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Phytase Rich Cereals for Animal and Plant Nutrition

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Cereals are major source of carbohydrate, protein, dietary fibers and minerals. However, they are not as rich in minerals and animal products. Further the mineral bioavailability is limited by certain antinutritional factors. Phytic acid is main storage form of phosphorus in plants. It alone forms the 65 to 80% part of total phosphorus in seeds. But, phytic acid is not the absorbable form of phosphorus. Plants uptake phosphorus in form of orthophosphates ions. Thus, hydrolysis of phytic acid by phytase enzyme is essential for release of phosphates groups. A characteristic property of phytic acid is its strong affinity towards cations especially divalent cations such as Ca^{2+} and Mg^{2+} as well as other cations such as zinc and iron. This reduces the bioavailability of these mineral ions in humans and monogastric animals which lack phytase activity and hence, limits the absorption of these minerals leading to poor mineral nutrition.

Non ruminants and humans whose main diet is food grains cannot hydrolyze this phytic acid and undigested part is excreted and that leads to another serious problem i.e., eutrophication. Phytic acid is organic form of phosphorus which can not be absorbed by plants thus phosphorus excreted by humans and animals as well as from degraded plant parts remains undegraded in soil and this is along with rain water reaches to water reservoirs leading to eutrophication. This phytic acid must be converted into inorganic form before uptake.

Phytic acid carry negative charged phosphate group due to this it remains associated with cations in salt form as phytate. In digestive tract it forms complexes not only with essential mineral ions but also with positively charged amino acids, proteins and carbohydrate. Poor mineral bioavailability is a serious threat in form of hidden hunger even after attaining self sufficiency in food grain production.

Phytase is the enzyme which hydrolyses phytic acid into phosphate group and *myo* inositol. Phytase activity is observed in seed during germination. This releases the phosphorus and ions essential for seed germination. But phytase activity is not that much significant in seeds and is unable to hydrolyze complete fraction. Further, phytase activity is confined to germination process only.

Table1: phytic acid content of major crops

Crop	Phytic acid g/100 g of dry weight
Maize germ	6.39
Wheat bran	2.1–7.3
Rice bran	2.56–8.7
Soyabean	1.0–2.22
Groundnuts	0.17–4.47
Chickpea	0.28–1.60

Phytic acid biosynthesis: Phytic acid (*myo* inositol hexakisphosphate) is synthesized from glucose 6-phosphate in sequential manner and stored in seed during seed developmental process. Cytosol is the site of phytic acid synthesis and it is synthesized by two pathways – lipid independent and lipid dependent pathway. Precursor in lipid independent pathway is *myo* inositol whereas, phosphatidylinositol (PtdIns) is substrate for phytic acid synthesis in lipid dependent pathway. In lipid independent pathway, sequential phosphorylation of inositol ring occurs by inositol phosphate kinases enzymes. This lipid independent pathway is mainly found in seeds whereas, lipid dependent pathway is prominent in organs other than seeds. In lipid dependent pathway, *myo* inositol is first converted to phosphatidyl inositol by enzyme phosphatidyl inositol phosphate synthase. Further, on synthesis, phytic acid is transported to vacuoles where it is accumulated as spherical bodies known as globoids.

Fine tuning of phytic acid: From above description it is evident that low phytic acid levels are desirable. This can be achieved by two different ways – one by decreasing the native phytic acid content by altering or down regulating genes involved in phytic acid biosynthesis and other is hydrolysis or degradation of phytic acid after seed development at the time of consumption. Followings are the few strategies to lower phytic acid level –

i) Soaking
ii) Food processing and milling
iii) Malting
iv) Germination
v) Cooking
vi) Fermentation
vii) Low phytic acid mutants
viii) Transgenics expressing heterologous phytase

Of above all processes only two approaches i.e., low phytic acid crops and crops expressing heterologous phytase are the only effective strategy. Other means such as cooking, milling and fermentation are neither suitable nor cost effective. Crop plants expressing heterologous phytase are effective mean for both animal and plant nutrition. Expression of phytase in roots will hydrolyze soil phytic acid and thus increases the inorganic phosphorus availability for plant to uptake whereas, plant expressing phytase in seed would be an effective mean for animal nutrition as it hydrolyzes phytic acid which cannot be done by mono gastric animals and humans.

Phytase: phytase hydrolyzes phytic acid into phosphoric acid and *myo* inositol in a sequential manner through the formation of myo-inositol phosphate intermediates such as inositol 3-phosphate. Phytases naturally occurs in plants and microorganisms, especially in fungi. However, phytase activity in microorganisms is prominent. Phytases has been classified on the basis of their catalytic properties, temperature and pH at which they catalyze the reaction. On the basis of catalytic properties, phytases are classified into following two main groups –

A.) **Histidine Acid Phytases:** Phytases from this group catalyzes removal of phosphate group at acidic pH. HAPs hydrolyzes five phosphate group from phytic acid giving rise to inositol monophosphate as end product. HAPs have a highly conserved RHGXRXP motif which is characteristic feature of HAPs. HAPs are further classified on the basis on optimal pH and position specificity os dephosphorylation into three group – *PhyA*, *PhyB* and *PhyC*. Phytases from *Aspergillus niger*, *Pseudomonas*, yeast etc. are some HAPs.

B.) **Alkaline Phytases:** This group of phytases differs from HAPs in their optimal pH, molecular mass and requirement of Ca2+ for catalytic function. In contrast to HAPs, end product of alkaline phytases is inositol triphosphate as this enzyme cannot use *myo* inositol phosphate with three or lesser phosphate residues as a substrate. Phytases from *Bacillus* species are example of alkaline phytases.

On the basis of specific position of hydrolysis, phytases are further classified into three types-

i) **3 Phytases:** Phytases from this group catalyzes the hydrolysis of ester bond at third position of phytic acid giving rise to *myo* inositol (1,2,4,5,6) petakisphosphate and one orthophosphate.

ii) **5 Phytases:** Phytases from this group catalyzes the hydrolysis of ester bond at fifth position of phytic acid giving rise to *myo* inositol (1,2,3,4,6) petakisphosphate and one orthophosphate.

iii) **6 Phytases:** Phytases from this group catalyzes the hydrolysis of ester bond at sixth position of phytic acid giving rise to *myo* inositol (1,2,3,4,5) petakisphosphate and one orthophosphate.

Phytase rich cereals: Food grains are main source of nutrition for animals and humans. Hence heterologous expression of phytase would not only releases phosphate but also enhances the bioavailability of essential minerals. But stable and high expression would be desirable. Moreover, phytase rich cereals would replace the supplementation of external phytase and external phosphorus to mono gastric animals. These cereals would also reduce the dependency on phosphatic fertilizers by effective hydrolysis of soil phytic acid and thus aids in plant nutrition and solves the problem of eutrophication.

Conclusion

Phytic acid, being the major storage form of phosphorus in seeds, holds a significant part of seed dry weight. In contrast to its useful characteristics such as role in signal transduction and antioxidant properties, it has antinutritional effect by chelating essential micronutrients with it. Since non ruminants and human lack phytase activity, consumption of phytic acid leads to not only loss of phosphorus but it takes the many micronutrients and proteins with it. Thus, it becomes essential to engineer crops with high phytase activity or reduce phytic acids in cereals *de novo.* Here the cereal crops expressing high phytase will not only hydrolyses phytic acid but they will also use phosphorus available in soil efficiently.