Inositol phosphates in plants and the development of low phytate crops

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From 65% to 80% of total P in mature seeds of the major grain crops is found in phytic acid. For basic cellular housekeeping functions, developing seeds need about 1 mg P per gram dry weight of tissue. During development nearly all additional P is synthesized into phytic acid (Ins P_6). Since mature seed produced by grains and legumes contain from 3.0 to 5.0 mg total P per gram, respectively, they therefore typically contain from 2.0 to 4.0 mg phytic acid P per gram. This represents from 7 to 15 mg of phytic acid per gram. Applied interest in phytic acid primarily concerns its role as the major form of P in seeds and its importance in human and animal nutrition. However, essentially all cells synthesize *myo*-inositol (Ins, the cyclitol backbone of phytic acid) and its phosphorylated derivatives such as phytic acid. With these two metabolites cells do many things important to basic housekeeping, the regulation of development, and signal-transduction. In plants this means that the pathways to Ins and Ins P_6 are active in both vegetative and reproductive tissues, and in addition to roles in seed P storage, are important in processes such as stress response and signal transduction. Therefore, genetic resources useful in manipulating seed phytic acid levels such as mutations that block Ins or Ins P_6 synthesis in seeds, or transgenic introduction of phytases, two approaches of potential value in dealing with issues important to human and animal nutrition, might also perturb other functions important to crop productivity. This presentation will review the genetics of seed P and phytic acid P, developments in breeding "Low Phytate" crops, and studies of the potential value of Low Phytate crops as human foods and animal feeds.

Seed P can be manipulated by altering the amount of seed total P, its chemical composition, or a combination of the two. While there has been progress in the physiology and genetics of P uptake by the plant, there has been little progress in aspects specific to P transport from the plant to the developing seed. Thus it is well known that increasing or decreasing the supply of P to the developing plant, results in increases or decreases (respectively) in the amount of plant and seed P. Increasing seed total P usually results in an increase in seed phytic acid. There has been substantial interest in P transport functions such as those encoded by the *Phosphate transporter* (*Pht*) genes important to P uptake from the soil, and to P transport within the plant. However, single genes or functions that if perturbed greatly alter the transport of P from the plant to the seed remain unidentified. For example, it might prove useful to identify a function that if perturbed reduces the transport of P from the plant to the seed by 50%, maintaining plant P concentration but greatly reducing seed total P.

In the cell-division phase of seed development, perhaps the first 14 days after pollination of most crops, sufficient P for basic cellular processes is acquired. After this, in normal seeds essentially all additional P is synthesized into phytic acid. In addition to an adequate supply of P, phytic acid synthesis requires seed-specific, *de novo* synthesis of Ins, and conversion of Ins to Ins P_6. Mutations that block the synthesis of Ins, or its conversion to Ins P_6, reduce the accumulation of seed Ins P_6. For example, Ins is synthesized via a two-step pathway: the conversion of glucose 6-phosphate to *myo*-inositol 3-phosphate (Ins(3)P_1), catalyzed by *myo*-inositol 3-phosphate synthase (MIPS); the breakdown of Ins(3)P_1 to Ins and phosphate

catalyzed by Ins monophosphatase (IMP). A mutation (LR33) in a soybean MIPS gene (Hitz *et al*., 2002. Plant Physiol. 128, 650) blocks Ins synthesis and phytic acid accumulation. Interestingly, the block in Ins synthesis in LR33 seed also reduces raffinosaccharides, from a nutritional standpoint also a desirable outcome. This is one example of how manipulating Ins or Ins phosphates can have multiple outcomes due to fact that they are part of complex interrelated metabolic pathways that have numerous biological functions. In addition to studying MIPS-encoding genes, we are currently studying IMP-encoding genes in our model system, barley, in order to have a complete tool box in terms of Ins synthesis.

A first example of plant mutations that reduce seed phytic acid and that are in the pathway that converts Ins to Ins phosphates would be maize *low phytic acid* 2-1 (*lpa*2-1; Raboy *et al*., 2000. Plant Physiol. 124, 355). *lpa*2-1 is a mutation in an Ins(1,3,4)P_3 5-/6-kinase that converts Ins(1,3,4)P_3 to Ins(1,3,4,5,6)P_5, an intermediate step in phytic acid synthesis (Shi *et al*., 2003. Plant Physiol. 131, 507). Plants homozygous for maize *lpa*2, or for one of several barley *lpa* mutations, or for a soybean *lpa* mutation, either have problems with germination and emergence, or are clearly more sensitive to stress. For example, we have developed near-isogenic lines in barley that are either homozygous for wild-type genes, or homozygous for one of four independent *lpa* mutations that reduce seed phytic acid by 50% (barley *lpa*1-1) to 90% (barley *lpa*-M955). In non-stressful (irrigated) production environments barley *lpa*1-1 yields were similar to those of wild-type, and barley *lpa*-M955 were reduced by about 13%. However, in stressful environments (dryland production during a drought), barley *lpa*1-1 yield was reduced by about 11% as compared with wild-type, but barley *lpa*-M955 yield was reduced by 34% (P. Bregitzer and V. Raboy, unpublished results). Two mutations, barley *lpa*2-1 and barley *lpa*3-1 that cause reductions in phytic acid intermediate between barley *lpa*1-1 and barley *lpa*-M955, had intermediate impacts on yield in non-stressful and stressful environments. The impact on stress tolerance of mutations that reduce seed phytic acid therefore appeared to be proportional to the level of phytic acid reduction. This represents a second example of the pleiotropic effects one observes when manipulating Ins and Ins phosphate pathways.

The maize *lpa*2-encoded Ins phosphate kinase, along with a number of other kinases, synthases like MIPS, phosphatases like IMP or phytases, and genes encoding several other functions such as transporters, all potentially represent targets for molecular engineering of the Low Phytate trait. The goal of this molecular engineering is to improve the nutritional quality of seed while maintaining agronomic quality such as stress tolerance and yield.

All *lpa* mutations isolated to date result in reduced phytic acid accompanied by matching increases in seed inorganic P, resulting in the so-called “High Inorganic P” (HIP) seed phenotype, but have little impact on seed total P. Thus these Low Phytate crops have greatly increased “available P”, at least when used in diets for non-ruminant animals such as poultry, swine and fish. A number of studies have now shown that use of Low Phytate crops in feeds for non-ruminants can help to reduce the level of phosphate or phytase supplementation needed for optimal animal productivity, and can reduce the level of P in animal waste. A series of studies using human subjects have now shown that iron, zinc and calcium are from 30 to 50% more available in foods prepared from Low Phytate maize as compared with normal maize, and that at least in the case of zinc, availability is proportional to the level of phytic acid reduction. The current status of nutritional studies using Low Phytate crop varieties, as well as the current status of plant genetics and breeding involving plant and seed phosphate and phytic acid, will be reviewed.