

# SOYBEAN GENETIC RESOURCES

## AND GENETIC ENHANCEMENT

### WHITE PAPER

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## I. EXECUTIVE SUMMARY

On February 23<sup>rd</sup> and 24<sup>th</sup>, 2000, twenty-two expert researchers with knowledge of plant breeding, plant physiology, plant pathology, entomology, nematology, molecular biology, functional genomics, and seed composition participated in a workshop hosted by the United Soybean Board Production Committee. Over the course of the two days, the scientists reached consensus on research priorities and time frames needed to conduct the research when funded in the area of soybean genetic resources and genetic enhancement. These are summarized below.

### A. Genetic Yield Potential

1. Identify genomic locations of yield quantitative trait loci (QTL or genes) and determine the parental source of positive alleles.
2. Validate yield QTL by evaluating their effect in other soybean breeding populations.
3. Identify and sequence yield genes, determine their function, and deploy appropriate transgenes.
4. Identify additional genetic variation for drought/heat tolerance.
5. Develop drought/heat screening protocols.
6. Discover QTL associated with drought tolerance.
7. Sequence drought/heat tolerance genes, determine their function, and deploy appropriate transgenes.

### B. Seed Composition

1. Develop genetic resources and prototype germplasm to meet objectives of the Better Bean Initiative.
2. Characterize the molecular basis for changes in seed composition.
3. Identify and quantify the effect of environment on seed composition and the impact of altered seed composition on agronomic performance.
4. Determine the value of altered genotypes on human and animal performance.

### C. Pest and Disease Resistance

1. Conduct a comprehensive evaluation of elite and exotic germplasm collections for new pest resistance genes.
2. Develop more efficient strategies to evaluate pest resistance.
3. Identify the molecular, cellular, and organismal bases of host-pathogen interactions.
4. Identify and evaluate novel genes for resistance.
5. Improve durability of pest resistance.

## II. INTRODUCTION

Presently the annual increase in yield of soybean in the United States is one-half bushel per acre. At least half of this increase is attributable to genetic improvement through breeding. As good as this accomplishment is, the industry must continue or even accelerate to keep U.S. soybean production globally competitive and to meet the demands of an increasing world population. Additionally, new traits will need to be incorporated into new varieties. These include tolerance to climatic extremes, which may occur more frequently due to human activity; resistance to emerging diseases, nematodes, and insects; and modified seed composition. Achieving these goals will require a broader gene pool. Genetic diversity is the basis of a sustainable agriculture and future improvements in soybean production. The genetic base of present soybean varieties in the United States is very narrow. Currently grown U.S. soybean varieties derive more than 70% of their genes from seven crosses that were made with nine unique parents. Soybean breeders have been extremely successful in exploiting this narrow germplasm base and this should continue. In addition, it is imperative that new genes for soybean improvement be identified and be made available so they can be readily incorporated into germplasm for soybean improvement.

The National Plant Germplasm System, operated by the United States Department of Agriculture, includes the soybean collection at Urbana, Illinois. This collection contains nearly 16,000 introduced accessions of *Glycine max* (domesticated soybeans), 1,100 accessions of *Glycine soja* (a close relative), and 1,000 accessions of several perennial *Glycine* species (more distant relatives). These genetic resources play a major role in soybean improvement and serve as the basis for introduction of new genes to improve productivity, crop quality, and to increase resistance to diseases, pests, and stresses imposed by natural environments. Since 1992, nearly 3,000 accessions have been added from China and several hundred lines have been added from North Korea, Vietnam, and Indonesia. Recent studies have shown that these Asian accessions have many desirable genes not currently in North American soybean varieties.

Developing and maintaining markets for U.S. soybeans is a high priority. Soybeans may lose market share to alternate sources of low-saturated vegetable oils if soybean oil composition is not improved. Concerned to maintain soybean oil is higher in saturated fatty acids, a health concern of many consumers. This may increase if, as expected, FDA guidelines are approved for the inclusion of trans-isomers as a part of total saturated fat on food product labels. Trans-isomers are produced during hydrogenation of vegetable oil, which reduces linolenic acid levels. Without hydrogenation, soybean oil is not stable when subjected to high temperatures, such as in frying applications. High levels of trans-fatty acids also are a health concern of many consumers. In addition, demand in both food and feed markets is increasing for higher protein soybean meal with greater digestibility, total metabolizable energy, and enhanced functionality in vegetable food applications. New intensive soybean production practices implemented throughout the United States during the past half century cause increased pressure from many disease pathogens, nematodes, and insects: with increasing soybean acreage, soybeans now appear more frequently in a given field; soybeans are grown with reduced tillage and frequently in higher plant densities; and new, more virulent genetic variants of already important pathogens and nematodes cause increased incidence of disease. As a result, formerly minor pathogens and nematodes are becoming increasingly important because they cause substantial yield loss. These are compelling reasons to continue searching for new sources of host-plant resistance.

The time lines identified subsequently in this report refer to time required to accomplish the science. Development of improved varieties based on these findings may require an additional five to seven years.

## III. PRIORITY RESEARCH TO INCREASE GENETIC YIELD POTENTIAL

The U.S. soybean grower's rapid adoption of improved genetics (new varieties) has contributed significantly to the continued climb of U.S. average soybean yields. Although previous attempts to mine exotic germplasm for yield genes has met with mixed results, the availability of new molecular tools and germplasm combined with private/public-sector cooperation in field evaluation and use of winter season soybean nurseries should allow for systematic identification of yield genes to enhance the rate of genetic improvement of soybean varieties. Another way to address increased productivity is to reduce yield losses from abiotic stresses (from environmental extremes). Current technology and new germplasm from Asia may allow development of varieties with improved performance when grown under sub-optimal environmental conditions. In the United States, drought and associated heat stress reduce soybean yields more than other abiotic stresses. Although other stresses are regionally important and justify research, e.g., iron and aluminum tolerance, flooding tolerance, and cold tolerance, the primary goal of research to reduce abiotic stress is the development of the genetic resources that will enhance the drought/heat tolerance and environmental stability of new varieties. Successful completion of this research will result in germplasm with enhanced yield and stress tolerance for use in development of elite varieties. This research will enhance breeding efficiency and maintain or accelerate genetic improvement in seed yield under both optimal and sub-optimal production conditions.

### A. Identify Yield Quantitative Trait Loci (Genes)

Yield is a complex trait involving many genes, each with a small effect. The genes conditioning a complex trait, such as yield, are described as quantitative trait loci (QTL). Robust field evaluation for seed yield must be combined with DNA marker analysis to identify genomic locations of yield QTL, and to determine the parental source of the positive alleles. The search for yield genes should include populations derived from matings of modern U.S. varieties with modern Asian varieties and exotic plant introductions. Public and private collaboration will allow yield testing of these populations in a wide range of environments. Another important approach is the application of powerful genomic surveys with recently developed DNA markers to determine the genetic differences between ancestral lines and current varieties (e.g., grandparents, parents, and offspring). This research will identify ancestral alleles that were preferentially fixed by selection in modern varieties.

#### TIME LINE: 3-5 years

### B. Validate Yield QTL

Newly identified yield QTL should be confirmed by evaluating their effect in other populations. In addition to verifying the magnitude of the QTL's breeding value and interaction with other yield genes, its stability in other genetic backgrounds should be determined. This will provide proof of concept prior to investing resources to introduce a yield QTL into multiple elite varieties. **TIME LINE: 5-10 years**

### C. Sequence Yield Genes, Determine Their Function, and Deploy Transgenes

Once QTL affecting yield have been established, it will be important to identify and sequence the genes causing the effects with the ultimate goal of relating allelic sequence with function (determine gene function). Definitive identification of yield genes is a scientifically challenging feat, which is yet to be accomplished. Nonetheless, it is critically important because they permit the discovery of alternative and superior forms (alleles) of these genes in the USDA Soybean Germplasm Collection and to guide the design or engineering of new genes for increased seed yield. Introgression of useful transgenes is another approach to increase yield. Using genes from other species or modifying genes in soybeans that can increase important plant functions should be evaluated for ability to increase yield. **TIME LINE: 5-10 years**

### D. Identify Additional Genetic Variation for Drought/Heat Tolerance

Some progress has been made in identification of plant introductions with slow wilting and drought tolerance in the later maturity groups. However, there has been only limited evaluation of plant introductions in the earlier maturity ranges. Because drought stress limits yield of soybeans in all production areas in the United States, there is a need to identify drought tolerant improved germplasm across a range of maturities. These lines may possess unique mechanisms of drought and heat tolerance. **TIME LINE: 3 years**

### E. Develop Drought/Heat Screening Protocols

One of the major limitations to developing new varieties with improved drought and heat tolerance is the lack of adequate methods for accurately screening large amounts of germplasm. Development of greenhouse, growth chamber, and laboratory methods should be examined. Prior to application, these methods will require validation for ability to predict field stress tolerance. **TIME LINE: 3 years or less**

### F. Discover QTL Associated with Drought Tolerance

This research will combine the earlier outlined approach for discovery of yield QTL - robust field evaluation for drought tolerance combined with QTL mapping of drought related traits in the newly identified germplasm. This research will identify yield QTLs in elite soybean breeders and physiologists in the public sector and public and private collaboration to achieve the field evaluation of these populations in a wide range of environments. **TIME LINE: 3-5 years**

### G. Sequence Drought/Heat Tolerance Genes, Determine Function, and Deploy Transgenes

Once QTL affecting heat/drought have been established, it will be important to identify and sequence the genes causing these effects with the ultimate goal of relating allelic sequence with function. Like the genes underlying yield QTL, the specific genes responsible for resistance to heat and drought will be difficult to identify. Nonetheless, their identification is needed to discover additional useful genetic variation for stress tolerance in the USDA Soybean Germplasm Collection. Introgression of useful transgenes is another approach that can increase important plant functions should be evaluated for stress tolerance in the laboratory and field. **TIME LINE: 3-10 years**

## IV. PRIORITY RESEARCH FOR SEED COMPOSITION

Recently, the United Soybean Board developed a strategic plan for a Better Bean Initiative. This plan calls for accelerated development of soybeans with enhanced seed compositional traits to maximize human and animal health benefits of food and feed with soybean ingredients. A lower saturated, naturally stable soybean oil substitute for partially hydrogenated soy oil is needed to meet consumer demand. The ultimate goal is an oil with 65-75% oleic acid, less than 3% linolenic acid, and less than 7% saturates, which would reduce trans-fatty acids produced by hydrogenation, and improve oxidative and flavor stability of soybean oil. The Better Bean Initiative also calls for accelerated development of soybean germplasm with superior meal attributes to reduce the negative environmental impact of livestock manure. Phosphorus and nitrogen excreted by livestock is a growing concern. Proposed meal improvement includes enhancing amino acid balance and reducing indigestible carbohydrates, increasing bioavailability of amino acids, and decreasing phytate phosphorus in soybeans. The current amino acid targets suggest increased methionine and cystine for poultry rations and increased lysine for swine rations. The initiative also calls for research to determine the impact of agronomic practices, handling, and processing on targeted oil composition; and for the development of methods for timely measurement of value-added characteristics of the Better Bean at first point of sale.

### A. Develop Genetic Resources and Prototype Germplasm to Meet Objectives of the Better Bean Initiative

Innovative genetic approaches are needed to improve soybean oil and protein utilization to maintain or expand the soybean market share. Although many of these traits may already exist in the private sector, it is important that they also be available in the public domain. Germplasm should be identified or created through traditional or transgenic methods. This material will establish the genetic stocks required to achieve the modification of soybean oil and protein composition to meet the objectives for the Better Bean Initiative and to meet additional needs for specific end users.

The primary target compositions for soybean oil are:

- i) A low-saturated substitute for partially hydrogenated soybean oil. Such an oil should ultimately contain 65-75% oleic acid, less than 7% total saturated fatty acids, and less than 3% linolenic acid. **TIME LINE: < 3 years**
- ii) A low trans-fatty acid substitute for hydrogenated base stocks. **TIME LINE: 3-5 years**
- iii) A high polyunsaturated substitute for use in industrial applications. **TIME LINE: 5-10 years**

The primary targets for improved soy-meal are:

- i) Low phytate, low indigestible carbohydrates. **TIME LINE: < 3 years**
- ii) Ensure greater than 48% crude protein meal. **TIME LINE: 3-5 years**
- iii) Enhance digestibility for specific end users. **TIME LINE: 3-5 years**
- iv) Enhance essential amino acid balance to support nutritional needs of poultry and swine. **TIME LINE: 3-5 years**

Develop analytical approaches to ensure the following:

- i) Rapid and accurate seed constituent analysis for both breeding programs and point-of-sale. **TIME LINE: 3 years**
- ii) Genetic markers for rapid movement of genes into elite germplasm and to preserve the identity of soybeans with altered traits. **TIME LINE: 5-10 years**

### B. Characterize the Molecular Basis for Changes in Seed Composition

To more efficiently create soybeans with improved quality for food, feed, or industrial use, the molecular basis for changes in seed composition must be characterized. Some information describing the inheritance of phytate, fatty acids, amino acids, and carbohydrates have been reported. Additional research is needed to elucidate the genetic basis of oil and protein modification and to determine the genetic basis of seed composition. In addition, more research is required to understand the genetic and metabolic interrelationships among the following seed constituents.

- i) Characterize molecular basis for changes in phytate. **TIME LINE: < 3 years**
- ii) Characterize molecular basis for changes in carbohydrates. **TIME LINE: 3-5 years**
- iii) Characterize molecular basis for changes in oil quality, protein quality (amino acids), and protein quantity. **TIME LINE: 5-10 years**
- iv) Characterize molecular basis for interrelationships among seed constituents, such as protein and oil interrelationships and interrelationships among fatty acid constituents. **TIME LINE: 3 to 5 years**

### C. Identify and Quantify the Effect of Environment on Seed Composition and the Impact of Altered Seed Composition on Agronomic Performance

Because genotype x environmental interactions may impact the altered seed compositional types, there is a need to assess the differences in response of the altered types versus the unaltered types across environments. This will help determine whether the altered types have wide or narrow adaptability and whether they have utility as commodity-wide varieties or if they will be more suited for specialty or contract production. Two strategies should be used. The first is to grow the altered and unaltered types over a wide range of environments (years and locations across the USA) employing the range of cultural practices used by U.S. soybean producers. Based on the initial results, the second strategy should look at specific factors that account for the major aspects of the genotype x environment interactions.

### TIME LINE: 3-10 years depending on trait

### D. Determining the Value of the Altered Genotypes for Human and Animal Consumption

Ultimate consumers of soybeans should test the genotypes developed with altered traits. For humans, the emphasis should be on the functionality, utility, quality, and nutritional aspects. For animals, feeding trials should be conducted on poultry and swine to determine and quantify the effect on animal performance. These studies will provide information on the utility of these improved germplasm and should serve as the basis for additional economic analysis. This information should be used to assess the market potential. **TIME LINE: 3 to 5 years depending on trait**

## V. PRIORITY RESEARCH ON PEST AND DISEASE RESISTANCE

Pathogens (such as nematodes and microbial agents) and pests (including insects and weeds) cause significant economic losses if uncontrolled. Soybean breeders have been highly successful in incorporating resistance to some of the major pathogens for which resistance genes are known. Today, genetic resistance is the primary tool used by producers for managing these pathogens and preserving soybean profitability. A few diseases, such as bacterial blight and pustule and northern and southern stem canker, are now well controlled that they are now rarely seen by producers. Other pathogens, such as *Heterodera glycines* (soybean cyst nematode) and *Phytophthora rot*, are the most important constraints on soybean breeders because they must select varieties with resistance to the prevalent races in their region. The durability of resistance to all pathogens and pests must be increased so that new races are not successfully breaking the available resistance in soybeans. Insects can produce substantial losses in soybeans, particularly in the southern U.S.A. Defoliation damage is produced by several species of Lepidoptera in the family Noctuidae, whereas stinkbugs (Hemiptera: Pentatomidae) can cause significant seed injury. No insect resistant varieties with competitive yield and other requisite qualities have been developed despite breeding efforts spanning 30 years. Thus, implementation of insect resistance is a major challenge that still confronts soybean breeders. Use of transgenic and DNA marker technologies should accelerate achievement of this goal. Separate breeding approaches for defoliators and stink bugs may be required.

Additionally, resistance genes do not exist or are unknown for a number of pests and pathogens. These include pathogens of regional significance, such as *Sclerotinia*, viruses, *Macrophomia phaseolina* (charcoal rot), *Septoria* brown spot, and *Meloidogyne spp* (root knot nematodes). In addition, new soybean pests and pathogens are emerging as economic threats. In the U.S.A., pathogens such as *Fusarium solani* (cause of sudden death syndrome) and *Sclerotinia sclerotiorum* (white mold) are increasing in importance. Pathogens, such as *Phakopsora melibioniae* (soybean rust) and *Dactylochaeta glycines* (red leaf blotch), cause serious disease loss in other soybean production regions of the world. The need to control these pathogens must be anticipated, even though they are not yet in the United States.

The identification of resistance genes is critical in both conventional and molecular soybean research. Soybean researchers are increasingly recognizing that soybean yield depends not only on host genetics, but also on those of the pathogens and pests.

### A. Comprehensive Evaluation of Elite and Exotic Germplasm Collections for New Pest Resistance Genes

The extent of disease damage depends on the genetics of both the soybean varieties and the pathogens; disease is an *interaction* between the two organisms. Considerable Federal, state, and private industry resources have been expended to protect diversity in soybean germplasm and to utilize it for genetic improvement of adapted varieties. In contrast, the preservation and assessment of diversity in soybean pathogens have been largely dependent on individual scientists. An extensive, genetically diverse collection of soybean pathogens is essential for identifying novel genes for resistance in soybean, understanding pathogen genomics, and improving disease resistance.

Genes for resistance to soybean cyst nematode (SCN) and phytophthora root rot have been incorporated into adapted soybean varieties. However, the threat of new biological isolates of SCN or *Phytophthora rot* as well as emerging diseases such as white mold and SDS are problems that need immediate attention. To ensure continuing resistance, the USDA Soybean Germplasm Collection and other collections of the world must be evaluated for new sources for use in breeding programs for pest resistance. **TIME LINE: 3 - 5 years**

### B. Develop More Efficient Strategies to Evaluate Pest Resistance

Consistent, reliable, and cost effective systems to assay soybean resistance need to be developed for many soybean pests. Sources of uniform, genetically defined strains of pathogens, nematodes, and insects need to be made available for soybean breeding programs. An important component of pest assay systems is the capability to rapidly evaluate many segregating lines. These systems may be most efficiently accomplished in a laboratory or greenhouse. Full-season screening of lines in protected or natural environments that are artificially or naturally inoculated with the pest may be the optimal evaluation system for certain pests/pathogens. Adequate containment protocols need to be developed in situations where resistant strains of pathogens/nematodes/insects are assayed. Development of molecular assay systems, including the use of high throughput marker-assisted selection, are important new technologies that need to be adapted for rapid pest resistance assays.

Funding for maintenance of pathogen/nematode strains in culture is needed so that they can be made available as needed for soybean breeding programs. For contemporary, *Sclerotinia* spp and *Phytophthora* spp strains are particularly important pathogens for soybean breeding and pathogen diversity objectives. Culturing of green, southern green, and brown stinkbugs, soybean looper, velvetbean caterpillar, corn earworm, bean leaf beetle, and Mexican bean beetle should be supported at strategic laboratories so that sufficient numbers can be made available as needed for soybean breeding. **TIME LINE: < 3 years**

### C. Identify the Molecular, Cellular, and Organismal Bases of Host-pathogen Interactions

To understand the complexities of signaling and interactions among host and pest genomes, initial research should be based on association studies between host germplasm resistance/susceptibility characteristics and pest germplasm virulence/avirulence characteristics.

- i) Molecular marker association studies based on pedigrees and segregating populations should be conducted to identify genomic regions (QTL) of resistance/susceptibility and virulence/avirulence phenotypes. **TIME LINE: < 3 years**
- ii) Candidate genes for resistance/susceptibility and virulence/avirulence from QTL regions and EST libraries should be identified. **TIME LINE: < 3 years**
- iii) Resistance/susceptibility or virulence/avirulence phenotypes should be associated with allelic sequence variants. **TIME LINE: 3-5 years**
- iv) Gene expression (messenger RNA, protein, metabolites) profiles should be associated with phenotypic variants for resistance/susceptibility and virulence/avirulence. **TIME LINE: 3-5 years;** and signal transduction pathways should be constructed from mRNA, protein and metabolite profiles. **TIME LINE: 5-10 years**
- v) Expression profiles should be compared with signaling pathways in the host and pathogen. **TIME LINE: 5-10 years**
- vi) Develop bioinformatic tools to facilitate association studies. **TIME LINE: < 3 years**

### D. Identify and Evaluate Novel Genes for Resistance

Molecular biology techniques will make it possible to modify resistance genes of soybeans to make them more effective for pest control and to transfer pest resistance genes from other organisms into the soybeans. Research currently is underway in soybeans to identify the DNA sequence of genes that confer resistance to the soybean cyst nematode, *Phytophthora rot*, and other pests. After the DNA sequence is identified, it should be engineered by molecular techniques to develop forms of the gene that are more effective for pest control. Similar research in other organisms will provide novel genes that can be transformed into soybeans and evaluated for their effectiveness. For example, the identification of genes for formation of the enzyme oxalate oxidase in other organisms has been transferred to soybeans and is being evaluated for its effectiveness in controlling white mold. The insertion of insecticidal genes for insect resistance has been accomplished and research activities should be increased in the future. **TIME LINE: 3-5 years**

### E. Improve Durability of Pest Resistance

Historically, resistance to pathogens, insects and nematodes has limited longevity due to genetic variability within these pests. Planting a resistant variety exerts selection pressure on the pest population, resulting in populations that can overcome resistance in soybeans. Research priorities in this area include:

- i) Identify and deploy specific genes in soybeans that overcome virulence genes in pathogens or pests. Develop effective rotation of varieties with different sources of resistance genes. **TIME LINE: 3-5 years**
- ii) Stacking or pyramiding of more than one gene for a specific pathogen or pest or groups of pests should be pursued. **TIME LINE: 5-10 years**
- iii) Engineered genes that affect the most vulnerable part of the pest life cycle should be targeted for development. **TIME LINE: 10 years or more**

## APPENDIX A

On February 23<sup>rd</sup> and 24<sup>th</sup>, 2000, twenty-two expert researchers with knowledge of plant breeding, plant physiology, plant pathology, entomology, nematology, molecular biology, functional genomics, and seed composition participated in a two-day workshop hosted by the United Soybean Board Production Committee. The workshop was planned by: Dr. Dwayne Buxton, National Program Leader for the Agricultural Research Service in Oilsseeds and Bioscience; Dr. Roger Boerha, Research Professor and Coordinator of the University of Georgia Center for Soybean Improvement; Maureen Kelly of AgSource, Inc., a soybean producer with the United Soybean Board focusing on Federal Research Coordination; and Kent Van Amburg, Production Committee Manager for the United Soybean Board of Smith, Bucklin and Associates. Elizabeth Vasquez of MCA Consulting facilitated the workshop.

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