Symposium: Plant Breeding: A New Tool for Fighting Micronutrient Malnutrition

Progress in Breeding Low Phytate Crops[1]

Victor Raboy[2]

U. S. Department of Agriculture, Agricultural Research Service, National Small Grains Germplasm Research Facility, Aberdeen, ID 83210

ABSTRACT Populations that depend on grains and legumes as staple foods consume diets rich in phytic acid (*myo*-inositol-1,2,3,4,5,6-hex*kis*phosphate), the storage form of phosphorus in seeds. This compound binds tightly to important mineral nutrients such as iron and zinc, forming salts that are largely excreted. This phenomenon can contribute to mineral depletion and deficiency. As one approach to solving this and environmental problems associated with seed-derived dietary phytic acid, the U. S. Department of Agriculture and others have isolated cereal and legume low-phytic acid mutations and have used these to breed first-generation low-phytate hybrids, cultivars and lines of maize (*Zea mays*), barley (*Hordeum vulgare*), rice (*Oryza sativa*) and soybean (*Glycine max*). Seed phytic acid is reduced in these crops by 50–95%. The progress in the genetics, breeding and nutritional evaluation of low-phytate crops are reviewed in this article. J. Nutr. 132: 503S–505S, 2002.

KEY WORDS: • *low-phytic acid mutations* • *phosphorus* • *mineral nutrition* • *plant breeding*

JN THE JOURNAL OF NUTRITION

A number of issues concerning the nutritional quality of grains and legumes revolve around the seed phosphorus (P)[3] storage compound called phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate) (1). Phytic acid represents from 1% to several percentage of seed dry weight and typically is deposited in seeds as mixed phytate or phytin salts of potassium and magnesium, although these salts can also contain other mineral cations such as calcium, iron and zinc (1,2). Phytic acid P typically represents from 65% to 85% of seed total P (1). As a polyanion at physiological pH, phytic acid is an effective chelator of positively charged cations. When consumed in feeds and foods, phytic acid will bind to nutritionally important mineral cations that it encounters in the intestinal tract, such as calcium, iron and zinc, and to proteins as well. Humans and nonruminants, such as poultry, swine and fish, excrete a large fraction of these salts.

This phenomenon can contribute to human mineral deficiency, particularly with respect to iron and zinc (3,4). Dietary phytic acid may also have beneficial health roles, for example as an antioxidant or anticancer agent (5,6). The relative merits of dietary phytic, therefore, must be evaluated on a case-by-case basis. For example, a subpopulation at greatest risk for mineral deficiencies caused in part by dietary phytic acid would be children and child-bearing women in rural communities in the developing world that depend on cereals and legumes as staple foods. A subpopulation that might benefit from dietary phytic acid may be aging adults in the developed world. In the context of poultry and swine production, because the bulk of grain P is phytic acid P and is excreted, to provide for an animal's nutritional requirement for P and optimal productivity, feeds must be supplemented either with an available form of P or with the enzyme phytase (7). Phytic acid-derived P in animal waste can contribute to water pollution, a significant problem in the United States, Europe and elsewhere (8).

Low-phytic acid 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*; **Fig. 1**) (9–12). Hybrids, cultivars and lines developed using these mutations represent a first generation of plant genetic resources useful in studying and possibly reducing problems associated with mineral malnutrition due to high seed phytic acid. Plants having the *lpa* characteristic produce seeds that have normal levels of total P but greatly reduced levels of phytic acid P. These mutations, therefore, do not affect the ability of a plant to take up P and transport it to a developing seed. Instead, *lpa* mutations block the ability of a seed to synthesize P into phytic acid P.

More than 20 independent *lpa* mutations have been isolated in both maize and barley, resulting in reductions in seed phytic acid P ranging from 50% to >95% (13). These reductions are matched either by increases in inorganic P (phosphoric acid, H_3PO_4 the *lpa1*-like mutations) or by increases in inorganic P and *myo*-inositol phosphates with five or fewer P

[1] Presented as part of the symposium "Plant Breeding: A New Tool for Fighting Micronutrient Malnutrition" given at the Experimental Biology 2001 meeting, Orlando, Florida, on April 1, 2001. This symposium was sponsored by the American Society for Nutritional Sciences. Guest editor for the symposium publication was Howarth E. Bouis, International Food Policy Research Institute, Washington, DC.

[2] To whom correspondence should be addressed.
E-mail: vraboy@uidaho.edu

[3] Abbreviations used: HIP, high inorganic phosphorus; *lpa*, low-phytic acid; P, phosphorus.

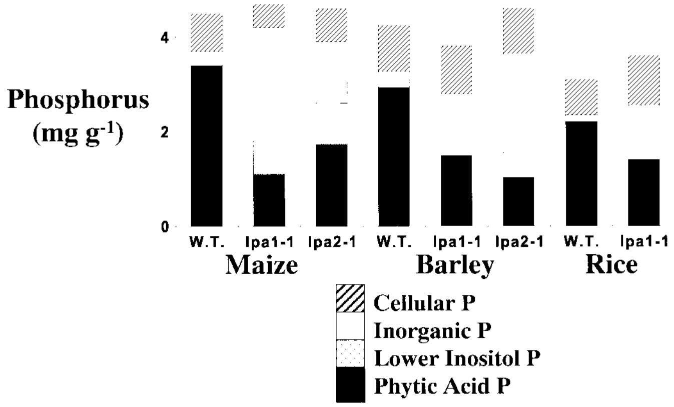

FIGURE 1 Seed phosphorus (P) fractions in normal or wild-type (WT) and low-phytic acid (*lpa*) isolines of maize, barley and rice. All fractions are given as their elemental P (atomic weight 31) contents.

esters per molecule (compared with phytic acid's six P esters per molecule, the *lpa2*-like mutations; **Fig. 1**). In the case of rice, only one *lpa* mutation has been reported to date, and as a homozygote confers a reduction of seed phytic acid P of ~50% (12).

Whereas normal seeds have consistently low levels of inorganic P at maturity, typically <0.5 mg P/g, *lpa* seed typically contain >1.0 mg/g. This high inorganic P (HIP) phenotype of *lpa* seeds provides the basis for a quick, sensitive, inexpensive and straightforward test for the trait, which is essential to make plant breeding practical (9). This is illustrated in **Figure 2**. Using tests for the HIP phenotype, the first maize *lpa* mutation, *lpa1-1* has been introduced into a number of maize inbred lines, using 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.

For maize, numerous pairs of near-isogenic lines, each pair consisting of two lines of the same parental genetic background, have been developed: one line that is essentially similar to the parent inbred line in most genes and homozygous for the normal or wild-type *Lpa* allele (normal phytic acid content) and the second line again essentially similar to the parent line in most genes but homozygous for the recessive *lpa1-1* allele (low-phytic acid content). These pairs of near-isogenic lines are then used as parents to produce pairs of near-isogenic maize hybrids. These sets of near-isogenic inbreds and hybrids represent a powerful experimental model to study the effects of *lpa* mutations on plant and seed growth and function and agronomic performance, as well as human nutrition and health.

The first trials that compared yield and other agronomic characteristics of normal vs. *lpa1-1* isohybrids evaluated 14 such first-generation isohybrid pairs (14). These trials indicated that homozygosity for the *lpa1-1* allele had little or no effect on germination in the field or in a cold-test, stand establishment, lodging, plant height, ear height, growth rate in the field and grain moisture. In 6 of the 14 hybrid pairs, no effect on grain yield was observed. In the remaining 8 pairs, the *lpa1-1* line had reduced yield compared with the normal hybrid, with an average yield loss of 5.5% for the *lpa1-1* hybrids vs. the normal hybrids.

These initial observations for maize and similar studies of barley *lpa* lines (13) provide a proof of principal; a classical genetics approach can be used to produce hybrids or cultivars that produce seed with greatly reduced (>50%) phytic acid while retaining good productivity for a first-generation technology (within 90% or more of sibling normal hybrids or cultivars). The yields of these first generation hybrids like maize *lpa1-1* or cultivars like barley Harrington M 422 or M 635 (13) compare well enough with their near-isogenic normal counterparts to suggest that simple plant breeding may improve these yields to the point where yield reductions are minimal, perhaps at an acceptable level for a nutritionally improved grain. A logical first step would be to determine whether approaches using classical genetics and breeding methods that only manipulate crop species' native genes are adequate (15). Still, common sense suggests that it is unlikely that this first-generation technology will represent the optimal technology, even with additional breeding efforts.

lpa mutations probably affect all the tissues of the plant, not just the seed. The yield losses observed could result from effects of these mutations on the vegetative processes of the growing plants. Also, little is known at present concerning the impact of these mutations on stress response or disease susceptibility. It, therefore, seems probable that if a low-phytate grain is desirable, a biotechnology approach might prove most successful. The best target genes are identified, and molecular methods are used to manipulate target gene expression only in specific tissues of the developing seed, thus, avoiding any undesirable effects on plant growth and productivity (15). A biotechnology approach might also allow us to achieve optimal reductions in seed phytic acid, on the order of 95%, while avoiding large effects on yield observed in lines like barley M 955 (13).

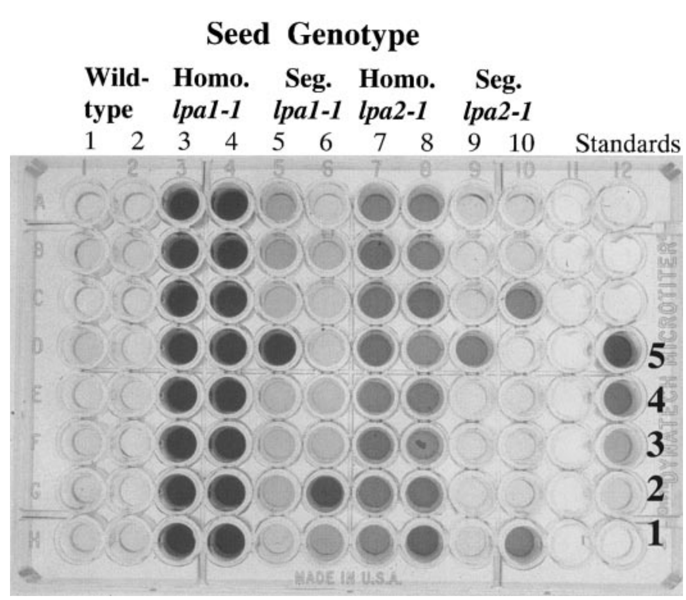

FIGURE 2 A simple assay for the high inorganic phosphorus (HIP) phenotypes of low-phytic acid (*lpa*) seed. Shown here are tests of 20 single seeds sampled from ears of maize that were either wild-type (columns 1 and 2), homozygous *lpa1-1* (columns 3 and 4), segregating (Seg) for *lpa1-1* (columns 5 and 6), homozygous *lpa2-1* (columns 7 and 8) or segregating for *lpa2-1* (columns 9 and 10). Single seeds are individually crushed and extracted overnight in 0.4 M HCl (10 v/w). Ten μL of extract is then assayed for inorganic P in microtitre plate wells. Reagent inorganic P is used as colorimetric standards. Reagent inorganic P is added to five standard wells to give: 0.0 μg P; 0.15 μg P; 0.46 μg P; 0.93 μg P; and 1.39 μg P. Using this test, normal seeds usually produce color development less than standard 3, whereas *lpa* seeds usually produce color development greater than standard 3.

Whether one is considering the improvement of diets for populations in the developing world or for livestock feeds for use in the United States or Europe, even if the cereal grain content of phytic acid is reduced by as much 95%, the legume dietary component may still contribute enough phytic acid to contribute significantly to mineral depletion or to phosphorus waste. An important, recent breakthrough, therefore, was the isolation of two soybean (*Glycine* max) *lpa* mutations, one of which reduced the phytic acid concentration by 80% (16). Although this is a fairly new development, initial indications are that seeds homozygous for this mutation germinate well and produce adequately productive plants.

Human nutrition studies

Animal nutrition studies of low-phytate types, involving poultry, swine and fish (14,17–19), first addressed the availability of phosphorus in low-phytate grains. These and other ongoing studies also address the effect of dietary phytic acid on mineral nutrition, such as calcium and zinc utilization and protein and energy utilization. One trend is that substantial reductions in feed phytic acid result in improvements in calcium utilization (14,19). Improvement in zinc utilization has been reported in one rat study of a low-phytate feed (20). However, if feeds are sufficiently fortified with additional minerals, such as iron and zinc, little or no differences in mineral retention from normal-phytate vs. low-phytate diets are detected (18).

The first use of normal-phytate and low-phytate isolines in a human nutrition study evaluated fractional absorption of iron from tortillas. Fractional absorption of iron was 8.2% of intake from the low-phytate tortilla, whereas it was 5.5% from the normal-phytate tortilla, an improvement of 49% (21). A similar study found that fractional absorption of zinc from an *lpa1-1* maize food was 30%, whereas it was 17% from normal maize (22). However, as observed in the studies using animal models, fortification with iron could offset any benefit of reduced phytic acid in a low-phytate grain (23). These results illustrate two of the major approaches to dietary problems associated seed phytic acid: genetic reduction of food or feed phytic acid; supplementation of foods or feeds with minerals or phytase enzymes (24). These and other alternative approaches can be complementary, used together to deal with these important nutrition and health problems (15,24,25).

Most previous studies of the health and nutritional effects of dietary phytic acid have been small-scale clinical or field studies that have evaluated short-term effects, involved small numbers of subjects, or looked at the effects on only one mineral nutrient. A major potential value to the low-phytate genetics technology discussed here is that it provides an experimental approach that permits large-scale field studies involving large numbers of subjects. Currently, grain produced by normal- or low-phytate isohybrids of maize or isocultivars of barley is readily available in sufficient amounts to supply large numbers of individuals in communities that traditionally rely on such grains as staple foods for extended periods. This provides an approach to study the long-term effect of consumption of phytic acid on steady-state mineral nutritional status in a global sense, in that the iron, zinc and calcium nutritional status of individuals within these populations then can be studied over time. This should allow the first truly definitive determinations of the effects of dietary phytic acid on phosphorus or mineral nutrition. The development of low-phytate variants of common food legumes like the black bean remains an unfulfilled objective critically important to the large-scale field studies described above.

LITERATURE CITED

1. Raboy, V. (1997) Accumulation and storage of phosphate and minerals. In: Cellular and Molecular Biology of Plant Seed Development (Larkins, B. A. & Vasil, I. K., eds.), pp. 441–477. Kluwer Academic Publishers, Dordrecht, The Netherlands.
2. Lott, J.N.A. (1984) Accumulation of seed reserves of phosphorus and other minerals. In: Seed Physiology (Murray, D. R., ed.), pp. 139–166. Academic Press, New York, NY.
3. Brown, K. H. & Solomons, N. W. (1991) Nutritional problems of developing countries. Infect. Dis. Clin. North Am. 5: 297–317.
4. Erdman, J. W. (1981) Bioavailability of trace minerals from cereals and legumes. Cereal Chem. 58: 21–26.
5. Graf, E. & Eaton, J. W. (1993) Suppression of colonic cancer by dietary phytic acid. Nutr. Cancer 19: 11–19.
6. Graf, E., Epson, L. L. & Eaton, J. W. (1987) Phytic acid: a natural anitoxidant. J. Biol. Chem. 262: 11647–11650.
7. Cromwell, G. L. & Coffey, R. D. (1991) Phosphorus—a key essential nutrient, yet a possible major pollutant—its central role in animal nutrition. In: Biotechnology in the Feed Industry (Lyons, T. P., ed.), pp. 133–145. Alltech Tech Publications, Nicholasville, KY.
8. Sharpley, A. N., Charpa, S. C., Wedepohl, R., Sims, J. Y., Daniel, T. C. & Reddy, K. R. (1994) Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23: 437–451.
9. Raboy, V., Gerbasi, P. F., Young, K. A., Stoneberg, S. D., Pickett, S. G., Bauman, A. T., Murthy, P.P.N., Sheridan, W. F. & Ertl, D. S. (2000) Origin and seed phenotype of maize low phytic acid 1–1 and low phytic acid 2–1. Plant Physiol. 124: 355–368.
10. Larson, S. R., Young, K. A., Cook, A., Blake, T. K. & Raboy, V. (1998) Linkage mapping two mutations that reduce phytic acid content of barley grain. Theor. Appl. Genet. 97: 141–146.
11. Rasmussen, S. K. & Hatzack, F. (1998) Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis. Hereditas 129: 107–112.
12. Larson, S. R., Rutger, J. N., Young, K. A. & Raboy, V. (2000) Isolation and genetic mapping of a non-lethal rice low phytic acid mutation. Crop Sci. 40: 1397–1405.
13. Raboy, V., Young, K. A., Dorsch, J. A. & Cook, A. (2001) Genetics and breeding of seed phosphorus and phytic acid. J. Plant Physiol. 158: 489–497.
14. Ertl, D., Young, K. A. & Raboy, V. (1998) Plant genetic approaches to phosphorus management in agricultural production. J. Environ. Qual. 27: 299–304.
15. Raboy, V. (2001) Seeds for a better future: "low phytate" grains help to overcome malnutrition and reduce pollution. Trends Plant Sci. 6. 458–462.
16. Wilcox, J., Premachandra, G. S., Young, K. A. & Raboy, V. (2000) Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci. 40: 1601–1605.
17. Spencer, J. D., Allee, G. L. & Sauber, T. E. (2000) Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 786: 675–681.
18. Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M. & Hardy, R. W. (1998) Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 170: 285–286.
19. Li, Y. C., Ledoux, D. R., Veum, T. L., Raboy, V. & Ertl, D. S. (2000) Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poultry Sci. 79: 1444–1450.
20. Poulsen, H. D., Johansen, K. S., Hatzack, F., Boisen, S. & Rasmussen, S. K. (2001) Nutritional value of low-phytate barley evaluated in rats. Acta Agric. Scand. Anim. Sci. 51: 53–58.
21. Mendoza, C., Viteri, V. E., Lönnerdal, B., Young, K. A., Raboy, V. & Brown, K. H. (1998) Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. Am. J. Clin. Nutr. 68: 1123–1128.
22. Adams, C., Raboy, V., Krebs, N., Westcott, J., Lei, S. & Hambidge, M. (2000) The effect of low- phytic acid corn mutants on zinc absorption. FASEB J. 14: A359.11.
23. Mendoza, C., Viteri, F. E., Lonnerdal, B., Raboy, V., Young, K. A. & Brown, K. H. (2001) Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. Am. J. Clin. Nutr. 73: 80–85.
24. Huff, W. E., Moore, P. A., Waldroup, P. W., Waldroup, A. L., Balog, J. M., Huff, G. R., Rath, N. C., Daniel, T. C. & Raboy, V. (1998) Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. Poultry Sci. 77: 1899–1904.
25. Lucca, P., Hurrell, R. & Potrykus, I. (2001) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor. Appl. Genet. 102: 392–397.