IOWA STATE UNIVERSITY

Plant Sciences Institute UPDATE

January 2009, Volume 9, Number 2

Soybean silencing seals success

Thomas Baum, Steve Whitham and John Hill survey soybeans outside a growth chamber.

A Plant Sciences Institute Innovative Grant powered up a project to develop a means for identifying genes to protect soybean plants from disease. That project has won funding from the Iowa Soybean Association, the United Soybean Board, the North Central Soybean Research Program, and now a $2.1 million grant from the National Science Foundation.

Using this technology to identify soybean defense genes is the goal of what is now a multi-institutional team led by Steve Whitham, associate professor; John Hill, professor; and Thomas Baum, professor and chair, all of the Department of Plant Pathology. The team's ultimate goal is to develop new broad spectrum pathogen resistance traits for targeted breeding efforts.

The team is drawing up networks of activated defense genes from a range of stress-inducing pathogens as well as the abiotic stress of iron chlorosis. They are also expanding their scope to targeted tissues that include leaf, stem and root.

The gene identification technology is a virus-induced gene silencing (VIGS) technique adapted to soybeans. Whitham and Hill have now morphed this technology into a DNA-based vector—creating important high throughput capability.

CONTINUED ON PAGE 2

Mobile RNAs move research to higher levels

The success of a Plant Sciences Institute Innovative Grant project investigating how a plant's RNA machinery transmits information from one part of the plant to another helped two Iowa State scientists secure a $ 0.4 million USDA-NRI grant and a $2.75 million grant from the National Science Foundation.

David Hannapel, professor in the Department of Horticulture, and Guru Rao, professor and chair of the Department of Biochemistry, Biophysics and Molecular Biology, are focused on understanding how potato plant RNA molecules transmit environmental information gathered in the leaf to below-ground organs called stolons, leading to tuber formation.

In response to day-length conditions, these RNAs travel through the vascular system from leaves to stolons by catching a ride on special protein escorts that Hannapel and Rao hope to identify. Once the RNAs arrive at the stolon, they are translated into proteins that promote tuber formation.

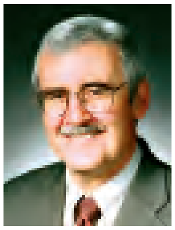
Hannapel determined that the RNAs encode a transcription factor called StBEL5 and demonstrated that its over-expression alone is enough to boost tuber formation. "Our experimental potato plant that was engineered to express StBEL5 throughout the plant at high levels caused tubers to form off-stem buds. This, of

CONTINUED ON PAGE 2

Spinning straw into gold

It is always gratifying when small startup projects we have supported turn into larger projects that attract substantial extramural funding. These cases are testimony to the quality of our researchers and are an endorsement of the research that we chose to support in the Institute.

Each year, we run an Innovative Grants Program—a competition within the university to support new research endeavors, which have the promise to develop into full-blown projects that can compete for extramural support.



Our newsletter highlights recent projects from the Innovative Grants Program that have captured major extramural funding or have resulted in significant publications. The projects are important because their success helps us to leverage our investment.

We also describe another project that has just gotten underway where we have been able to leverage our investment up front through a partnership with ConocoPhillips Company.

The only problem with our Innovative Grants Program is that we have more ideas than resources, so some very good proposal may go wanting.

The ideal project is one that makes a scientific or technological advance and has an opportunity to be adopted in the marketplace or contribute to the welfare of Iowans. Not all projects meet those ideals—some might have potential as a scientific or technological advance, but might not have an obvious application in the marketplace.

Nonetheless, we need to support some of these research projects, because it is often that kind of basic research that fuels truly transformative events in the marketplace.

Stephen Howell
Director

Soybean silencing seals success/CONTINUED

VIGS vectors provoke host plant cells to specifically degrade RNA from the gene that matches sequences each VIGS vector is designed to carry—silencing the target gene. Starting with a DNA vector instead of RNA reduces cost, and most importantly, it speeds the process along, as DNA is more stable and can be directly introduced into the young plant leaves using a gene gun.

The team is now building a library of VIGS vector constructs that can silence each of a thousand genes suspected to have roles in soybean defenses or susceptibility to various pathogens. Partner researchers at other institutions are developing disease-specific assay systems—making use of sets of these gene silencing vectors to pinpoint each gene's function.

A novel aspect of this work is to expand the VIGS technology to work on roots. "We know a lot about when and where the vector works for VIGS in leaves, but we don't have these details yet for roots," says Whitham.

Baum will use a VIGS construct carrying a piece of the green florescent protein (GFP), along with a soybean plant that uniformly expresses GFP in roots, "to nail down the spatial and temporal gene silencing activity in roots."

Silencing GFP in roots will lead to a loss of green florescence that will provide a visual marker for the vector's functional presence. Baum is interested in roots, as this is where the soybean cyst nematode feeds.

"We expect that if these studies are successful, then the VIGS vector can be applied for studying other soybean traits ranging from development to nitrogen fixation," says Whitham.

Mobile RNAs move research to higher levels/CONTINUED

David Hannapel and Guru Rao discuss tuber production in potato plants—a crop that provides more calories for human consumption per acre than any other food crop on the planet.

course, is not normal and was due to expression of BEL5 protein in places where it normally does not occur," says Hannapel.

When sunlight hits the plant, StBEL5 RNA synthesis is activated in the leaf. When days shorten late in the growing season, the RNA from this gene starts migrating away from the leaf and down to the stolon. Because RNA is vulnerable to attack by degradative forces, it must be protected. As shown by studies on mobile RNAs in other systems, a protein escort is the logical transport partner.

While Hannapel characterizes the genetic control of the transcription factor, Rao will work to isolate and characterize the proteins that protect the StBEL5 RNA. "We'll use both a targeted and random approach to do this," says Rao, who will be extracting total protein from different parts of the potato plant during different photoperiods.

Rao will run these extracts over a column of StBEL5 RNA to capture proteins that bind to the RNA. He will also use the high throughput protein screening technique called phage display to capture and identify peptide sequences that bind the RNA.

Understanding the fundamentals of this mechanism and the research protocols that result will advance an understanding of the metabolic interplay between plants and sunlight and provide molecular tools to enhance crop production.

"StBEL5 is a member of the BEL1 gene family, which is ubiquitous in plants," says Hannapel, suggesting the researchers' discoveries will be applicable to more than just potato plants.

One-stop cropping

An Innovative Grant from the Plant Sciences Institute helped launch a project for the sustainable production of lignocellulosic feedstocks. These feedstocks will play a large role if the United States is to succeed in producing the 36 billion gallons of domestic biofuel by 2022, mandated by the Energy Independence Security Act of 2007.

To this end, bioenergy double-cropping systems that include winter biomass cover crops offer a viable alternative to the single row crop systems currently dominating Iowa's landscape. Their advantage is the ability to produce more biomass by capitalizing upon the entire growing season.

Matt Liebman, Rob Anex and Andy Heggenstaller.

A three-year study initiated in 2005, led by then Ph.D. student Andy Heggenstaller; Matt Liebman, Wallace Endowed Chair for Sustainable Agriculture and professor in the Department of Agronomy; and Rob Anex, Bioeconomy Institute associate director for research and associate professor in the Department of Agricultural and Biosystems Engineering, put the double-cropping theory to the test.

In addition to a conventionally managed single crop of corn, "our double-cropping systems included winter triticale, which was planted in the fall after soybeans and harvested in spring the following year. Depending on the cropping system, triticale was followed directly by corn, sorghum-sudangrass or the tropical legume, sunn hemp," explains Heggenstaller.

The researchers measured total biomass yield, potential ethanol yield, nutrient capture and nutrient export. Comparing results, the team discovered total biomass yields were twenty-five percent higher with two of the three double-cropping strategies—triticale/corn and triticale/sorghum-sudangrass. In addition, estimated ethanol production capacity for the triticale/corn system outperformed the next best candidate—sole-cropped corn.

Another benefit confirmed by the study was successful nitrogen sequestration in crops, particularly "during periods of the year when it is most susceptible to loss from the soil," says Heggenstaller.

One downside to the double-cropping systems tested was that biomass harvest resulted in the removal of significant amounts of nutrients—including nitrogen— from the soil. These nutrients would need to be returned to the soil in some fashion to maintain superior biomass yields. Heggenstaller and his colleagues are now addressing several sustainable approaches to achieve this, including recycling residual materials from biorefineries back to fields used for crop production.

The results of this three-year effort appear in the November-December 2008 issue of *Agronomy Journal.*

Partnering on pyrolysis

A project jointly funded by the Plant Sciences Institute and the ConocoPhillips Company will define the most desirable feedstock traits for optimum bio-oil production through fast pyrolysis.

Bio-oil can be produced from renewable biomass sources, such as the non-grain portions of corn and perennial grasses via a thermochemical process called fast pyrolysis. But some feedstock properties appear able to yield better bio-oil than others.

In fast pyrolysis processing, ground biomass is rapidly heated to a high temperature (450 degrees C) in an oxygen-free environment—pyrolyzing the biomass constituents of lignin, cellulose and hemicelluloses—then rapidly cooled to yield bio-oil and bio-char.

Bio-oil quantity and quality is influenced by the reactor and the process conditions under which the thermo-chemical conversion occurs.

But biomass qualities, such as the relative amounts of cellulose, hemicelluloses, lignin and minerals, also play a large part. Thomas Lübberstedt, director of the Raymond F. Baker Center for Plant Breeding, Kenneth J. Frey Professor and associate professor in the Department of Agronomy, will be identifying these qualities with the goal of applying them to a feedstock breeding program.

Thomas Lübberstedt and Marjorie Rover.

"Traits we identify in corn should be easily transferrable to other bioenergy grasses, as these are related species," says Lübberstedt.

Using a micro-pyrolyzer attached to a gas chromatograph/mass spectrometer for instant chemical analysis, "significant progress will be achieved with the ability to screen 30 samples per day," says Marjorie Rover, one of the scientists at the Center for Sustainable Environmental Technologies, who will pyrolyze biomass for the project.

The initial study will involve 3,200 samples of well-characterized corn plants, including a set bred for high and low lignin content and others representing exotic germplasm. Different plant parts, including cobs, above-ear and below-ear stover, will also be analyzed.

From these analyses, Lübberstedt will work to identify genomic regions and specific genes controlling desirable bio-oil promoting traits and look to develop genetic markers that will speed along a bio-oil breeding program, be it corn or related bioenergy grasses.

Preliminary analyses suggest high lignin and low mineral content are qualities the group is expecting will be important for yielding the best quality oil.

Recent research grants

The following 23 new grants totaling $8 million were awarded recently to plant science researchers at Iowa State.

TRPGR: Cyberinfrastructure for (Comparative) Plant Genome Research Through PlantGDB
National Science Foundation—$839,681
(V. Brendel, genetics, development and cell biology)

IGERT: Computational Molecular Biology Training Group
National Science Foundation—$599,622
(D. Dobbs, genetics, development and cell biology)

Protein Utilization, IA: Advanced Soybean Biorefineries
USDA, CSREES—$581,522
(L. Johnson, food science and human nutrition)

Agricultural Trade Analysis
USDA, CSREES—$410,816
(D. Hayes, economics)

A New Open Reading Frame in the Potyviridae Genome
USDA, NRI—$399,620
(W. Miller, plant pathology)

Food and Agricultural Policy Research Institute
USDA, CSREES—$362,526
(D. Hayes, economics)

Post-Transcriptional Regulation of Host Genes in Potyviral Infections at the Shoot Apex
Binational Agricultural Research and Development Fund—$154,000
(S. Whitham, plant pathology)

CPA-ACR: Parallel Algorithms and Software for Large Scale Microarry Data Analysis and Gene Network Inference
National Science Foundation—$246,197
(S. Aluru, electrical and computer engineering)

The Sudden Death Syndrome Research Alliance
University of Illinois—$62,174
(M. Bhattacharyya, agronomy)

TRMS: High-Throughput Functional Analysis of Soybean Defense Pathways by Virus-Induced Gene Silencing
National Science Foundation—$512,975
(S. Whitham, plant pathology)

Human Nutrition, IA
USDA—$447,253
(D. Birt, food science and human nutrition)

The Long-Distance Signaling Pathway that Controls Tuber Formation
USDA—$400,000
(D. Hannapel, horticulture)

Biotechnology Test Production, IA: Advanced Corn Biorefineries
USDA, CSREES—$319,467
(C. Glatz, chemical and biological engineering)

Food and Fuel Initiative: Iowa
USDA, CSREES—$277,797
(M. Misra, agricultural and biosystems engineering)

Enzymatic Cyclization to Labdanes and Related Diterpenoid Natural Products
National Institutes of Health—$269,964
(R. Peters, biochemistry, biophysics and molecular biology)

Control of Cap-Independent Translation by a Viral 3′ UTR
National Institutes of Health—$223,606
(W. Miller, plant pathology)

GEPR: Functional Genomics of Bud Endodormancy Induction in Grapevine
National Science Foundation—$215,700
(J. Dickerson, electrical and computer engineering)

Regulation of Chloroplast Biogenesis: The Immutans Mutant of Arabidopsis
U.S. Department of Energy—$160,000
(S. Rodermel, genetics, development and cell biology)

Bioinformatics and Computational Systems Biology Summer Institute at Iowa State University
National Science Foundation—$150,000
(R. Jernigan, biochemistry, biophysics and molecular biology)

Biofuels and the Hydrologic Cycle
National Science Foundation—$149,324
(R. Anex, agricultural and biosystems engineering)

SCA No. 58-3625-7-631 Development of Novel Maize Sequence Access and Analysis Methods for MaizeGDB
USDA, ARS—$80,000
(V. Brendel, genetics, development and cell biology)

Transferring Research from a Model System to Uncover the Network that Regulates Long-Distance Signaling in Potato
National Science Foundation—$694,269
(D. Hannapel, horticulture)

Popcorn—A Project Portal for Corn
National Science Foundation—$451,356
(C. Lawrence, genetics, development and cell biology)

Plant Sciences Institute UPDATE

The Plant Sciences Institute Update is published four times each year by the Plant Sciences Institute at Iowa State University, 1060 Roy J. Carver Co-Laboratory, Ames, Iowa 50011-3650; phone 515 294-5255.

The Plant Sciences Institute at Iowa State University is dedicated to becoming one of the world's leading plant science research institutes. More than 200 faculty from the College of Agriculture and Life Sciences, the College of Liberal Arts and Sciences, the College of Human Sciences, and the College of Engineering conduct research in nine centers of the institute. They seek fundamental knowledge about plant systems to help feed the growing world population, strengthen human health and nutrition, improve crop quality and yield, foster environmental sustainability and expand the uses of plants for biobased products and bioenergy. The Plant Sciences Institute supports the training of students for exciting career opportunities and promotes new technologies to aid in the economic development of agriculture and industry throughout the state. The institute is supported through public and private funding.

To be added to our mail list, e-mail psidir@iastate.edu.

On the Web at http://www.plantsciences.iastate.edu/

Printed with soy inks

Printed on recycled papers

IOWA STATE UNIVERSITY
Plant Sciences Insitute
1060 Roy J. Carver Co-Laboratory
Ames, Iowa 50011-3650