February 2012
Virginia Soybean Board Project Proposal

Project Title: Breeding Soybean Varieties Adapted to Virginia

Term of Project: July 1, 2012 to June 30, 2013

Project Leader: (Temporary until a new soybean breeder is hired)
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Funds requested: $35,217 for wages and supplies.

Project Objective: To develop soybean varieties adapted to Virginia that are high-yielding, herbicide tolerant, nematode resistant, lower cost of production, and possess other economically valuable traits.

Locations and Facilities: The Eastern Virginia Agricultural Research and Extension Center in Warsaw is the main location for the soybean breeding program field activities. Tests of advanced breeding lines are conducted at Tidewater, Northern Piedmont, and Southern Piedmont ARECs, with additional research at the VCIA Farm in Mt. Holly, Kentland College Farm in Blacksburg, and in the over 1000 square feet of greenhouse space in Blacksburg. Molecular marker and wet chemistry data are collected in the Molecular Crop Genetics Lab in Latham Hall in Blacksburg.

Budget: (see attached VT OSP budget)

Salary Personnel	$15,000 (Special Research Faculty)
Wage Personnel	$ 4,000
Fringe benefits	$ 5,218
Materials and Supplies	$ 8,999
Contractual Services	$ 2,000
Total	$35,217

Estimated Total Cost of Research: $120,000 (including funds requested from VSB)

Justification: The soybean breeding program selects high-yielding varieties through extensive testing in diverse locations in Virginia in order to address regional factors reducing yields. This is achieved by screening thousands of distinct genetics lines every season. Funds are requested for labor and supplies associated with field-testing at EVAREC. The soybean breeding position is currently open following the departure of Dr. Rainey. However, the department and college are committed to the short-term and long-term success of the soybean breeding program. Therefore, we are conducting a national search, have identified top candidates, and will soon be conducting interviews. In the interim, the program will remain fully staffed and the 2012 field season will go forward.

Soybeans are grown on over half a million acres in Virginia, and annually are worth around $100 million in the state. In support of this vital industry, the primary objective of the soybean breeding program is development of improved public soybean varieties for Virginia. The Commonwealth represents a distinct market from major U.S. soybean production areas, and the vast majority of soybean breeding in the private and public sector is focused on the Midwestern states. This amplifies the significance of the breeding program for developing varieties targeted to unique local growing conditions such as day length, soils, and rainfall. In particular, distinct local pathogens and pathogen races (variants) can reduce yield on a regional basis. Soybean cyst nematode and frogeye leaf spot are two such pathogens in Virginia that are addressed by the soybean breeding program. However, in recent years Monsanto and Pioneer have opened breeding stations in North Carolina that will eventually target Virginia maturity groups and testing environments.

Expected Outcomes: 1. New conventional and herbicide-tolerant soybean varieties are grown on widespread acreage in the region. 2. Germplasm incorporating new traits for use in soybean breeding, complemented with genetic, genomic, and quantitative information for deployment of traits regionally and nationally. 3. Students trained in crop breeding.

Specific Objectives:

1. Develop high-yielding soybean cultivars adapted to the climatic and edaphic conditions and cultural practices in Virginia, the mid-Atlantic, and Southeast region that lower cost of production and possess resistance to economically important diseases and pests, in both herbicide-tolerant and conventional classes of soybean.

2. Develop superior germplasm and varieties with modified oil quality, higher protein content, enhanced quality for feed and food, and other high value traits.

3. Emphasize development of RM 4.0-5.0 soybean germplasm and varieties.

4. Targeted development of varieties resistant to races of soybean cyst nematode specific to Virginia.

Procedures:
The general procedures that are followed for genetic improvement of soybean include:

A.   Field Research Methods

The soybean breeding program emphasizes high yield and good seed quality along with other important traits. High-yielding cultivars, as appropriate, are used as checks in all stages of the selection and evaluation processes. An experimental line must show superiority to these standards in repeated testing before it is considered for release as a new cultivar.

Parental materials include released cultivars, advanced breeding lines with desirable traits, plant introductions, and other appropriate germplasm for meeting the stated objectives and providing a broad germplasm base. Approximately 150 new crosses (750 pollinations) are made each year. Most F1 plants are grown in winter nurseries in Costa Rica and Argentina. F2 populations are advanced by a modified single seed descent procedure. Where possible winter nursery services are used to advance F2 and F3 populations, to reduce the time elapsed between making a cross and obtaining stable lines for evaluation. This is one of the most critical areas in increasing the rate of genetic progress in a breeding program. Winter nursery services are also an economical means of growing early generations. Seeds that are returned from the winter nursery are planted at Warsaw or Blacksburg, Virginia. About 20 to 100 single plants are selected at maturity from each population, depending on the general appearance of the population and/ or special traits that might be present. Depending on objectives, early generations (F1 to F4) are selected using automated high- or medium- throughput Marker Assisted Selection (MAS), eliminating unwanted genotypes early in the breeding process.

Short F5 progeny rows are planted at Warsaw or Blacksburg from the seeds of each selected plant. About 8000 to 10,000 progeny rows are grown each year; about 10% to 20% are selected for advancement to preliminary yield trials. Selection at this point is visual and is based on pod set, maturity, plant height, and freedom from undesirable traits such as lodging and disease. Visual seed quality (freedom from imperfections and disease) is evaluated after harvest. Some F5 progeny rows are further selected after harvest for fatty acid content, protein and oil content, carbohydrate content, and seed size.

About 400 to 500 new selections are placed in preliminary yield tests each year at the Warsaw location only. The usual experimental design is a randomized complete block with two replications or modified augmented design. About 20% to 25% of lines are then advanced to Virginia advanced line tests. The latter normally are planted in both full-season and double-crop plantings at Warsaw utilizing randomized complete block with three replications. Each experiment is also planted at one or two additional locations as resources permit. Superior lines are advanced to the Southern Regional Cooperative Soybean Tests, which are grown at 25 to 30 locations throughout the Southeast.

The purpose of these tests is to obtain performance data over a wide geographic area and to quickly obtain a broad database to assist in cultivar release decisions. Most publicly developed soybean cultivars released in the South have been evaluated in these tests. The experimental design for all of the regional tests is a randomized complete block.

The standard plot size in yield tests listed above that are grown in Virginia is four rows, 5.4 m long, spaced 0.76 m apart. The center rows of each plot are end-trimmed, so that two 3.6 m rows are harvested for yield estimates. Other observations to be made on each plot include mature plant height, maturity date, lodging score, visible disease symptoms, seed shattering, and any other traits deemed to be of significance. Observations made on the seed after harvest include a seed quality score, seed size where appropriate, percent purple-stained seeds, and percent mottled seeds when present. Yield is a major factor in selecting lines to be advanced, but all observations are considered. Since most lines are close to the acceptable limits of seed protein and oil content, these determinations are typically not made before lines are entered in one of the regional tests. Seeds from all entries in the regional tests are routinely tested for oil and protein content.

All the procedures described are carried out each year, although the emphasis on individual traits varies somewhat from year to year, depending on environmental conditions (e.g. in a year when all lines have good seed quality, very little selection can be done for that trait).

B. Procedures for selecting various high value traits:

Selection of modified FA populations for lines which contain 3% linolenic, 6.5% palmitic + stearic, and/ or <:!:35% oleic fatty acids occurs in early generations as resources allow. The method of selection and the generation in which it takes place is dependent on resources available to the program. FA content can be measured via Near Infrared spectroscopy (NIR). This was formally done at National Center for Agricultural Utilization Research Soybean Uniform Test Analysis Program, Peoria, IL. However, this service is no longer available; therefore we are looking for a new source. Fatty acid content is measured more precisely using Gas Chromatography (GC), which is more costly than NIR. This program can apply GC to numerous early-generation samples with adequate funding. MAS can also be used for certain FA traits to select populations in early generations, but must be used in combination with NIR or GC because FA content is a quantitative trait. MAS for FA traits will be applied only if sufficient funding is obtained from VSB and other sources.

The low phytate and stachyose trait is measured several ways: high pressure liquid chromatography (HPLC), colorimetric phytate or Pi assays and by measuring sucrose content indirectly with a glucose monitor after digestion of sample with invertase. Whatever method is used, the best time to measure is in F5 progeny row stage, when low phytate and stachyose lines with good germination can be selected with stand counts.

C. Marker Assisted Selection:

Molecular markers linked to the trait of interest are screened in various generations using polymerase chain reaction (PCR) and automated DNA electrophoresis on DNA extracted from young trifoliate. Current markers include: SCN resistance, FLS resistance, low phytate trait (SNP genotyping).

D. Procedures for selecting tolerance and resistance to SCN

Advanced breeding lines will be screened in SCN-infested fields in collaboration with VCIA Foundation Seed Farm in Mt. Holly, VA, David Holshouser, Keith Balderson, and others. SCN-resistant and tolerant parents from public breeding programs are used in every round of crossing.

Selecting for SCN tolerance within breeding populations also requires MAS and fee-based greenhouse assays, which we are able to do as resources allow. In 2009 and 2010, we used MAS

to select SCN resistant individuals from segregating populations. In 2010, advanced lines were screened for nematode resistance at Iowa State University (Tylka Nematology Lab) and University of Arkansas (Kirkpatrick Lab).

E. Statistical procedures

Data from all field experiments are analyzed by appropriate statistical procedures for the experimental design that was used, followed by multiple comparison procedures as needed. Typically, this is ANOVA of a RCBD, followed by a Duncan's multiple range test, generated using General Linear Model procedure of SAS software, or Agrobase software. Field-plot techniques, experimental design, and data analysis are standard procedure for breeding soybeans and other crops.

F. Dissemination of results

Variety and germplasm releases and the results of screening commercial cultivars with FLS races prevalent in Virginia will be communicated to growers in the following ways:

•	Experimental lines deemed worthy of release as new cultivars are handled according to the policies of the Virginia Agricultural Experiment Station.
•	Virginia Soybean Update.
•	Presentation at the VCIA seed conference.
•	Virginia AG Expo, Virginia Tech Crop Genetics web site, and Virginia Soybean Field Day.