Phytic acid is the major storage form for Phosphorous in plants - 80% of the P in cereal seeds is present as phytic acid. Unfortunately, phytic acid is extremely poor as a dietary source of phosphate both in humans and in animals - particularly monogastric livestock which, like humans, lack phytase enzymes in their gut to breakdown phytic acid. Moreover, phytic acid chelates vital mineral nutrients - in particular iron and zinc, but also calcium and magnesium. The iron and zinc deficiency of cereal grain diets affecting millions of people in developing parts of the world is largely due to phytic acid. In developed world agriculture, phosphate rich discharge from pig, poultry and fish farms as a major environmental pollutant. As well as the human and environmental afflictions cause by phytic acid, because the phosphate in cereal grains so unavailable, livestock feed is routinely supplemented with inorganic phosphate, or alternatively manufactured microbial phytase enzyme is added in diets to breakdown the cereal phytic acid after ingestion. Both supplementation approaches are costly, and global inorganic P sources are rapidly being depleted. An alternative solution could be to reduce the levels of phytate, and increase the levels of bioavailable P, in cereal seeds, however this has met with little success due to negative effects on plant growth.

Instead of genetic approaches to decreasing grain phytic acid levels, attempts have been made to increase the level of phytase in the seed. While phytase is destabilised rapidly above 80°C (eg in feed manufacture), a pre-soaking at c.27°C of seeds (or flour) will destroy most or all phytic acid in some plant species. Moreover, with sufficient endogenous preformed seed phytase, even if some activity would be lost during processing, the need to add synthetic phytase to animal feed could nonetheless be drastically reduced or avoided altogether. Transgenic soybean and canola overexpressing phytase have been shown to reduce phosphate discharge from poultry and pigs by 50%. To understand phytase accumulation in cereal seed, Dr Henrik Brinch-Pedersen and colleagues at the University of Aarhus, Denmark have characterised the phytase enzyme families in a wide range of different cereal accessions. Of the two major classes of phytases - Purple Acid Phytases A and B – the PAPhyA’s are the predominant phytase isoforms preformed in mature grain, while the PAPhyB’s are expressed primarily de novo in germination. Maize, oats and rice have extremely low levels of seed phytase and do not express the PAPhyA isoforms, relying solely on de novo PhyB synthesis during germination. Now, the Aarhus team has, by detailed genomic sequence analysis, determined key signature “enhancer” sequences in the promoter region of the PAPhyA enzymes that determine the level of mature grain phytase achieved in the Triticeae. Using breeding and selection to accumulate the appropriate alleles at each PAPhyA locus, they have now provided a strategy to increase the level of preformed phytase in wheat grains by 4-8x the levels of commonly grown wheat varieties.

Not only have the inventors identified source accessions - “HighPhy Wheat” - from which to breed for enhanced seed phytase, they have also developed molecular markers suitable for low-cost high throughput genetic screening. They have identified the chromosomal location of these genes for wheat and barley, to better inform marker assisted breeding of the optimal conformations of phytase genes. The screening technology also can be applied to survey existing germplasm, or for example mutagenized material to identify and select newly created alleles of the enhancer region conferring the “HighPhy” trait.

The inventors have screened many accessions of wheat, including elite hexaploid bread- and feed- wheat cultivars, other diverse hexaploid accessions, durum wheats, ancestral diploids and other wheat relatives. They have found a very limited number of hexaploid wheats, but notably no modern hexaploid wheat, with seed phytase levels of over 6000 FTU/Kg DM (Fig 1). This “HighPhy” phenotype is completely explained by the sequence signature that they have identified in the PaPhyA gene promoter enhancer region.

References:
How much grain phytase is necessary?

One FTU is the amount of phytase activity which liberates 1 micromole of inorganic phosphorous per minute from an excess of sodium phytate at a pH of 5.5 at 37°C. For poultry and pigs, finished feed phytase level is recommended to be 300-500 FTU/Kg. Typical seed phytase activities of cultivated wheat are in the region of 1000 FTU/Kg dry matter, which is insufficient to avoid adding phytase (or inorganic P) to the feed. It is estimated that at least 2500, and preferably 5000 FTU/Kg DM would be necessary to avoid adding exogenous phytase. In bread making, phytic acid is associated with high ash content (and correspondingly low available mineral nutrient content), and while rye, which has grain phytase levels of around 2000–5000 FTU/kg DM is seen to have sufficient levels of preformed phytase to reduce phytic acid during dough preparation, wheat with only around 1000–1500 FTU/Kg DM, does not. With 3000–6000 FTU/Kg DM, HighPhy Wheats provide the solution.

The inventors simulated feed preparation using heat treatment at 80°C at 100% RH. The results suggest that very little if any industrial phytase would need to be added to feed prepared from HiPhy wheats.

The Aarhus team also looked at reduction of phytic acid during dough preparation from HiPhy wheat, and found a linear relationship between fermentation time and phytic acid reduction such that, without any optimisation of fermentation protocol, phytic acid levels in HiPhy dough were reduced about twice as much as in ordinary wheat, such that eventual phytic acid levels were just 55% of starting levels. This is expected to be sufficient to have potential for increasing mineral bioavailability in human diets, and combatting such conditions as iron-deficiency anemia.

HighPhy Wheat and related breeding technology opens the way to:
- Improved diets for monogastric livestock
- Significant economic benefits in animal feed regimes, through reduced additive costs
- Environmental benefits through reduced phosphate pollution
- Enhanced nutritional status of humans with grain-based diets

Patent applications cover:
- HighPhy cereal mutants
- Methods for making HighPhy alleles
- Methods and markers for detecting and selecting HighPhy alleles
- Feed and food compositions derived from HighPhy cereals

**HighPhy Technology – including rights under the patents, the HighPhy Wheat materials and associated molecular marker packages are available for licensing from PBL.** Also, use of HighPhy’s cereal grain specific expression enhancer element for transgenic uses.