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Phytin: A Nutritional Inhibitor in Food and Feed - Review of Strategies and Challenges to Overcome the Menace in Maize

Vimla Singh[1], Rakesh Mehra[2*], Sunaina Bisht[3], Meena Shekhar[1], and Arvind Kumar[1]

[1]ICAR-Indian Institute of Maize Research, Pusa campus, New Delhi (Delhi) - 110012, India
[2]C.C.S. Haryana Agricultural University, Regional Research Station, Karnal (Haryana) - 1320001 (India)
[3]Division of Plant Pathology, Indian Agricultural Research Institute, Pusa Campus, New Delhi (Delhi) - 110012, India

**Corresponding author*

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A B S T R A C T

Phytin, or myo-inositol hexakisphosphate (InsP6), is stored form of phosphorus (P) seeds of cereals and legumes. It has a strong tendency to chelate metallic cations Ca, Fe, K, Mg, Mn and Zn and form insoluble complexes which renders them unavailable to animals or humans fed on seed diet. The undigested P is released in excreta and spreads as manure into the soil causing eutrophication of water bodies also due to run off. In maize (*Zea mays*) 90% of the phytin occurs in the germ portion of the kernel, while in wheat and rice it is stored in aleurone layers of the kernel and the outer bran and acts as anti nutritional factor. Apart from reducing the dietary nutritional value, phytin plays several positive roles in metabolism, *viz.*, protein and carbohydrate metabolism, responding to oxidative stress, draught tolerance by inducing stomatal closure, prevents senescence and apoptosis, regulates phosphate homeostasis and acts as shelter factor for maturing embryo in the seeds. Application of phytin is also reported in paper preservation, meat preservation, body metabolism and also cancer therapy. Phytin also has probable role in disease resistance in plants; however, much information is not available. Inspite of so many positive roles for plants, the economic value of feed and food is greatly reduced due to the anti nutritional attribute of the seed phytin. In this review we discuss the approaches and challenges to overcome the menace of phytin in food and feed to improve bioavailability of minerals and maintaining seed phytin to desired levels for normal plant metabolism.

Introduction

Maize (*Zea mays* L.) seeds forms an important ingredient of feed for poultry and swine and also a food for humans. In seeds stored phosphorus (P) largely occurs as *phytin* or Phytic acid (PA) a myo-inositol hexakisphosphate, *Ins (1,2,3,4,5,6) P6*, or *InsP6*. PA content is a major concern for nutritive value of maize. It is the storage compound of phosphorus in seeds accounting for up to 80% of the total seed phosphorus and contributing as much as 1.5% to the seed dry weight. The negatively charged phosphate in

PA strongly binds to metallic cations of Ca, Fe, K, Mg, Mn and Zn making them insoluble and thus unavailable as nutritional factors (Bohn *et al.,* 2008). Also, PA reduces the phosphorous availability required for growth in monogastric animals, which digest PA poorly. Moreover, undigested PA eliminated by the monogastric animals into the environment leads to an increase in phosphorous level in the environment and contributes to water pollution through eutrophication (Cromwell and Coffey 1991). When released during food or feed processing or in the gut, PA binds minerals and makes them unavailable and hence PA is an anti-nutritional factor, which causes malnutrition in human (Zhou and Erdman 1995). Hence, phytin-P is poorly available to poultry and swine. Enzyme *phytase* releases phosphate groups from *phytin* and P is available to the animal, thereby reducing P excreted from poultry and swine. Phytase is the only recognized enzyme that can facilitate the release of phosphate from phytin (IUB, 1979).

Phytic acid plays several positive roles in plant metabolism hence it is not beneficial to completely eliminate it from food. Phytic acid concentrations in seeds depend upon cultivars, soil conditions, fertilizer applications, available moisture content and other climate factors (Nelson et. al., 1968). Lott et al., (2000) have reported that the range for phytic acid in cereal grains is 0.86-1.06%, while Reddy et al., (2002) have obtained values between 0.50 and 1.89%. PA is also an important mineral reserve in seeds, and it is stored in protein storage vacuoles in the aleurone cell layer or the embryo of the seed.

Feed supplemented with inorganic phosphate or with industrially produced phytase enzyme, which breaks down PA and releases phosphorous for animal use, can address the phosphorous requirement for animal growth and reduce phosphorous pollution. However, phosphate and phytase supplementation increase the animal feed costs. In maize (*Zea mays*) 90% of the phytin is stored in the germ portion of the kernel, while in wheat and rice aleurone layers of the kernel and the outer bran are the primary storage sites (O'Dell *et al.,* 1976). The means that within relatively closely related grasses different control points exist for PA biosynthesis and assimilation exist. PA biosynthesis initiates after flowering and it accumulates during development up to seed maturation and desiccation.

In this review we discusses the physical and chemical properties of Phytin, it role in plant metabolism and the approaches to overcome this menace to improve the nutritional value of food and feed. This review presents a compilation of all available approaches to improve the nutritional value of food and feed with maize as ingredient.

Phytin: Healthy or harmful?

Phytic acid originates in plant seeds from natural mineral sources in soil or from fertilizers (Fig. 1)

Phytin-P content in feedstuffs varies from 72 and 60 % of total seed P in corn and soybean meal (SBM), respectively, which form the poultry and swine diets. Phytic acid chelates with Ca, Fe, Mg, Cu, Zn, carbohydrates, and proteins and forms less soluble complexes in the small intestine which are not hydrolyzed to release phosphorus for nutrition. Hence, phytin acts as an anti-nutrient factor. The unutilized P is excreted by the animal. Phytin in feedstuffs is relatively thermostable (O'Dell 1962). The phytate phosphorus is calculated from the ferric ion concentration assuming a 4:6 iron: phosphorus molar ratio:

$$The\ phytate = \frac{6}{4} \times \frac{A \times mean\ k \times 20 \times 10 \times 50 \times 100}{1000 \times 2} mg/100g\ sample\ ,$$

Where, *A* is optical density.

Phytin ($C_6H_{18}O_{24}P_6$) is chemically *myo*-inositol (1, 2, 3, 4, 5, 6) hexa-*kis*phosphate, structurally consisting of hydroxylated inositol ring and at least one phosphate group sterically stable at pH range 0.5~10.5. Agranoff (1978) described the structure as a turtle's limb. The four limbs and tail of the turtle are coplanar and represent the five equatorial hydroxyl groups (Fig.2).

The order of forming complexes with mineral cations *in vitro* with inositol phosphates is found to be $Cu^{2+}>Zn^{2+}>Cd^{2+}$ for all InsP3~InsP6 at pH 3~7, but binding strength is weaker for the lower inositol phosphates (Persson *et al.,* 1998). Vohra *et al.,* (1965) also reported the order $Cu^{2+}>Zn^{2+}>Ni^{2+}>Co^{2+}>Mn^{2+}>Fe^{3+}>Ca^{2+}$(Boh n *et al.,* 2007, 2008; Raboy, 2003). Thus, iron uptake is inhibited by strong chelators such as PA and some polyphenols having a chatechol group in their structure which form very stable chelates (Tuntawiroon *et al.,* 1991).

Studies on germinability, free iron level, free radical relative abundance, protein carbonylation level, damage to DNA, degree of lipid peroxidation, α- and β-tocopherol amount and antioxidant capacity of maize seeds from low phytic acid mutant revealed that, phytin provides protection against oxidative stress and antioxidant action (Berjak and Pammenter, 2003). Phytic acid is signal transducer for appropriate response to oxidative stress, and detoxifies reactive oxygen species during seed maturation thus, protects the embryo. It is directly involved in signal transduction events in guard cells (Lemtiri-Chlieh *et al.,* 2003), plays a role in the abscisic acid (ABA) induced stomatal closure, conserving water and ensuring plant survival. Over increase in free phosphate level may perturb phosphorus homeostasis during seed maturation and interferes with phosphorylation cascade involved (Pilu *et al.,* 2003). In the aerobic cell environment, if iron cations are not chelated or sequestered by other molecules it can give rise to reactive oxygen species which may damage cell molecules and structures causing cell senescence and apoptosis phenomena. Therefore, phytic acid maintains the phosphate homeostasis in maturing seed and acts as a 'shelter factor'.

The chelating ability of PA is also used in preserving paper, having ink made from gallic acid derived from the tannins. Phytic acid prevents iron catalysed oxidation of the cellulose in the paper, thus enhances the half-life of the documents (Neevel, 1995). InsP5s inhibits free radical formation (Hawkins *et al.,* 1993), which also preserves paper (Sala *et al.,* 2006). The PA mediated inhibition of Fe-dependent reactions is also used for storage of meat (Stodolak *et al.,* 2007). In mammals, PA has helps in starch digestion and blood glucose response (Lee *et al.,* 2006), prevents dystrophic calcifications in soft tissues (Grases *et al.,* 2006) and kidney stone formation (Selvam, 2002), lowers cholesterol and triglycerides (Onomi *et al.,* 2004). PA is reported to inhibit transcription of the viral genome from HIV-1 (Filikov and James, 1998), and tested in toothpaste for preventing plaque formation (Vasca *et al.,* 2002). At the cellular level, PA is involved in gene regulation, efficient export of mRNA, RNA-editing and DNA repair (York, 2006). The lower inositol phosphates take part in cell signaling cascades (Berridge and Irvine, 1989) and pathways involved in Ca^{2+} mobilisation and signalling (Efanov *et al.,* 1997; Larsson *et al.,* 1997). It also helps in protein folding (Macbeth *et al.,* 2005) and trafficking (Shears, 2004), endo- and exocytosis (Efanov *et al.,* 1997; Saiardi *et al.,* 2002), oocyte maturation (Angel *et al.,* 2002), and cell division and differentiation (Berridge and Irvine, 1989).

Phytic acid has role in cancer therapy also (Vucenik and Shamsuddin, 2006). The lower

inositol phosphates act as an antioxidant by inhibiting iron mediated oxidative reactions, enhancing immunity by its 'Natural Killer cell function' activity. Also, they normalise abnormal cell proliferation, induce cell differentiation and apoptosis inhibiting angiogenesis. The inositol phosphates modify Phases I and II metabolising enzymes by causing G_0/G_1 arrest in cancer cells, thus, modulating oncogene expression, and prevent tumor metastasis formation. Mammalian cells can synthesize the inositol phosphates themselves (York, 2006), however, endogenous synthesis of phytic acid is minor. Hence, cancer therapy using phytic acid would require daily intake of phytic acid and will be influenced by the absorption rate of PA (Grases *et al.,* 2006).

Regarding the negative functions of phytic acid, a high PA diet causes mineral deficiency and malnutrition. The chelating ability of phytic acid renders it to be an anti nutritional factor. The phytic acid: metal-complexes are weakly soluble in major part of the intestines (Sandberg *et al.,* 1999). Phosphorous in the form of phytic acid is largely unavailable as a nutritional factor to the monogastric animals. Animal feeds therefore supplemented with inorganic phosphate to meet the nutritional requirements for optimal growth of the animals. The excess of phosphorous in phytic acid is excreted through the faeces and spreads as manure into the soil. This leads to eutrophication of fresh water streams, lakes and near coastal areas causing cyanobacterial blooms, hypoxia, death of aquatic animals and production of nitrous oxide, a greenhouse gas (Vats *et al.,* 2005). In the laboratory tests, phytic acid in faeces also inhibits polymerase chain reactions (PCR), thereby preventing PCR-based diagnostic. Murphy *et. al.* (2008) reported probable role of phytic acid in resistance against TMV, turnip mosaic virus, cucumber mosaic virus and cauliflower mosaic virus as well as to the fungus *Botrytis cinerea* and to *P. syringae* suggesting that a specific pool of InsP6 involed in salicylic acid pathway regulates defense against phytopathogens.

Approaches for lowering Phytin in food and feed

(a) Dephosphorylation by Phytases

The enzyme phytase is a novel and cost effective tool in poultry and swine for improving P utilization from phytin diets. Phytases are phosphatases (myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolase) which can release phosphate from PA. Microbial phytases discovered from *Aspergillus ficuum* (Ullah and Phillippy, 1988) are in use now-a-days. Monogastric animals, including humans, lack phytases for hydrolysis of PA in their digestive tract (Lei and Porres, 2003). Phytases potentially improve mineral bioavailability in food applications due to their higher thermostability favouring efficient degradation of phytic acid. For a 'phytate-free' after ingestion several factors are considered: form of the diet (pelleted, mash, or liquid), phytate concentration, microbial source, solubility, protein concentration and type, phytase type, location of addition of phytase (post-pelleting or mixer), dose, vitamin D status of the animal, water characteristics, dietary calcium concentration and the need exogenous enzymes supplement (coated, size of the particle, etc.), temperature, and pH optima of the enzyme, diet mineral concentration (Ca, Fe, Mg, Cu, and Zn), ingredients used in the diet, diet manufacturing methodology, disease status of the animal, and other factors (Cowieson *et. al.,* 2014). Feedstuffs made of corn, oats, sorghum, and oilseeds have little or no phytase activity (Eeckhout, and de Paepe, 1994). Adding fungal phytase in diets for poultry reduces P excretion by the animal. Kornegay,

(1999) reported that, 1000 U/kg of fungal phytase included in corn/SBM-based diets of pigs, increased P retention from 52 to 64% and 50% - 60% in boilers. Commercial phytases are produced using recombinant DNA technology for improved functional use of phytases better thermostability, pH specificity, and resistance to break-down by other digestive enzymes in the animal.

Removal amounts of P for 500 units of phytase / kg diet varies from 0.06% to 0.10% for broilers, turkeys, and swine (500-1000 U/kg). Phytases are used in the nursery with 250 to 1000U used in grow/finish and sow diets), ~ 300 units of phytase / kg diet for laying hen. In swine, typically higher levels are required. The *E. coli*- based phytases are more efficient in the animal than the *Aspergillus* and *Peniophora* derived phytases (Applegate *et al.,* 2003). There is limited data available regarding the efficacy of the different phytases at concentrations above those suggested by the manufacturer. Hence, it is difficult to give an accurate estimate of P released when phytases are used beyond their recommended levels. Plant phytases work better at 45 to 60° C (113 to 140° F) whereas microbial phytases work on a wider temperature range (35 to 63° C; 95 to 145° F) (Wodzinski and Ullah, 1996). One unit of phytase is defined as the amount of enzyme required to liberate 1 µmol of orthophosphate from phytin per minute at pH 5.5 and 37° C (Zyla *et al.*, 2013).

The maize roots phytases are isoformas of D/L- 6-phytases and are completely different from the 3-phytase or 5-phytase from *Aspergillus ficuum* (Ullah and Phillippy, 1988). Currently, 3 classes of phytase enzymes are characterized (IUB 1979) which initiate the dephosphorylation of PA at different positions on the inositol ring, and produce isomers of the lower inositol phosphates. *EC 3.1.3.8: the 3-phytases,* is the largest group found in fungi and bacteria, structurally homologous to β-propeller phosphatase (BPP), or histidine acid phosphatases (HAP). BPPs chelate three Ca ions. Most microbial and plant phytases belong to the HAPs which initiate hydrolysis of phytic acid on either the C3 or the C6 position of the inositol ring (Greiner and Carlsson, 2006). *EC 3.1.3.72: the 5-phytase* is the only known 5-phytase (EC 3.1.3.72) so far isolated from lily pollen with highest activity at pH 8.0 and temperature 55 °C (Jog *et al.,* 2005). *EC 3.1.3.26: the 4/6-phytases* are 4/6-phytases (EC 3.1.3.26) also called a 6-phytase, include plant phytases. They are most active in weakly acidic environments (pH 4~6) with a temperature optimum in the range 40~60 °C.

The first commercially available phytase was isolated from *Aspergillus niger* (NatuphosTM, BASF) in 1991, but now several phytases are commercially available, from e.g., *Peniophora lycii* (RonozymeTM, DSM/Novozymes), *Escherichia coli* (QuantumTM, Diversa/Syngenta) and *Schizo saccharomyces pombe* (PhyzymeTM, Diversa/ Danisco). However, a cost-effective and efficient production strategy is lacking for production of plant phytases.

(b) Production of phytase in transgenic plants

For *in planta* production of phytases in the feed, thermostability of the enzyme is a constraint (Brinch-Pedersen *et al.,* 2006). "Biofarming" of the phytase can be a cost-effective approach to its production. Commonly, cauliflower mosaic virus (CaMV) 35S promoter is used for the construct which gives enzymes with almost similar characteristics as the fungal phytase, except minor changes in pH optima and sizes. Heat stable *A. fumigates* phytase expressed in tobacco leaves and *Pichia pastoris* also has great potential (Wang *et al.,* 2007). *Bacillus*

mucilaginosus, in soil expresses high phytase activity extracellularly and degrades PA in the soil, thereby potentially limiting eutrophication (Li *et al.*, 2007).

(c) Phytase as feed additive

Exogenous phytases in feed increase mineral, phosphorous and energy uptake and decreases phosphorous excretion thereby, reduces eutrophication in water supplies. Supplements of microbial phytase increased P availability by 38%, 12% and 15% in pig diets containing maize, wheat and triticale, respectively (Dungelhoef *et al.*, 1994), and up to 60% reduction in manure P (Nahm, 2002). In broiler chickens also, exogenous phytase supplement reduces the excretion of endogenous amino acids, calcium, sodium, phytate phosphorus and sialic acid significantly (Cowieson *et al.*, 2004; Nahm, 2002)

(d) Phytase as food additive

Phytase potentially of decreases phytate content, releasing calcium thereby, promotes the activation of endogenous α-amylase. Iron absorption from porridges from rice, wheat, maize, oat, sorghum and wheat-soy flour is improved by cereal porridges prepared with water but not with milk, with addition of ascorbic acid (Haros *et al.*, 2001).

(e) Transgenic livestock

Creating transgenic livestock is another approach. A transgenic pig that constitutively secretes microbial phytase from the salivary glands shows up to 75% reduction in phosphorous excretion. Also, its requirements for inorganic phosphorous supplements are almost zero. Similarly, another group has experimented with expressing an avian phytase in chickens (Ward, 2001; Cho *et al.*, 2006).

Fig.1 Schematic representation of absorption of phosphate from soil and biosynthesis of phytate

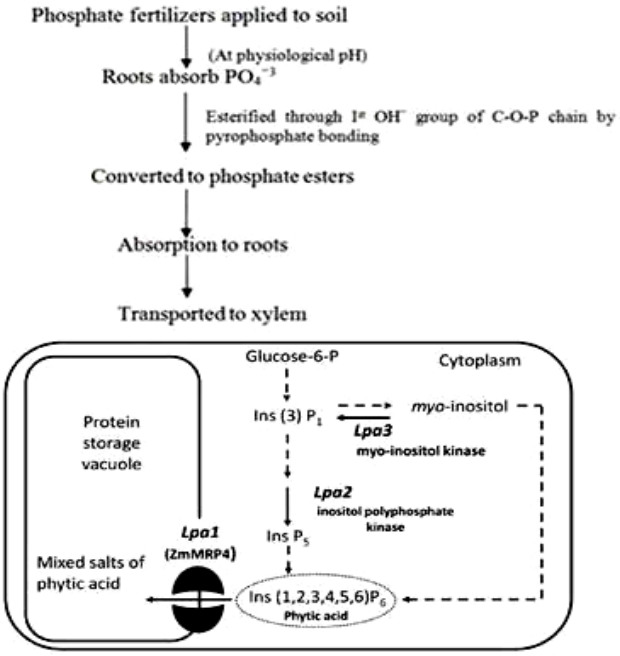
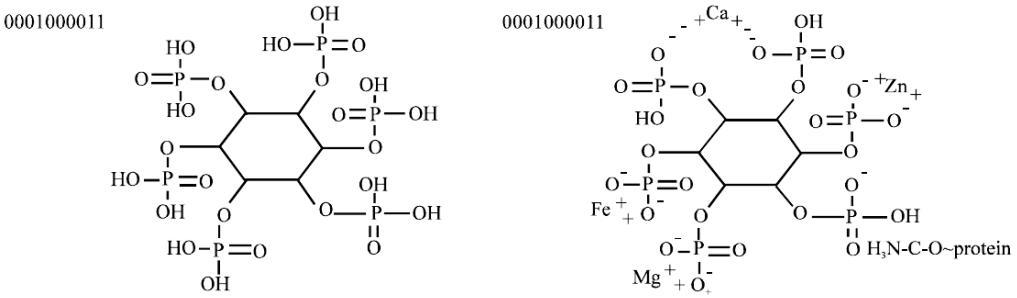


Fig.2 Structure of phytic acid with different possibilities to chelate with cations (Source: Coulibaly et. al. 2011, Am. *J. Plant Nutr. Fert. Technol.)*



(f) Low-phytic acid (lpa) genetics and breeding

Low-phytic acid (*lpa*) mutations were first isolated in the cereal grains maize (*Zea mays*), barley (*Hordeum vulgare*) and rice (*Oryza sativa).* More than 20 independent *lpa* mutations have been isolated in both maize and barley, showing reduction in seed phytic acid P ranging from 50% to 95%. The high inorganic P (HIP) phenotype of *lpa* seeds provides the basis for a quick, sensitive and inexpensive test for the trait, for plant breeding purposes. Using HIP phenotyping, the first maize *lpa* mutation, *lpa1-1* has been introduced into a number of maize inbred lines, by traditional backcrossing breeding methods. Currently, the most work and progress concerning low-phytate crops has been accomplished with this first maize mutant, with 55–66% lower phytic acid content compared with normal seeds (Chen. *et. al*. 2008).

Several pairs of near-isogenic lines have been developed for maize inbreds for use as parents to produce pairs of near-isogenic maize hybrids. These sets of near-isogenic inbreds and hybrids are a powerful experimental model to study the effects of *lpa* mutations on plant, seed growth and function, agronomic performance, as well as human nutrition and health. Studies reveal no effect on germination in the field or in a cold-test, stand establishment, lodging, plant height, ear height, and growth rate in the field and grain moisture. However, low yield compared with the normal hybrid, is recorded. This opens classical genetics approach to produce hybrids or cultivars with seeds having reduced (~50%) phytic acid and good productivity for a first-generation technology. But, more data is needed on the impact of these mutations on stress response or disease susceptibility. The biotechnology approach can be successful for characterizing target manipulating target gene expression only in specific tissues of the developing seed, avoiding any undesirable effects on plant growth and productivity.

(g) Transgenic approaches

To overcome the menace of phytate, phytase-transgenic (PT) maize has been developed by achieving seed-specific over-expression of *Aspergillus niger* phytase (*phy*A2), an enzyme catalyzing the release of phosphate from phytate (Chen. et. al. 2008). Tan *et. al.*, (2017), studied comparative proteomics of the seeds of phytase transgenic (PT) and non-transgenic (NT) maize using 2-DE and iTRAQ techniques and identified 148 differentially expressed proteins (DEPs) (42 up-regulated and 106 down-regulated) in PT maize seeds compared with NT maize seeds. Most of the DEPs were involved in post-transcriptional regulation and modification

functions in PT maize seeds. Many studies were performed to ensure the safety of PT maize, *viz.,* nutritional value (Gao et. al., 2012), used as livestock feed (Li et. al., 2013), and effect on associated arthropod communities in the field (Tan et. al., 2016).

Omics-based studies, including transcriptomics (mRNA profiling), proteomics (protein profiling) and metabolomics (metabolite profiling), conducted in maize by Gong and Wang, (2013) revealed a comparison of the proteomic profiles of GMCs and corresponding wild-type lines. Detailed information on DEPs (differentially expressed proteins) involved in metabolism and cellular development or those involved as toxins, anti-nutrients and allergens could be generated. Protein profiles revealed significant differences between GMCs and their control lines (Albo *et al.,* 2007; Zolla *et al.,* 2008; Balsamo *et al.,* 2011; Vidal *et al.,* 2015). BVLA430101 transgenic maize line, which over-expresses an *Aspergillus niger* phytase (*phy*A2) has been approved as a potential biosafety species by the Chinese Academy of Agricultural Sciences (Chen *et al.,* 2008). This phytase GM line expresses the 60 kDa *phy*A2 protein in its seeds, showing a higher phytase activity than non- transgenic maize seeds (Chen *et al.,* 2008). Kuiper *et al.,* (2001) studied potential unintended effects of 57 identified proteins by global profiling technique and reported that 40% proteins were related to carbohydrate transport and metabolism, 12% related to post-translational modification and 11% regulated to coenzyme metabolism. Several proteins regulated other pathways, including energy production and conversion, inorganic ion transport and metabolism, translation, ribosomal structure and biogenesis, the cytoskeleton, amino acid transport and metabolism, cell cycle control, cell division, chromosome partitioning, signal transduction, and lipid transport metabolism. The differences were not homogeneous hence; unintended effects are not unique to GM plants (Ladics *et al.,* 2015).

(h) Breeding, germplasm screening and biotechnological approache

Low phytin-P corn genotypes (LPA) have been developed to overcome the menace of phytin. In LPA corn only 35% of the total P is phytate-P compared to 75 - 80% in normal corn. The P in LPA corn and soybean meal is more available (Cromwell *et al.,* 2000a, b; Spencer *et al.,* 2000) and can reduces litter P from broilers by 58% (Applegate *et al.,* 2003b) and P excretion from pigs by 37% (Spencer *et al.,* 2000b). De-hulled and de-germed corn (DDC) is a produced by dry milling industry with Phytin-P deposited in protein bodies of the aleurone (10 %) and scutellum (90 %) of corn grain (O'Dell *et al.,* 1972). Removal of the germ layer during dry-milling, removes a large amount of phytin-P located in the scutellum. In diets formulated with DDC corn grain has 20% less P being excreted by broilers and 30 to 35% less in swine. However, there is risk of stomach ulcers in swine.

Vitamin D_3 metabolites can also reduce P excretion by stimulating P transport mechanisms in the intestine which enhances the activity of supplemented phytase. Breeding for low phytate maize genotypes can be effectively used for reducing the content of kernel phytic acid (which chelates mineral cations such as K, Ca, Fe, Mg and Zn) and improving bioavailability of nutrients in human and animal diet as well as decreasing the environmental pollution by P released from undigested and unutilized phytic acid (Ertl *et al.,* 1998; Mendoza *et al.,* 1998).

Many low phytic acid mutants have been developed by disrupting phytic acid

biosynthesis through mutation breeding in maize, rice, barley, and soybean (Raboy *et al.*, 2000) for use in genetic breeding for low phytate lines. So far, in maize, three low phytic acid (*lpa*) mutants have been isolated, viz. *lpa1, lpa2* and *lpa3* which are valuable genetic resources to develop low phytin maize crops. The *lpa1* mutant is derived from mutation in gene that encodes trans-membrane transporter protein (ZmMRP4), which loads phytic acid into protein storage vacuoles of maize seed. The *lpa2* mutant is obtained by a mutation in inosotol phosphate kinase gene (ZmIpk4), which along with other kinases leads to phytic acid synthesis. Compared with wild-type kernels, the *lpa 1, lpa2, lpa3* mutations achieved 66%, 50% and 50% reduction in phytic acid content, respectively (Raboy *et al.*, 2000; Shi *et al.*, 2005). The mutant lines perform well in temperate and not adapted to local tropical and subtropical conditions. Therefore, there is a need to have the *lpa* locus introgressed into locally adapted agronomically superior lines to improve their nutritional benefit. Marker assisted backcross breeding (MABB) is an effective strategy for developing low phytate maize which may provide success for transfer of desirable trait of interest into recipient by recurrent backcrossing and recovery of the recurrent parent genome efficiently. Thus, the development of a co-dominant molecular marker can enable quick selection for LPA maize breeding for faster release of *lpa* varieties. Reducing phytate content through *lpa* mutants have been attempted through knock-out of genes involved in PA biosynthesis. The mineral composition does not change in *lpa* mutants, indicating that there is no direct link between mineral distribution and phytic acid biosynthesis (Liu *et al.*, 2004; 2007). However, this can be a reasonable approach to enhancing the bioavailability of micronutrients.

Due to the involvement of the lower inositol phosphates in plant cell metabolism, developing a perfect *lpa* mutant has is a challenge as, many kinases and tranferases are involved in the synthesis of phytic acid (Josefsen *et al.*, 2007). Furthermore, the yield or germination ability is affected if PA content is reduced more than 50% (Raboy, 2002), thereby making this approach less economic. The best result so far is the maize *lpa1* mutant, which is mutated in an embryo-specific ATP binding cassette (ABC)-transporter and it is able to hold up to 90% reduction of PA without compromising seed viability. The effect on mineral distribution in this mutant is not evaluated yet (Shi *et al.*, 2007). Maize, millet and sorghum have low initial phytase activity that increase rapidly after germination (Egli *et al.*, 2002). Estimation of inorganic phosphorous by 'high inorganic phosphorous' (HIP) assay can help to indirectly estimate the phytic acid content of maize kernels. However, *lpa2-2* allele specific marker is lacking for selecting plants with *lpa2-2* alleles. Therefore, SSR marker, that is tightly associated and hence co-segregating with *lpa2-2* allele needs to be developed.

(i) Biofortification (Genetic modification in maize)

Anemia due to iron deficiency afflicts an estimated 2 billion people worldwide, particularly in developing countries such as Asia and Africa, whose populations are sustained on a few staple food crops. Biofortification programs based on conventional breeding consider existing genotypic differences in seed Fe concentrations to identify cultivars with improved Fe content. Considerable progress has been made in recent years to modify seed iron content via genetic engineering by over-expressing different Fe-regulated proteins in target food crops. Ferritin has been widely used to enhance Fe content of staple food crops, due to its high-Fe binding capacity (~ 4,500 atoms of Fe/molecule). These

biotechnological efforts show only marginal success. *Lpa1-1* mutant of maize has been developed as a tool for iron biofortification to combat anemia with iron biofortification of staple food crops. Studies reveal that there was no significant correlation between Fe content and bioavailability. Furthermore, the transgenic approaches in maize led to a little improvement in Fe content (~30-35 µg/g). Variation in transgenic maize is a common phenomenon due to issues like germplasm used for transformation, transgene copy number, or changes in the expression of genes (epigenetic effects). The *lpa* mutants have poor seed quality and crop yield. Thus, a better understanding of plant iron homeostasis and the probable limiting factors is required. Studies revealing the significance of phytase-mediated improvements in iron absorption and iron status is still low along with a more detailed understanding of uptake mechanism for iron released from partially dephosphorylated phytate chelates, the affinity of microbially derived phytases towards insoluble iron phytate complexes, and the extent of phytate dephosphorylation required for iron release from inositol phosphates is required.

(j) Other conventional method

Phytic acid prevents the absorption of phosphorus as well as Fe, Mg, Ca, Zn and negatively affects absorption of lipids and inhibits enzymes viz., pepsin, amylases and trypsin. To reduce phytic acid menace following conventional techniques are followed

Germination and sprouting

Soaked seeds imbibe moisture which allows mobilization of primary and secondary metabolites favouring germination. Surface sterilization with 0.1 % lemon extract can avoid mould contamination. Phytic acid reduction ~28 % to 60 % can be achieved. However, in maize seeds the reduction is quite low (Poiana et. al., 2009).

Fermentation

Fermentation provides optimum pH for hydrolysis of phytic acid and release of polyvalent cations Fe, Zn, Ca, Mg, proteins in soluble form. Reale *et. al.*, 2004 studied effects of *Lactobacillus Plantarum, L. brevis, L. curvatus* and *Saccharomyces cerevisiae* strains on IP_6 hydrolysis by Italian Sourdough technique and reported a positive correlation between lactic acid and endogenous phytase activity at optimum pH 5.5.

Enhancing Iron Absorption

Pretreatment of grains by soaking, malting, germination and addition of organic acids, *viz.*, ascorbic acid and citric acid, enhances iron absorption. Such classical pretreatments serve to activate the endogenous cereal phytases.

In conclusion, high phytate content in staple food crops is a major barrier to successful iron biofortification. Low phytic acid (lpa1-1) mutant of maize has been exploited to generate transgenic plants with up-to 70 µg/g seed iron through the endosperm specific overexpression of soybean ferritin, with enhanced iron bioavailability. To combat the menace of phytin in diet approaches for genetically modified crops, with low phytate content / higher phytase activity, have revealed little success. Direct fortification of foods and iron supplementation are still potential solutions to improve the iron status of vulnerable populations. However, iron supplementation works in combination with phytase-mediated degradation of phytate. Options for improving phytase stability in the gut need to be investigated, including genetic engineering and/or formulation approaches.

To improve our understanding the research emphasis should be on estimating inositol phosphate contents as well as phytate: iron ratios in the final digested/pretreated food. These parameters crucially determine iron absorption *in vivo*. Further, research on the exact chemical form of iron and phytate in the stomach and cellular uptake from loosely chelated iron to lower inositol phosphates needs to be elucidated to study the mechanism of phytase-mediated iron release and absorption in the human body. A deeper understanding of the significance of phytase dosage, kinetics (*i.e.*, rates of dephosphorylation), and stability under gastrointestinal conditions may help in identifying the intelligent solutions for *in vivo* phytase catalysis and defining the efficacy of phytase-mediated iron release. With more emphasis on these issues, engineering of better dietary solutions for improved iron absorption from cereal and other plant foods can be possible.

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