

SUMMARY

VIRGINIA SOYBEAN BOARD PROJECT PROPOSAL

TITLE: Use of Genomics to Develop Disease Resistant Soybeans

PROJECT PERIOD: July 1, 2012 to June 30, 2013

PROJECT LEADER:

M. A. Saghai Maroof, Professor
Department of Crop and Soil Environmental Sciences
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061
540/231-9791

OTHER PERSONNEL: Other personnel involved with project are: a laboratory manager, postdoctoral fellows, graduate students and part-time helpers working with the project leader. Furthermore, we are collaborating with Dr. Alison Roberson (Plant Pathology Department) of Iowa State University on screening soybean for reaction to *Phytophthora* root rot disease.

LOCATION: All laboratory and greenhouse work and some of the field activities will be performed at Blacksburg. Some of the crossing and growing of plant materials will be conducted at the Eastern Virginia Agricultural Experiment Station.

PROJECT OBJECTIVES:

The long-term goal of the proposed project is to develop disease-resistant and high-yielding soybean cultivars adapted to the Virginia growing conditions. The major objectives for the current phase of the project are:

1. To develop breeder-friendly and easy to use DNA markers for soybean virus resistance genes.
2. To initiate a marker-assisted selection (MAS) program aimed at pyramiding or stacking of genes conferring resistance to soybean virus and *Phytophthora* root rot diseases.

FUNDS REQUESTED:

We are requesting funds (\$11,000) for the second year of the same project funded (\$10,000) by VSB last year. The funds requested from VSB are to supplement our large multi-institutional project initiated last year through funds from the USDA's National Institute of Food and Agriculture (NIFA) program on Global Food Security (for more details please see Previous Work Section below).

February 2012

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TERM OF PROJECT: July 1, 2012 to June 30, 2013

PERSONNEL AND FACILITIES:

Personnel:

M. A. Saghai Maroof, is a soybean molecular geneticist in the Crop and Soil Environmental Sciences (CSES) Department at VPI&SU. Field and laboratory personnel from his program will conduct DNA sample preparations, detection of DNA sequence variation, development of molecular markers, making genetic crosses, screening for disease reaction and statistical analyses of data. A major emphasis of his program is to adapt biotechnology methods and genomics tools to solving plant breeding problems.

Facilities:

A modern, fully equipped Plant Molecular Genetics Laboratory is available for conducting DNA marker assays and various genomic analyses. Furthermore, we have access to sequencing technologies through the Core Laboratory Facility of the Virginia Bioinformatics Institute. There is adequate greenhouse space for growing soybean plants for DNA extraction, performing controlled crosses, and screening for disease reaction. Identification of progenies to be used in crosses will be done in the field. We have equipment and the machinery to thresh seeds of individual plants to use for various assays and preservation of selected genotypes. Ample field space is available to carry out this project.

BUDGET (VSB):

Salary:

Dr. Ruslan Biyashev (Lab Manager/Classified Staff)	
2% salary (0.18 months)	\$876
Fringe Benefit (38.5%)	\$337

Hourly Wages:

Student Wages	\$ 3,000
Fringe Benefits (9.5%)	\$ 285

Supplies and Materials:

Laboratory Supplies	\$ 3,002
Field Supplies	\$ 500
Contractual Services	\$ 3,000

TOTAL Direct Costs	\$11,000
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Budget Justification:

Salary: 2% of Dr. Ruslan Biyashev's salary (classified staff personnel) is being requested. He will participate in the experimental conduct as well as supervision of the project. (2% salary with FB @ 38.5% = \$1,213).

Wages: \$3,000 with FB @ 9.50 % (\$285) is being requested to recruit an undergraduate student to help with field and laboratory work.

Contractual Services: \$3,000 is being requested for work to be performed by VBI or service companies (for genomics work such as DNA sequencing), to cover publication cost, or contribute towards equipment maintenance contracts.

Materials & Supplies: \$3,502 is requested for the purchase of molecular biology reagents and disposable laboratory materials. **Total** fund requested: \$11,000

BACKGROUND, JUSTIFICATION AND SIGNIFICANCE:

Soybean is a major crop in the US. In 2009, it had a total crop value that exceeded \$31.7 billion. Soybean mosaic virus (SMV) is considered as one of soybean's most widespread virus diseases globally with direct yield losses of 8 to 35%. Additionally, SMV interacts synergistically in soybean with *Bean pod mottle virus* (BPMV), causing drastic reductions in yield and seed quality. SMV has been controlled through deployment of three resistance genes designated as *Rsv1*, *Rsv3* and *Rsv4*.

Phytophthora root and stem rot, caused by *Phytophthora sojae*, is even a more serious disease of soybean. Losses from this disease to US soybean producers have quadrupled over the last 10 years and are estimated to be over \$300 million annually. Host resistance is the most cost-effective way to manage the disease. *Phytophthora* has been controlled through the deployment of single resistance genes called *Rps* (resistance to *Phytophthora sojae*). However, such resistance is not durable and is easily overcome by the changing pathogen population. Therefore, it is of paramount importance to search for and identify new *Phytophthora* resistance genes and stack them together with virus resistance genes in new soybean cultivars.

DNA molecular markers are powerful tools in selecting for traits of interest in breeding programs. They are especially important in breeding for complex traits such as resistance to *Phytophthora* root and stem rot disease of soybean. Therefore, we propose to employ molecular marker and genomics technologies to manage *Phytophthora* and virus diseases in soybean. Successful execution of this study should provide disease resistance DNA markers for rapid screening and identification of high-yielding soybean lines with high levels of disease resistance.

PREVIOUS WORK:

Over the years, we have had a very extensive and active soybean disease resistance project funded by Virginia Soybean Board, United Soybean Board, Iowa Soybean Promotion Board, USDA Competitive Grants Program (NRI), USDA/National Institute of Food and Agriculture (NIFA), and National Science Foundation.

The proposed activities in this VSB proposal are to supplement/complement our large (\$9.28 M) multi-disciplinary (employs various approaches including GMO) and multi-institutional (18 institutions) project, which is funded by USDA/NIFA (April 2011). This project is led by Brett Tyler along with 27 Co-PIs from 18 institutions. A major objective of the project is to identify NEW and novel *Phytophthora* resistance genes using essential *Phytophthora sojae* effectors. Discovery of highly conserved effector proteins, essential for virulence in soybean, has only recently been possible after the completion of the *Phytophthora* genome sequencing projects. A number of *Phytophthora* essential effector proteins will be used to screen a large number of soybean lines to discover novel and durable resistance genes to be deployed in high yielding elite cultivars. All results, including resistant germplasm, will be publically available.

The USDA project is for a period of five years starting April 2011 and the fund is allocated on yearly basis. Funds for years one and two have already been made available. In addition to the funds VSB provided us last year, we (VT and ISU) also received \$78,183 from USB for year one of our project. We (VT and ISU) have a pending proposal with USB requesting \$120,000 for year 2 of the collaborative project with ISU.

PROJECT OBJECTIVES:

The specific objectives of the VSB project are listed below.

1. To develop breeder-friendly and easy to use DNA markers for soybean virus resistance genes.
2. To initiate a marker-assisted selection (MAS) program aimed at pyramiding or stacking of genes conferring resistance to soybean virus and *Phytophthora* root rot diseases.

PROCEDURES:

All laboratory and greenhouse procedures including DNA extraction, marker assay and disease screening will follow established and published protocols. DNA sequencing will be performed through service companies such as the Core Laboratory Facility at Virginia Bioinformatics Institute. Initial experiments will rely on several existing soybean populations and unique genetic materials developed through our on-going projects. New crosses will be made among lines containing single resistance genes for different diseases. Segregating populations will be developed with the goal of stacking multiple resistance genes in single lines. Various genomics tools including SNPs (Single Nucleotide Polymorphisms) and SSRs (Simple Sequence repeats) will be used for mapping and development of easy-to-use markers. For marker development, we will make efficient use of the soybean genome sequence which became available only over a year ago. Virus resistance genes are located on soybean chromosomes 2, 13 and 14. However, more emphasis will be placed on chromosome 13 and 14. Chromosome 13 contains a region with a cluster of genes conferring resistance to several diseases including cyst nematode and *Phytophthora*. Mapping and data analysis will be performed using computer programs and bioinformatics tools currently available in our program and through our project collaborators. Final output from this project will be germplasm with resistance to multiple diseases, and DNA markers to accelerate development of new high-yielding soybean cultivars for domestic and export markets.

PROGRESS:

- Planted 600 lines (rows) resulting from an advanced generation of a disease-resistant by a disease-susceptible cross. This population segregates for the virus resistance gene on soybean chromosome 14.
- Planted 230 lines from an early generation of a population segregating for the virus resistance gene on soybean chromosome 2.
- Leaf tissue samples were collected from the above 800+ lines for DNA extraction and chromosomal mapping disease resistance genes.
- Identified 400 *Phytophthora* root rot resistant soybean lines/accessions from the USDA soybean germplasm collection, requested seed, and planted in the field to increase seed for a collaborative project with Iowa State University.
- 600 accessions of *Glycine Soja* (wild soybean) were obtained from USDA/GRIN and planted in the field to identify lines with new sources of resistance genes.
- Made genetic crosses among lines containing genes conferring resistance to various diseases in order to develop multiple-disease resistant lines.

More detailed and up to date progress will be presented to the board during the March meetings.