress and Zn deficiency in soil (Cantrell and Joppa 1991; Cakmak et al. 2010)

Reducing phytic acid content through low phytic acid (lpa) mutants

This involves the creation of gene knockout mutants by knocking out genes involved in phytic acid biosynthesis pathway (Fig. 1).

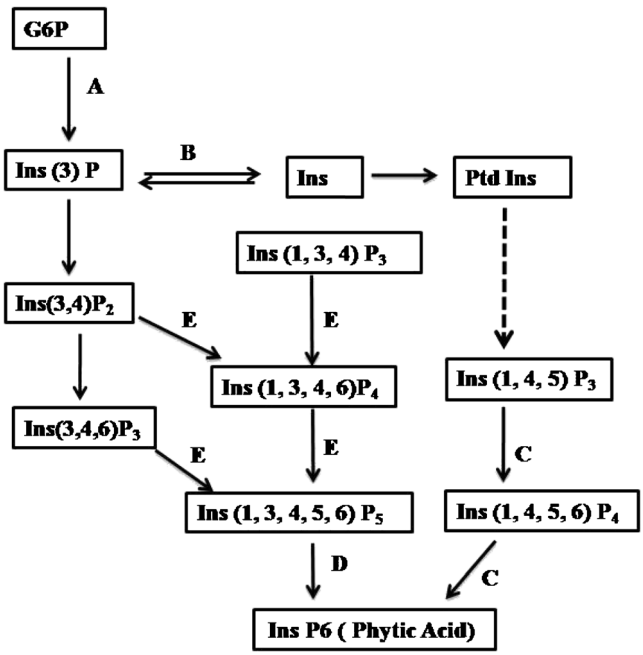

Fig. 1 The phytic acid biosynthetic pathway in plants where A, B, C, D and E are the genes involved in phytic acid biosynthesis pathway. A.MIPS(myo-inositol 3-phosphate synthase) B. IMP(inositol monophosphatase) C. IPK2(inositol1,4,5-tris phosphate kinase) D. IPK1(inositol 1,3,4,5,6-pentakis phosphate 2-kinase) E. ITP5/6 K(inositol 1,3,4-trisphophate 5/6-kinase). G6P:glucose 6-phosphate, Ins:myo-inositol, PtdIns:phophatidyl inositol (Suzuki et al. 2007)

The TILLING population was developed by Random mutations using Ethyl methanesulfonate (EMS) chemical mutagen agents for generation of low phytic acid content as well as high endogenous phytase activity showing mutants in Pusa Basmati rice (Shukla and Singh 2012). RNAi technology has been used to reduce maize phytic acid by silencing MRP4 ATP- binding cassette (ABC) transporter (Shi et al. 2007; Gupta et al. 2011).

Conclusion and perspective

Abolition of micronutrient malnutrition remained a widespread global health problem in developing countries. Increasing micronutrient intake in food through food based approaches is a sustainable method of prevention of micronutrient malnutrition which should be achieved through food diversification. Biofortification offers a long-term, sustainable, food-based solution for a world population (Jorge et al. 2008). Breeding programs designed to improve grain Zn and Fe concentrations. In low-income countries breeding for mineral solidity may remain the only agricultural involvement to improve the nutritional content of staple crops (Cakmak et al. 2010). Genetic improvement as well as several pre-treatment methods such as fermentation, soaking, germination also improves nutritional quality. The ability of any given phytase to hydrolyze the anti nutrient phytic acid in the digestive tract is determined by its enzymatic property such as catalytic efficiency, substrate specificity, temperature stability, pH optima and resistance to proteolysis. Research is needed to discover new phytases and to engineer them to develop desired characteristic for specific purpose. Cost effective process for commercial production should be developed. The antinutritive properties and its values as a possible phosphorus source have encouraged researchers to develop a safe method to remove phytic acid. Future research is needed to determine the optimal dose and appropriate delivery of phytase to human foods.

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