Review

Food Crops Low in Seed-Phytate Improve Human and Other Non-Ruminant Animals' Health

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ABSTRACT

Background: Low phytic acid crops may offer improved nutrition for human population that largely depend upon on cereals- and legume-based staple foods, reduce the risk of eutrophication, but may compromise crops productivity and nutritional quality.

Methods: Google search was conducted for the period between 2000 to 2021 to search for published literature in high impact factor journals focusing key words/phrases such as 'genes and diagnostic markers', 'genetically modified low-seed phytate crops', 'high-seed phytase activity', 'low-seed phytate mutants', 'phytate and minerals bioavailability and absorption', 'phytate and stress tolerance', 'phytate-human-nonruminant livestock's health', 'seed phytate, plant growth, development and yield', 'seed phytate and germination & seedling establishment', 'seed phytate and nutritional quality', and 'seed phytate and baking and nutritional quality'.

Results: Low phytate mutants (except with few exceptions in barley and common bean) often carry negative pleiotropic effects in grain crops. Oil and protein contents in soybean *lpa* mutants were not affected, but some mutants relative to the wild type (WT) had greater sucrose and isoflavone and lower raffinose. Predominance of crossing parents on the metabolite profile and imprinting of a specific mutation induced metabolites— consistently expressed in the homozygous *lpa* mutant offspring—were noted across generations and environments. A few functionally characterized genes and many putative candidate genes associated with low seed phytate or seed phytase have been discovered in grain crops. Both crossbreeding and biotechnology-led genetic improvement with *lpa* led to offspring combining high yield and low seed phytate in maize, rice, soybean, and wheat. Crossbreeding has shown that it is possible to combine lower seed phytate with greater iron and zinc in the offspring. A few *lpa* cultivars are commercially grown in USA and Canada, while such developments are yet to occur in the developing world. A fine balance between yield-nutrition-stress tolerance may be achieved by deploying modern biotechnology. Accumulated evidence suggests more bioavailable

iron in biofortified and *lpa* grains than normal phytate grains. The lack of phytic acid however perturb Ca distribution, which as a consequence may alter cooking time and stability of storage proteins, thereby causing serious gastrointestinal discomfort, and should be factored while developing biofortified or *lpa* beans. The low phytate-based products by and large were not associated with detrimental effects on nutritional and baking quality or retention of nutrients in the food. A long-term assessment may be necessary to assess bioavailability and absorption of minerals from diets differing in phytate concentrations and its effect on human health. Low-phytate-based feed has demonstrated substantial health and productivity benefits to nonruminant animals. Enabling policy for taxing high phytate animal waste may encourage more investments on private agricultural research programs to deal with excess phytate in food and feedstocks.

Conclusions: This review highlights advances regarding seed phytate or phytase activity in cultigen gene pools and suggests how to organize cost-effective breeding programs for developing low seed phytate cultivars. The use of modern biotechnology effectively untangled negative pleiotropic effects associated with low phytate grains. A moderate reduction of seed phytate should be achievable to combining crops productivity and adaptation across environments. Effects of low phytate diet on human and nonruminant livestock's health are also highlighted.

KEYWORDS: agriculture for nutrition; biofortification; biotechnology; eutrophication; human and nonruminant livestock's health; phytate; plant breeding

SEED PHYTATE, CROPS PERFORMANCE, ANIMAL AND HUMAN HEALTH, AND ENVIRONMENT POLLUTION

Phytic acid (inositol hexaphosphoric acid, InsP6), the predominant form of phosphorus storage, accounts for 65–85% of the total seed phosphorus. It is worth highlighting that phytic acid is a ubiquitous molecule, but we refer in this article to the effects of dietary phytic acids in the gut [1]. High concentration of phytic acid in foods limits micronutrient bioavailability as it binds minerals to form mixed salt phytin [2,3]. The undigested phytin excreted by humans and nonruminant animals significantly pollute soil and cause eutrophication in rivers and lakes [4]. Phytic acid is however critical for seed development, crops establishment and productivity [5]. Phytic acid contributes positively to human health as antidiabetic, antioxidant, anticancer or anti-inflammatory agent, prevent kidney stone formation, protect against atherosclerosis and coronary heart disease or in managing neurodegenerative diseases [6]. InsP6 molecule relative to citric acid exhibit significantly greater inhibitory effect against foodborne pathogens and therefore safe to use as for food preservation, food safety, and for greater shelf-life of the food products [7].

Globally about 2 billion people suffer from malnutrition causing substantial burden to society. Elevated CO_2 in atmosphere increases phytic acid content and reduces the concentrations of essential micronutrients such as Fe and Zn in the grains, resulting nutritionally inferior crop produce, which may further aggravate malnutrition amongst the people largely dependent on grain-based food products [8].

The main biological constraint before crop-genetic enhancers is how much phytate *per se* can be reduced that is not detrimental to plants and produce is beneficial to human and animal health. They face selecting for low phytate that may reduce overall nutritional quality of seed, as well as if it can be combined with increased grain Fe and Zn. Should they block the phytate biosynthetic pathway or degrade phytate in the developing seeds? Is there genetic variation for seed phytase activity to the extent that can be exploited to develop high seed phytase crops as alternative to fungal phytase to reduce feed cost? How to minimize the negative pleiotropic effects between low phytate—agronomic defects—human non-communicable diseases? Whether feed with varying phytate concentrations impact carcass characteristics of growing pigs or productivity of milking cows and poultry's egg laying and quality? Whether any adverse effect on flavor and sensory characteristics of bread made from low phytate whole grains? Whether consumers prefer and willing to buying and eating low-phytate-based bakery products? This review article provides some answers to these questions by highlighting the advances in quantifying seed phytate or phytase activity, and how this knowledge assists developing low seed phytate cultivars.

LOW PHYTATE-INDUCED CHANGES IN SEED CHEMISTRY

Sparvoli and Cominelli [9] summarized low phytic acid (*lpa*) mutants originated through mutagenesis in grain crops with significant alteration in seed-phytate and metabolites, most with few exceptions associated with negative pleiotropic effects (e.g., low seed germination, stunted growth, reduced seed development and weight), thus with limited value to breeders. Crossbreeding between *lpa* mutants and wild types (WTs) showed predominance of crossing parents on the metabolite profile and imprinting of a specific mutation induced metabolite signature, consistently expressed in the homozygous *lpa* mutant offspring across generations, genetic backgrounds, and environments [10−15]. Disruption of *OsSULTR3;3* produced significant reduction in both phytic acid (PA) and total phosphorus (P) accompanied by changes in other metabolites and increased concentrations of nutritionally relevant compounds in two brown rice *lpa*-derived mutants [16].

Sakai et al. [17] noted no changes in total P and other minerals in mature seeds between two different *lpa* mutants and WT rice. They however observed significant changes in mineral localization within *lpa* seeds. P and K substantially decreased in the aleurone layer and diffused into the endosperm, while Zn and Cu localized in the narrower space

around the aleurone layer. In WT, minerals were broadly distributed from the aleurone layer to the inner endosperm. The transgenic rice with the *lpa* phenotype also had similar pattern, indicating the role of phytate (InsP6) for the accumulation and distribution of minerals during seed development in rice.

The *lpa* mutants had no adverse effect on oil and protein contents or any individual amino acids and saturated fatty acids in soybean. Some mutants however consistently showed higher sucrose (47–86%) and lower raffinose (about 25%, 74–84%) and higher total isoflavone content (3039–4305 μg g^{-1}) than WT across environments [17,18].

The *lpa1-1* mutation in maize exerted pleiotropic effects, unrelated to phytic acid biosynthesis, on seed density, ions content and on antioxidants in the seed. The flour contained high lignin and protein content, while total starch remained the same, but structure and granule sizes changed [19].

Reducing the seed phytate means more bioavailable minerals for absorption. For example, there were significantly higher Zn absorption by 47–52% in maize and 35–52% in rice as compared to WT [20]. Fe absorption from *lpa* beans was significantly higher than from their parental lines with normal phytate [21], while Ca absorption enhanced from low phytate maize-based tortillas as compared to WT [21].

Clearly, *lpa* mutants are useful genetic resource to enhance the bioavailability of minerals. However, some changes in seed chemistry and localization of minerals within *lpa* seeds were noted in cereal and grain legume crops, thus requiring careful selection of *lpa* mutants with no adverse impact on nutritional seed quality.

ASSAY FOR IDENTIFYING *lpa* SEEDS IN MUTAGENIZED OR BREEDING POPULATIONS

A simple and cost-effective high throughput assay is needed to support breeding for low seed phytate crops. The currently available seed phytate assay protocols are destructive in nature and based on quantification of P in the flour obtained by milling the seeds [22−24]. More recently, a seed density-based method directly identifies the putative *lpa* mutant seeds in a mutagenized maize (*Zea mays*) population. It is based on the principle that *lpa* seeds are lower in density than WT. The *lpa* seeds float in concentrated sugar solution (density 1.218–1.222 g cm^{-3}) due to their lower density, unlike WT sibs that sink. This is non-disruptive, cost effective and timesaving assay that may be used to identify putative *lpa* seeds when screening mutagenized or early generation breeding populations [25]. In the absence of data available to demonstrate it could work with any *lpa* mutation in any species, the low-phytate phenotype should, however, initially be evaluated by FT-NIR for faster though destructive assessment [26] or nondestructive assessment by EDXRF [27], and later confirmed by wet seed chemistry in advanced generations.

GENES AND DIAGNOSTIC MARKERS ASSOCIATED WITH LOW SEED-PHYTATE OR HIGH SEED-PHYTASE ACTIVITY IN GERMPLASM POOLS

To date a number of low phytate mutants as well a few germplasms with high seed phytase activity were reported in cereal and food grain legume crops [9,25,28−35]. Livestock's raiser often adds fungal phytase to enhance feed value, however, it increases feed cost. Diagnostic markers are genetic tags (a string of DNA) which may be indirectly used to select for specific phenotypes (i.e., plant height, seed weight and quality or disease resistance, among other characteristics) in plant breeding. A few functionally characterized genes associated with low seed phytate, such as *Pvmrp1* (orthologous to the *lpa* genes *AtMRP5* and *ZmMRP4*) in common bean [18], *dlMIPS* in dolichos bean [36], *Mf3*, *DH2* and *CB5* in maize [37], *OsITP5/6K-1, MIPS,* and *SPDT* in rice [38−40], *ABCC5* and *MIPS* in soybean [41,42], and *TaIPK1* in wheat [43], or their homologs in other crops, have potential to accelerate the development of food or feed crops low in seed phytate. In addition, the function of putative candidate genes associated with low-seed phytate or high seed-phytase activity in cereals and food grain legumes [44−50] must be validated prior to use in crop breeding.

DEVELOPING LOW SEED PHYTATE BREEDING POPULATIONS/ADVANCED LINES AND CULTIVARS

Developing low phytate crops that yields well in optimal and stressed field environments is a significant breeding challenge. Plant breeders often face the dilemma about the extent of phytate to reduce in the grains that does not adversely impact growth and productivity of new cultivars but at the same time the produce is beneficial to human health. Progress in breeding or genetically engineered resource use efficient cultivars continues. While there are alternative approaches to deal with the problems posed by seed-derived dietary phytic acid, for example, use of phytase enzyme as feed additive or biofortification breeding. The proof of concept has been demonstrated that breeding for low (moderate reduction) phytate crops is achievable given adequate time and support. Populations at risk for iron and zinc deficiency, especially in developing economies, will benefit from production and consumption of low phytate grains, and find acceptance among farmers for sustainable agricultural production [51]. Both conventional (mutation or crossbreeding aided by applied genomics) and biotechnology (genetic engineering or gene editing) approaches have led to development of several low seed phytate lines yielding at par or 5–10% lower than the normal phytate cultivars as detailed herewith.

Mutation Breeding

Physical and chemical mutagens were used to obtain stable low phytate mutant lines with moderate to severe reduction in seed phytate. When assessed the performance of sets of barley sib lines homozygous for WT or

homozygous for one of the four independent *lpa* mutations (*lpa1-1*, *lpa2-1*, *lpa3-1*, and M955) in 'Harrington', Bregitzer and Raboy [28] noted that WT sib sets yielded at par with 'Harrington', thereby suggesting presence or absence of *lpa* alleles as the major constraint to productivity in WT and low phytate lines. All mutations except *lpa1-1* yielded low in rainfed environments, thus suggesting that reduced stress tolerance accounts for low yield in other low phytate mutants. The *lpa1-1* mutation being aleurone specific avoided yield loss under rainfed. The superior yield performance of *lpa1-1* was further confirmed when six low phytate barley mutants including *lpa1-1* were evaluated in multi-environment trials. *lpa1-1* had no adverse effects on yield while *lpa4-1* resulted in low yield loss (3.5%) across all locations. In optimal environments, *lpa1-1*, *lpa3-1*, and *lpa4-1* had yields similar to or within 5% range of that for WT sibling isolines [30]. The desirable effect of *lpa1-1*—limited to aleurone layer— may be directly introduced in breeding programs to develop cultivars for stress-prone sites while *lpa1-1*, *lpa3-1*, and *lpa4-1* may be exploited for developing barley cultivars adapted to optimal environments.

The *lpa* trait increase the nutritional quality of foods and feeds and improve P management in agriculture. These mutations are often accompanied by negative pleiotropic effects leading to agronomic defects —either seed viability and germination or plant growth and development or increase the resistance to cooking—limiting value to the breeders. Thus, achieving *lpa* mutants endowed with no negative pleiotropic effects on seed germination and field emergence or productivity is still a significant breeding challenge [52].

Crossbreeding

Low phytic acid enhances bioavailability of micronutrients but may reduce seed germination and yield. Thus, improving seed yield and germination is the major breeding target while developing low phytate grain crops. High and low phytic acid maize populations isolated after three cycles of recurrent selection differed significantly for phytic acid concentration. The *lpa* population showed improved seed germination and bioavailable Fe was at par with *lpa* inbred lines. Recurrent selection for phytic acid level is thus a viable option for improving Fe bioavailability while maintaining good seed germination in *lpa* population [53]. Crossbreeding of *lpa* mutants with cultivars led to offspring combining high yield and low seed phytate in rice. Phytic acid content among the offspring was however dependent on environment and phytic acid contents of the crossing parents [54]. In a field test involving low phytate offspring derived after three successive backcrosses involving CX1834 (low-phytate) and B019 (normal-phytate) soybean lines, Spear and Fehr [54] identified one low phytate progeny with improved seed germination (equivalent to normal phytate) while other low phytate offspring had reduced germination. Low phytate (LP) soybean lines (56CX-1273; 56CX-1283) had field emergence and seed yield at par with cultivars (normal

phytate, NP) '5002T' and 'Osage', but with greater seed protein and ultrahigh soymeal protein. No crossover interaction or change in ranks for inorganic P (Pi) suggests that it is achievable to differentiate LPs from NPs irrespective of test environments [55]. The mutated gene may have also different effects in different species. The *lpa1* mutant, the only characterized mutant in common bean, is devoid of negative pleiotropic effects normally associated with *lpa* mutants. It affects the *PvMRP1*, the gene coding for a putative tonoplastic phytic acid transporter [56]. Further, it was shown that loss of *PvMRP1* is compensated by *PvMRP2*, however, its effect was seen in leaf tissues instead in seeds. The GUS activity in different organs of transgenic *Arabidopsis thaliana* and *Medicago truncatula* harboring 1.5 kb portion of the intergenic 5ʹ sequences of both *PvMRP* genes suggests a refined species-specific mechanism regulating gene expression for *PvMRP* genes [57].

Genomic-Led Breeding

Low phytate trait in rice has been successfully introgressed into otherwise high phytate 'Super Basmati' genetic background (*lpa12-3*, *lpa111-1*, *lpa141*, *lpa56-3*, *lpa53-4*, *lpa99-2*, *lpa205-4*), with improved seed germination and 4–18% yield advantage over parent cultivar [58].

In maize, a mutant plant with a *lpa2-2* allele accumulates less phytic acid in seeds. The microsatellite marker 'umc2230' co-segregate with low phytate conferred by *lpa2-2* alleles and may be deployed to develop low phytate maize [59]. Using umc2230 and marker-aided backcrossing, four low phytate maize lines with agronomic traits similar to its recurrent parent were bred [60]. A co-dominant cost-effective SNP marker for *lpa1-1*, caused by a single amino acid change from alanine to valine, differentiated maize inbred lines varying in phytic acid, and upon validation successfully distinguished homozygous dominant (WT) from homozygous recessive (*lpa*) or heterozygous genotypes at early vegetative phase [61].

A high throughput molecular marker assay detected a perfect association between recessive mutations in soybean genes homologous to the maize *lpa1* linked with low seed phytate and identified for the first time a novel *lpa2-b* allele in a soybean line M766 which may be deployed in breeding for developing low phytate soybean germplasm. Two recessive MRP4/MRP5 mutant loci together confer low phytate phenotype in soybean unlike one single recessive mutant locus sufficient for expression of low phytate in maize. *MIPS1* causes modifications to seed phosphorus and carbohydrate contents and improve nutritional value of soybean food and feed [62]. A Kompetitive allele specific PCR (KASP) assay-based marker BARCSOY_11_1495 was 93–97% effective in identifying low phytate mutation, which also differentiated advanced lines varying on seed phytate [63]. KASP assays are simple and cost effective and therefore may be deployed in soybean breeding.

To date a few low seed phytate cultivars such as barley 'Herald', 'Clearwater', and 'Sawtooth' [64−66] and soybean 'USDA-N6003LP' and 'N6202' [67,68] have been released for cultivation in USA. These cultivars were developed by crossbreeding, and combine moderate reduction in seed phytate, multiple fold increase in Pi, adaptation, productivity (yielding at par or up to 90% of the control), seed quality, and good field emergence. 'CDC Raezer' and 'CDC Limerick' field peas combining low seed phytate and resistance to diseases have been released in Canada [69,70].

Nutritional Quality

Quality refers to the variation in physical and chemical characteristics of seeds associated with low seed phytate. A multilocation evaluation of two *lpa* soybean mutants revealed that *lpa* did not affect oil and protein contents or protein and oil quality. However, *Gm-lpa-TW75-1* had consistently higher sucrose (+47–86%) and low raffinose (−74–84%) contents than WT, while *Gm-lpa-ZC-2* had higher total isoflavone content (+3039–4305 µg g^{-1}) than WT. Furthermore, F$_3$-derived *lpa* lines had total isoflavone content greater than WT, suggesting that *lpa* mutant lacks negative effects on seed quality [18].

The *lpa* soybean mutant *Gm-lpa-TW-1-M* relative to WT unraveled remarkable metabolic differences. The raffinose oligosaccharides and galactosyl cyclitols were reduced while sucrose and free amino acids were increased. Such induced metabolite changes were nearly unaffected by the crossbreeding involving *lpa* and WT and consistently expressed over generations and environments, indicating that this mutant is the ideal genetic resource to produce soybean seeds stably exhibiting low phytate trait with no adverse impact on seed metabolite changes in the offspring [10].

Soybean seeds low in phytate, high in sucrose, and low in raffinosaccharides are highly desirable from a nutritional and environmental standpoint. Seed phytate levels are not reduced significantly after *mips* mutation in double mutant *mrp-l*/*mrp-n* as revealed by the analysis of recombinant inbred lines (RILs) resulting from crossing two soybean *lpa* mutants bearing *mips* mutation on chromosome 11 (VP-5089), and *mrp-n* plsu *mrp-1* mutations on chromosomes 3 and 19, respectively. The mutant *mrp-l*/*mrp-n* reversed, however, the desired high sucrose/low stachyose/low raffinose of homozygous soybeans for the *mips*/*mips*). The homozygous *mips/mrp-l/mrp-n* had 7.7% sucrose, 4.2% stachyose and 0.94% g^{-1} in dry seed. The *myo*-inositol flux in *mips* seeds may be changed by blocking phytic acid transport—which is affected by mrp-l/mrp-n—from the cytoplasm site where its synthesis occurs to the storage site, thus restoring to the levels noticed in the wild type the raffinosaccharides [71]. Such results demonstrates the effects of these low

phytic acids related to mutations regulating seed phytate and oligosaccharides.

Hard-to-cook (HTC) defects of *lpa* beans relative to the wild types is due to presence of lectin phytohemagglutinin L (PHA-L) in seed. The lack of phytic acid perturb Ca distribution that as a consequence alters the cooking time and the stability of a bean storage protein, a lectin, causing severe gastrointestinal discomforts [72]. Whether *lpa* beans provide more bioavailable iron than a biofortified bean? The biofortified and *lpa* beans provide more bioavailable iron (421 and 431 μg vs 278 μg) than control beans (regular iron and PA concentrations) to reduce iron deficiency in healthy Rwandese women with low iron status [73]. Thus, adverse gastrointestinal effects of reduced iron bioavailability should be investigated to factor while developing biofortified or *lpa* beans.

A pronounced effect on phytic acid and metabolite profile was reported when two *lpa* rice mutants each carrying *myo*-inositol kinase mutated genes *OsMIK* or *OsMRP5* were intercrossed. The phytic acid content in resulting double mutant was reduced by −63% relative to −26% or −47% in single mutant, while the metabolite profile was predominated by the *OsMIK* mutation effect. Crossbreeding of two single-gene *lpa* rice mutants may generate offspring with significant reduction in phytic acid and a mutant gene-specific metabolite signature [74].

In summary significant progress have been achieved that generating *lpa* mutants and subsequently transferred the *lpa* trait into elite genetic background, which may be further used as a genetic resource for crossbreeding with the aim of releasing newly bred cultivars. Availability of genetic tags (DNA markers) and associated high throughput genotyping assay to breeding programs in National Agricultural Research Systems (NARS) may accelerate development and release of low seed phytate cultivars in low- and middle-income countries (LMICs).

GENETICALLY MODIFIED LOW SEED PHYTATE CROPS

Breeding for low phytate either through induced mutations or crossbreeding has been frustrating due to negative pleiotropic effects between low phytate and desirable agronomic traits, including seed yield and germination. Alternative to both is either blocking the phytate biosynthetic pathway or degrading phytate in developing seeds to produce low phytate crops through genetic engineering.

Maize *lpa1* mutants are defective in MRP ATP-binding cassette (ABC) transporter that is highly expressed in embryos, but also in immature endosperm, germinating seed, and vegetative tissues. Silencing this transporter in an embryo-specific manner produced maize transgenic seeds that were low in phytic acid and high in Pi that germinate well, and

were not associated with reduction in seed weight. Furthermore, suppressing the homologous soybean MRP gene also produced low-phytic acid seed, paving the door that this technology may be possibly applied in other crops as well [74].

RNAi-mediated down-regulation of *OsITP/6K-1*, which altered the transcripts of essential phytic acid pathway genes without affecting the transcript of other *OsITPK* homologs, led to 46% reduction in seed phytate and 3-fold increase in Pi. The low phytate seeds exhibited 1.3- and 1.6-folds increase in Fe and Zn in polished seed endosperm, respectively [39], while RNAi-mediated embryo-specific downregulation of *ITPK-2* produced rice seeds in relation to WT reduced phytic acid by 37%, nearly 3-fold increase in Pi, and increased iron and zinc in polished rice grains. Importantly, the reduced phytic acid content had no adverse impact on seed germination or seedling growth of transgenic rice [75]. A decrease in *IPK1* transcript levels in RNAi-mediated silencing of *IPK1* at T_4 generation wheat seeds resulted in 28–56% low phytate, and significant increases in Pi, and Fe and Zn. However, lowering seed phytate adversely impacted seeds spikelet^{-1} [43]. More importantly, the silencing of these genes in homozygous transgenic lines did not lead to any defect in seed development including low germination or overall agronomic performance as often evidenced in mutagenesis. CRISPR/Cas9-mediated disruption of *TaIPK1A* gene in cv. 'Borlaug 2016' reduces phytic acid and improves iron and zinc accumulation in wheat grains [76], while knocking out three functional paralogs of *BnITPK* resulted low phytate mutants with an increased Pi in the canola (rapeseed) grade spring cultivar 'Haydn' [77]. These are thus important genetic resources for the development of biofortified cultivars of wheat and rapeseed.

The targeted disruption of the expression of *MIPS1* using seed specific promoter in soybean resulted in T_4 generation lines with 39–41% reduction in seed phytate as compared to WT. Significantly Fe, Zn and Ca, respectively, increased by 15–22%, 7–11% and 10–15% in low phytate lines [78]. Transgenic lines expressing *inositol polyphosphate 6-/3-/5-kinase* gene specific RNAi construct in seeds of 'Pusa-16' soybean cultivar had exceptionally low seed phytate levels, moderate accumulation of Pi, and elevated mineral contents [79].

Clearly, a few transgenes are now available as well new mutants through CRISPR/Cas9 mediated gene editing for targeted reduction of seed phytate without adverse impact on plant growth, development and reproduction in cereals and grain legumes.

COMBINING HIGH IRON AND ZINC DENSITY WITH LOW PHYTATE TO ENHANCING MICRONUTRIENTS BIOAVAILABILITY

Widespread malnutrition, caused by deficiency of Fe and Zn, afflicts more than two billion people globally. A high phytate diet is associated with reduced absorption of Fe and Zn in the gastrointestinal tract [80]. Molar ratios of Phytate:minerals are used to estimate micronutrient bioavailability. Phyt:Fe molar ratio above 1 causes negative effect on Fe absorption, while a Phyt:Zn molar ratio above 15 cause low Zn bioavailability [81,82]. Biofortification has been successful to improving seed Fe and Zn contents in grain crops [83]. While breeding for low seed phytate is often (with few exception) mirrored with negative pleiotropic effects [5], combining it with seed biofortification another dimension of conflict for the plants and human health [9,84].

Double mutant offspring originating from a cross between high- and low-density mutants displayed in grains significantly higher Zn and low phytic acid contents, and lowest molar ratio of phytic acid to Zn across all environments. Such progenies contained relatively high content of bioavailable Zn in brown rice [85]. The homozygous *lpa* and WT offspring and double *lpa* mutants originating from crosses involving *MIPS1* and *IPK1* soybean mutants with WT or crossing the mutants among themselves revealed that *lpa* trait of the *MIPS1* mutant was not altered by crossbreeding with a WT cultivar. The *lpa* offspring had InsP6 reductions of about 44% compared to WT. *IPK1* offspring had up to 12.4 mg g^{-1} accumulation of specific InsP3-InsP5 reduction compared to the progenitor *lpa* mutant (4.7 mg g^{-1}). The extent of InsP6 reduction (43–71%) depended on the WT crossing parents, with double mutants exhibiting up to 87% InsP6 reductions. *IPK1* offspring had substantial accumulations of specific InsP3-InsP5 isomers (up to 12.4 mg g^{-1}) compared to the progenitor *lpa* mutant (4.7 mg g^{-1}), while the extent of InsP6 reduction (43–71%) depended on the WT crossing parent. Double mutants exhibited up to 87% InsP6 reductions accompanied by moderate accumulations of InsP3-InsP5 (2.5 mg g^{-1}). Crossbreeding therefore modulates the amounts of both InsP6 and InsP3-InsP5 contents in *lpa* soybean mutants and improve their nutritional quality [86]. In wheat, gamma rays induced mutants showed more than two-fold higher grain Fe and Zn concentrations, 1.5-fold lower phytic acid concentration, and 3.8- and 6.6-folds lower Phyt:Fe and Phyt:Zn molar ratios compared to WT [87]. Thus, valuable genetic resources for use in breeding to select for increased Fe and Zn concentrations and lower phytic acid, a win-win combination, to minimize the risk of micronutrients malnutrition in humans.

BAKING AND NUTRITIONAL QUALITY, NUTRIENT RETENTION, IN VIVO BIOAVAILABILITY AND ABSORPTION OF MINERALS

Biofortification often alters the flavor, taste, appearance, and other feature of the foods, which may limit the consumer acceptance. Equally important is whether poor consumers prefer and are willing to buy nutritious foods. A few surveys and choice preferences experiment from Asia and Africa regions revealed that poor consumers prefer nutritionally enhanced (Fe, Zn, or Vitamin A) food. Food with little or no effect on taste and appearance is regarded more positively than that with significantly altered characteristics. However, willingness to pay for nutritional attributes is small [5,88].

Baking and Nutritional Quality

The *lpa* hard wheat-derived germplasm was not associated with detrimental effects on flour protein concentration, dough mixing properties, or bread loaf volume, but showed substantial increase in flour ash concentration relative to WT wheat. Higher flour ash is an indication of higher proportion of aleurone and bran fragments and Asian noodles prepared from such flour have dull appearance. The *lpa* germplasm had significant redistribution of minerals from the bran to the endosperm. The *lpa* soft wheat had greater sodium carbonate and sucrose, which is an indication that *lpa* wheat had greater apparent starch damage or pentosan content than its WT sibs [89].

A comparative study involving WT and *lpa* lines in the genetic background of 'Grandin' red spring wheat revealed no differences in total P concentration, however, the distribution of P between phytic acid and Pi was altered [90]. This research shows that Pi in *lpa* grain was up to 340% of WT grain, while phytic acid phosphorus in *lpa* grain reduced to as low as 65% of the concentration in WT grain. Moreover, such differences in P were also reflected in flour: Pi in break and reduction flours of *lpa* wheat was 3 to 4 times the concentration in break and reduction flours from WT wheat, with total P concentration in *lpa* flour 20% greater than in WT flour. Furthermore, mineral concentrations in bran and shorts of *lpa* and WT wheats were similar. However, magnesium (Mg) concentrations in *lpa* break and reduction flours were significantly greater than in WT flour. The *lpa* wheat had little effect on concentrations of other minerals, while *lpa* grain flour had on average greater ash (4.38 g kg^{-1}) than WT grain flour ash (3.86 g kg^{-1}). The protein content of *lpa* and WT flours was similar. The *lpa* flour had a longer time to mixograph peak and greater mixograph peak height than WT flours, while bread loaf volume between *lpa* and WT flours was similar. Thus, *lpa*-derived germplasm can produce flours with greater Pi and Mg concentration and little effect on bread flour functionality.

Nutrient Retention

In a comparative study of Fe, Zn, and phytate retention after preparing common household recipes Hummel et al. [3] noted greater average Fe retention (77–91%) after boiling than Zn (41–84%) or phytate (50–86%). Soaking led to a significant decrease in Zn and phytate after boiling and refrying, but no change in Fe. The *lpa* beans relative to other group of beans had not shown a consistent pattern of difference in Fe and phytate retention. However, *lpa* beans had significantly lower retention of Zn compared to conventional and biofortified beans. This result suggests that combining the *lpa* plus increased Fe and Zn may further enhanced the nutritional benefits of biofortified beans, by decreasing its Phyt:Fe and Phyt:Zn molar ratios [3].

Minerals (Ca, Fe, Zn) Absorption

Bioavailability of minerals is compromised by high phytate in the food. Seed grains constitute the major source of dietary energy. About 80% of the seed phosphorus in food grains is in the form of phytate. Phytate binds minerals such as Ca, Fe and Zn and therefore these minerals are unavailable for absorption in nonruminant animals including human digestive tract. Maize is the major source of dietary food to millions of people in developing countries. Hambidge et al. [91] noted greater mean Ca absorption, by 42.8% (0.50 ± 0.03), from tortilla meals prepared from low-phytate as compared to high-phytate maize tortilla meals (0.35 ± 0.07). The molar ratios of phytic acid to Zn in polenta prepared from low- and high-phytate maize were 17:1 and 36:1, which respectively corresponded with fractional absorption of Zn to 0.30 ± 0.13 and 0.17 ± 0.11 [92]. In another experiment involving tortilla prepared from low phytate maize hybrids and their WT isohybrids revealed that fractional absorption of Zn from tortilla is positively related to the extent of phytate reduction in low-phytate hybrids [93].

A study involving Swedish women under free-living conditions and single meal intervention of eating whole grain rye bread with high or low phytate revealed no change in any of the Fe status biomarkers detected after 12 weeks of intervention in high-phytate-bread group, while in low-phytate-bread group, both ferritin and total body Fe were decreased, indicating that single meal intervention increase in Fe bioavailability from dephytinization of cereals [94]. In low phytate pea lines, bioavailable Fe as assessed by cell culture bioassay was reported 1.4–1.9-fold higher than that of the normal phytate cultivars [95]. However, it should be noted that several factors contribute to iron absorption and human body adapts to absorbing higher percentages of sources of iron over time; any conclusion obtained from a short period may give a false measure too low a

bioavailability [96]. Although positive effect of low phytate diets on Ca, Fe and Zn bioavailability were unfolded, a long-term assessment, both in vivo and on healthy subjects, are needed to assess bioavailability and absorption of minerals from diets differing in phytate concentrations.

Crop biofortification has resulted in development and release of high Fe biofortified beans in Africa (www.harvestplus.org). The consumption of biofortified-, *lpa-*, and non-biofortified-beans among Rwandese women with low Fe status in the body showed that biofortified- and *lpa*-beans provided more bioavailable Fe than non-biofortified beans. The total amount of Fe absorbed from biofortified and *lpa*-beans was 50% greater than non-biofortified beans. The *lpa*-beans were, however, hard to cook and their consumption caused adverse gastrointestinal symptoms among most who ate them [73].

An in vivo study involving red jungle fox (*Gallus gallus*), Warkentin et al. [97] noted that certain biofortified low phytate field pea cultivars provided greater Fe bioavailability and modest increase in Fe status, had significant effect on gut microbiota and duodenal brush border membrane (BBM) functionality. BBM is the major site of nutrients transport and absorption in the small intestine [98].

Natto, a traditional food prepared from fermented soybean and popular dish amongst Japanese, prepared from low phytate (LP) soybean revealed improved color, lower in stickiness, hardness, and taste, but with greater Pi concentrations than those prepared from normal-phytate (NP) natto. Ca, Mg, and K concentrations were the same between LP and NP natto. A slightly higher protein in NP natto than that in LP natto. Thus, improved qualities of LP-soybean natto may increase its acceptability overseas and thereby increase its commercial value and contribute to sustainable food and nutritional security to people in Southeast Asia [99].

In brief, no significant changes were noted in bread functionality from grains differing in seed phytate contents. Noodles prepared from low phytate grains had, however, dull appearance due to high ash content in flour that may limit its consumption, while improved quality of natto from low-phytate soybean may be commercialized. Greater nutrition retention and absorption was noticed in household recipes made from low-phytate flours. Biofortified- and *lpa*-beans provided more bioavailable Fe than non-biofortified beans. Consumption of low-phytate bean however causes stomach discomfort. Subjects eating low-phytate products showed significant effect on gut microbiota and BBM functionality. Overall, consumption of low phytate grains (or flours) may contribute healthy livelihood to inhabitants in LMICs.

ASSESSING IMPACT OF PHYTATE ON HUMAN HEALTH

Although phytate may affect the bioavailability of trace elements, dietary phytate may prevent formation of kidney stones, or protect humans against diabetes mellitus, caries, atherosclerosis, coronary heart maladies, and various cancers [6,100−105]. Hence, phytates provides an example of being both good and bad for human nutrition and health, particularly for vegans and vegetarians who eat a lot of high-phytate diets based on cereals and legume grains or nuts. Further, it has been reported that the majority of the InsP6 found in organs and tissues has a dietary origin and is not a consequence of endogenous synthesis, as evidenced from rat to human, and health benefits linked to extracellular InsP6 relate to dietary InsP6 [106,107]. However, Irvine and colleagues demonstrated that InsP6 does not and cannot exist in mammalian serum or urine. Therefore, any physiological effects of dietary InsP6 can only be due either to its actions in the gut as a polyvalent cation chelator or to inositol generated by its dephosphorylation by gut microflora [1]. A purification protocol based on Titanium dioxide (TiO_2) beads bind inositol phosphates which enabled Wilson et al. [108] quantify InsP6, its pyrophosphate derivatives InsP7 and InsP8, and the nucleotides ATP and GTP from cell or tissue extracts. They further visualized InsP6, InsP7 and InsP8 in *Dictyostelium* extracts and a variety of mammalian cell lines and tissues, as well InsP6 in human plasma and urine. Hence, active InsP6 phosphatase is noted in human plasma but undetectable in either fluid, thereby challenging reports that InsP6 is found in human biofluids and the related advice of using it as supplement in diets. A capillary electrophoresis (CE) coupled to electrospray ionization mass spectrometry (ESI-MS) was used to analyze complex mixtures of *myo*-inositol phosphates (InsPs) and *myo*-inositol pyrophosphates (PP-InsPs) with high sensitivity using stable isotope labeled (SIL) for matrix-independent quantitative assessment. SIL-CE-ESI-MS accurately monitored InsPs and PP-InsPs in wild type and knockout mammalian cell lines and in model organisms arising from compartmentalized cellular synthesis pathways by feeding cells with either $[^{13}C_6]$-*myo*-inositol or $[^{13}C_6]$-D-glucose. It thus provides evidence for the existence of unknown inositol synthesis pathways in mammals, highlighting the potential SIL-CE-ESI-MS to dissect inositol phosphate metabolism and signalling [109].

ASSESSING THE IMPACT OF LOW SEED PHYTATE (or HIGH PHYTASE) FEED ON NONRUMINANT ANIMALS

Phytic acid reduces the bioavailability of minerals in case of monogastric livestock's, such as pigs and poultry, as they lack phytase enzyme in their digestive tract. Livestock's raisers often add fungal or

bacterial-phytase to enhance feed value. Assessing the impact of low phytate- or high phytase-based diets on nonruminants may help livestock's farmers develop efficient and cost-effective feeds to optimize animals' health and productivity.

Comparative Assessment of Phytate in Animal Feces

There are various factors affecting feed phytase efficiency in gastrointestinal tract on non-ruminant or monogastric domestic animals such as pig (mainly in the stomach and upper small intestine) and poultry (mostly in upper part of digestive tract). The antinutritional effects of phytate (InsP6) are eliminated through hydrolysis as quickly it is in the upper part of the digestive tract. They are related to the phytase itself, the diet or the animal [110]. Type, optimum pH and resistance to endogenous protease are the factors associated to the phytate *per se*, while the dietary phytate content, feed ingredient composition and feed processing, and total P, Ca and Na content are those factors influencing its activity. Species, gender and age are the animal related factors. As indicated by Dersjant-Li [110] both phytate content and activity vary between crops over farming seasons and across regions due to changes in climate or weather and physical environments. Dusˇková et al. [111] indicated that phytic acid P amounts were 112, 153 and 185 g kg^{-1} total P in the faeces of young pigs (40–60 kg) after feeding them with supplemental phytase (490 FTU kg^{-1}), finishing pigs' and non-lactating sows, respectively, whereas the excreta of laying hens had 362 g kg^{-1} total P (or 23.7 g phytic acid kg^{-1} dry matter). Schlemmer et al. [112] found that phytate degradation was nearly completed throughout the whole gut and very similar in non-heated or extruded diets, but total P availability was higher in the former. Contrarily, poultry research showed incomplete phytate degradation (remarkably in broiler chickens) even if augmented with phytase [113,114]. Thus, addressing phytate in animal feces should minimize the risk of eutrophication of water ways and water bodies in LMICs, while such an effort in developed economies will reduce the feed cost and phosphorus pollution.

Carcass and Meat Characteristics in Growing and Finishing (Slaughter Age) Pigs

A comparative feeding experiment on growing swine's fed on low- and normal-phytate maize feed revealed that low-phytate maize feed with reduced levels of Pi supplementation had no adverse effects on pig performance, bone strength, or carcass characteristics, which suggests that P in low phytate maize is available and adequate to growing-finishing swine. The improved carcass characteristics in pigs fed with low phytate

feed further suggests more nutrients bioavailable to absorption in the digestive tract [115]. In another feeding experiment involving NutriDense (ND) and NutriDense Low-Phytate (NDLP) maize hybrids relative to yellow dent (YD) maize in swine diets, Hastad et al. [116] noted 4.5% and 2.5% greater metabolizable energy (ME) of ND and NDLP than that of YD maize in growing swine, while in finishing pigs, it was 5.3% greater than that of YD maize, suggesting ND or NDLP-based maize feed resulted in enhanced feed efficiency. ND and NDLP hybrids are a high-protein and high-oil type or a high-protein, high-oil and low-phytate maize hybrids.

In an experiment involving growing and finishing pigs fed with five maize-soybean meal-based diets, positive control (PC, supplemented with Pi and Ca), negative control (NC, with 0.13% and 0.11% reduction in available P and Ca, respectively), and three NC diets supplemented with bacterial phytase of 1000, 2000, 3000 FTU kg^{-1} in the feed, da Silva et al. [117] observed that supplementing increasing levels of phytase to a maize- and soybean meal-based diet with Pi and Ca reduction improved daily weight gain and feed intake of growing pigs, which maintained even in finishing pigs.

A study on supplementation of very low protein diets with maize-phytase in pigs decreased the adverse effects of such diets on weight gain and protein efficiency ratio, increased Ca and P digestibility, improved bone characteristics and produced diverse effects on fecal bacterial population, suggesting that maize-phytase (4000 FTU kg^{-1} phytase vs 2000 FTU kg^{-1} phytase) is potentially useful for enhancing the growth performance of weaned pigs fed with low-protein diets and should be considered for sustainable swine production [118].

Previous research has shown that phytases derived from Escherichi coli are more effective on nutrient digestibility and reduction of P excretion than fungal phytase in broiler chicks and in pigs [119,120]. The pigs fed on phytase + low-phytate relative to adequate-P diets were more efficient at reducing the P and Ca excretion and restoring average daily gain. The P released by phytase was absorbed and contributed to improved bone growth, greater rates of tissue accretion, and increased body weight, but no change in tissue P concentrations. This, however, has limit beyond which the excess P is excreted in urine [121].

Minerals and Protein Digestibility and Bone Integrity of Broilers

A feeding experiment involving broiler chicken feeds containing grains from low-phytate barley and maize isolines or near isogenic WT barley and maize, Jang et al. [122] noted greater mean growth and bone responses for barley compared to maize diets, greater for 60% grain inclusion compared to 40%, and higher for low phytate compared to WT grains. The

low phytate and P-supplemented type-grain diets were comparable. The chickens fed with low-phytate-based diets relative to WT or P-supplemented diets, respectively, exerted 33% and 43% less P in the environment. Young broiler chickens fed on low phytate pea-based diet showed lower weight gain and feed intake but greater bone strength than those fed on normal pea or soybean meal [123].

More recently, super dosing of phytase in the chicken's diet improved bone development and morphometric measurements of the femur and tibiotarsus in broilers of 1 to 21 d of age. The phytase super dosing also improved mineral matter and the protein digestibility coefficients, which probably relate to phytate degradation and enhanced myo-inositol availability [124]. Continuous application of phytase in the feed however may deliver more or less than expected benefits therefore its judicious use is recommended as to date our knowledge on how phytase enzyme interact with feed ingredients is limited [125].

Egg Production and Quality

An assessment of five type of poultry feed, positive control (PC) and negative control (NC) diets with recommended P levels in layer diets and NC diet supplemented with three super doses (10,000, 20,000, 30,000 FTU kg^{-1} feed) of phytase, revealed that a diet containing 20,000 FTU kg^{-1} phytase had positive effect on egg production rate, but dietary treatments including 20,000 FTU phytase had no beneficial effect on egg quality as measured by variation in egg weight, egg mass, eggshell strength, eggshell color, egg yolk color, and egg protein quality (Haugh unit) in laying hens [126]. Rice bran is often added in poultry feed. However, it is poor source of plant proteins but contain highest amount of phytate-bound phosphorus. The phytate-bound phosphorus is poorly utilized by poultry. Laying hens fed with maize-soybean-based feed supplemented with 250 FTU Kg^{-1} microbial phytase and 25% rice bran had improved performance, egg composition, and shell quality [127].

Clearly, low phytate- or high phytase-based feeds are not only efficient in P utilization and growth and productivity of nonruminant livestock's, but also for reduction of P excreta to minimize environmental pollution. Enabling policy environments for taxing high phytate animal waste may encourage more investment on private agricultural research programs to deal with excess phytate in food and feedstocks [128].

ASSESSING BENENFIT/COST OF PURSUING BREEDING FOR LOW PHYTATE CROPS

Very recently and after four decades of research, Raboy explains the pros of breeding low phytate crops from a social and economic perspective

[51]. It has been indicated that about 20% reduction in soil P mining during rice farming—achieved by reducing seed total P through breeding—could save growers several hundred million US dollars annually in fertilizer inputs [129]. In this regard, the barley cultivar 'Herald' [64] exhibited up to 15% reduction in seed total P due to the *lpa1-1* mutant. Such a cultivar may allow limiting the use of non-renewable rock phosphate reserves.

CONCLUDING REMARKS

The SDG2—known as Zero Hunger—aims to end hunger by both achieving food security and improving nutrition while promoting sustainable agriculture. Boosting nutrition sustainably through healthy diets is therefore key for succeeding in this endeavor. In this regard, people eating mostly grain crops (cereals and legumes) that are rich in phytic acid are affected by Fe and Zn deficiency because this compound binds to them to form salts that their body excretes. Hence, breeding low phytate crops appears as an interesting approach for tackling this mineral shortcoming in the human diets, thus reducing human malnutrition. Indeed, the genetic enhancement of crop using various germplasm sources and breeding methods, as described above, allows developing nutritious low-phytate cultivars, particularly in cereals such as barley, maize and rice, or legumes such as soybean. Combining a *lpa* seed phenotype with increased Fe and Zn is an achievable breeding target, while food products prepared from low phytate flour has similar functionality and acceptance as those from normal phytate flour. However, a long-term assessment must be pursued to assess bioavailability and absorption of minerals from diets differing in phytate concentrations. Phytic acid, though antinutrient, plays an indispensable role directly or indirectly in preventing human diseases. The medicinal significance of phytic acid outweighed its negative impact. Hence, there is a critical need for LMICs to improve on the dietary pattern of its people in addition to nutrition education. Reducing a nutrient in the diet may be risky rather than increasing the amount. Hence, pro- and cons- should be carefully investigated before developing low-seed phytate crops, especially in LMICs. A moderate reduction of seed phytate should be beneficial to untangle negative complexity involving environment-food/feed production-animal/human nutrition triangle.

AUTHOR CONTRIBUTIONS

CONFLICT OF INTEREST

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