The Use of Genomics and Biotechnology to Reduce Environmental Pollution and Agricultural Production Cost

Virginia Tech
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

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ABSTRACT

Phytic acid is the major storage form of phosphorous (P) in plant seeds. Typically 65% to 80% of total P in mature seeds exists as phytic acid or its anion form, phytate. However, the phytate form of phosphorous in seeds poses several nutritional, agricultural, and environmental problems. Phytate present in animal feed or human food is unavailable to swine, poultry, fish, and human because non-ruminant animals lack sufficient phytase activity to metabolize phytate. Therefore, phytate is largely excreted in manure and accumulates in soil and water, which contributes to eutrophication in freshwater ecosystems and becomes an environmental concern. To meet phosphorous nutritional requirements for optimal productivity, animal feed must be supplemented with an available form of P or to be treated with the enzyme phytase. All these lead to extra production cost in poultry and swine industries. In addition, phytate strongly binds essential minerals such as zinc, calcium and iron, resulting in decreased bioavailability of trace minerals and contributing to iron deficiencies suffered by over two billion people worldwide. Therefore, development of low phytate crops has become an important objective in many crop improvement projects.

Our research focus at Virginia Tech is on soybean improvement for phosphorous bioavailability and nutrient management purposes. Soybean is the most important legume crop and plays a significant role in the U.S. economy as animal feed, human food, new source of bio-energy and bio-products, and source of export income. We have discovered genes controlling low phytate content in soybean seeds, developed high throughput phytate assay protocols, and identified DNA molecular markers to assist the selection and breeding process. We are integrating biotechnology with field practices to accelerate development of low-phytate soybeans, to reduce crop and livestock production cost, and to eliminate adverse environmental impacts of phosphorous.

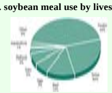
1. Significance of soybeans in the U.S. economy

- Animal Feed

Soy-fed chickens

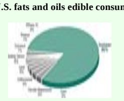
Soy-fed cattle

U.S. soybean meal use by livestock



- Human Food

U.S. fats and oils edible consumption 1999



Soy food

- Biodiesel

Soy bio-diesel operated bus

Bio-diesel pump

- New Uses

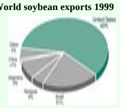
Soy based cleaners

Soy based insulation

Soy carpet adhesive

- Export Income

World soybean exports 1999



U.S. soybean and soy products exports 1999

U.S. soybean transport abroad

2. Why to develop low phytate soybeans for animal feed?

- Almost 65% to 80% of soybean phosphorus exist as phytate form, which is unavailable to non-ruminants.
- Bio-unavailable phosphorus in animal feeds is excreted in manure, which accumulates in soil and water and becomes an environmental concern.

Phosphorus pollution accelerates a process called Eutrophication, causing Chesapeake Bay's biological death due to algal blooms

3. Development of low phytate soybeans via genomic tools

(1) Discovering and using available low phytate soybean mutants

Table 1. Characterization of low phytate mutants vs. a normal phytate soybean line, V99-3337

Parental lines	Total P (mg/g)	Pi%	Phytate-P%	Other P%	Field Emergence (%)
V99-5089	6.06	51	49	0	51.6 ± 3.2
CX1834	5.07	74	20	6	49.5 ± 4.9
M766	7.14	50	43	7	16.0 ± 4.8
V99-3337	5.25	8	79	13	79.2 ± 3.2

- Low phytate mutants contain normal levels of total phosphorus (P), but 50% -75% reduction in phytate –P.
- These mutants are viable but seedling emergence in the field is reduced.

(2) Making crosses and generating segregating populations

Making crosses by hand pollination

Field planting to develop segregating populations

- Low phytate mutants were crossed with soybean lines that contain normal level of phytate but possess other desirable agronomic traits.
- Their progenies segregate for phytate content and other traits of interest.

(3) Developing high throughput protocols for phytate assays

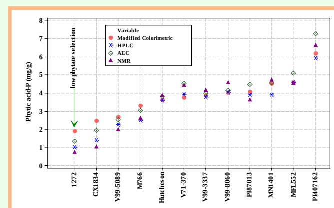


Figure 1. Ranking of phytate-P content among 12 soybean genotypes with the modified colorimetric, HPLC, AEC (anion exchange column), and ^{31}P-NMR (nuclear magnetic resonance spectroscopy) methods.

- A simple, reliable, and quantitative phytate assay protocol was developed.
- This new protocol produces highly correlated results as three well-established methods, including anion exchange column (AEC), high performance liquid chromatography (HPLC), and nuclear magnetic resonance spectroscopy (NMR)

(4) Genetic inheritance study and selection of low phytate lines

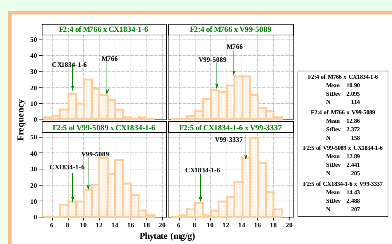


Figure 2. Segregation of phytate content in the progenies derived from low phytate mutants

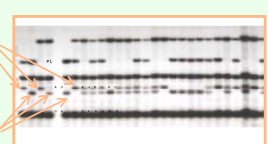
- Progenies derived from crosses between low phytate mutants segregate for phytate content.
- The three low phytate mutants confer at least two low phytate genes from each other.
- Progenies with lower phytate content than the low phytate mutant parents were selected.

(5) Accelerate selection of low phytate plants via biotechnology

Leaf tissue collection for DNA finger printing

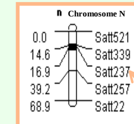
Low phytate plants have the upper band

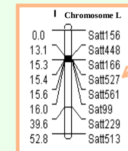
High phytate plants have the lower band



DNA fingerprinting of large segregating soybean lines

(6) Locate the low phytate genes on chromosomes by genomic tools





Genetic map location for low phytate genes

Figure 3. Location of phytate related genes on two soybean Chromosomes N and L

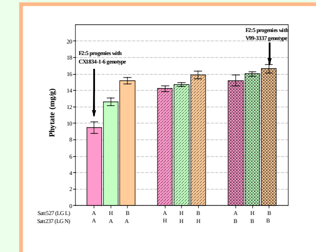
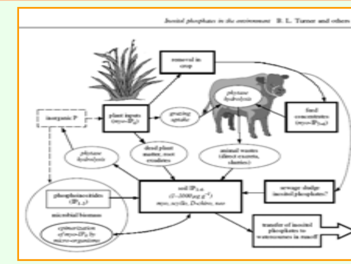


Figure 4. Phytate levels for various Satt 237-Satt527 marker classes in $F_{2:5}$ line seed from CX1834-1-6 x V99-3337 population (A = alleles from CX1834-1-6, B = alleles from V99-3337, H = heterozygotes, and bars are one standard error from the mean)

4. Animal feed trials and field emergence tests on the low phytate soybean lines developed



Conceptual biogeochemical cycle of phytate in terrestrial systems

- Soybean lines with phytate level lower than the low phytate mutant parent were selected.
- These selections had up to 90% of emergence.
- These low phytate selections are used in animal feed trails.
- Phosphorus in the animal wastes will be analyzed.

CONCLUSIONS

- Existing and novel low phytate soybean mutants were used in the program as donors to develop low phytate soybean lines (Table 1 and Figure 2).
- A simple, reliable and high throughput phytate assay protocol was developed in our program which enables screening of low phytate soybeans for inheritance studies (Figures 1 and 2).
- Progeny from crosses between three low phytate mutants display a wide range of phytate levels, suggesting at least two different low phytate genes are segregating (Figure 2).
- Two major genes, Satt 237 and Satt 527, located on chromosomes N and L were identified to be associated with low seed phytate content (Figures 3 and 4).
- Low phytate soybean lines developed in our program segregate for field emergence and low phytate lines with up to 90% of emergence can be selected.

IMPLICATIONS

- Reduce pollution of phosphorus runoff in water sheds (e.g., Chesapeake Bay)
- Produce environment-friendly soybeans for animal feed and human food
- Lower commercial production cost for the animal industry
- Overcome trace mineral (e.g., iron) deficiency problems of high phytate soybeans

FUNDING SOURCE

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