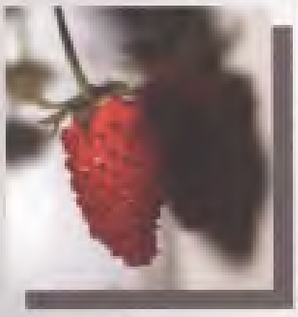
26th annual

Mid Atlantic Plant Molecular Biology Society Meeting

20-21 August, 2009



Patuxent National Wildife Refuge
Beltsville, Maryland

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WELCOME

On behalf of the Organizing Committees we welcome you to the twenty-sixth annual meeting of the Mid-Atlantic Plant Molecular Biology Society, MAPMBS 2009. MAPMBS was formed more than 25 years ago to provide a high quality, accessible and affordable plant molecular biology meeting each year for scientists in the Mid-Atlantic region. Our goal continues to be to bring some of the best minds in the country to the Mid-Atlantic region to present interesting advances in plant molecular biology at a reasonable price and an accessible location. We hope to encourage students, post-docs, and senior scientists to actively participate in presentations and discussions. In addition, the meeting is designed to promote interaction among scientists in an informal atmosphere. Therefore, we provide on-site lunches and breaks so that each participant has the opportunity to meet invited speakers and other presenters.

The meeting encompasses a broad range of research areas, which we hope will stimulate participants by informing them of advances outside of their own immediate interests. Please contact members of the organizing committee if you have any thoughts or comments for consideration in the planning of future meetings. Also, if you are interested, please join next year's organizing team and volunteer your services to improve upon this year's meeting. All are welcome at any stage of the planning and organizing process.

Many people were involved in the planning and organization of this meeting, and we give them our hearty thanks. We wish to especially thank our sponsors and exhibitors who furnish us with up-to-date products, and help to defray costs.

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society.

Benjamin F. Matthews and Leslie A. Wanner

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THURSDAY, AUGUST 20

9:00 Registration and poster set-up

9:20 Welcome (Ben Matthews and Leslie Wanner)

SESSION I Science Policy and Regulation
Moderator, **Bret Cooper**, USDA-ARS Soybean Genomics and Improvement Lab

9:25 **Caroline Wagner**, Senior Science and Technology Policy Analyst, SRI International and George Washington University
The New Invisible College of Science: Policy in a Globalizing Era

10:00 **Kenneth Haymes**, US EPA Office of Science Coordination and Policy
EPA and the Products of Biotechnology

SESSION II Genomics Tools for Agricultural Plants
Moderator: **Reid Frederick** USDA-ARS Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD)

10:20 **Janet Slovin**, USDA-ARS Genetic Improvement of Fruit and Vegetables Lab, Beltsville, MD
A genome-enabled reference species for fruit development, the diploid strawberry Fragaria vesca

10:40 **COFFEE BREAK: POSTER SESSION and EXHIBITORS**

11:10 **Kevin Folta**, University of Florida Horticultural Sciences Dept & Graduate Program in Plant Molecular and Cellular Biology
Strawberry Genomics: New Tools for Basic and Applied Studies in Crop Plants

11:45 **Chris Town**, J. Craig Venter Institute, Rockville, MD
Applications of Next Generation Sequencing in Plant Genomics

12:20 MAPMBS business meeting and planning for next year

12:30 **LUNCH/POSTERS/EXHIBITORS**

SESSION II, (cont.) Genomics Tools for Agricultural Plants
Moderator: **Dave Hyten**, USDA-ARS Soybean Genomics and Improvement Lab

1:40 **Zhan-Bin Liu**, DuPont Crop Genetics, Pioneer, Trait Discovery and Technology, Wilmington DE
Amino Acid Improvement in Soybean Seeds

2:15 **Thomas E. Clemente**, University of Nebraska Center for Biotechnology, Center for Plant Science Innovation & Department of Agronomy & Horticulture
Development and evaluation of novel input and output traits in soybean

2:50 **COFFEE BREAK: POSTER SESSION and EXHIBITORS**

3:20 **Introduction of Keynote Speaker, Bret Cooper**, USDA-ARS Soybean Genomics and Improvement Lab

3:25 **KEYNOTE ADDRESS**

GARY STACEY
National Center for Soybean Biotechnology, Divisions of Plant Sciences and Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri

Soybean genomics: Construction of a physical platform to support biological research

4:30 Close of Day (Building closes)

FRIDAY, AUGUST 21

9:00 **REGISTRATION, POSTER SESSION and EXHIBITORS**

SESSION III Molecular Biology Applied to Plant and Fungal Pathosystems
Moderator: **David Puthoff**, Frostburg State University, Frostburg, MD

9:30 **Christie Williams**, USDA-ARS Crop Production and Pest Control Research Unit and Department of Entomology, Purdue University
Hessian fly-induced resistance and susceptibility in wheat

10:05 **Petra Wolters**, DuPont Agricultural Biotechnology, DuPont Experimental Station, Wilmington, DE
Disease resistance in maize: the road from QTL to gene discovery and commercial product

10:40 **COFFEE BREAK: POSTER SESSION and EXHIBITORS**

11:10 **Daniella Thomazella**, Sustainable Perennial Crops Laboratory, USDA Beltsville/MD and Departamento de Genética e Evolução, Universidade Estadual de Campinas – Campinas/SP/Brazil
T***he mitochondrial alternative oxidase of*** **Moniliophthora perniciosa*: Its possible function in fungal metabolism and pathogenesis***

11:30 **Ralph Dean**, Center for Integrated Fungal Research, Dept. Plant Pathology, North Carolina State University
Beyond the genome sequence: Unraveling the complex biology of rice blast disease

12:25 **LUNCH: POSTER SESSION and EXHIBITORS**

SESSION III, cont. Molecular Biology Applied to Plant and Fungal Pathosystems Moderator: **Mark Holland**, Biology Dept, Salisbury University, Salisbury MD

1:30 **John Hammond**, USDA-ARS, Beltsville, MD
Shedding light on viral movement and pathogenicity through expression of fluorescent fusion proteins of Alternanthera mosaic virus

2:05 **Zongrang Liu**, USDA-ARS Appalachian Fruit Research Station, Kearnysville, WV
Enhancer-promoter interactions and insulation in plants: A neglected factor affecting tissue-specific engineering of gene function and genetic traits in crops

2:20 **Bret Cooper**, USDA-ARS, Beltsville MD, Soybean Genomics and Improvement Lab
Nuclear Proteomic Changes Linked to Soybean Rust Resistance

2:45 Close of day: Thanks for your participation! Please take down your poster

Poster #	Abstract Page	**Author**(s); affiliation: ***TITLE***
01	19	**Ron Collins** USDA- ARS, Sustainable Perennial Crops Laboratory, BARC, Beltsville, MD ***VERIFYING THE SUCCESSFUL APPLICATION OF FUNGAL BIOCONTROL AGENTS WITH DIFFERENT FORMULATIONS***
02	19	**Chuck Davis** and Mark A. Holland Department of Biology, Salisbury University, Salisbury, MD ***METHYLOTROPHIC BACTERIA IMPROVE THE GROWTH OF ALGAE CULTIVATED FOR BIODIESEL PRODUCTION***
03	20	**Karen Gau**[1], Erick Breathwaite[1], Jonathan Levin[1], Jeff Elhai[1,2], and Wan-Ling Chiu[1]* [1]Department of Biology; [2]Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA ***WHAT DOES IT TAKE FOR GUNNERA PLANTS TO FORM N2-FIXING SYMBIOSIS WITH CYANOBACTERIA***?
04	20	**Dapeng Zhang**[1], Michel Boccara[2,3], Lambert Motilal[1,2], Sue Mischke[1], Elizabeth S. Johnson[1], David R. Butler[2], Bryan Bailey[1], & Lyndel Meinhardt[1] [1]USDA, ARS, PSI, SPCL, Beltsville, MD, USA; [2]Cocoa Research Unit, The University of the West Indies, St. Augustine, Trinidad and Tobago; [3]Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France (CIRAD) ***CHARACTERIZATION OF AN ORIGINAL CACAO COLLECTION USING MICROSATELLITE DNA MARKERS***
05	21	**Arianne Tremblay**[1], Parsa Hosseini[1,2], Shuxian Li[3], Benjamin F. Matthews[1] [1]Soybean Genomics & Improvement Laboratory, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Beltsville, MD 20705, U.S.A., [2]Towson University, 8000 York Road, Towson, MD 21252 and [3]USDA-ARS, CGPRU, Stoneville, MS ***GENE EXPRESSION STUDY OF PHAKOPSORA PACHYRHIZI-GLYCINE MAX INTERACTION IN SUSCEPTIBLE PLANT USING NEXT GENERATION SEQUENCING***
06	22	**C. Srinivasan**, Zongrang Liu and Ralph Scorza USDA-ARS-Appalachian Fruit Research Station, Kearneysville, WV ***ECTOPIC EXPRESSION OF MALUS DOMESTICA CLASS 1 KNOX GENES ALTERED GROWTH AND DEVELOPMENT OF NICOTIANA TABACUM AND PRUNUS DOMESTICA AND INDUCED ADVENTITIOUS SHOOT REGENERATION FROM LEAF EXPLANTS WITHOUT EXOGENOUS CYTOKININ***
07	22	**Jelena M. Savic**[1, 2] and Ann C. Smigocki[1]. [1]USDA-ARS, Molecular Plant Pathology Laboratory, PSI, 10300 Baltimore Ave., Beltsville, MD 20705; [2]Institute for Biological Research "Sinisa Stankovic", Department of Plant Physiology, University of Belgrade, 142 Bul. despota Stefana, Belgrade, 11070, Serbia ***BIOTIC AND ABIOTIC REGULATION OF A SUGAR BEET SERINE PROTEINASE***

INHIBITOR GENE (BvSTI) IN INSECT RESISTANT SUGAR BEET

08 **Maximo Rivarola**[Ψ], Agnes Chan[ϕ], Janet Slovin*, and Pablo Rabinowicz[Ψ]
[Ψ]Institute for Genome Sciences, School of Medicine, University of Maryland, 801 W Baltimore St., Baltimore, MD;[ϕ]J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD; *Genetic Improvement of Fruits and Vegetables, USDA 10300 Baltimore Blvd, BARC-WEST Beltsville, MD

EST DISCOVERY AND CHARACTERIZATION OF ABIOTIC STRESSED STRAWBERRY PLANTS

09 Stephen Neyens[1], Michael Matthews[1], Benjamin F. Matthews[2] and Nadim W. Alkharouf[1]
[1]Towson University, 8000 York Road, Towson, MD 21252; [2] Soybean Genomics and Improvement Laboratory, ARS - USDA, Beltsville, MD 20705

THE SOYBEAN GENOMICS & MICROARRAY DATABASE, AN UPDATE

10 Heba M. M. Ibrahim[1,3], Nadim Alkharouf[2], Susan L. F. Meyer[3], Manar Sanad[1], Abd El Kader Gamal El-Din[1], Ebtissam Hussein[1] and Benjamin F. Matthews[3]
[1]Genetics Department, Faculty of Agriculture, Cairo University, Giza, Egypt; [2]Department of Computer and Information Sciences, Towson University, Towson, MD 21252; [3]USDA-ARS, Plant Sciences Institute, Beltsville, MD

POST TRANSCRIPTIONAL GENE SILENCING OF ROOT-KNOT NEMATODE IN SOYBEAN

THE NEW INVISIBLE COLLEGE OF SCIENCE: POLICY IN A GLOBALIZING ERA

Caroline S. Wagner

Senior Science and Technology Policy Analyst, SRI International & George Washington University

Science—defined broadly as knowledge about the natural world—offers humanity the promise of a better life. Scientific advances throughout history have helped save millions of people from disease, famine, and poverty. But the benefits of science have been unevenly distributed, and this has contributed to a widening gulf between the developed and developing worlds. This talk seeks to explain why that is so and offers a new framework for science policy that can help bridge the gap between the scientific haves and have-nots.
The twentieth century was the era of "big science." Driven by strategic rivalries and fierce economic competition, wealthy governments invested heavily in national science establishments. Today the organization of science is undergoing a fundamental transformation. In her book, The New Invisible College, Caroline Wagner combined quantitative data and extensive interviews to map the emergence of global science networks and trace the dynamics driving their growth. Her talk will focus on the shift from big science to global networks creates. This shift offers unprecedented opportunities for developing countries to tap science's potential. Rather than squander resources in vain efforts to mimic the scientific establishments of the twentieth century, developing country governments can leverage networks by creating incentives for top-notch scientists to focus on research that addresses their concerns and by finding ways to tie knowledge to local problem solving. The New Invisible College offers both a guidebook and a playbook for policymakers confronting these tasks—this will be the topic of presentation and discussion.

EPA AND THE PRODUCTS OF BIOTECHNOLOGY

Kenneth M. Haymes

U.S. Environmental Protection Agency, Office of Science Coordination and Policy
1200 Pennsylvania Ave. NW, Washington, DC 20460
Email: haymes.kenneth@epa.gov

The U.S. Environmental Protection Agency (EPA) is the Federal agency responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). The Agency's role in biotechnology is to ensure that sound scientific decisions are made for products registered for use as biopesticides and in industrial applications

Under the Coordinated Framework for Regulation of Biotechnology (51 FR 23302, June 26, 1986), EPA works closely with the U.S. Department of Agriculture (USDA), which has responsibilities under the Plant Protection Act (PPA), and the U.S. Food and Drug Administration (FDA), which has responsibilities under FFDCA. These Agencies consult and exchange information when such consultation is helpful in resolving safety questions. The three agencies also strive for consistency between programs following one of the basic tenets of the Coordinated Framework, in that the agencies composing the Framework adopt consistent approaches to the extent permitted by the respective statutory authorities. A consistent approach between agencies is easier for the regulated community to understand, and it likely conserves resources because data developed for one agency may meet some of the requirements posed by another agency for the same or similar products. In regulating products of biotechnology with other federal agencies, EPA works with international organizations on biotechnology and science-related issues and the development of

policy as it relates to biotechnology. EPA is also strongly committed to transparency in making its regulatory decisions by making its science policies and procedures available to the public.

A GENOME ENABLED REFERENCE SPECIES FOR FRUIT DEVELOPMENT; THE DIPLOID STRAWBERRY, FRAGARIA VESCA

Janet Slovin[1], Vladimir Shulaev[2], Kevin Folta[3], Thomas Davis[4], Daniel Sargent[5], N. Bassil[6] and Otto Folkerts[2]

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[2] VBI, Virginia Tech, Blacksburg, VA 24061
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Janet.slovin@ars.usda.gov

Strawberry is the only major Rosaceous crop plant that has an efficient transformation system for rapid elucidation of gene function. Fresh and processed products of the Rosaceae plant family (almonds, apples, apricots, blackberries, peaches, pears, plums, cherries, strawberries, raspberries, roses) in the U.S. are valued at over $7 billion. Understanding the genomics, genetics, and germplasm base of flower, fruit, and nut development, ripening, senescence, and microbial contamination is essential for maximizing and maintaining quality of these crops.

The diploid woodland strawberry, *F. vesca*, has been developed as a system for rapid discovery in strawberry genetics and genomics, and as a reference plant for the Rosaceae family. The octoploid (2n=8x=56) genome of the cultivated strawberry, *Fragaria* x *ananassa* is among the most complex of any crop species, making functional genetic studies difficult. However, the ~200 Mb size of the basic (x=7) strawberry genome ranks among the smallest of any cultivated crop species. Advantages of *F. vesca* include its self-fertility, fecundity, small plant size, short generation time (~3.5 months), amenability to genetic transformation, diverse germplasm base, and very small genome. *F. vesca* has been sequenced to greater than 30x coverage with 454 technology. In addition to a sequenced genome, a diploid genetic map is rapidly being populated with markers, documented inbred lines are available, and a highly efficient transformation system facilitates insertion mutagenesis and direct assessment of gene function with overexpression or rnai. A well-characterized *F. vesca* system enables us to develop useful assays to evaluate genes for their function in plant stress responses, flower development, fruit quality, disease resistance and a host of other horticulturally relevant traits. It will also facilitate study of the hormonal systems involved in growth and development of a non-climacteric fruit.

STRAWBERRY GENOMICS: NEW TOOLS FOR BASIC AND APPLIED STUDIES IN CROP PLANTS

Kevin Folta

Horticultural Sciences Department and the Graduate Program in Plant Molecular
And Cellular Biology Program, University of Florida

The cultivated strawberry (*Fragaria ×ananassa*) is a valuable crop with substantial acreage in the United States. Over the last several years great progress has been made in understanding the mechanisms underlying important traits of interest, as well as the genetic origin of this octoploid

organism. These milestones have been enabled by the growing body of genomic resources in the genus. Our laboratory has explored structural, functional and translational genomics in strawberry using octoploid strawberry and the related diploids. Analysis of intergenic regions in various cultivars and comparison to diploids provide strong evidence of subgenome origins in the octoploid materials. Our efficient transgenic systems permit in planta validation of gene function. Functional genomics work analyzing the strawberry "unknown-ome" have identified new genes with functional roles in plant biology. Examination of the genes associated with flowering time in *Arabidopsis* have been tested in strawberry, and show evidence of participation in the process with some notable exceptions. Together the new structural, functional and translational tools in strawberry make it a rich system to explore new areas of plant biology and their potential relevance to a wide array of high value crops.

APPLICATIONS OF NEXT GENERATION SEQUENCING IN PLANT GENOMICS

Chris Town

J. Craig Venter Institute, 9704 Medical Center Drive, Rockville MD 20850

Next generation sequencing is rapidly changing the face of plant genomics. At the time of writing, three technologies are actively in play and to some extent competing for the limelight. 454-Roche pyrosequencing techniques are now producing read lengths up to 700 bp with a median of over 400 bp. Paired end reads with 3 kb, 8 kb and 20 kb are available. 454 sequencing has been used for whole genome shotgun sequencing, for BAC pool sequencing and for transcriptome sequencing and examples of each will be discussed. The capabilities of the Illumina-Solexa Genome analyzer are advancing rapidly with read lengths of up to 100 bases and various paired end insert sizes either in production or promised in the near future. Single runs are now generating in excess of 50 Gb, although up to now most have been in the 10-15 Gb range. Already whole genome shotgun assemblies of genomes up to 500 Mb are being reported although the results have not yet been rigorously analyzed by third parties. The Illumina-Solexa technology is also being used for quantitative transcription profiling, chip-seq and epigenomic analyses. The third method is the ABI solid sequencing by ligation. This method is currently generating over 20 Gb per run with read lengths of 50 bp and paired end insert sizes of up to 10 kb. Current applications for solid are mainly in the areas of re-sequencing and expression profiling. In this presentation, I will review the principles underlying each of the methods and provide examples of applications in genomics and transcriptomics drawing both upon recent public data and our own experiences at JCVI.

AMINO ACID IMPROVEMENT IN SOYBEAN SEEDS

Zhan-Bin Liu, Byung-chun Yoo, Shirong Zhang, Bruce Schweiger, Tim Ward, Allison Haug, Bob Croes, Kevin Stecca, Tony Kinney

Dupont Crop Genetics, Pioneer, Trait Discovery and Technology, Wilmington, DE 19880

Efforts to improve the quality of food seed grains have long been an objective of mankind. Soybean seeds are limited for several essential amino acids, especially sulfur amino acids, tryptophan and lysine. Our research has been focused on improve all these essential amino acid composition in soybean seeds by combining both amino acid biosynthetic pathways as a source and a protein expression in seed as a sink approaches. We over-expressed a synthetic protein, BHL8, in soybean seeds. The BHL8 protein is rich in sulfur amino acids, lysine and tryptophan. At the same time, a feedback insensitive Cystathionine gamma synthase (CGS) or Serine acetyltransferase (SAT) gene was over-expressed constitutively for sulfur amino acid improvement.

A lysine feedback insensitive *Corynebacterium dapa* gene was over-expressed in seeds to increase lysine content. I will describe our experimental results to achieve the balanced amino acid composition in soybean seeds for feed application.

DEVELOPMENT AND EVALUATION OF NOVEL INPUT AND OUTPUT TRAITS IN SOYBEAN

Thomas E. Clemente

Center for Plant Science Innovation, Center for Biotechnology and Department for Agronomy & Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588-0665

The soybean biotechnology program at the University of Nebraska-Lincoln has developed soybean germplasm with a diverse set of input and output traits. With respect to the former, some of the novel traits include soybean events with resistance to the broadleaf auxin-type herbicide dicamba, and resistance towards two viral agents soybean mosaic virus and bean pod mottle virus. The output traits primarily target oil modification. Perturbing fatty acid biosynthesis in a seed-specific fashion, we have successfully developed soybean with high oleic acid (>85%) and low palmitic acid (<4%). This trait has been stacked with an elevated stearic acid (app. 15%) transgenic event, with the resulting germplasm producing oil with approximately 70% oleic acid and 17% stearic acid. A third type of soybean oil developed produces over 60% omega-3 fatty acids, composed of approximately 30% linolenic acid and 30% stearidonic acid. While soybean oil with high omega-3 fatty acids has value in food applications, we are currently investigating its utility as a feed ingredient for aquaculture.

KEYNOTE ADDRESS

SOYBEAN GENOMICS: CONSTRUCTION OF A PHYSICAL PLATFORM TO SUPPORT BIOLOGICAL RESEARCH

Gary Stacey

National Center for Soybean Biotechnology, Divisions of Plant Sciences and Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211

Plant genomics is revolutionizing our understanding of basic plant biology and, yet, the impact on major crop plant species is still limited. Until recently, emphasis has been placed on 'model' plant species (e.g., Arabidopsis). However, if these are models, then what are they models of? Where will we apply the knowledge obtained from the 'models'? Clearly, the targets must be crop plants, which ultimately provide the benefit to mankind. However, why work with models and then test these discoveries in crop plants, when the resources are available to make the original discoveries in the crop? In this scenario, application is direct and immediate.

The day is fast approaching when crop plants will have the resources available for molecular plant research that are now found only with model plant species. An example is soybean. The recent completion of the soybean genome sequence now enables a wide variety of molecular studies. This fact, coupled with a detailed genetic map, tremendous genetic resources and a dedicated research community make soybean an attractive system for both basic and applied plant research. In my presentation, I will outline the advances that have been made to develop soybean into a good research system and some of the struggles that took place to get to this point. Using my own research, as well as that of others, I will highlight how these resources are now being used to make significant discoveries in soybean.

HESSIAN FLY-INDUCED RESISTANCE AND SUSCEPTIBILITY IN WHEAT

Christie Williams[1], Subhashree Subramanyam[2] and Richard Shukle[1]

[1]USDA-ARS Crop Production and Pest Control Research Unit at Purdue University, Department of Entomology and [2]Departmant of Biological Sciences, 901 West State Street, West Lafayette, IN
Christie.Williams@ars.usda.gov

Through a gene-for-gene interaction, Hessian fly larvae induce in their wheat plant hosts either resistance or susceptibility. If the plant carries an appropriate resistance gene, it recognizes larval effectors and initiates a low-cost resistance response that includes production of toxic lectins and reinforcement of its surface cells. As a result, microvilli in the larval midgut are damaged and the larvae are unable to establish permanent feeding sites. However, if larvae are able to attack without triggering the plant's surveillance system, they can block resistance, even if plant resistance has already been induced in adjacent cells by a previous larval infestation. The induction of susceptibility leads to changes in plant cell permeability associated with loss of cuticular waxes and cutins, blockage of cell repair mechanisms and activation of pathways that deliver nutrients to the larvae. The spreading of this nutritive tissue is able to rescue even genetically avirulent larvae if they are located in close proximity to virulent larvae that induced the plant susceptibility. Molecular events leading to these changes will be discussed.

DISEASE RESISTANCE IN MAIZE: THE ROAD FROM QTL TO GENE DISCOVERY AND COMMERCIAL PRODUCT

Petra Wolters

Dupont Agricultural Biotechnology, Dupont Experimental Station
P.O. Box 80353, Wilmington, DE 19880-0353

Each year, infection of maize with fungal pathogens results in significant yield losses. At Pioneer Hi-bred we are improving disease resistance in maize hybrids through Marker Assisted Breeding for major QTL regions that confer resistance to fungal pathogens. An example of a QTL region that was fine-mapped, cloned and is currently utilized in product development is the Rcg1 region which significantly increases anthracnose stalk rot resistance in Pioneer hybrids. This locus harbors two NBS-LRR class genes that are both required for anthracnose stalk rot resistance. I will discuss our approach from QTL region to gene discovery and product development and will highlight several unique features of the *Rcg1* locus.

THE MITOCHRONDRIAL ALTERNATIVE OXIDASE OF Moniliophthora perniciosa: ITS POSSIBLE ROLE IN FUNGAL METABOLISM AND PATHOGENESIS

DPT Thomazella[1,2*], Rincones JR[2], Teixeira PJ[2], Toni IM[2], Pereira GAG[2], Meinhardt LW[1]

1) Sustainable Perennial Crops Laboratory, United States Department of Agriculture – Beltsville/MD
2) Departamento de Genética e Evolução, Universidade Estadual de Campinas – Campinas/SP/Brazil
*danidanibio@gmail.com

The hemibiotrophic fungus *Moniliophthora perniciosa* is the causal agent of witches' broom disease of cacao. As a consequence of this disease, great losses have occurred in many cacao producing countries of South America and the Caribbean islands. The *M. perniciosa* Genome Project has led to the identification of a putative gene homolog of an alternative oxidase protein (AOX). AOX bypasses complex III and IV and provides an alternative route for electrons passing through the electron transport chain to reduce oxygen. This oxidase is extensively studied because its activity accounts for the resistance of many phytopathogens to fungicides containing Cytochrome-dependent Respiratory Chain (CRC) inhibitors (e.g. Azoxystrobin). AOX is also believed to confer resistance against plant defense molecules (e.g. nitric oxide). In the present work, the expression profile of *aox* gene from *M. perniciosa* was analyzed *in vitro* under stress-related conditions and throughout the life cycle of *M. perniciosa* by qPCR. The *aox* gene was up-regulated under conditions of low nutrient availability and exposure to substances associated with the impairment of CRC pathway. In addition, the results showed AOX transcripts were considerably higher in the biotrophic phase of the fungus. Complementing these data, inhibitors of CRC (Azoxystrobin) and AOX (salicyl hydroxamic acid – SHAM) were tested on mycelia growth *in vitro*. Biotrophic and saprotrophic mycelia were able to grow in the presence of azoxystrobin. However, a higher sensitivity to SHAM was observed in the biotrophic mycelia. Overall, these results indicate AOX might play an important role during the biotrophic phase of the fungus. The enzyme may therefore be considered as a potential drug target to disrupt the life cycle of this pathogen by impairing its resistance to fungicides and plant defense mechanisms.

BEYOND THE GENOME SEQUENCE: UNRAVELING THE COMPLEX BIOLOGY OF RICE BLAST DISEASE

Ralph A. Dean

Center for Integrated Fungal Research, Dept. Plant Pathology, North Carolina State University, Raleigh, NC 27606, USA

Magnaporthe oryzae is the causal agent of rice blast, the most devastating disease of rice worldwide and is a seminal model to elucidate the basis of pathogen–host interactions. Following the completion of the genome sequence of both the fungus and its host, rice, research is focused on functional and other high throughput approaches to uncover the molecular and evolutionary foundation of fungal pathogenesis. Whole genome microarray analysis of appressorium formation induced by hydrophobic surfaces and by camp revealed a core set of genes that were differentially expressed when compared to conidial germination under non-inductive conditions. In addition to genes involved in lipid, carbohydrate and secondary metabolism, numerous genes involved in protein turnover and amino acid catabolism were significantly induced. I will show that protein catabolism, including endo-proteases and key enzymes involved in shuttling carbon back into the Krebs cycle, is critical for successful host infection. Other research efforts in my laboratory are currently focused on examination of novel non-coding transcripts, transcriptional networks using protein arrays and protein-protein interactions to define the circuitry regulating rice-rice blast interactions. I will present results from these ground-breaking studies and conclude with future research directions.

SHEDDING LIGHT ON VIRAL MOVEMENT AND PATHOGENICITY THROUGH EXPRESSION OF FLUORESCENT FUSION PROTEINS OF ALTERNANTHERA MOSAIC VIRUS

Hyoun-Sub Lim, Anna Maria Vaira, Gary Bauchan, and **John Hammond**

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The host range of viruses is determined in part by their ability to move cell-to-cell and long distance. Potexvirus movement functions are provided by coordinated action of the Triple Gene Block (TGB) and Coat (CP) proteins. We have examined subcellular localization of these proteins of *Alternanthera mosaic virus* (AltMV) by *Agrobacterium*-mediated transient expression and confocal microscopy and observed differences between AltMV and *Potato virus X* (PVX), the type member of the genus *Potexvirus*. Neither yeast two-hybrid assays nor confocal microscopy of co-expressed TGB2 and TGB3 fluorescent fusion proteins revealed interactions reported for *Potato virus X* (PVX). Agro-infiltrated AltMV GFP:TGB2 was clearly associated with the ER in both the epidermal and mesophyll layers, whereas AltMV GFP:TGB3 was not ER-associated, and accumulated preferentially in mesophyll cells, associating with the chloroplast membrane. In contrast PVX GFP:TGB3 localized to ER, mainly at the periphery of epidermal cells. Infectious clones of AltMV with either TGB2 or CP expression disrupted were unable to spread beyond the initially infected cells, whereas a clone with TGB3 expression ablated (AltMVΔTGB3) retained limited cell-to-cell movement within the epidermis, but was unable to move into the mesophyll; complementation of AltMVΔTGB3 with agro-infiltrated wild-type TGB3 restored the ability of TGB3-deleted AltMV to spread through the mesophyll to the opposite epidermis throughout the agro-infiltrated area. Over-expression of TGB3 from infectious AltMV caused veinal necrosis. These results suggest that TGB3 contributes to both cell-to-cell and long distance vascular movement. TGB3 deletion mutants revealed that a central domain of twelve of fewer amino acids directs targeting either GFP or DsRed to the chloroplast membrane; deletions lacking this region accumulated in the epidermal layer. The mesophyll and chloroplast targeting of TGB3 distinguishes AltMV from previously characterized potexviruses.

ENHANCER-PROMOTER INTERACTIONS AND INSULATION IN PLANTS: A NEGLECTED FACTOR AFFECTING TISSUE-SPECIFIC ENGINEERING OF GENE FUNCTION AND GENETIC TRAITS IN CROPS

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Transgenic technology is an important tool for both basic plant biological research and the improvement of agronomic traits in a wide variety of crop species through the stable expression of foreign genes. Often, this necessitates the use of tissue-, organ- or developmental stage-specific and strong constitutive promoters to drive transgene expression exclusively in targeted tissues. However, the presence of multiple promoter and enhancer elements within a single vector might, due to the inherent orientation-independent nature of enhancers, provoke enhancer-promoter or promoter-promoter interference, thereby altering the specificity and strength of discrete promoters in transgenic plants. We demonstrated that both tissue-specific and constitutive enhancer elements can effectively activate adjacent promoters in non-targeted tissues. Our detailed analyses revealed that enhancer-mediated gene activation occurred through the non-specific activation of gene transcription at multiple sites. Furthermore, we took advantage of this enhancer-promoter interaction and developed a new assay strategy for the identification of enhancer-blocking

insulators in plants. Our study showed that at least two of the DNA fragments tested were able to function as enhancer-blocking insulators in plants. Potential applications for the identified insulators in the remediation of enhancer-promoter interactions and crosstalk during plant transformation and in transgenic plants will be discussed.

NUCLEAR PROTEOMIC CHANGES LINKED TO SOYBEAN RUST RESISTANCE

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Approximately 4,975 proteins from nuclear preparations of soybean leaves were detected using a high-throughput liquid chromatography-mass spectrometry method. Statistics of summed spectral counts revealed sets of proteins with differential accumulation changes between isogenic soybeans susceptible and resistant to the soybean rust fungus. These protein accumulation changes were compared to previously reported gene expression changes and very little overlap was found. Many of these proteins have predicted nuclear localization signals, have homology to transcription factors and other nuclear regulatory proteins, and are phosphorylated. These results suggest that numerous plant proteins are post- translationally affected in the nucleus after infection. It is possible that some of these proteomic changes influence defense responses that ultimately confer resistance to soybean rust. This is the first indication of large-scale proteomic change in the nucleus of any plant after infection. In addition, a new concept, termed proteogenetics, is introduced whereby proteomics information is used to complement DNA marker information to map a genetic trait.

01 *VERIFYING THE SUCCESSFUL APPLICATION OF FUNGAL BIOCONTROL AGENTS WITH DIFFERENT FORMULATIONS*

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Verifying the successful application of fungal biocontrol agents consist primarily of measuring spore germination and colonization of the plant. The effectiveness of the fungal biocontrol agent and the long term colonization of the host plant are not the preliminary concern of the verifying the successful application.

Spore germination for different formulations has historically been conducted in Petri dishes or on the plant surface in the laboratory. Spore germination test in Petri dishes give maximum potential spore germination. Spore germination test on plant surfaces in the lab give a more realistic picture of spore germination in relation to plant surface. The spore germination on the plant surface has two inherent sources of variability. First, finding the exact position on the plant surface where the spores were applied. Second, the timing of the spore extraction from the plant surface is critical. If the spores are extracted too early, germination percentage will be low. If the spores are extracted too late, many of the spores have germinated and the mycelia are attached to the plant surface. The germination percentage is very low.

A method was developed combining the proceeding two methods with finger nail polish and two sided tape. This new method eliminated the previous problems. As well as allowed for non-destructive plant sampling in the field after biocontrol agent application.

02 *METHYLOTROPHIC BACTERIA IMPROVE THE GROWTH OF ALGAE CULTIVATED FOR BIODIESEL PRODUCTION*

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In order to reduce America's dependence on foreign oil, biodiesel has been suggested as an alternative that can be produced on the home front. Traditional sources such as corn, however, take vast land requirements and influence the price of staple food supplies for much of the world's population. Lipid rich algae are an alternative source of biodiesel that can be produced in large quantities in a very small area. In order to investigate a strategy for increasing the productivity of algae farms, both *Neochloris oleoabundans* and *Chlamydomonas* were grown experimentally in the presence of pink pigmented facultatively methylotrophic bacteria (PPFMs). One of two types of PPFMs, wild type *Methylobacterium mesophilicum* and a B-12 overproducing mutant, were co-cultivated with each species. After measuring the growth of the algae for two weeks, significant differences were noted between those grown with added bacteria and those grown without, as well as between the two strains of bacteria. Analysis of the results shows that the addition of even a small amount of PPFMs can enhance the growth of algae dramatically. Data from the experiments above as well as economic ramifications will be discussed.

03 *WHAT DOES IT TAKE FOR GUNNERA PLANTS TO FORM N_2-FIXING SYMBIOSIS WITH CYANOBACTERIA?*

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Gunnera is the only angiosperm genus known to form N_2-fixing symbioses with cyanobacteria. Cyanobacteria (primarily *Nostoc*) enter a *Gunnera* host through mucilage-secreting glands on the stem and form intracellular colonies in tissue behind the glands. Insight into the means by which *Gunnera* cells attract and maintain cyanobacteria may enable us to extend biological N_2-fixation to other plants.

Unlike legume-rhizobia associations, those between *Gunnera* and cyanobacteria make use of symbiotic structures that can develop in the absence of the bacterial partner. Our investigation suggests that *Gunnera* evolved the ability to utilize factors associated with N-deprivation in other plants, such as sugar- and flavonoid-accumulation, to initiate symbiotic gland development. To test the role of flavonoids in gland development, we have introduced RNAi constructs into *Gunnera* plants in order to knockdown the expression of several genes for chalcone synthase (CHS), the first enzyme in the flavonoid biosynthesis pathway. Preliminary results indicate that, instead of forming glands, putative transgenic plants develop adventitious roots on their stems.

As a first step in the characterization of *Gunnera-Nostoc* symbioses at the molecular level, we have sequenced a normalized cDNA library representing genes expressed in mature *Gunnera* glands, using both traditional and 454 sequencing technologies. Currently, we are analyzing genes expressed in the symbiotic glands to identify those that may enable *Gunnera* cells to initiate and maintain cyanobacteria for the purpose of N_2-fixation.

04 *CHARACTERIZATION OF AN ORIGINAL CACAO COLLECTION USING MICROSATELLITE DNA MARKERS*

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The chocolate tree (*Theobroma cacao* L.) originated in the Amazon River basin, and this region of South America harbors a large number of diverse cacao populations. Several collections from the present-day Peruvian Amazon, dating from the 1930s, have been maintained in ex situ germplasm repositories in various countries. However, the efficient conservation and use of this historic cacao germplasm is greatly hindered by lack of information on population structure and pedigree relationship, and by the inaccuracies in labeling the accessions that have accumulated over the years. The present study assessed individual identity, sibship, and population structure in cacao populations collected from the present-day Loreto Region, Peru during the 1930–1940s. Using a capillary electrophoresis genotyping system, we analyzed simple sequence repeat variation of 612 cacao accessions. A Bayesian clustering method for admixture detection identified 180 cases of

mislabeling. Maximum likelihood methods indicated the pods collected in the 1930s were from a maximum of 48 mother trees. Likelihood simulation also identified eight probable parents that are responsible for 117 pairs of mother–offspring relationships in this collection. Despite the high level of allelic diversity in this collection, it was composed of a large number of related family members collected from a relatively small area. The vast majority of the Peruvian Amazon, especially the upper Marañon River and its tributaries, has not been sampled by collecting expeditions. In addition to demonstrating a pronounced structure of genetic diversity, stratified by the river systems of the Peruvian Amazon, this study provides important baseline information to guide future expeditions intent upon collecting diverse cacao germplasm in the Peruvian Amazon.

05 *GENE EXPRESSION STUDY OF PHAKOPSORA PACHYRHIZI-GLYCINE MAX INTERACTION IN SUSCEPTIBLE PLANT USING NEXT GENERATION SEQUENCING*

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Soybean is one of the top five agricultural products in the United States. Protection of soybean from present and new exotic pathogens is very important for soybean production. Soybean rust is caused by the obligate fungus *Phakopsora pachyrhizi* Sydow, an exotic pathogen. This pathogen causes yield losses due to premature defoliation, fewer seeds per pod and decreased number of filled pods per plant. From this perspective we want to analyze the expression pattern of *P. pachyrhizi* genes and its soybean host genes during the infection process. Thus, we constructed and analyzed cDNA libraries to identify candidate genes that may be useful to provide resistance to *P. pachyrhizi*. Libraries were constructed from RNA isolated from soybean palisade cells infected by *P. pachyrhizi* at different time-points during the infection course. Infection sites were visualized by immunofluorescence using an antibody raised against a *P. pachyrhizi* secreted protein. Infection sites were isolated by laser capture microdissection. The libraries were sequenced using Next-Generation Sequencing. DNA sequences were aligned to the soybean genome (Glyma1 version from DOE JGI) and homology searches were conducted to determine the identity of the genes. From sequences without similarity to soybean genome, contigs were formed and homology searches were conducted to determine the identity of the genes. In our first experiment on 10 days after inoculation, we obtained 14,869,540 sequences that align on the soybean genome. These sequences correspond to 16,487 annotated mRNA regions from annotation release by DOE JGI. The number of times a gene is sequenced reflects the abundance of the mRNA and the level of transcription of the gene. Sequence counts from these expressed genes ranged from 1 to 4,545. We found 7,909,330 sequences without any homology to the soybean genome. These are expected to be *Phakopsora pachyrhizi* sequences. These sequences formed 10,674 contigs. Thirteen percent of these contigs had homology (e-value ≤ 10-5) with proteins in the NCBI database. Most of them (82%) were homologous to fungal proteins. Although a number of hits described hypothetical or predicted proteins, we found interesting genes such as those involved in signal transduction, sporulation and germination and intracellular communication. In the future, target pathogen and host genes will be studied to determine if they can be used to control ASR in soybean.

06 *ECTOPIC EXPRESSION OF* MALUS DOMESTICA *CLASS 1* KNOX *GENES ALTERED GROWTH AND DEVELOPMENT OF* NICOTIANA TABACUM *AND* PRUNUS DOMESTICA *AND INDUCED ADVENTITIOUS SHOOT REGENERATION FROM LEAF EXPLANTS WITHOUT EXOGENOUS CYTOKININ*

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Transgenic tobacco (*Nicotiana tabacum* L.) and plum (*Prunus domestica* L.) plants were regenerated by transforming with apple class 1 *KNOX* genes (*MdKNP1* and *MdKNP2*) or corn *KN1* (*ZmKN1*) gene. Transgenic tobacco plants were produced in vitro from transformed leaf discs in the absence of cytokinin in the culture medium. Ectopic expression of *KNOX* genes retarded shoot growth by suppressing elongation of internodes in both tobacco and plum plants. When over-expressed, all three *KNOX* genes reduced leaf lamina expansion and induced extensive lobing and malformation of leaves. In vitro culture of leaf explants and stem sections excised from in vitro grown *MdKN1* expressing tobacco shoots regenerated adventitious shoots on Murashigi and Skoog basal medium without exogenous cytokinin. However, leaf explants excised from in vitro grown shoots of *MdKN1* over-expressing plum required 7.5 µM thidiazuron in the culture medium for adventitious shoot regeneration. In situ regeneration of adventitious shoots was observed from leaves and roots of transgenic tobacco plants expressing apple *KNOX* genes.

07 *BIOTIC AND ABIOTIC REGULATION OF A SUGAR BEET SERINE PROTEINASE INHIBITOR GENE (BvSTI) IN INSECT RESISTANT SUGAR BEET*

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BvSTI (*Beta vulgaris* serine protease inhibitor) is a gene we cloned from the F1016 sugar beet breeding line that has moderate levels of resistance to one of the most devastating insect pests of sugar beet in North America, the sugar beet root maggot (SBRM, *Tetanops myopaeformis* Roder). *BvSTI* codes for a serine (trypsin-type) proteinase inhibitor, a protein that targets the major digestive enzymes in root maggot midguts. Expression of the *BvSTI* gene was analyzed in resistant F1016 and susceptible f1010 3- and 6-month old plants. Leaves and roots were collected after mechanical wounding or fall armyworm (FAW, *Spodoptera frugiperda*) feeding. FAW, a pest that feeds on hundreds of vegetable crops, was used since SBRM cannot be reared *in vitro*. In the resistant F1016 line, *BvSTI* gene expression was up-regulated by mechanical wounding. Transcript levels were always higher in the wounded F1016 tissues. The highest level of transcription was reached 6 hours after wounding both in roots and leaves. The opposite pattern was observed in the susceptible F1010 line. Levels of *BvSTI* transcripts decreased in mechanically wounded F1010 tissues. Insects feeding on the F1016 tissues also induced expression of the *BvSTI* gene, however, the response was delayed with highest transcript levels observed after 24 hours. In the susceptible F1010 line, the response to FAW feeding was similar to that observed for the F1016 line. Trypsin proteinase inhibitor activity in f1016 and F1010 was analyzed on polyacrylamide gels. High level of correlation between *BvSTI* gene expression and its product *BvSTI* protein was observed. FAW feeding bioassays on F1016 and F1015, a similarly resistant line, produced smaller larvae with higher mortality rates relative to the susceptible F1010 line. Our results demonstrate that in 3- and 6-month old F1016 and F1010 plants, the level of *BvSTI* gene

expression coincides with pest-insect resistance. In addition, since we observed different patterns of *BvSTI* gene expression in response to the biotic and abiotic induction, our findings suggest that insect-specific wounding may be important for regulation of *BvSTI* gene expression.

08 *EST DISCOVERY AND CHARACTERIZATION OF ABIOTIC STRESSED STRAWBERRY PLANTS*

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Strawberries (*Fragaria*) are eudicotyledon plants that belong to the Rosaceae family. In addition to raspberries and blackberries, this family contains a number of deciduous fruit trees of agricultural importance, including apples, peaches, plums, pears, apricots, nuts and cherries. Strawberries alone have an annual market value of ~ $1.1 billion in the United States. In contrast to its importance, few stress-related expression characterizations have been performed to date. Abiotic stresses pose a serious problem for agriculture and its effects may become even more prominent as global climate change continues. Abiotic stresses can account for substantial yield losses; as a result, some strawberry growers are beginning to irrigate their crops with reverse osmosis water to prevent high salinity deterioration. In this study, we set out to identify new stress-related genes by sequencing cDNAs in *Fragaria vesca* from different tissues from strawberry plants treated with several abiotic stresses. The outcomes of this project will advance multiple areas of *Fragaria* research, such as development of molecular markers, gene discovery, and comparative genomics. Four different abiotic stresses (cold, high salt, heat, and drought) and a combination of high salt and heat stress were selected as treatments of *F. vesca* strain PI551574 Hawaii 4. Several RNA samples form either seedlings or mature plants were taken for every condition and a total of ~ 41500 expressed sequence tags (ESTs) were successfully sequenced. After clustering and assembling the sequences, a total of 11,836 unique sequences were obtained. The putative function of the consensus sequences was assigned using Blast2GO, which assigns gene ontology (GO) terms to the sequences using a combination of similarity searches and statistical analysis. GO terms could be assigned to 5112 EST assemblies among which, 361 were identified as 'stress-related'. Six of these stress-related assemblies showed no similarity to existing Rosaceae ESTs. We also found 91 genes that show at least a 20-fold difference in the level of expression between treatments, indicating stress-specific expression. Moreover, our results suggest that heat (either by itself or in combination with high salt) give the highest expression of 'stress-related' genes. Furthermore, our approach identified ~ 710 (6%) unique expressed sequences that do not show any similarity to existing Rosaceae ESTs, expanding the genomics research framework for *Fragaria*.

09 *THE SOYBEAN GENOMICS & MICROARRAY DATABASE, AN UPDATE*

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The Soybean Genomics and Microarray Database (SGMD), was developed in 1999 to serve soybean functional genomic research, specifically related to soybean-pathogen interactions. In 2009 the database is undergoing a major face lift. The search pages have been redesigned based on the .NET framework using ASP.NET access and login features. The user will have the power to browse the database to view gene names, fold changes, p-values and probe IDs for a given experiment and\or time point. The user will also be able to find commonalities between two experiments and their respective time points to determine whether or not fold change or p-values differ. For power users, the ability to edit gene annotations in the database will be given; however, these powers are only given to those working locally with the database. Online users will, however, be able to propose an edit of the data for our power users to review. New visualization tools are also expected to be incorporated into the database allowing a user to view data for a time course experiment in a line graph instead of tables. Work is also underway on incorporating pathway information to the database, so a user can visually see what genes are being differentially expressed in a given pathway. All this is expected to help scientists better understand the complex reactions that soybeans, and plants in general, go through when fighting off a pathogen.

10 *POST TRANSCRIPTIONAL GENE SILENCING OF ROOT-KNOT NEMATODE IN SOYBEAN*

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Plant parasitic nematodes cause about $100 billion in crop losses annually. Root-knot nematodes (RKN; *Meloidogyne spp.*) are sedentary endoparasites, and the genus has been found on more than 3000 host plant species. The most economically important species in this genus is *Meloidogyne incognita,* which is widespread and considered to be an important crop pathogen worldwide.
RNA interference (RNAi) has recently been used to knock down gene expression or silence genes in plant-parasitic nematodes. It is a newly discovered gene regulatory mechanism that can be used to determine gene function. Therefore, the present investigation was conducted with two main objectives. The first objective was to study gene expression and product functions of those genes that are turned on during infection in comparison to the non-infected controls. The second objective was to design RNAi constructs to silence specific nematode genes that are expected to have essential functions in RKN, so that the nematode can no longer complete its life cycle.
In this work, RKN genes having high similarity with essential soybean cyst nematode (*Heterodera glycines*) and *C. elegans* genes were identified using BLAST. Four RKN genes were chosen according to their expected essential function in the life cycle. The four RKN genes were amplified, cloned, and used to transform soybean to obtain roots expressing RNAi to silence these RKN genes. The transformed roots were recognized by the presence of green fluorescent protein. The transformed roots were challenged with RKN and at different time points we took root samples for staining to monitor nematode infection inside the RNAi transformed roots compared to control roots. After 28 days post infection, we found that two constructs interfered with the life cycle of RKN. The number of galls formed on these roots was significantly reduced by 92% and 94.7%. Also the development of the nematodes inside the transformed roots was retarded and the diameters of the nematodes were noticeably smaller compared to the control. These results indicate that silencing these genes can be a valuable approach for broadening resistance against this plant-parasitic nematode.

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