



February 3, 2013

VDACS/Virginia Soybean Board  
102 Governor's Street, Room 319  
Richmond, VA 23219

Attention: Mr. Phil Hickman, Program Director

Subject: Virginia Tech Proposal 13-1610-02

Dear Mr. Hickman,

Enclosed please find Virginia Polytechnic Institute and State University (Virginia Tech) proposal entitled "Use of Genomics to Develop Disease Resistant Soybeans" which is being submitted by Dr. M. Saghai-Marooof in our Department of Crop and Soil Environmental Sciences.

All correspondence related to this proposal should reference Proposal Number **13-1610-02**.

This proposal is considered confidential/proprietary information of Virginia Tech and is being provided for the Virginia Soybean Board to evaluate funding this work at Virginia Tech. No other use of the information contained in this proposal is authorized until such time as an award is made.

Virginia Tech reserves the right to negotiate mutually agreeable terms and conditions at the time of award.

Virginia Tech appreciates the opportunity to submit this proposal. If contractual or budgetary questions arise, please contact me by phone at (540) 231-4471 or email at [rcb05@vt.edu](mailto:rcb05@vt.edu). Technical questions should be directed to Dr. Saghai-Marooof at 540-231-9791 or email at [amarooof@vt.edu](mailto:amarooof@vt.edu).

Sincerely,

Robert C. Blackwell  
Pre-Award Administrator  
Office of Sponsored Programs

Enclosures  
Cc:  
University File  
Dr. Saghai-Marooof

## SUMMARY

### VIRGINIA SOYBEAN BOARD PROJECT PROPOSAL

**TITLE:** Use of Genomics to Develop Disease Resistant Soybeans

**PROJECT PERIOD:** July 1, 2013 to June 30, 2014

**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 this project include 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 diseases. We have also initiated a recent collaboration with Dr. Aardra Kachroo (Plant Pathology Department) of University of Kentucky on soybean virus resistance gene discovery.

**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:

- To develop breeder-friendly and easy to use DNA markers for soybean virus resistance genes and their incorporation into breeding lines.
- To initiate a marker-assisted selection (MAS) program aimed at pyramiding or stacking of genes conferring resistance to soybean virus, *Phytophthora* root rot, and *Pythium* diseases. The latter soybean root disease is becoming more prevalent and 54 species have recently been identified.

**FUNDS REQUESTED:**

We are requesting funds (\$21,194) for the third year of the same project funded by VSB for the last two years. The funds requested from VSB are to supplement our large multi-institutional project which was initiated through funds from the USDA's National Institute of Food and Agriculture (NIFA) program on Global Food Security (more detail is given below).

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**VIRGINIA SOYBEAN BOARD  
PROJECT PROPOSAL**

**TITLE:** Use of Genomics to Develop Disease Resistant Soybeans

**TERM OF PROJECT:** July 1, 2013 to June 30, 2014

**PERSONNEL AND FACILITIES:**

**Personnel:**

M. A. Saghai Maroof, is a soybean molecular geneticist in the Crop and Soil Environmental Sciences (CSES) Department at Virginia Tech. 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 solve plant breeding problems. He collaborates with 30 scientists to develop disease-resistant soybean cultivars.

**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 DNA sequencing technologies through the Core Laboratory Facility of the Virginia Bioinformatics Institute (VBI). 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 genetics and breeding aspects of this project at the Blacksburg campus or the Warsaw Experiment Station.

**BUDGET (VSB):**

Salary:

Dr. R. Biyashev (Lab Manager)	
2.65% salary (0.32 months)	\$1,516
Fringe Benefit (40%)	\$606

Hourly Wages:

Student Wages	\$ 7,296
Fringe Benefits (7.75%)	\$ 565

Supplies and Materials:

Laboratory Supplies	\$ 3,000
Field Supplies	\$ 711

Contractual Services	\$ 4,500
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Travel:	\$ 3,000
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<b>TOTAL Requested Budget</b>	<b>\$21,194</b>
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## **Budget Justification:**

**Salary:** 2.65% 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. (2.65% salary including FB @ 40% = \$2,122).

**Wages:** \$7,296 with FB @ 7.75 % (\$565) is being requested to recruit an undergraduate student to help with field and laboratory work.

**Contractual Services:** \$4,500 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.

**Travel:** \$3,000 is requested for trips to Warsaw station and for attending Annual Soybean Breeders Workshop in St. Louis, Missouri.

**Materials & Supplies:** \$3,711 is requested for the purchase of molecular biology reagents and disposable laboratory materials as well as field supplies.

**Total** fund requested: \$21,194

## **BACKGROUND, JUSTIFICATION AND SIGNIFICANCE:**

Soybean is a major crop in the US, with a total crop value that exceeded \$35.7 billion in 2011. In Virginia, soybean was planted in 560,000 acres and generated 262 million dollars in 2011. 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. After cyst nematode, it is the most important disease problem for 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.

*Pythium* damping-off and root rot is another soybean disease which results in poor stands and reduces yield. The disease is common in all soybean production areas. Its control is complicated as the pathogen is persistent in the soil for several years and has wide host ranges including corn, cotton and wheat. Recent disease screenings have identified 54 *Pythium* species. So far, only a single resistance gene for *Pythium* control has been identified in the soybean cultivar 'Archer'.

Therefore, it is essential to develop strategies for incorporation of this resistance gene into breeding lines.

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 and *Pythium* damping-off diseases of soybean. Therefore, we propose to employ DNA molecular marker and genomics technologies to manage *Phytophthora*, *Pythium* 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 resistance to these important diseases.

### **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), National Science Foundation, and recently the USDA/National Institute of Food and Agriculture (NIFA).

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 since April 2011. This project is led by Brett Tyler (Project Director), John McDowell (Project PI) 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* effector proteins. Discovery of highly conserved effector proteins, essential for virulence in soybean, has only recently been possible after the completion of the DNA sequencing of the *Phytophthora* genome or chromosomes. Several *Phytophthora* essential effector proteins, from among 400, are being used to screen over 1000 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 2011 and the fund is allocated on yearly based on project performance. Funds for years one, two and three have already been allocated for all participating universities through Virginia Tech. In addition to the funds VSB provided us for the last two years, we (VT and ISU) also received \$78,183 from USB for year one and \$120,000 for year two of our collaborative project with ISU. The fund being requested from VSB is for year three of the VT project.

### **PROJECT OBJECTIVES:**

The specific objectives of the VSB project are listed below.

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

diseases. The latter root disease is becoming more prevalent and 54 species have recently been identified.

## **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 DNA markers. For marker development, we will make efficient use of the soybean genome sequence which became available only a couple of years ago. Virus resistance genes are located on soybean chromosomes 2, 13 and 14. However, more emphasis will be placed on chromosomes 13 and 14. Chromosome 13 contains a region with a cluster of genes conferring resistance to several diseases including cyst nematode, *Phytophthora* and *Pythium*. 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:**

Significant progress has been made towards achieving the stated objectives of the project in identifying new DNA markers for disease resistance genes and their subsequent use in breeding for resistance to multiple diseases:

- During the 2012 growing season, over 1000 rows of genetic materials were planted in the field for seed increase. This germplasm included populations segregating for disease resistance genes, advanced breeding lines, and cultivars containing known resistance genes for root rot, *Pythium* and virus diseases.
- We also planted a set of 33 soybean lines which were selected because of being resistant to *Phytophthora* root rot pathogen. The lines were identified after disease screening of 514 soybean accessions at Iowa State University through a USB-funded collaboration. A combination of three isolates of *Phytophthora* pathogen was used for disease screening. These pathogen isolates, in combination, overcome (defeat) seven of the eight known root rot resistance genes in soybean. Therefore, the selected lines are likely to contain new and novel resistance genes. The set of 33 lines were planted in the 2012 field at three different times and were crossed with a susceptible line to develop segregating populations for inheritance and gene mapping studies. They were also crossed to each other (resistance by resistance) and to the Archer soybean, which contains the only known gene conferring resistance to *Pythium*, another root disease. The resultant breeding populations will be used in subsequent experiments to identify lines conferring resistance to multiple diseases.

- DNA samples from a population of 283 individuals resulting from crossing a resistant by a susceptible line were used to develop several new DNA markers closely linked to the virus resistance gene on soybean chromosome 14. Such markers should facilitate breeding for disease resistance.
- Soybean line L29, which contains a virus resistance gene, was planted in the greenhouse. Leaves were inoculated with the most virulent strain of soybean mosaic virus disease. Tissue was collected from virus-infected and healthy-control plants 0, 1, 2, 3 and 24 hours after inoculation. Total RNA was extracted from the samples and several libraries were constructed and sequenced at the Virginia Bioinformatics Institute. The amount of data generated (1.97 billion sequence reads) was almost twice as much as any other sequencing project. In order to analyze this massive data (acquiring 300 Gigabytes of computer memory), we obtained funds from other sources to purchase a powerful computer that can do all the required data analysis, which is currently under way. Results from such analysis of the genomic sequences will aid in the identification of the virus resistance gene in the soybean cultivar L29 and should facilitate its subsequent deployment in susceptible varieties.
- As an additional approach to identify and isolate the resistance gene in L29 soybean, we conducted a detailed analysis of a candidate gene, Glyma14g38540, which resides in the chromosomal region of the virus resistance gene. We compared the structure of this resistance gene candidate in a set of susceptible and resistant soybean lines. Results indicated that a set of 39 base-pair of DNA was missing from this gene in all of the nine resistant lines we tested, compared to the susceptible lines. To confirm our observation, we ordered seed for 14 additional known virus resistant soybean cultivars from the USDA germplasm collection bringing the total number of resistant lines to 23. Seeds were planted in the greenhouse, DNA extracted and the same gene was sequenced from all lines. Results indicated that all but two of the 23 resistant lines were missing the same 39 base-pair of DNA. Interestingly, the two lines which were not missing the 39 base-pair of DNA but still were resistant, had previously been reported to contain a different allele (form) of the same resistant gene! Therefore, we not only have discovered a DNA marker for the resistance gene itself, we can also tell apart the different forms of the same resistance gene. This information should facilitated marker-assisted selection for resistance and help understand the nature and mechanism of the resistance.
- We secured additional funds from USB (Iowa State University collaboration): \$78,000 (2011-2012) and \$120,000 (2012-2013) for VT and ISU for *Phytophthora* research.
- We initiated a new collaboration with University of Kentucky for virus resistance work. This collaboration will require no funds from us. However, it will be crucial for confirmation of identity of resistance genes resulting from our work.
- Virginia Soybean Board fund (from past and present) was acknowledged in one scientific paper and one poster presentation in 2012 as listed below:
  - Wen, R. H., B. Khatabi, T. Ashfield, M. A. Saghai Maroof, and M. R. Hajimorad 2012. The HC-Pro and P3 cistrons of an avirulent *Soybean mosaic virus* are

recognized by different genes at the complex *Rsv1* locus. A paper published in: *Molecular Plant Microbe Interaction*. 26:203-215.

- Redekar N., M. Laskar, B. Bowman, R. Biyashev, R. Jensen, G. Cormier, S. Jeong, S. Tolin, M. Saghai Maroof. 2012. Towards Identification of Soybean Mosaic Virus Disease Resistance Gene, *Rsv3*. Poster presented during a mini symposium (November 2012) organized by the Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech.

More detailed and up to date progress will be presented to the Board during the March 2013 meeting.