

SoyCAP: Roadmap for Soybean Translational Genomics.

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Executive Summary

Soybean is the most valuable legume crop, with numerous nutritional and industrial uses due to its unique chemical composition. Recently, a group of legume specialists voted soybean as the 'representative species' of the phaseoloid legumes, the group that contains most legume crop species. Elucidating the genome sequence of soybean was chosen as the top priority. Through a series of past workshops and discussions, the soybean community developed and refined a cogent plan for soybean genomics. Many of the preliminary goals identified have already been accomplished. Thus, soybean is well positioned as a key model for translational genomics.

With this in mind and with funding from the USDA National Research Initiative, a SoyCAP planning conference was held in St. Louis, MO, from December 16-17, 2004. A largely elected steering committee organized the meeting, which was attended by 30 soybean experts, as well as observers representing industry, commodity groups and the USDA. Attendance at the conference was purposely slanted toward soybean breeders, since it is this group that will ultimately develop improved soybean germplasm, aided by genomic tools and information. Over a two-day period, with much give and take, the following three objectives, in order, were chosen as representing the most critical needs, as well as the areas to which translational genomics could be effectively applied:

- I. **Biotic Stress:** Efforts should be mounted to develop breeder-friendly molecular markers to identify QTL regions encoding resistance to a variety of soybean diseases and pests. A special focus should be placed on developing markers to aid breeders in developing soybean germplasm with decreased susceptibility to Asian soybean rust. Finally, transgenic approaches should be considered to develop soybean germplasm resistant to priority pathogens and pests, especially in those cases where genetic resistance has proved elusive.
- II. **Seed composition:** Breeder-friendly molecular markers also should be developed towards seed target traits, in order to increase seed protein quality and quantity, without reducing seed yield or oil. Given the growing importance of biodiesel and the importance of oil quality in nutrition, efforts should be made to develop markers to allow breeders to engineer soybean with optimal oil composition. Finally, since soybean farmers are paid based on soybean seed yield, there is a need to broaden the soybean genetic base and foster technological innovations for sustainable yield improvements.

- III. **Abiotic stress:** Environmental stress is among the leading causes of soybean yield reduction. Drought, iron limitation, heat and cold stress, and flooding were identified as key areas in need of additional research with the goal of developing breeder-based tools for soybean improvement. Among these, increased drought tolerance was chosen as the top priority for soybean improvement through translational genomics.

In addition to these research priorities, participants at the SoyCAP planning conference also set priorities with regard to information transfer, outreach, education and extension. Perhaps most notable, the participants called for the development of a novel, Web-based “**Soybean Breeder’s Toolbox**”. This resource is envisioned as an interactive database to deliver genomic and genetic information in a manner targeted for and conducive to breeding and crop improvement strategies. The Toolbox also will play an educational role.

Specific timelines were set to achieve the consensus goals identified. A management plan was developed, as well as policies on public release of information and intellectual property. A coordination committee was chosen and charged with preparing a SoyCAP grant proposal following the guidelines set during the planning conference.

Introduction and Rationale

The advent of genomics is resulting in an in-depth understanding of plant biology and the development of an unprecedented number of tools for molecular biology. Yet, the impact of genomics on major crop species remains limited (Thro et al., 2004).

The concept of “Translational Genomics” is emerging in recognition of the need to apply genomics tools to plant improvement. The Webster dictionary states that “translation implies the rendering from one language into another”, while ‘genomics’ is generally considered the use of high-through-put methods to study genomes—both form and function. Therefore, translational genomics implies going from the language of genomics to that of breeding, or in other words, the direct application of genomic resources to make plant breeding programs easier and more efficient.

A practical system for translational genomics will require that traits handled by breeders all be mapped and tagged with DNA markers that lend themselves to easy assays and automation, such as SNPs (single nucleotide polymorphisms) and SSRs (simple sequence repeats). Furthermore, a breeder must have user-friendly access to this information. Ideally, any breeder could go to a website, find the traits s/he wishes to breed, the germplasm source of desired alleles, and the necessary molecular markers to use. In addition, such a site would have instructions, not only for the use of the markers, but on how to conduct any necessary phenotypic assays—such as for protein content or resistance to a given pathogen. In essence, the breeder must have access to a “**Breeder’s Toolbox**” that will seamlessly integrate genomics and breeding information together. This paper describes a strategic vision towards the development and deployment of such a **Breeder’s Toolbox** for soybean.

Translational genomics and legumes

To date, considerable attention has focused on two model legumes, *Medicago truncatula* and *Lotus japonicus* (reviewed in Young et al., 2003). Genome sequencing efforts are currently underway in both of these species. Yet, the legumes are the third largest

family of angiosperms, behind only orchids (Orchidaceae) and asters (Asteraceae). In form, habitat and in every other character, legumes far outstrip these other groups in total diversity (Doyle and Luckow, 2003). Therefore, it is likely impossible to focus on only a few legume species that would serve as omniscient "models" for all legumes. Though *M. truncatula*, *L. japonicus*, and soybean (*Glycine max* (L.) Merr.) are members of the papilionoid subfamily, the group that contains nearly all crop legumes (Doyle and Luckow, 2003), *Medicago* and *Lotus* fall within the mainly temperate Hologalegina lineage, while soybean is a member of the generally tropical phaseoloid group.

Experts on a variety of legume systems met December 14-15, 2004 in Santa Fe, NM, for a conference entitled "Cross-Legume Advances through Genomics" to draft a research plan for comparative legume genomics. A key recommendation of this conference was that soybean should be targeted for extensive genome sequencing as the 'representative species' of the phaseoloid legumes.

Soybean is the most valuable legume crop, with numerous nutritional and industrial uses due to its unique chemical composition. With its high protein content (40%) and moderately high oil (20%), the soybean seed is the world's main source of vegetable protein and oil, accounting for 55% of all oilseeds produced. In 2004 soybeans were grown on over 74 million acres in the U.S., and the crop was valued at over 17 billion dollars (USDA.NASS). The enormous agronomic importance of soybean, coupled with the development of modern molecular biology, has led to an increasing level of activity to develop soybean genomics. Numerous collaborative public research endeavors targeted towards soybean genomics have been supported by the United Soybean Board (USB), North Central Soybean Research Program (NCSRP), state soybean and promotional councils, along with panels within NSF and USDA. These efforts by the public soybean research community have led to substantial progress in the areas of molecular marker development, expressed sequenced tag (EST) databases, BAC end sequences, microarrays and efficient genetic engineering capabilities (Stacey et al., 2004). A physical map for cv. Forrest was completed with NSF support (Wu et al., 2004, 2004a). The physical map of cv. Williams 82, developed with USB funding, is nearing completion (unpubl.). Thus, soybean is poised for the genome sequencing effort recommended in Santa Fe but, more importantly, is also positioned as a key model for translational genomics.

Efforts to organize the soybean community and set priorities.

The U.S. soybean research community has worked to develop and refine a cogent plan for soybean genomics. A series of workshops was held, starting with the first in October 21-22, 1999, to identify key goals. This workshop was hosted by the United Soybean Board (USB) Production Committee and is indicative of the continuing support that soybean has received from the various commodity groups. A white paper was produced as a result of that meeting and laid out consensus priorities for molecular markers, transformation and structural/functional genomics. A second workshop, again sponsored by USB, was held on February 23rd and 24th, 2000, involving twenty-two expert researchers with knowledge of plant breeding, plant physiology, plant pathology, entomology, nematology, molecular biology, functional genomics, and seed composition. Over the course of two days, a consensus was reached on priorities and time frames needed to conduct the agronomically related research on soybean genetic enhancement (http://129.186.26.94/Genetic_Resources/Soybean_Genetic_Resources.html)

Subsequently, a U.S. Legume Crops Genomics Workshop was convened on July 30-31, 2001, in Hunt Valley, Maryland. This meeting was hosted by the United Soybean Board, National Peanut Foundation, USA Dry Pea and Lentil Council, and the USDA-ARS. Twenty-six scientists participated and a white paper was developed

(http://129.186.26.94/Legume_Initiative/LegGenomicsPaper10Oct01.html). This meeting differed from the 1999 meeting in taking a broader view and focusing on genomic needs for all legumes. An important development arising from this workshop was the organization of the U.S. Crop Legume Genome Initiative and the establishment of a steering committee composed of representatives of the major legume commodity groups, as well as scientists representing each of the major legume crops. A key recommendation of this workshop was the goal of complete genome sequencing of the model legume, *M. truncatula*. As noted above, an international consortium is now pursuing this objective.

Sequencing of *M. truncatula* and targeted sequencing of soybean were two recommendations echoed in a report of the Workshop on the National Plant Genome Initiative: 2003-2008 held on June 6-7, 2002, and sponsored by the U.S. National Academy of Science (<http://books.nap.edu/books/0309085292/html/R1.html>).

In 2003, USB again hosted a soybean genomics workshop with a white paper developed that outlined the current status of soybean genomics and identified potential goals for resource development. A subcommittee appointed during the workshop produced a short document that laid out a proposed consensus path for the soybean scientific community, U.S. Crop Legume Genome Initiative and a soybean resource development initiative. This and the various preceding workshops, due to the status of soybean genomics at the time they were held, focused primarily on the technological and resource needs of soybean genomics. Therefore, funds were sought from the National Science Foundation to host a subsequent workshop in 2004 that focused primarily on the biological opportunities for soybean and the unique opportunities that exist for soybean research. A white paper was developed from this meeting and subsequently published (Stacey et al. 2004). Another notable development was the establishment of a Soybean Genetics Executive Committee (SoyGEC) in the summer of 2003, whose mission is to serve as a communication focal point for the development of soybean research priorities and as a liaison between the soybean research community and granting agencies. Information on SoyGEC members and actions may be found from a link on the SoyBase web page (<http://129.186.26.94/>).

Unique opportunities in soybean.

The soybean is a member of the tribe Phaseoleae, the most economically important of the legume tribes. Other legumes within the tribe include pigeon pea, common bean, lima bean, tepary bean, winged bean, cowpea, mung bean, black gram, adzuki bean, and Bambarra groundnut (Hymowitz and Singh, 2004). The extensive genetic resources of soybean and the associated physiological tools available for soybean present a set of unique opportunities to study everything from seed development to the biology of polyploidization to a considerable array of pathogenic and symbiotic plant-host interactions. The large plant size of soybean is an advantage for such studies, permitting the use of techniques not easy or possible with smaller plants.

Soybean is paleopolyploid, with $2n = 40$ as its base chromosome number with an estimated genome size of 1.1 Bbp/C (Arumuganthan and Earle 1991). This makes it about seven and one-half times larger than the genome of *Arabidopsis* but less than half the size of the maize genome (Arumuganthan and Earle 1991). The diversity of ploidy within legumes and the extensive level of genome duplication in soybean make it an excellent model for comparative studies of genome evolution (Stacey et al., 2004).

Large and accessible soybean germplasm collections, exhibiting broad phenotypic diversity, are maintained by USDA. The Soybean Genetic Type Collection maintains over 300 phenotypic mutants (Palmer et al., 2004) encompassing a broad array of traits. Perhaps most importantly, there is a wealth of mutants in key metabolic

enzymes (Palmer et al., 2004), which has long made soybean a model for plant metabolism.

Stacey et al. (2004) outlined the wide variety of genetic and genomic resources available for soybean that can facilitate ‘translational genomics’. This list includes a well developed genetic map (encompassing 20 linkage groups and over 2500 cM ; Soybase; <http://129.186.26.94/>), a wide variety of mapping populations, bacterial artificial chromosome (BAC) libraries (>33-fold coverage), physical maps (both Forrest and Williams 82), over 300,000 soybean expressed sequence tagged (EST) sequences in Genbank (<http://129.186.26.94/soybeanest.html>), a variety of functional genomics tools (including DNA microarrays and proteomic resources), an efficient system for genetic transformation and regeneration (e.g., that can be applied for transposon tagging), and, perhaps most importantly, an enthusiastic and capable soybean research community.

Soybean Coordinated Agricultural Project (SoyCAP)

As described above, the soybean community has worked hard to organize its genomic efforts and define priorities, and has achieved many of these objectives. Previous efforts did an excellent job in identifying the technology and experimental resources needed to conduct basic soybean genomic research. Because of this, soybean genomics experienced a rapid period of growth and discovery. However, as with other crops, this work has been largely the purview of genomicists, couched within the language of genomics, with little translation to the breeding community. Given the excellent foundation already laid for soybean genomics and genetics, a pertinent key question becomes: what is the consensus view of how this developing resource should be further developed and applied to practical benefit? How can we translate between the language of genomics and soybean breeding?

With funding from the USDA National Research Initiative, a SoyCAP planning conference was held in St. Louis, MO on December 16-17, 2004. Planning for this conference began much earlier with submission of the request for funding and then a public discussion at the 2004 Molecular and Cellular Biology of Soybean Conference (Soy2004) held at the University of Missouri August 8-11, 2004. A steering committee was established to help plan the conference. In addition to the PIs of the planning grant request, this steering committee was also composed of the 5 standing members of the SoyGEC. An election was held of the ~250 participants at the Soy2004 meeting to elect two soybean breeders and two non-breeders to the steering committee. After the Soy2004 meeting, an additional internet election was held strictly among the soybean breeding community to elect additional members to the steering committee. Thus, the great majority of members on the steering committee were chosen by direct election from the soybean community. Moreover, the steering committee purposefully targeted majority membership by soybean breeders to facilitate effective translational genomics. One of the first actions of the steering committee was to establish a SoyCAP website (<http://digbio.missouri.edu/soycap/>) and a list server to serve as a conduit for community discussion about SoyCAP. Information about SoyCAP, the website and list server was disseminated through a series of mailing lists that targeted University, government, and industry soybean researchers, soybean breeders, and extension agents.

A series of conference calls was held by the steering committee to set the overall agenda of the meeting. Additional discussions led to a list of invited participants, which was subsequently augmented by individuals who provided special expertise and/or desired participation due to applicable research interests. The net result of these activities was the hosting of the meeting that was attended by over 30 scientists (see list

attached). Breeders were well represented. The major soybean industries were represented, as well as USDA-ARS and the United Soybean Board.

The meeting was held over two days with the first day focused on the identification of key goals in three target areas (biotic stress, abiotic stress, and seed traits). These three areas were judged by the steering committee as those most limiting soybean production and value. This consensus view was also mirrored by the ad hoc discussions and postings to the SoyCAP list server. The subcommittees for these three groups met again on the second day to refine the identified goals and develop a specific research plan to address them. In addition, a subcommittee tackled the issues of bioinformatics, education, outreach and extension. During the discussions, it also became clear that issues related to intellectual property and data release should be addressed, and an ad hoc subcommittee was constituted to address those needs. Finally, all participants met to discuss the subcommittee reports and to set overall priorities. After much discussion and give and take, it was decided that the priorities, in order, were the first three objectives listed below. The fourth objective, dealing with information transfer, was considered of equal priority to Objective 1.

Overall the objectives identified were:

- I. **Biotic Stress**
- II. **Seed Traits**
- III. **Abiotic Stress**
- IV. **Information transfer**

At the close of the meeting, a coordinating committee was chosen and instructed to prepare a full grant proposal for submission to USDA-NRI for a SoyCAP project. Additional follow-up discussions were conducted during the Plant and Animal Genome Conference, held January 15-20, 2005, in San Diego, CA and at the annual Soybean Breeders' Workshop, to be held February 21-22, 2005 in St. Louis, MO. In addition the SoyCAP list server remains functional to provide for soybean community feedback.

Specifics of the roadmap for soybean translational genomics.

We realize that the projects listed here far surpass the funding available through SoyCAP. Nevertheless, we are convinced that efforts funded by SoyCAP should be part of, and consistent with, a larger, coordinated plan for soybean genomics.

Objective 1. Biotic stress

When first introduced into the U.S., soybeans were grown with relatively few problems from pathogens and pests. However, as soybean acreage increased over the years, the crop "matured" and is now host to numerous pathogens and pests. Soybean cyst nematode and *Phytophthora sojae* are the primary cause of yield loss in most of the soybean production regions followed by charcoal rot, virus diseases, sudden death syndrome, seedling diseases and a mix of leaf blights, stem rots, and other nematodes (Wrather et al., 2003, Grau et al., 2004). In addition to these soybean diseases, eight major insect pests also contribute to yield losses due to feeding and damage to seed quality. This damage is more consistent in the southern U.S. and sporadic in the Midwest. A new insect threat has emerged with the newly introduced soybean aphid in the Midwest, where insecticide applications now are becoming a routine practice.

Asian soybean rust, a disease that causes serious losses in many parts of the world, was first detected in the continental United States in November 2004. Soybean rust is caused by the fungus, *Phakopsora pachyrhizi*. Long known to occur in Asia, the

fungus spread to Zimbabwe, South Africa, Paraguay, Brazil, Columbia, and now the U.S. during the last 10 years. Yield losses in other parts of the world due to soybean rust are reported to range from 10-90%. Annual yield losses for U.S. soybean production are predicted to be at least 10% in the upper Midwest and Northeast, and 50% or greater in the Mississippi Delta and Southeastern states. However, losses in hard-hit areas anywhere in North America could exceed 80% if effective management tactics are not deployed. Management costs are expected to be high. Current U.S. cultivars are universally susceptible to Asian soybean rust. Four dominant alleles at loci *Rpp1-Rpp3* (Hartwig and Bromfield 1983) and *Rpp4* (Hartwig 1986) that condition resistance to specific races of the rust were identified in unimproved soybean germplasm. None of these resistance alleles has been incorporated into production cultivars. Therefore management will rely on fungicide applications. Current cost estimates range from \$10 to \$60 per acre depending on the material and number of applications needed to manage this disease. At current prices (~\$5.00/bu) chemical control measures will have a very negative impact on many U.S. soybean producers. Soybean cultivars with partial resistance to soybean rust are needed to reduce the rate of the epidemic and reduce the number of required fungicide applications. As is the case with wheat leaf rust and common corn rust, two similar diseases where partial resistance often reduces disease and decreases the need for fungicide usage, soybean germplasm apparently exhibiting partial resistance (slow rusting) has been identified. Along with seeking additional sources of resistance, efforts are needed to incorporate partial resistance into well adapted U.S. soybean cultivars.

With the emergence of soybean rust as a backdrop, participants at the SoyCAP planning conference established the following five priorities during the first day of discussions:

1) Development of tools for breeders to identify and maintain resistance to pathogens and pests in the threat of soybean rust. Targeted markers are required for concise introgression and maintenance of resistance QTLs or genes to biotic stresses, especially as public and private breeding programs scramble to incorporate resistance to Asian soybean rust in their germplasm pools. History has shown that when a trait (e.g., glyphosate herbicide resistance) is rapidly incorporated into the U.S. germplasm pools, resistance traits are inadvertently lost if a specific effort is not made to maintain them. In addition, soybean has several R-gene and QTL clusters to biotic pathogens already identified and mapped in the soybean genome. It is highly likely, based on other host-pathogen systems, that QTLs for the slow rusting (partial resistance) phenotype for soybean rust may map to these regions as well. Thus, a high priority should be placed on SNP development in those regions where resistance to plant pathogens is well documented.

2) Evaluate association mapping as a means to identify new genes/sources of resistance to biotic stresses. There have been numerous efforts in the past 10 years to identify new genes and sources of resistance to many of soybean's biotic pests. For example over 120 and 400 plant introductions were identified with resistance to SCN and *P. sojae*, respectively. Association mapping is a means to facilitate gene discovery in germplasm pools to identify superior alleles, assess the genetic potential of specific genotypes, and facilitate high resolution genetic mapping (Gebhardt et al., 2004; Thornsberry et al., 2001).

3) Evaluate transgenic approaches to developing resistant germplasm for soybean rust. Classical breeding approaches for development of soybean lines with partial resistance

to soybean rust may not be sufficient to provide high levels of protection for yield. Therefore, we should immediately consider exploring transgenic approaches through gene silencing for critical host/pathogen traits, as well as expression of candidate genes. Other novel technologies (e.g., use of antimicrobial peptides) should also be explored to evaluate their efficacy.

4) Development of a bioinformatics Breeders' Toolbox. Soybean breeders need a readily accessible database that they can use. This resource should allow breeders to easily target information on the sources of resistance and available markers and thus make use of the "vast hordes of genomics tools" currently in existence or under development.

5) Continue and expand facilities for management of genomic resources. Currently, the soybean germplasm collection center at Urbana has 20,608 accessions. However, there is no infrastructure in place or sufficient funds available to maintain soybean genetic stocks, such as mapping populations, TILLING populations, RILs, insertional mutagenized populations, etc. In addition, stocks for four of the major soybean pathogens are also maintained at Urbana for use by companies for screening germplasm. However, there is no master plan for the long-term maintenance of additional soybean pathogens, particularly diagnostic strains used in breeding programs.

The second day of discussions was used to develop a more specific research plan to address these five priorities areas. This plan is outlined below, as well as in other parts of this document (e.g., bioinformatics).

Goal 1: Develop more refined markers for known QTLs for resistance to soybean pathogens and pests.

Evaluate and validate known QTLs based on the availability of populations segregating for these traits. In a preliminary survey of public plant breeding programs, J. Specht (Univ. of Nebraska) identified 31 populations segregating for QTLs for resistance to insect feeding, SCN, brown stem rot, and white mold. In addition, numerous populations also exist which are segregating for resistance to *P. sojae*, soybean viruses, bacterial leaf spot and root knot nematodes. Stakeholders will be surveyed to develop a prioritized list of QTLs to target for SNP development. It was proposed at the NSF-sponsored workshop on Soybean Genomics that the number of markers for the soybean genome be doubled to 4,000 (Stacey et al., 2004). Taking a targeted approach to specific loci of high interest will make this more useful for breeders as they attempt to incorporate resistance to multiple pathogens and pests into high yielding varieties. The approach will be to focus first on those QTLs or regions shown to exist in multiple populations and to be associated with R gene clusters. The expected outcome or target will be confirmed genome positions of QTLs conferring resistance to a variety of important soybean diseases and pests.

The next objective is to utilize genomic information to develop SNPs flanking these QTLs. The approach will be to exploit BAC end sequences, BAC subclone sequences, mapped ESTs and other information to facilitate SNP marker discovery. SNP markers will be mapped into a common mapping population to verify their genome position followed by subsequent validation in populations segregating for specific diseases and pests. The resulting SNP markers can be used to identify resistant breeding lines and at the same time select for reduced linkage drag; thereby, allowing introgression of specific resistance traits into new cultivars with greater accuracy. .

The result will be a validated, practical toolbox of markers for a variety of important soybean diseases and pests.

Goal 2. Evaluate the feasibility of developing markers for rust resistance or partial resistance using existing populations with known resistance to other diseases.

Slow rusting or partial resistance is characterized as reduced area under disease progress curves, which can be the result of fewer lesions developing from standard spore concentrations, longer latent period, decreased sporulation and/or decreased sporulation period. At present, clear sources of slow rusting or partial resistance have not been validated. Approximately 700 soybean plant introductions and 10-15 commercial soybean varieties were identified as promising based on greenhouse inoculations. Field evaluations will be necessary to verify resistance. Once sources of partial resistance are identified, markers for QTLs associated with other pathogens and pests will be examined for correlation with QTLs for partial resistance to soybean rust. Using this approach, validated, practical toolbox markers for rust resistance will be immediately available to the soybean community for rapid incorporation of soybean rust resistance loci into elite lines. In addition, populations with the four known R-gene based resistance loci (*Rpp1→4* genes) will be evaluated with these markers. In some host-pathogen systems, R-genes for different diseases are known to occur in gene clusters.

Goal 3. Utilize and develop new populations segregating for partial resistance to soybean rust.

The focus will be to evaluate the “**Breeder’s Toolbox**” markers to known soybean diseases against populations segregating for partial resistance to soybean rust. In addition, it was judged cost effective to sub-contract to industry high-throughput development of SNP mapping for these sources of resistance. The expected outcomes will be new QTLs and tightly associated markers for new and previously identified loci. This research will further enhance efforts to incorporate this resistance in combination with known resistance to other pathogens and pests.

Goal 4. Association mapping in populations of plant introductions identified with resistance to important soybean diseases.

The specific objective for this goal will be to rapidly identify novel putative QTLs associated with resistance/tolerance to pathogens. The approach will identify populations of plant introductions suitable for association mapping using SSR and SNP marker-based, complete genome scans. The expected outcome of this approach will be the identification of putative novel sources of resistance genes to pathogens, as well as markers for these novel QTLs.

Timeline:

Year 1-2.

Identify QTLs to target for SNP development and develop SNP markers (Goal 1)
Identify and evaluate soybean plant introduction populations suitable for Association Mapping (Goal 4)

Through other projects, identify sources of partial resistance to soybean rust.

Year 3-4.

Validate SNP markers in segregating populations for several plant pathogens.

Incorporate markers into “**Breeders Toolbox**” as they become validated.

If sources of resistance to soybean rust have been identified, then validate markers associated with QTLs for other diseases to determine if these are associated with resistance to soybean rust.

Objective II. Seed Composition

Improvement in soybean protein quantity and quality is an immediate priority for the U.S. soybean industry, which currently faces international competition from expanding production areas in Brazil. Moreover, a current perception in the international market is that the protein quality of South American soybeans is better than that of U.S. soybeans. Rapid applications of translational genomics will help to protect the U.S. soybean industry by targeting improvements in soybean protein quantity and quality. Priority areas of national need include improvements in essential amino acid balance, enhanced protein digestibility of the meal, and reduced environmental impacts of livestock production (Wilson, 2004).

Global demand for soybean meal drives the soybean market because soybean provides nearly 70% of the world's livestock feed protein meals. Soybean provides the most well balanced amino acid composition and the highest level of crude protein compared with other sources of vegetable protein. A wide array of food and nutritional products, such as soy milk, tofu, texturized soybean protein, protein isolates, meat analogs, and energy bars are produced from soybean and soybean meal. Industrial soybean meal products include composite building materials, adhesives, caulking compounds, and pharmaceuticals. An important by-product of meal production is soybean oil, which is extracted from the crushed meal. Soybean oil is utilized worldwide as cooking oils, spreadable margarines, and in other food applications. Soybean oils also provide important industrial applications such as inks, paints, plastics, lubricants, hydraulic fluids, and biodiesel fuel.

The SoyCAP planning conference enabled participants in its 'Seed Composition' subcommittee to establish the following three research priorities during day one:

1. Increase Seed Protein Quality and Quantity without reducing seed yield or oil. Soybean is the world's principal source of vegetable protein. Yet the quality of its protein can be enhanced by improving the amino acid balance, resulting in improved digestibility and nutritional value of livestock feeds and soy foods for human consumption. Moreover, enhanced protein quantity and quality will protect U.S. agriculture by increasing the competitiveness of the U.S. crop in domestic and international soybean markets. Enhancement of germplasm, and genetic, and genomic resources for soybean protein improvement has been endorsed by the Legume Genomics community. This research would provide a bioinformatics system focused on seed protein composition.

2. Increase Oil Quality and Functionality. Current political unrest in the petroleum producing regions of the Middle East and interest in renewable energy sources is prompting more research on biobased products. Biodiesel production is now receiving major national attention. Soybean oil is an important component of biodiesel fuel.

From the standpoint of human health and nutrition, all food processors will be required to list the levels of trans isomers of fatty acids on product nutrition labels by 2006 (Pantalone et al., 2004; Wilson, 2004). Therefore, U.S. processors need an improved quality vegetable oil from a domestic crop, namely soybean, so that the process of hydrogenation (which forms trans fatty acids) can be reduced or eliminated. Other fatty acids, such as the saturated fats, could be reduced to favor health-conscious consumers. Increased monounsaturated fat (oleic acid) is desirable to reduce cholesterol and improve cardiovascular fitness. Increased oleic acid content also would

improve greatly the stability of soybean oil for food and industrial processing applications. In that regard, though transgenic approaches have already been demonstrated to be practical, and additional improvements through transgenic technologies could be readily accomplished, the ability of conventional breeding to alter oil composition has yet to be exhausted, and would be greatly facilitated if the appropriate molecular markers were available.

3. Increase Seed Yield. Soybean farmers are currently paid for the total amount of seed produced and delivered to grain facilities. Given that the U.S. soybean germplasm genetic base is very narrow (Sneller, 1994; Gizlice et al., 1996), a need exists to broaden the soybean genetic base and to foster technological developments and innovations for sustainable yield improvements (Specht et al., 1999). Research would target improvements in genetic gains for seed yield, which would expand opportunities for improving seed composition traits in high-yielding genetic backgrounds.

During the second day of the SoyCAP planning conference, the seed composition subcommittee established the specific goal 'to increase soybean seed protein quantity and quality without decreasing yield' as the greatest priority area, and a plan was developed to target accomplishment of five research objectives:

Goal 1. Clone the linkage group 'I' protein locus.

The expected outcome would be the creation of a perfect marker for soybean protein. Variants of that allele would be detectable in different germplasm. Pleiotropic effects would be identified. The approaches will include positional cloning, and confirmation through transgenic expression.

Goal 2. Determine gene function of the 'I' protein locus.

The expected outcome would be gains in knowledge of protein improvement, and understanding of the impact of the 'I' protein locus on seed yield. Approaches targeting the achievement of this objective will include transcript profiling, proteomics, and metabolomics.

*Goal 3. Utilize the **Breeders Toolbox** to create better protein markers on linkage groups E and H, and define their associations with seed yield.*

The expected outcome would be increased knowledge and understanding for protein improvement in high-yielding backgrounds. Approaches will include SNP development for creation of superior markers, and field phenotyping over multiple environments. We will utilize existing populations known to segregate for protein QTLs on linkage groups I, E, and H.

Goal 4. Identify additional protein QTLs.

The expected outcome would be to have available to breeders alternative protein QTLs which may accelerate genetic gains for protein in high-yielding genetic backgrounds. The approach will be to utilize known existing populations which do not have the linkage group I, E, or H protein QTLs. In that regard, a recent paper (Hyten et al., 2004) describes validated and novel protein QTLs on a linkage group basis, providing useful information to target detection of additional protein QTLs.

Goal 5. Develop perfect markers for protein subunits: 2S, 7S, and 11S.

The target of this objective would be to position the genes on revised soybean maps and identify markers for soybean protein quality improvement. Recent work (Panthee et al.,

2004) documents the feasibility of detecting QTLs governing the 7S and 11S storage fractions of soybean protein. An expected outcome will be knowledge gained in protein quality regulation. Approaches will include SNP development and EST sequence analyses.

Timeline

Year 1-3: Conduct positional cloning of the linkage group 'I' locus, and determine its gene functionality through transcript profiling, metabolomics, and proteomics. Identify additional protein QTLs on other linkage groups utilizing SSR and SNP markers

Year 2-5: After the first year of development of the Breeder's Toolbox, protein QTLs on LG E and H will be targeted for the creation of perfect markers through SNP development.

Year 3-5: The cloned LG I locus will be transferred to other genetic backgrounds via transgenic technologies. Stability of expression of the protein transgene will be evaluated. Perfect markers for the 2S, 7S, and 11S fractions of soybean protein will be developed utilizing SNPs from the Breeder's Toolbox.

Objective III. Abiotic stress

The yield gap between average on-farm soybean crop yield and the yield potential in optimized conditions is attributable to the following causes: (1) diseases and insects (see above), which are normally sporadically devastating at the local level, but generally do not depress average U.S. yields by more than 4.1 and 2.6%, respectively. However, these problems are increasing and Asian soybean rust may change this perception in the coming years; (2) weed competition, which depresses yield by 4%, and (3) inappropriate soil conditions and unfavorable environmental conditions, which were estimated to decrease yield potential by as much as 69%.

Various abiotic stresses such as drought, water logging and high temperatures are the major environmental factors limiting soybean yield. Among them, drought is the major stress. Water is an increasingly limited resource, and water availability limits crop productivity in many parts of the U.S. Most soybean production areas experienced severe drought conditions over the past few years.

During the first day of discussions at the SoyCAP planning conference, an "Abiotic Stress" subcommittee established the following five priorities after active discussion:

1. Utilize existing germplasm and molecular tools to develop high yielding germplasm with improved drought tolerance. Genetic resources with phenotypic differences for yield under drought stress and deep root development are available. Molecular marker and functional genomics technologies are useful tools for the genetic dissection of complex morphological, physiological, and biochemical traits associated with drought tolerance. In soybean, QTLs related to drought tolerance were identified for deep rooting and water use efficiency. Near-isogenic lines segregating for specific QTLs are under development. These genetic materials are useful for the determination of their possible role in improving yield under drought conditions and for functional genomic analysis leading to the identification of the genes responsible for drought tolerance. Research is needed to map additional QTLs and to identify genes for drought tolerance using existing soybean germplasm resources. Public and corporate efforts are underway, using translational genomics approaches, to genetically engineer soybean plants with improved drought tolerance. The identification of QTLs and genes will help cultivar improvement by marker assisted selection, breeding and genetic engineering.

2. Utilize existing germplasm and molecular tools to develop high yielding germplasm tolerant to iron stress. Soybean cultivars differ in their ability to absorb and translocate iron. The uptake of iron appears to result from metabolic changes that occur in iron-deficient (iron-stressed) plants that favor iron uptake. QTLs controlling iron stress could be deployed for marker-assisted selection.

3. Find or create genetic variation for heat tolerance to develop new, heat-tolerant, high-yielding germplasm. Limited genetic variation has been reported for heat tolerance traits. For example, it was shown that temperature variation has a significant effect on reserve mobilization and partitioning during seed development but the molecular and regulatory mechanisms are not well understood. Seeds from soybean plants that experience heat stress during seed fill often have reduced germination. The largest reduction in germination occurs when high air temperatures and severe water stress occur at the same time. In one study the average germination dropped from 91% to 69% when plants were exposed to high air temperatures along with severe water stress (Keigley and Mullen, 1986). The changes in mineral concentrations resulting from high temperature may alter soybean nutritional quality and modify its end-use properties for oil processing.

4. Find or create genetic variation for low temperature germination, emergence, and early vigor to develop germplasm better suited for planting in cold soils. Cold temperature causes delay in germination and seedling emergence. Soybean seedlings also can be exposed to cold stress after emergence. Physiological processes such as photosynthesis and translocation are influenced by low temperature resulting in reduced growth. The oil and protein composition is affected by temperature variation and varies between soybean germplasm. The extent of genetic variation in germination and seedling emergence under low temperature needs to be studied and mapping populations have to be developed.

5. Utilize existing germplasm and molecular tools to develop high-yielding germplasm with tolerance to flooding. Flooding tolerance can be classified into submergence tolerance and soil waterlogging. Tolerance to flooding is a quantitative trait that is apparently controlled by a small number of genes in soybean and other crops. Quantitative trait loci and DNA markers for flooding tolerance were identified in soybean using the recombinant inbred population of Minsoy x Noir (Vantoai et al. 2001). Confirmation of the existing QTLs and discovery of new QTLs for flooding tolerance along with the functional analysis of genes are needed to develop molecular strategies for soybean improvement.

The “Abiotic Stress” subcommittee met again on the second day to refine these objectives into a specific research plan, giving highest priority to drought tolerance.

Proposed objective: *Utilize existing germplasm and molecular tools to develop high-yielding germplasm with improved drought tolerance.*

In soybean, QTLs related to drought tolerance were identified for prolific rooting, water use efficiency, and seed yield in stress environments have been identified. QTLs for the prolific rooting trait were identified from the Benning x PI 416937 population (Boerma, personal communication). QTLs for water use efficiency and aluminum tolerance were identified from the cross of Young x PI416937 (Mian et al. 1996; Bianchi-Hall et al., 2000) and an additional water use efficiency QTL in S100 x Tokyo (Mian et al. 1998). In a population of Hutcheson x PI471938, QTL for slow wilting and seed yield in drought-

prone environments were identified (Boerma, personal communication). Near-isogenic lines segregating for specific QTLs are under development. These genetic materials will be useful for the determination of their possible role in improving yield under drought conditions and for functional genomic analysis leading to the identification of the genes responsible for drought tolerance. One of the major objectives for this goal is to identify additional QTLs and genes associated with drought tolerance. Confirmation of drought related QTLs are needed before they can be deployed in a marker-assisted selection program.

The contrasting growth responses of the different soybean organs to water deficits, and the genetic variation in the responses, provide important tools for making molecular comparisons. An in-depth analysis of the physiological responses during drought stress and their genetic control is essential to solve several key questions such as the identification of regulatory genes, loci associated with the discovery of the initial proteins contributing to stress tolerance, the identity of genes that may serve as molecular markers for the physiological stage of the plant, and the gene products that can be considered as secondary stress-induced metabolites. Proteomic and metabolomic analyses will be essential for a complete understanding of gene functions and metabolic networks associated with drought tolerance.

Timeline

Year 1-3.

Confirm existing drought QTLs through analysis of near-isogenic lines.

Identify additional new QTLs associated with drought tolerance.

Year 3-5

Saturate the target QTL regions with SNP markers.

Identify and isolate genes using functional genomics and map-based cloning approaches for the development of perfect markers or transgene deployment.

Incorporate markers into the Breeders Tool Box when they become validated.

Release new germplasm tolerant to drought

Objective IV. Information delivery. During the SoyCAP planning conference, a specific subcommittee met to focus on issues of bioinformatics, education, extension and outreach. The recommendations made by this subcommittee were discussed by all attendees, approved, and are described below.

1. Bioinformatics.—Development of the “Soybean Breeders Toolbox”

A viable and project-focused database is essential to ensure fast and seamless access to information. The interface for such an information exchange should be web-based to take advantage of public bioinformatic and computational resources. The database serving as the interchange interface will deliver genomic and genetic information in a manner targeted for and conducive to breeding and crop improvement strategies.

The successful database serving crop improvement/plant breeding efforts should be in a relational structure to facilitate complex queries. It should be populated with data types of interest to modern breeders such as germplasm (source of mutants, traits, etc.), phenotypes, QTLs (trait description, LOD scores, P-values or R-square values, map location, references, etc.) maps, markers (SNPs, SSRs, RFLPs, and classical traits) and associated candidate genes as they become available. Additionally, the database should be accessible from numerous entry points, depending upon the type of question being asked by the user. And finally, the database should serve an educational role and should include tutorials, instructions for phenotyping, references, etc. If successfully developed,

such a database structure would be portable among all plant breeding programs, of all crops.

SoyBase, a USDA-supported soybean genetic database is the ideal foundation from which to build the new interface. To accomplish this, key components of SoyBase will need to be restructured from its current ACeDB format into a relational database with well-defined connections among data types; an activity already begun. Building upon SoyBase strengths will allow soybean breeders to take advantage of an existing resource, to leverage USDA-ARS activities, and to tap into an existing linkage with LIS (Legume Information System; <http://www.comparative-legumes.org/>).

2. Outreach

The need to communicate science to the public is gaining greater importance as science gets more complicated. There is increasing recognition that failure to properly communicate science to the public leads to situations where “ignorance often leads to fear” (Willems, 2003). Nowhere is this phenomenon clearer than in the area of plant biotechnology.

Lack of effective science communication has other ramifications as well, including a distorted view of science which serves as a disincentive for students to choose careers in science. A recent NSF report (National Science Board, 2002) indicates that one fourth of all respondents perceive scientists as “odd and peculiar,” and half perceive working in science as dangerous. Yet, most scientists have difficulty explaining their work in language comprehensible to the lay public. Others dread reporters taking their words out of context, or worse yet, being on the receiving end of a hostile interview.

SoyCAP should and will be an avenue to help fill the current void in science communication to the public. First, SoyCAP will develop a training module for effective public communication skills and participants will make themselves available to the media as resource persons.

SoyCAP funds should be targeted for the hosting of a communications skills workshop involving all SoyCAP participants and taught by a well-qualified media expert. The workshop will cover the basic principles of communication with the public. It will coach participants in the proper way to give interviews to prevent their messages being taken out of context, and will prepare participants to handle a hostile media interview, should they ever find themselves in that situation. Participants will get a chance to do a radio and TV interview as well.

Secondly, SoyCAP investigators will be expected to write a minimum of two articles per year for the popular press. Participants will make use of the communication infrastructure that exists within each College of Agriculture and the USDA, ARS. This includes the assistance of professional writers and the use of the distribution system that colleges and universities have for issuing press releases. These press releases will be archived in the SoyCAP web page.

SoyCAP participants will also serve as an Information & Data Analyses and Response Team (I-DART) and will be available as media contacts for reporters in their vicinity. Contact information for all the participants will be posted on the SoyCAP web page and disseminated through the various Colleges of Agriculture press offices. To assist the participants in their task, one PI will take the lead in preparing ‘talking points’ for emerging and newsworthy issues. These talking points will then be disseminated to each of the participants.

3. Education

A vision of graduate education is embodied within the NSF Integrative Graduate

Education and Research Traineeship Program

(<http://www.nsf.gov/crssprgm/igert/intro.jsp>). Among the objectives of this program is the training of scientists with “deep knowledge in chosen disciplines, and technical, professional, and personal skills” which will result in “the development of a diverse, globally-engaged, science and engineering workforce.” SoyCAP should and will be structured so that graduate student training is an integral component of the program.

Graduate student participation in the Communications Outreach section should foster global-engagement and communications skills among the students. Furthermore, development of the “**Breeder’s Toolbox**” will present opportunities for student participation in the development process. Most importantly, students should be able to use the Toolbox itself in their thesis research projects, thus fomenting technical skills and deepening the students’ knowledge in the discipline.

Development of the toolbox itself will entail an information exchange meeting between the Toolbox developers and breeders (to be held in conjunction with the annual Soybean Breeders’ Meeting). Subsequently, at least one ‘Breeder’s Academy’ will be held to ensure breeders are comfortable with the Toolbox. These training workshops would be held in conjunction the annual Soybean Breeders’ and Crop Science Society of America meetings. Graduate students and postdoctoral associates are expected to be a key component of these meetings. Note that since the Toolbox is being developed as a community tool, participation in these workshops will be open to all interested parties.

4. Extension.

Information generated through SoyCAP will be disseminated through the International Certified Crop Advisor Program (ICCA). ICCA is administered by the American Society of Agronomy and, at present, there are over 14,000 certified crop advisors (CCA) from the USA and Canada. ICCA is a voluntary program that certifies a basic level of understanding in crop production and management and maintains standards through continuing education. ICCA members are required to take a minimum of 40 continuing education units (CEU) over a two year period to maintain their status as a certified crop advisor (CCA). SoyCAP will offer opportunities for CEU throughout the soybean growing regions of the USA and Canada via the ICCA program so that CCA have the opportunity to be educated in soybean biotechnology.

Graphic, computer presentations will be developed that will provide information for CEU for certified crop advisors. These presentations will be formatted to follow ICCA guidelines including learning objectives and quizzes. Information guides will be prepared for extension agents, industry representatives and soybean growers desiring information about the project. It is expected that this information can be disseminated at field days, grower meetings, soybean extension events, etc. In addition, interactive workshops will be a vital part of the extension programming efforts which will provide education on soybean biotechnology to growers, extension personnel, industry representatives, crop consultants and others needing such information.

Management Plan:

During the SOYCAP planning meeting, a coordination committee was established. The role of this committee is to select the PI, lead institution and writing committee for the SOYCAP proposal submission. The overall management plan organization will be modeled after the RICECAP project, which includes a Project Director (PI), an Executive Committee, an appointed external Scientific Advisory Board, and an external Stakeholder Advisory Board. The Project Director will allocate 20% of his time to the management of this project for the project duration. The Project Director will be assisted by a full-time project manager/administrative assistant for the duration of the project. In

addition, fiscal management and budget oversight will be provided by the lead institution. The project's Executive Committee will be composed of team leaders who will coordinate different research objectives and outreach and extension activities. The Scientific Advisory Board will be composed of 4-5 nationally and internationally-renowned plant scientists, representing the areas that are related to the project such as plant breeding, genetics, bioinformatics, and plant pathology. The Stakeholder Advisory Board will be composed of 4-5 leaders in commodity group, industry, government, soybean production and processing, and media. One scientific board member will serve as a liaison to provide continuity and facilitate communication between the Scientific and Stakeholder Advisory Boards. Both the Scientific and the Stakeholder Advisory Boards will provide input, advice, and oversight to the Project Director on a regular basis for the duration of the project via conference calls and meetings. The Executive Committee will work in concert with the Project Director and the co-PIs and collaborators to assist in project evaluations, progress reports and charting future directions based on accumulated data and accomplishments. Program direction and funding decisions will be determined based on the direct input of the two external advisory boards. Annual reports will be submitted to the USDA-CSREES program director and presented at the Soybean Breeders' Workshop. The project activities will be communicated and coordinated with other outside groups including professional societies (e.g. Crop Science Society of America, American Society of Plant Biologists), USDA-ARS, industry (e.g. American Soybean Association and the United Soybean Board), and other CAP projects.

Data release and intellectual property

In modern society, especially within the context of a vibrant and competitive soybean industry, SoyCAP issues cannot be discussed without some reference to intellectual property and without a well designed data release policy. Therefore, a subcommittee met during the planning conference to address these issues and to make the following recommendation.

Recommended policy on intellectual property and data release.

The overall philosophy will be that information and tools from the "**Breeder's Toolbox**" will be made available immediately after verification and will be disseminated as widely as possible without impediments. Traits and tools developed will be disseminated widely for the purpose of advancing biotechnology in soybean. If patents are filed, we encourage investigators to grant non-exclusive licensing for the purpose of enhancing distribution. Tools contained in the "Breeder's Toolbox" will be publicized via the internet and made available to the public.

Maintaining international cooperation.

SoyCAP will seek to encourage international collaboration and cooperation to the extent that it stimulates U.S. agriculture and marketing.

Acknowledgements: The Planning Conference was supported by a grant (#2004 35606-14859) from the USDA National Research Initiative. The authors express their gratitude to all participants of the SoyCAP planning conference.

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