Harnessing Plant Breeding and Genetics to Identify and Develop Economically Important Crop Traits

A. Scope/audience

Plant breeding and genetics are fundamental to a secure and abundant supply of food, feed, fibers for clothing and shelter, and biomass for conversion into renewable energy. Research in this area in the Department of Agronomy involves broad and extensive activities and collaborations, including: education and hands-on research development for students for BS, MS and PhD degrees, and postdoctoral training; research interactions/collaborations with federal government agencies and businesses in the seed industry; partnering with government agencies and industry in the education/training of students and providing conduits for graduates for continued career development; building professional relationships with alumni and industry; and providing professional leadership at national and international levels.

B. Overview & contribution in the past 5 years

Four new faculty have been hired - two in the areas of soybean genetics/genomics and breeding, and two in maize breeding and genetics – significantly strengthening our research in the two most important agronomic crops in the state and region, and significantly strengthening our student education capabilities. Twenty five BS, 12 MS, and 23 PhD degrees have been awarded in plant breeding and genetics in the Department in the past 5 years. Since 2002, nine wheat cultivars, four oat and five soybean cultivars have been released for commercial production and 20 inbred lines of sorghum have been distributed to commercial companies. Several DNA markers that co-segregate with important genes were developed in several crops and marker assisted selection has been integrated as an important component of germplasm and cultivar development. Large and genetically diverse populations of maize, soybean and sorghum were developed here and others were acquired from around the world as outcomes of diversity survey projects and of establishing a reverse genetic service of targeting induced local lesions in genomes (TILLING) in the department. Virus induced gene silencing (VIGS) has been developed here as a method for studying and engineering traits into wheat and, potentially maize. Analytical infrastructure at the university has been significantly expanded, including high throughput genotyping with excellent service and collaborations with crop improvement research programs, massively parallel DNA sequencing, 454 transcript profiling, and NIR reflectance spectroscopy. Improvements were made in facilities for long-term storage of seeds in the Plants & Soils building. Irrigation facilities were added at the agronomy center for research and education (ACRE) as well as global positioning system (GPS)-guided precision planting equipment.

C. Current challenges

There is an urgent need in the seed industry, government agencies and academia for more contemporary plant breeders and geneticists with new interdisciplinary skills and broad based knowledge to replace those that are retiring and to meet new demands. Changing climate, increased population with increased food, fiber and fuel requirements and dwindling resources have created needs for increased total metabolizable energy produced per unit area through improved crop disease resistance, abiotic stress tolerance, new and improved food, feed and energy qualities, harnessing soil microbes for greater crop productivity and biocontrol of plant diseases and nutrient use efficiency. Multidisciplinary research has played a pivotal role in improving our understanding of plant-microbe and plant-insect interactions, plant development, physiological and biochemical plant processes, understanding of the rhizosphere, and field performance and productivity. An important challenge is that we effectively exploit knowledge obtained from model systems into useful applications. This has been achieved in the areas of lipid metabolism, flowering time control, disease resistance, and plant stress responses. The faculty will have to determine which traits are most amenable to an integrated approach that includes model systems, crop physiology, and molecular marker-based plant breeding technology. There is also increased pressure to increase the efficiency of nutrient uptake and productivity of the major agronomic crops. Tremendous opportunities exist to mine the explosion of data from genome sequencing in development of genetic technologies that provide solutions to the array of problems encountered in agricultural production, end-users of our products, and stewardship of the environment. We need to increase our understanding at the molecular and whole-plant levels of plant-microbe and plant-insect interactions, plant development, physiological and biochemical plant processes and plant development. We need to expand collaborations with scientists in related areas including plant pathology, plant physiology, soils and entomology as well as “end-user” areas such as nutrition and process engineering. There is also a need to explore potential new, useful crops and cropping systems for increasing grain and biomass productivity, and ways to deliver food/feed nutrients with decreased input requirements per unit output.

D. Fundamental issues for the next 10 years

Developing capacity to educate/train a larger number of post doctoral fellows and plant breeding graduates with BS, MS and PhD degrees, and providing comprehensive education in the many related areas of science including several areas of genetics and plant physiology (as well as other areas of science for which we rely on other departments – biology, plant pathology, statistics, bioinformatics, horticulture, forestry) is a primary issue for our department. This relates, not only to limited funding for graduate student support, but also we need to strengthen our faculty research capability in plant physiology at the whole plant and molecular levels, and our soybean breeding capability.

There is a significant need for improving methodologies for accurate phenotyping of traits, to maximize the potential and efficiency of DNA marker technologies in plant breeding, especially for quantitatively inherited plant traits. There is also a need to develop a better understanding of the physiology and biochemistry of complex plant traits. To do this we need to develop dynamic research collaborations involving plant breeders, geneticists, physiologists and biochemists. There is need to seamlessly integrate molecular genetics into real-world crop improvement to more fully deliver on the promises of molecular technologies. There are environmental issues and needs, and opportunities for research on cover crops for soil conservation, "trap crops" for N, legumes to fix N, and alternative cash crops.

A fundamental challenge for the Department and Purdue College of Agriculture is to build capability to provide funding for new research initiatives to enable faculty to be competitive for federal and other sources of research funding in developing areas of research. Seeding good ideas is the most direct mechanism to new sources of external funding and interactions with industry.

E. Projects that Purdue and especially Agronomy can develop to address these issues

1. ***Crop biotic stress resistance:*** An internationally recognized research program in small grains breeding, with strong research collaborations among faculty in Agronomy, Botany and Plant Pathology, Entomology, and USDA-ARS scientists focuses on fusarium head blight, new virulence in fungal organisms that cause stem, leaf, and stripe rusts and Hessian fly in wheat, and yellow dwarf viruses disease in wheat and oat, and crown rust in oat. There is ongoing research in soybean for broad based resistance to soybean cyst nematode and phytophthora root rot, and combining newly identified genes for resistance to sudden death syndrome and frogeye leaf spot. The corn breeding and genetics group was recently strengthened by the addition of two mid-career faculty. This group collaborates on research with faculty in Botany and Plant Pathology to develop better understanding of fundamental processes of disease and insect resistance as well as a class of mutations called disease lesion mimics to elucidate genes and pathways that may be of relevance to maize's interactions with pests. One example includes understanding how a conserved growth control pathway in Arabidopsis and maize contributes to biotic and abiotic stress resistance/tolerance. This work will lead to a molecular understanding of plant resistance mechanisms, and therefore the tools that are needed to engineer plants with enhanced resistance properties. In addition to traditional forward and reverse genetics approaches to identify major genes (e.g. transposon tagging, tilling… etc.), a new emphasis includes efforts to understand epistasis and the effects of genetic background. The sorghum breeding and genetics program studies fungal diseases such as leaf rust and grain mold, but focuses heavily on resistance to the parasitic weed Striga and development of tools for Striga management in Africa.

2. ***Improved food/feed quality:*** Soybean researchers at Purdue are involved in research to improve quality traits for food and feed. The breeding research includes the development of soybean lines with increased protein and sucrose content for tofu and soymilk production and oil with 1% linolenic acid. Research is being conducted to stack genes and to develop molecular markers

for reduced levels of three major allergens to improve soy protein for human and swine nutrition. Basic research is also underway to determine how fatty acid and protein composition are regulated at the genetic level, in part utilizing the soybean TILLING population located in the department.

Corn and sorghum research is underway examining nutrient synthesis, availability and control processes in the grain (protein, starch, oil, carotenoids, tocopherols). There is a long history of research in wheat for milling and baking qualities for a wide range of soft wheat products including cakes that require low protein/low gluten and crackers and other products that require strong gluten, as well as other products that require other milling and baking characteristics. In oat, research is focused on increasing ß-glucan, percentage protein and percentage groat. The model plant Arabidopsis is being used to understand cellular processes that impact nutrient deposition and to understand cellular processes that impact nutrient deposition, and these results are being extended to corn.

3. ***Improved biofuel feedstock quantity and quality:*** Projects in this area utilize maize (both for improving existing feedstocks and as model systems for other grasses), sorghum grain and biomass production and quality, and soybeans as well as second and third generation feedstocks such as switchgrass and Miscanthus. Major areas of emphasis are development of stacked traits including brown mid-rib, low lignin, high biomass (and lodging resistant) varieties of sweet sorghum, high starch concentration, high grain yielding temperate experimental maize hybrids which integrates knowledge from QTL and expression analysis, high stalk sugar varieties of tropical maize, rapidly digesting and high extraction corn starch mutants that reduce energy input and grain requirements, assessment of genetic diversity and reverse genetic tools are being used to accelerate switchgrass breeding, and growing and providing materials to feedstock processing studies across campus and nationwide. The cellular mechanism of cell wall synthesis and wall remodeling is also being investigated in both Arabidopsis and maize. The soybean project for increased oil content has increased oil percentage by up to 20% above the Midwest average through transgressive segregation from elite x elite crosses. A mapping study to identify the major QTLs involved in oil synthesis is underway.

4. ***Improved abiotic stress tolerance:*** It is anticipated that impending climate change will increase frequency and severity of abiotic stresses, including heat, drought, flooding and cold periods during winter. Currently, crop losses to abiotic stresses are estimated to exceed 30% of U.S. production annually. An integrated approach inclusive of breeders/geneticists and physiologists/ecologists working together to identify genotypes adapted to these stresses is essential if we are going to meet the food, feed, fiber and fuel demands of the world. Studies are underway to characterize the nature of genes positively associated with alfalfa winter hardiness. In perennial

ryegrass, drought tolerance traits are being evaluated in a globally distributed germplasm collection at different locations in Indiana. Assessment of drought tolerance in perennial ryegrass will lead to identification of genes/markers that are associated with drought tolerance. The research of gene/marker and trait association will provide important information for perennial grass improvement by selecting desirable genes. Abiotic stress research in perennial grasses also includes flooding tolerance and gene identification. The potential physiological traits could be used for developing rapid and reliable methods for screening a wide range of perennial grass germplasm for flooding tolerance. The selected candidate genes could be used as markers for assisting a breeding program. Other research focuses on flooding tolerance genes in grasses. For many of these traits, agronomic performance is inversely associated with high stress tolerance.

In wheat, large differences in root volume have been identified among adapted winter wheat lines, and a landrace from Iran has been identified that has unusually large roots, and research is underway to identify and map genes associated with large root volume, and to document that large root volume is associated with tolerance to low soil moisture stress and to increased efficiency of nutrient uptake under low soil fertility conditions. Current studies suggest that plants with large root volume are more tolerant to low soil moisture stress given that the recent cultivar release, INW0731, which has larger root volume than most other adapted winter wheat cultivars, consistently ranked first in performance trials in Indiana, Ohio, and Illinois in 2007, a moderate drought season, and in the top 10% of entries in other recent seasons, but not at the top.

Abiotic stress research on sorghum includes work on drought tolerance and early season seedling cold tolerance, traits highly sought by the sorghum seed industry. In both traits the work focuses on identifying molecular markers associated with these traits to aid in breeding and characterizing genes and genetic effects expressed in specific physiological mechanisms. Sorghum genetic populations created from deliberate crosses or well characterized germplasm panels are utilized in identification of putative markers and in implementing marker associated selection with robust markers to develop improved germplasm for use against these major abiotic stresses of sorghum.

5. ***Genomics research:*** In soybean, researchers have developed a number of resources and tools that have led to the recent completion of the sequencing of the soybean genome. They are now working to develop tools and methodologies to be able to leverage diversity in annual and perennial soybeans that has, heretofore, not been utilized. Research is underway to develop sequence-based resources in six perennial and one annual relative of soybean that can be aligned to the soybean genome sequence. Moreover, they are in discussions with collaborators in Korea, Mexico, China and Japan to develop interspecific recombinant inbred line (RIL) populations to map

domestication genes and further leverage genetic diversity for soybean improvement.

The maize research group and collaborators in the departments of Botany and Plant Pathology and Horticulture and Landscape Architecture are focusing on maize germplasm characterization and genetic resource development. The Maize TILLING Project is one of the premier functional genomics resources utilized by labs worldwide. The TILLING group is expanding the mutant populations used in the project, the seed for these lines has been deposited at the maize genetics co-op, and copies of the collection were provided to consortia at IRRI and in Spain for large-scale forward screening of photosynthetic mutants and cold tolerance, respectively. Current work is aimed at using the massively parallel sequencing capacity at Purdue for re-sequencing of several thousand specific target genes in mutagenized lines to identify all mutations present. In addition to creating new alleles and genetic variation through mutagenesis, studies of natural genetic variation as represented by the Maize Diversity Panel and populations of recombinant inbred lines developed from them are being conducted to identify and characterize genes and networks of genetic interactions that influence abiotic stress tolerance including tolerance to heat, cold, drought, and oxidative stresses and genes that modify plant architecture. The goal of this research is to expand our understanding of the genes controlling quantitative variation to better enable modeling networks relevant to all agriculturally significant grass species. Purdue is now one of only a few sites in the U.S. with the field, technical, and faculty resources and willingness that enables properly growing and evaluating these large-scale resources. Greater capacity in translational genomics and breeding with relevance to the commercial hybrid seed industry is being developed. Progenies, testcross progenies and populations derived from recent public release of former PVP protected commercial inbreds are being developed and evaluated. These materials are quite promising and it appears that they will provide a more contemporary and relevant genetic platform for graduate student research projects. The ability to make crosses between former Pioneer, Holdens, and Dekalb elite inbreds is already creating experimental testcross progenies that are not dramatically different in yield performance than non-GMO commercial hybrids. Futhermore, these experimental progenies were interesting enough to prompt funding by Dow AgroSciences for a graduate student research project.

6. ***Improving nutrient use efficiency:*** Industry partners hope to deploy transgenic maize possessing genes that in model systems have shown promise for improving N use efficiency (NUE). Repeated failures in demonstrating greater NUE in the field has prompted recent conversations with faculty working in plant nutrition, soil fertility, and physiology aimed at understanding the intricacies of N cycling in the soil-plant-atmosphere continuum. It is anticipated that these conversations will lead to research that will increase the likelihood of successful deployment of genes associated with

NUE, drought, and other abiotic stresses.

Nutrient use efficiency improvements in maize are presently focused on understanding plant N uptake dynamics, N partitioning to various plant parts, and optimum N rates for maize hybrids with and without transgenic (i.e. stacked traits) at progressively higher plant densities in multiple environments. The overall goal of this research is to investigate the potential interactions of hybrids with new traits (pest resistance, etc.) and various degrees of abiotic stress tolerance (as tested under high population and limited moisture environments) with N use efficiency. Although tremendous strides have been made in increasing maize grain yield over the past three decades with less fertilizer N requirement per unit yield, continued research is required to ensure that future higher maize yields can be achieved with even greater N use efficiency.

F. The science team (expertise needed)

Maize, sorghum, soybean, wheat, oat, alfalfa and perennial ryegrass are represented by ongoing research programs. There is also effort in the use of model systems to understand the cell biology of plant responses to fungal pathogens and fundamental growth mechanisms. Work is beginning in several labs on switchgrass and on Miscanthus, but still needs to be developed into a coordinated effort. Soil microbial genetics is also represented by ongoing research in two laboratories. However, there is a gap in our ability to address and make best use of the potential of emerging crops. Federal and Foundation funding for biomass-related research and nutrition related research exists, and industry support for biotic and abiotic stress in major commodities is good; however, funding in most other areas is becoming scarce. The soybean breeding position, currently an A/P level position, is funded by the Department of Agronomy. The breeding program has an operational budget that is primarily funded by the Indiana Soybean Alliance. The five cultivars released by the program since 2005 exhibit high yield and excellent disease resistance. Their impact on soybean acreage is showing an increasing trend. The program is currently in a transition from its focus of primarily variety development to a program that involves more breeding and genetics research activities that lead to peer-reviewed publications, acquisition of external funding, and involvement in graduate student education. If this AP position were to be replaced with a tenure-track position, competitiveness for research support would be expected to increase significantly and the faculty member would be expected to contribute to classroom instruction and mentoring of undergraduate and graduate students.

Our departmental staffing plan should include replacing the current Associate Professional soybean breeding position with a tenure-track soybean breeding/genetics research/instruction position to expand our competitiveness for research support and expand our capacity to educate students in plant breeding.

G. Time frame

Discussions among faculty throughout the college of Agriculture and the College of Science (Biology Department) are already underway to develop improvements in our curriculum in the areas of plant genetics and breeding and related sciences, and in collaborative research initiatives. We are also engaged in discussions with industry for graduate student support and expanded research collaborations. We need to take a leadership role to develop a center of excellence with partnering universities as soon as possible, to strengthen our curriculum and to be more attractive to excellent potential students.

H. Evaluation of success

Increasing the number of graduates with BS, MS and PhD degrees and that are competitive for career positions in industry, government and academic settings will be indicative of our efforts to strengthen our faculty and research funding capacities in various areas related to plant breeding, and crop physiology. Increased research funding from a wide array of sources including industry, federal and state government programs will be indicative of our effectiveness in participating in ongoing and developing relevant research needs, our capacity to carry out competitive research, and our ability to develop collaborations to address complex research challenges. Positioning a research program and collaborations to be competitive for basic research funding can provide support for long term research for germplasm and cultivar improvement, and significant research publications in high impact international journals.

I. Dissemination of information to decision makers/scientific community/public

We need to participate much more at regional and national levels in ***planning*** research agendas and funding decisions rather than responding to them. In this process, we need to carry out research that tries to anticipate changing needs and convey those results to decision makers at the state and national levels, to foundation directors and to the public on a regular and timely basis.

J. Resources needed

Leveraging of government and industry funding will maximize research capability, productivity and relevance. It is critical that we strengthen our research capability in plant physiology at the whole plant and molecular levels with additional faculty position(s). Concrete action needs to be taken to begin building permanent seed and biomass handling and storage capacity that will both accommodate existing programs and allow them to grow. Existing facilities are inadequate and even the interim improvements underway will only be a temporary solution that may be outgrown before they are even completed. We need to expand our collaborations with industry to include new research activities and funding for graduate student assistantships. We must create strategic partnerships with other universities to create a center of excellence for educating/training plant breeders for the 21st century. We need to work with the Purdue Ag Development Office to attract funding from alumni and other sources of endowments for graduate fellowships.

K. **Conclusion/recommendations/vision for the future**

There are incredible opportunities: demand for excellent BS, MS, and PhD graduates and scientists with post-doctoral research experience in industry, government and universities, with education/training in plant genetics and breeding. There are many needs for new knowledge and plant genotypes with new traits. There are funding opportunities from multiple sources for new and innovative research that is relevant to the many needs of society. We need to develop and participate in ongoing discussions among the broad community of researchers in and across departments on campus and with researchers at other institutions to develop relevant and dynamic research initiatives that integrate research, education and engagement, and that are competitive for funding by new emerging federal programs, like the Agricultural and Food Research Initiative (AFRI), and some by leveraging of funding by industry.

We need to expand relationships/collaborations with industry in research and education/training of students, particularly a next generation of modern plant breeders. We need to take a leadership role in developing educational partnerships with other universities that complement our institutional (broader than Agronomy – includes other departments in the College of Agriculture) weaknesses and expertise in providing faculty capacity to educate/train students in plant breeding/genetics in a broad range of field, vegetable, horticultural and forest species. We need to develop in-depth workshops and intensive short-courses of one to several days involving various experts in developing areas of science related to plant breeding/genetics as a facet of graduate student education. There is significant need to expand available seed funds at the university and college levels to invest in and stimulate new or 'high risk' areas of research to improve our competitiveness for federal and industry funding for further development. Interim improvements in seed storage and handling capacity need to be followed up with permanent capacity that can accommodate the large number of existing programs and absorb expected growth and demand. We need to develop our overall research capacity, encompassing all of our research programs in the various areas/commodities for food, feed and bio-energy, and at all levels of very basic to applications research, to be competitive for federal, state and industry funding.