

**2015 DOE Bioenergy Technologies Office (BETO)  
Project Peer Review**

**Research and Technology Development for Genetic  
Improvement of Switchgrass**

**Albert Kausch and Richard Rhodes, University of Rhode Island  
Award # DE-FG-36-08GO88070**



Date: March 24, 2015

Technology Area Review: Feedstock Supply & Logistics

Principal Investigator: Albert Kausch

Organization: University of Rhode Island

# Goal Statement



## *Long Term Goals and Specific Objectives*

### *Long Term Goals*

*The overarching goal of this project is the development of technology leading to commercial switchgrass hybrid varieties engineered for enhanced, low-cost conversion of cellulosic biomass to liquid biofuels. Another goal is the development of intellectual property that is widely applicable to bioenergy and agricultural crops generally.*

### *Specific Objectives*

- Development of hybrid plant systems using male and female sterility*
- Development of advanced breeding strategies utilizing wide crosses, advanced tissue culture and genomics to produce new Non-GMO hybrids*
- Development of robust transgenic and gene confinement strategies*
- Enhance education, student training and internship research opportunities in biofuels crop improvement and plant biotechnology*

# QUAD CHART OVERVIEW

2015 DOE Technology Area Review: Feedstock Supply & Logistics  
Research and Technology Development for Genetic Improvement of Switchgrass  
Albert Kausch and Richard Rhodes, University of Rhode Island  
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## Timelines

Project start date 11/30/08  
Project end date (NCE) 05/30/15  
Percent complete 98% (sunset)

## Budget

	Total Costs FY 10 –FY 12 \$3,155,809	FY 13 Costs \$899,242	FY 14 Costs \$572,900	Total Planned Funding (FY 15- Project End Date 05/30/15) \$237,841
DOE Funded	\$3,435,500	\$0.00	\$0.00	\$0.00
Project Cost Share (Comp.)*	\$738,617	\$0.00	\$0.00	\$0.00

## Barriers addressed MYPP 2.1.3

**Ft-A Feedstock Availability and Sustainable production and yield in Switchgrass and related species**

**Ft-C Feedstock Genetics and Development – New varieties; Breeding and genetics; Transgenics and gene confinement**

## Partners

University of Rhode Island

Yale University

Plant Advancements LLC

Ernst Conservation Seeds Inc

## Project Management

Dr. Albert Kausch, Director, PBL

Dr. Richard Rhodes, III

# PROJECT OVERVIEW

**History:** *This project was conceived through the collaboration of academic and industry researchers at the University of Rhode Island, Yale University and Ernst Conservations Seeds Inc to meet the need for technology development related to new bioenergy cultivars and gene confinement for GMO trait improved crops.*

**Accomplishments/Context:** *Development of hybrid plant systems is important for both advanced breeding and gene confinement purposes. In this project we have discovered new technologies to develop hybrid plants and non-GMO wide crosses. Also of significance, this project has developed robust transgenic and gene confinement strategies to allow genetically improved varieties to be deregulated more quickly through the USDA and eventually released for commercial production on a large scale.*

**The High Level Objectives of this project, dubbed ‘Project Golden Switchgrass’** *are to create hybrid systems, advanced genomics assisted breeding, and gene confinement platforms that are broadly applicable to bioenergy and agricultural crops. This enabling technology aims to improve the efficiency and throughput of breeding programs directed at development of high performance crop varieties*

# **PROJECT MANAGEMENT PLAN (PMP)**

**Bioenergy Technologies Office (BETO)**

**UPDATED PMP August 20, 2014**

## **Task M. Technology for New Varietal Development**

**Subtask M.1 Wide cross hybrids**

**Subtask M.2 Genomic assisted breeding**

**Subtask M.3 Evaluation of field grown accessions**

## **Task N. Technology for Hybrid Plant Systems**

**Subtask N1. Evaluation of molecular constructs**

**Subtask N2. Production and evaluation of transgenic lines**

**Subtask N3. Screen for Transgenic events**

**Subtask N4. Greenhouse test of transgenics**

## **Task O. Development and introduction of new trait genes into switchgrass**

**Subtask O.1 Establish collaborations with industrial and academic partners for access to trait genes for improved biofuels traits.**

**Subtask O.2 Create the transgenic populations with selected traits.**

**Subtask O.2.1 Wide cross development relating to Task M**

## **Task P. Project Management and Reporting**

# Research and Technology Development for Genetic Improvement of Switchgrass



## 1- Approach

### *I. Hybrid Systems:*

- *Development of male (pollen) and female (seed) sterility systems*
- *Wide crosses recovered through a novel embryo rescue technique*
- *Recovery of Non-GMO hybrids*

### *II. Genomics Assisted Breeding*

- *Genomic characterization of Non-GMO hybrids (F1BC1 population)*
- *Development of male (pollen) and female (seed) sterility systems*
- *Wide crosses recovered through a novel embryo rescue technique*
- *Recovery of Non-GMO hybrids*

### *III. Transgenic Trait Improvement*

• *Gene Confinement and Development of GM Traits for Biofuel Crop Improvement*

### *IV. Patent and other IP development*

*Seeking industry partnerships for collaboration and introduction of novel transgenic traits*

# Research and Technology Development for Genetic Improvement of Switchgrass

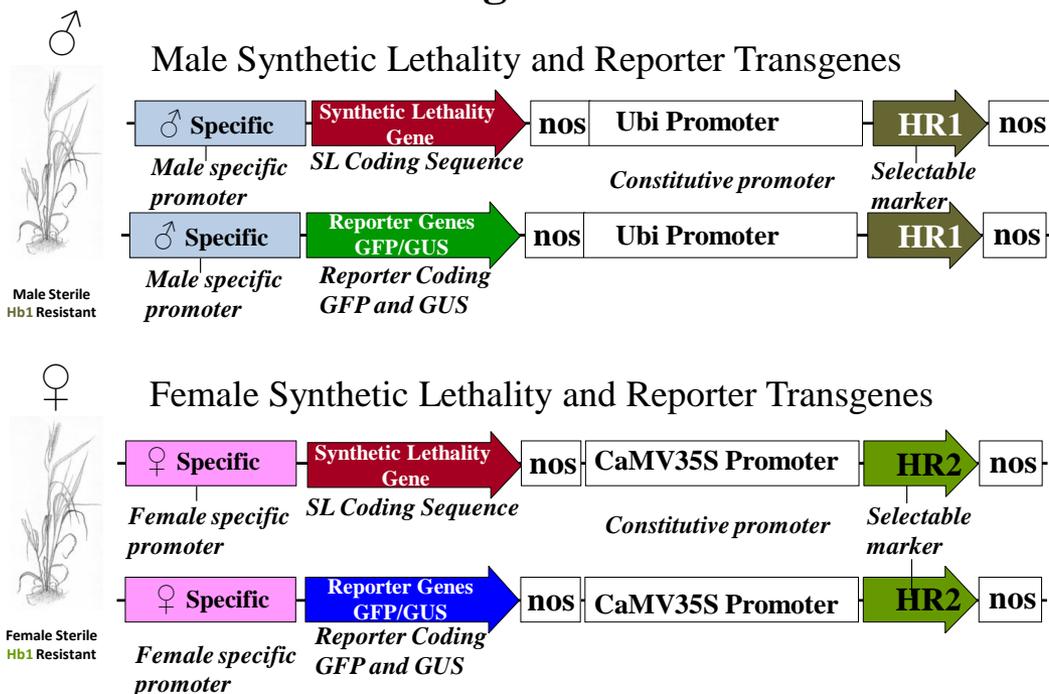


## 2 - Technical Accomplishments/Progress/Results

### I. Hybrid Systems:

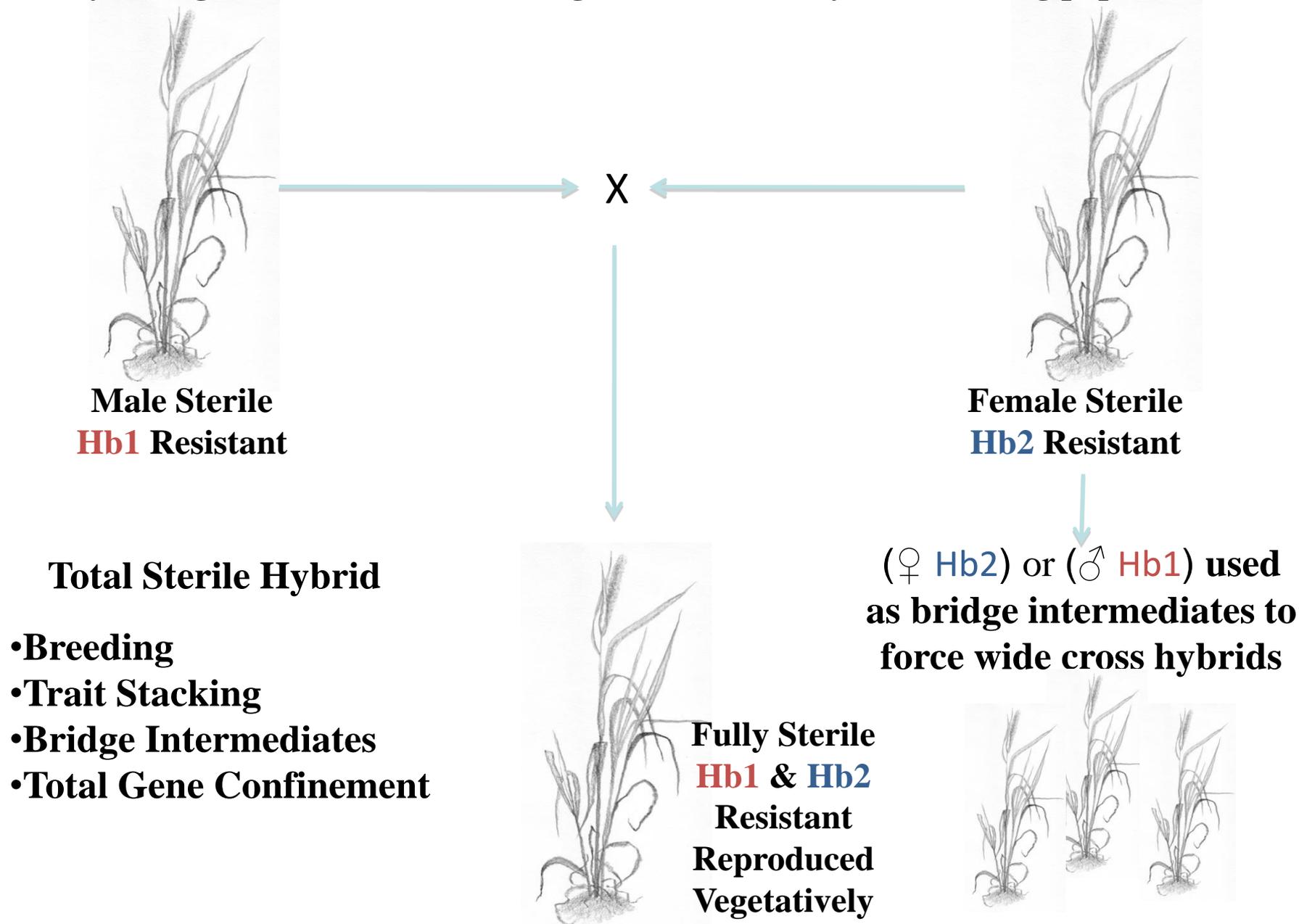
- *Development of male (pollen) and female (seed) sterility systems*

## Transgenes for Male and Female Sterility Useful for Breeding and Gene Confinement



Male and female sterile lines useful for hybrid plant breeding or gene confinement. Male and female lines are created through the application of the promoters and/or the coding sequences.

# Physical linkage of herbicide resistance (HR1 and HR2) with male- and female-sterility transgenes for creation of bridge intermediate hybrid breeding populations



# Research and Technology Development for Genetic Improvement of Switchgrass

## 2 - Technical Accomplishments/Progress/Results

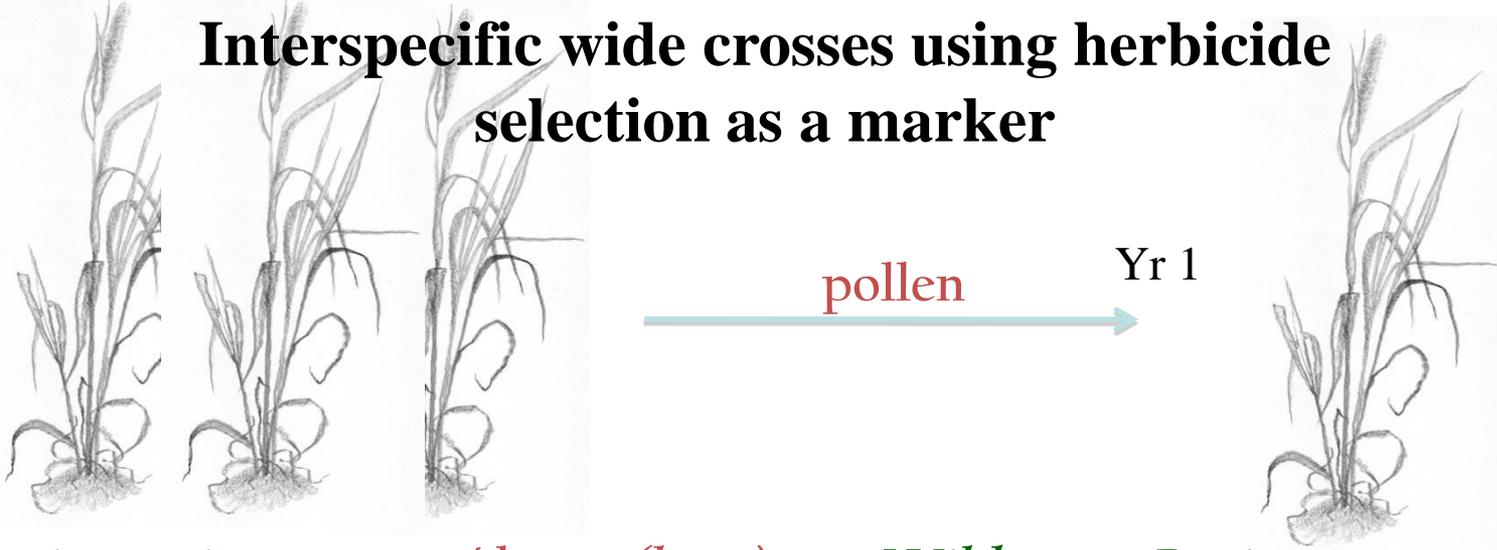
### *I. Hybrid Systems:*

- *Wide crosses recovered through a novel embryo rescue technique*
- *Recovery of Non-GMO hybrids*

**Can We Use Herbicide Resistant Lines for Recovery of Wide Crosses?**

**Can We Use Wide Crosses to Create Non-GMO Hybrids?**

# Interspecific wide crosses using herbicide selection as a marker



*GM Panicum virgatum* cv Alamo (*bar+*)  
Switchgrass 4X  
Hbl Herbicide Resistant

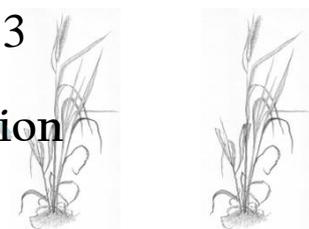
*Wild -type Panicum amarum* Ell.  
Atlantic Coastal Panicgrass (ACP) 4X  
Herbicide Sensitive

New Yr 4  
Commercial  
Variety



*(bar+)* population

Selection  
Biomass



*(bar)* population

non-GMO Hybrid [Alamo X ACP] X Alamo

*Wild -type Panicum virgatum* cv  
Alamo  
Switchgrass 4X  
Herbicide Sensitive

Yr 2

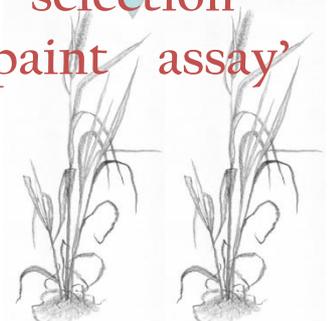


backcross



*(bar+)*

Germination and herbicide selection 'paint assay'

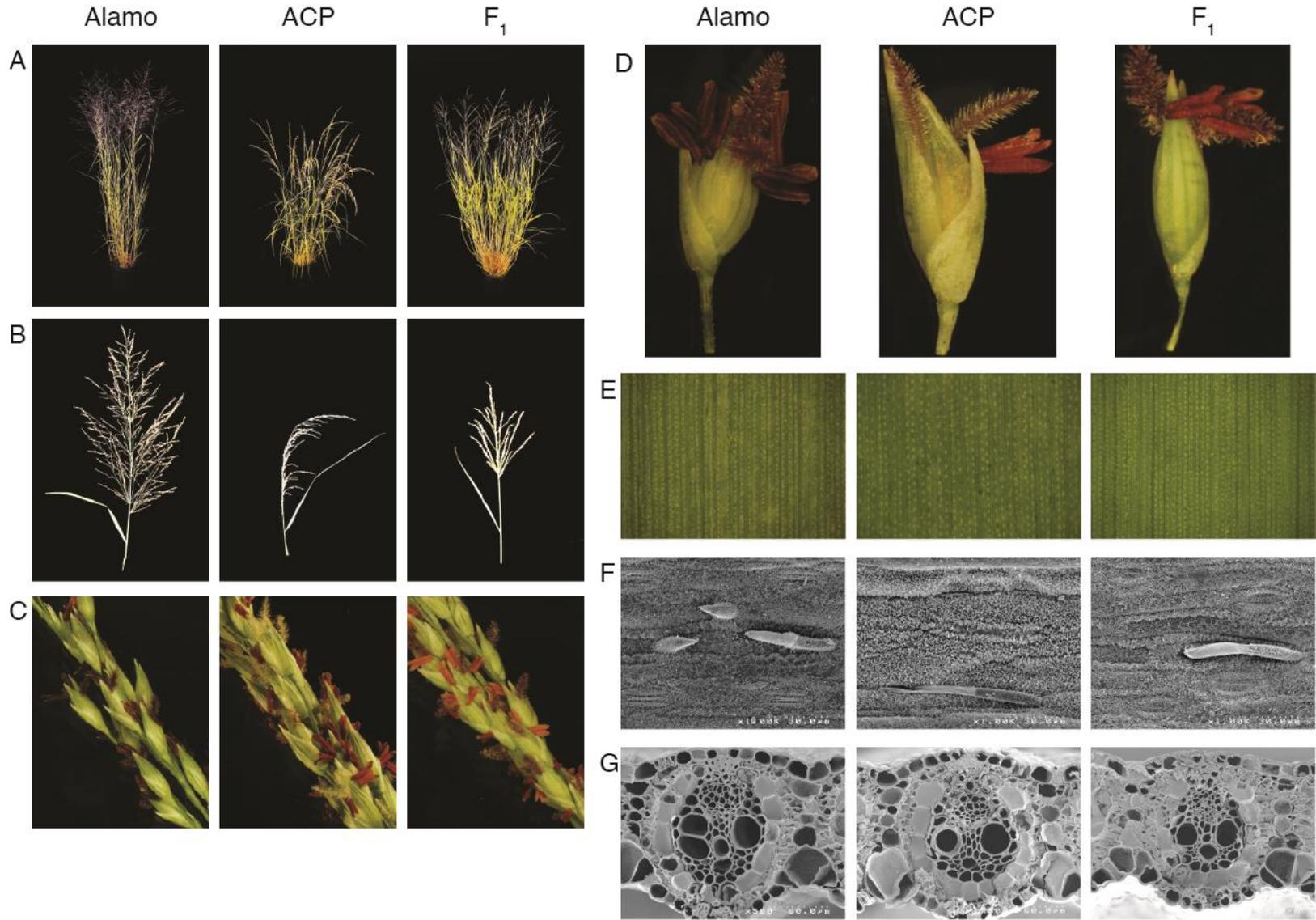


*(bar+)*

*(bar)*

*(bar+)* Hbl Herbicide Resistant Alamo X ACP

# Characterization of Parental and F1 Hybrids

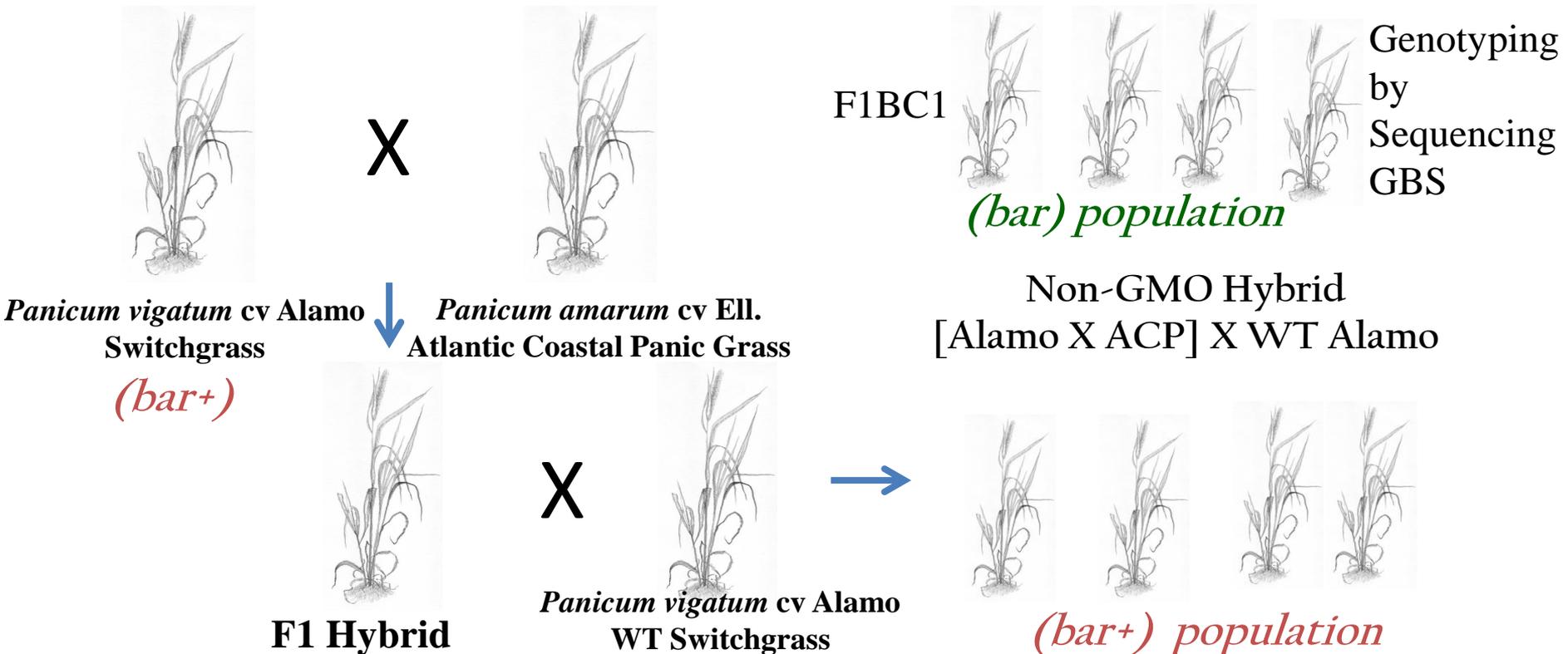


# Research and Technology Development for Genetic Improvement of Switchgrass

## 2 - Technical Accomplishments/Progress/Results

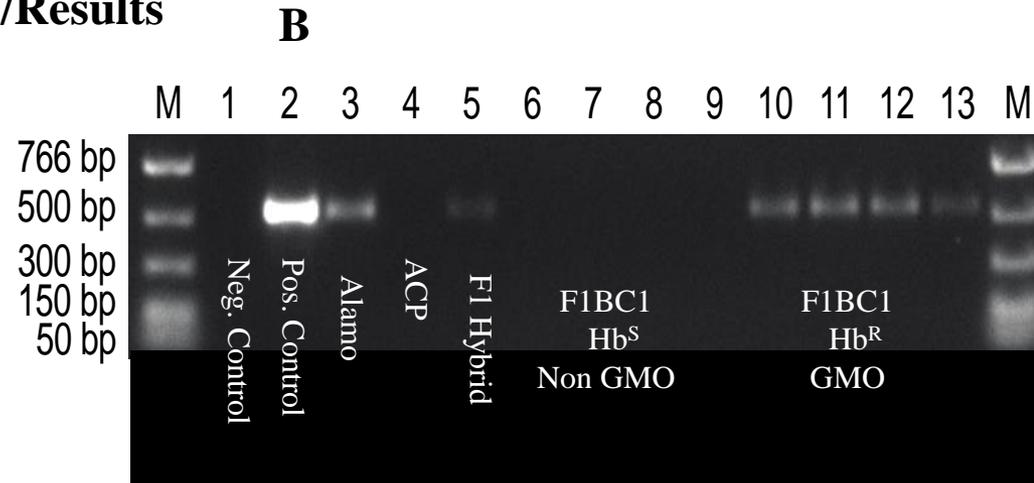
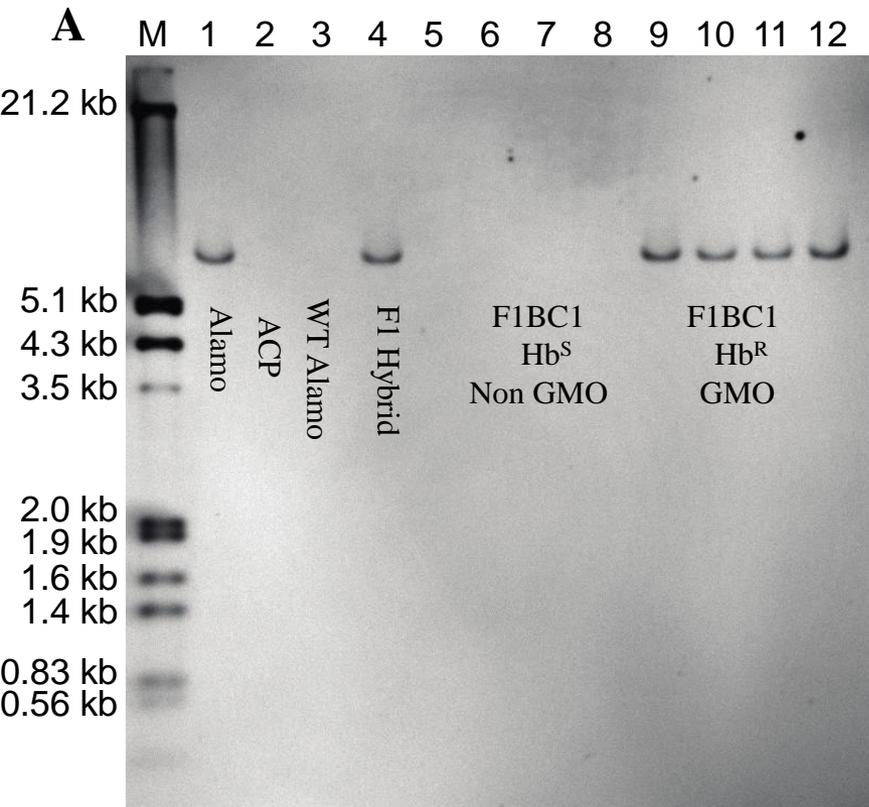
### II. Genomics Assisted Breeding

- Genomic characterization of Non-GMO hybrids (F1BC1 population)
- Development of male (pollen) and female (seed) sterility systems
- Wide crosses recovered through a novel embryo rescue technique
- Recovery of Non-GMO hybrids



# Research and Technology Development for Genetic Improvement of Switchgrass

## 2 - Technical Accomplishments/Progress/Results

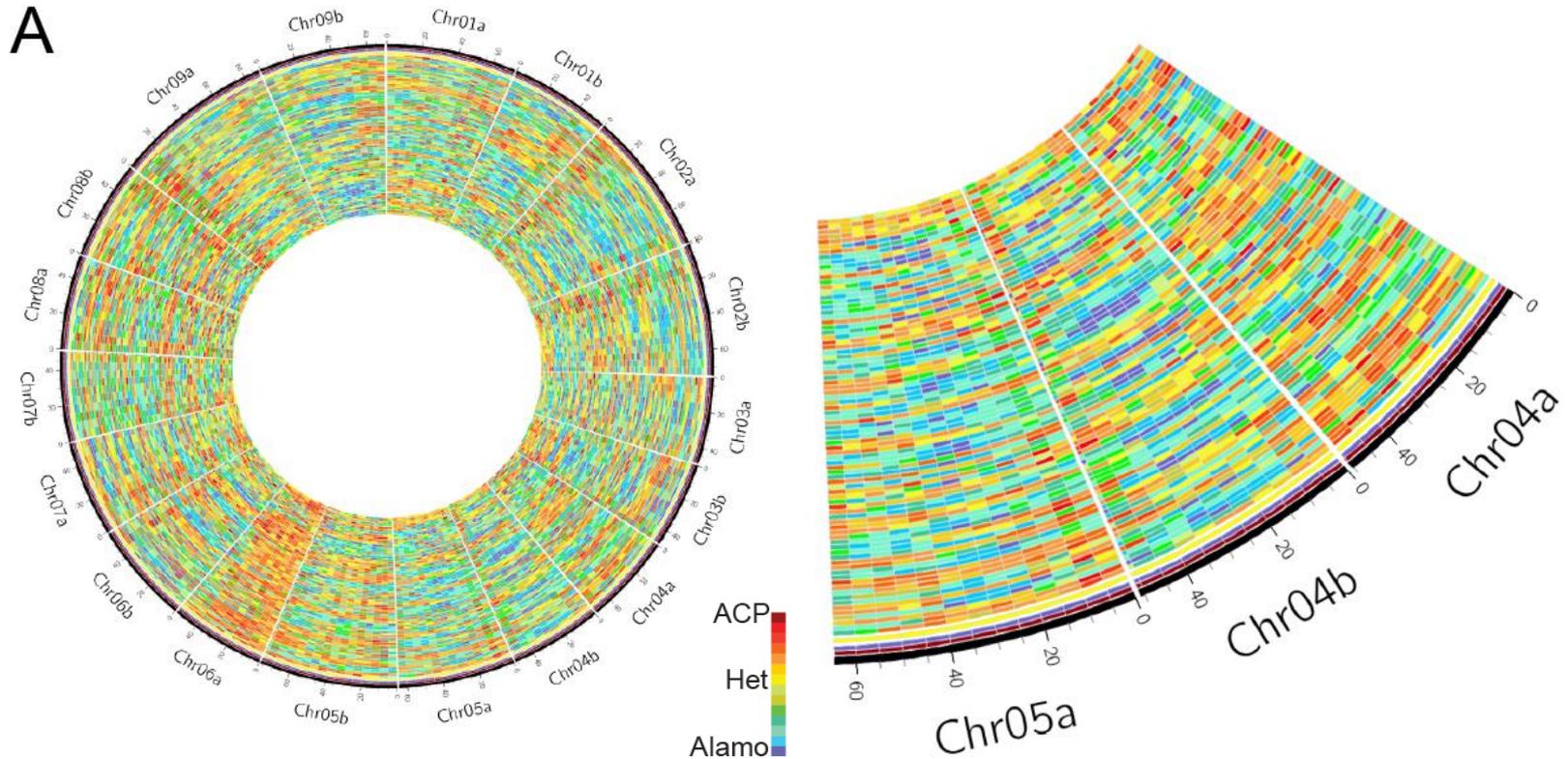


### Southern blot (A), PCR (B) of *bar* transgene amongst resistant and sensitive switchgrass

A single transgene insertion event is identified in the transgenic Alamo parent, F<sub>1</sub>, and Hb<sup>R</sup> F<sub>1</sub>BC<sub>1</sub>. The transgene was not identified in any Hb<sup>S</sup> individual. 1) T85-2 Alamo paternal parent, 2) ACP maternal parent, 3) Wild-type Alamo 4) F<sub>1</sub>, 5) Hb<sup>S</sup> F<sub>1</sub>BC<sub>1</sub>-1, 6) Hb<sup>S</sup> F<sub>1</sub>BC<sub>1</sub>-2, 7) Hb<sup>S</sup> F<sub>1</sub>BC<sub>1</sub>-3, 8) Hb<sup>S</sup> F<sub>1</sub>BC<sub>1</sub>-4, 9) Hb<sup>R</sup> F<sub>1</sub>BC<sub>1</sub>-1, 10) Hb<sup>R</sup> F<sub>1</sub>BC<sub>1</sub>-2, 11) Hb<sup>R</sup> F<sub>1</sub>BC<sub>1</sub>-3, 12) Hb<sup>R</sup> F<sub>1</sub>BC<sub>1</sub>-4. (B) PCR matches

## II. Genomics Assisted Breeding

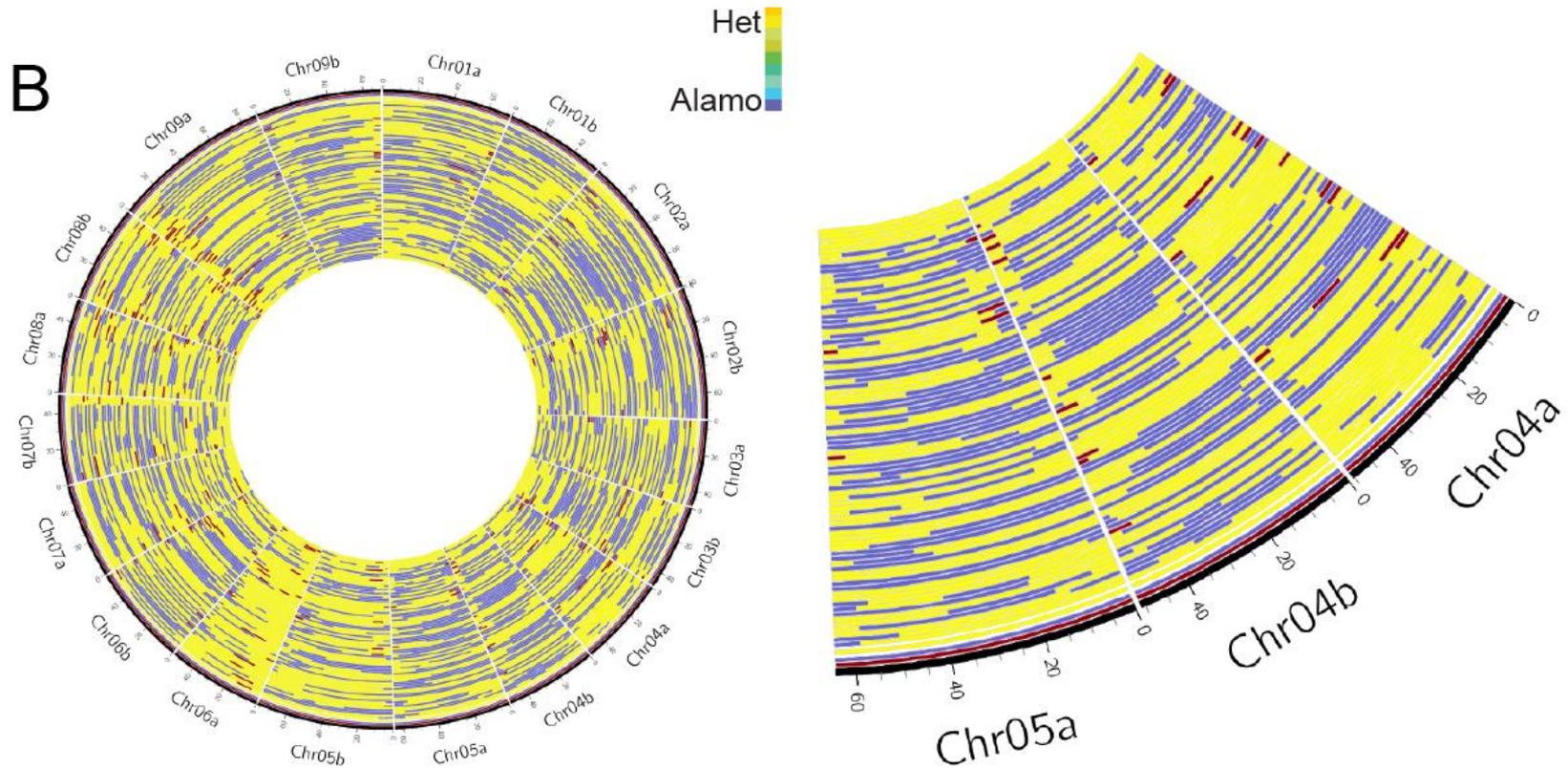
- *Genomic characterization of Non-GMO hybrids (F<sub>1</sub>BC<sub>1</sub> population)*



**Parental genetic contribution in a switchgrass F<sub>1</sub>BC<sub>1</sub> population.** Genome-wide parental contribution to eighty-three F<sub>1</sub>BC<sub>1</sub> offspring was measured using RFLP 26,131 markers. Outermost to innermost, the rings represent the ACP parent, Alamo parent, F<sub>1</sub>, and finally the 83 F<sub>1</sub>BC<sub>1</sub> offspring. Graphing was done via Circos (Krzywinski, M. *et al.* 2009. A) Warm heat map colors indicate high ACP contribution and cool colors indicate high Alamo contribution.

## II. Genomics Assisted Breeding

- *Genomic characterization of Non-GMO hybrids (F1BC1 population)*



**Parental genetic contribution in a ACP X switchgrass X switchgrass  $F_1BC_1$  population.** Genotypes were imputed via a least-squares methodology, with recombination breakpoints resolved. Regions of the genome were called as either homozygous Alamo, homozygous ACP, or heterozygous. Due to the nature of the  $F_1$  backcross to Alamo, only homozygous Alamo and heterozygous genotypes were possible. Blue indicates contribution from Alamo switchgrass; yellow indicates heterozygotes. The contribution from the ACP parent across all  $F_1BC_1$  individuals was found to be 31.62% ( $\pm 6.35%$  (*SD*))

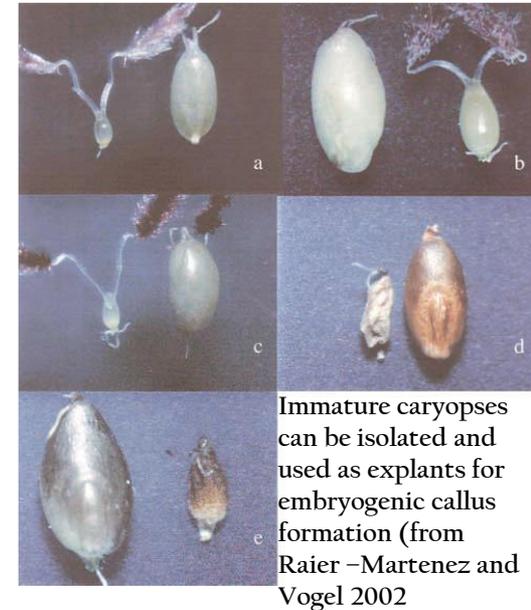
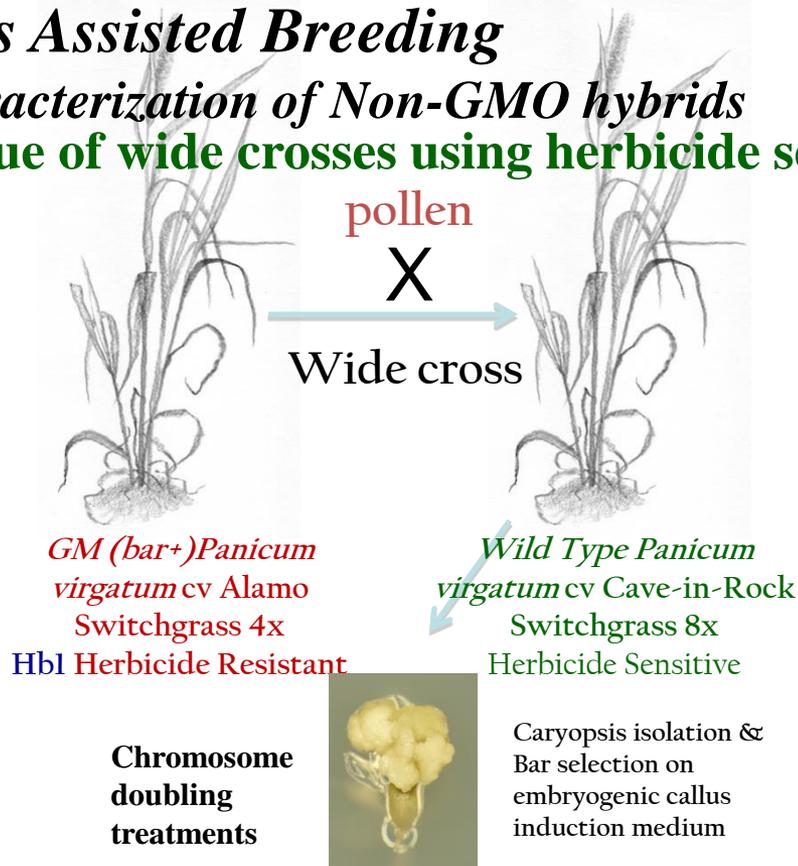
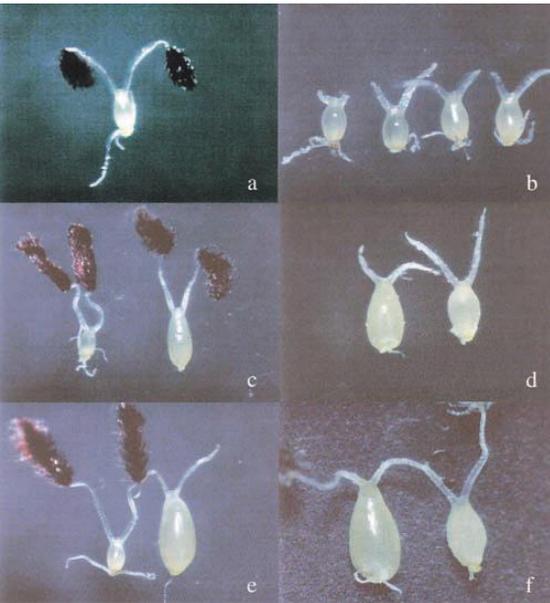
## Publication

### **Genomic Characterization of Interspecific Hybrids and an Admixture Population Derived from *Panicum amarum* × *P. virgatum***

Christopher Heffelfinger, Adam P. Deresienski, Kimberly A. Nelson, Maria A. Moreno, Joel P. Hague, Stephen L. Dellaporta, and Albert P. Kausch\* *The Plant Genome* (in Press)

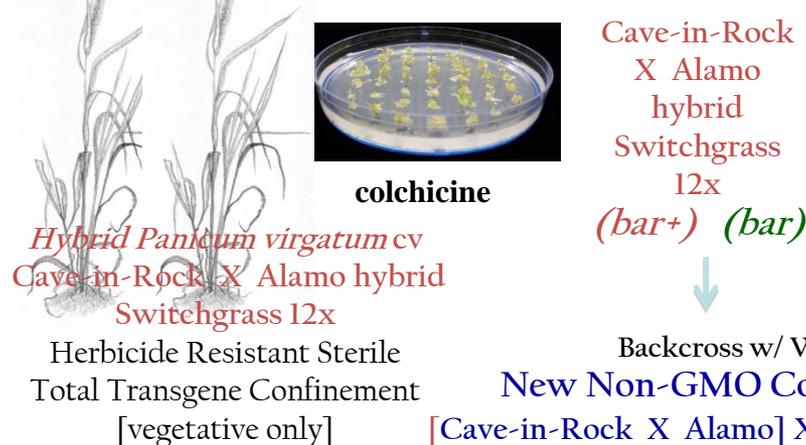
## II. Genomics Assisted Breeding

- Genomic characterization of Non-GMO hybrids
- In situ embryo rescue of wide crosses using herbicide selection as a marker**



### In situ embryo rescue and

II. sterile herbicide selection I. fertile



**Examples of caryopsis development of tetraploid by octaploid crosses 15 d after pollination (right)**

**Caryopsis development of tetraploid by octaploid switchgrass cross n days after pollination.**

# Research and Technology Development for Genetic Improvement of Switchgrass



## 2 - Technical Accomplishments/Progress/Results

### *III. Transgenic Trait Improvement*

- *Gene Confinement and Development of GM Traits for Biofuel Crop Improvement*

### *IV. Patent and other IP development*

*Seeking industry partnerships for collaboration and introduction of novel transgenic traits*

*Plant Advancements LLC*

*Ernst Conservation Seeds Inc*

# Research and Technology Development for Genetic Improvement of Switchgrass



## 3 - Relevance

- **This project addresses the following goals and objectives of Biomass Program Multi-Year Program Plan (updated November 2014).**

**BETO MYPP Section 2.1.3 *Feedstock Technical Challenges and Barriers***

**Ft-A Feedstock Availability and Cost Sustainable production and yield in Switchgrass and related species**

**Ft-C. Feedstock Genetics and Development: The productivity and robustness of terrestrial feedstock crops used for biofuel production improved by selection, screening, breeding, and/or genetic engineering.**

**This project considers applications of the expected outputs:**

- **New commercial varieties**
- **Technology for development of new varieties**
- **Intellectual property to support commercial development**

# Research and Technology Development for Genetic Improvement of Switchgrass



## 3 - Relevance

**Objectives regarding the relevance of this project to the Bioenergy Technologies Office, alignment with MYPP goals, and relevance for the overall bioenergy industry**

- *Development of hybrid plant systems (Ft-A and Ft-C)*

**Increased yields, new breeding and gene confinement technology for the future**

- *Development of advanced breeding strategies utilizing wide crosses, advanced tissue culture and genomics to produce new Non-GMO hybrids (Ft-A and Ft-C)*

**Increased yield, new bioenergy specific cultivars, and new technology for the future crop improvements of perennial biofuels crops**

- *Development of robust transgenic and gene confinement strategies (Ft-C)*

**“Any genetically modified organisms deployed commercially will also require prior deregulation by the appropriate federal, state and local government agencies” and gene confinement will hence be required.**

- *Enhance education , student training and internship research opportunities in biofuels crop improvement and plant biotechnology*

**Facilitating public education resource development and public perception**

# Research and Technology Development for Genetic Improvement of Switchgrass



## 4 - Critical Success Factors

- **Critical success factors (technical, market, business) which will define technical and commercial viability.**

**This project has demonstrated significant technical success**

**The market, business and commercial viability is currently dependant on end use for cellulosic biofuels**

**Patents generated during this project will be broadly applicable to the bioenergy crops field and agricultural biotechnology generally.**

**There is a large business and commercial viability for the licensing of IP in this area**

# Research and Technology Development for Genetic Improvement of Switchgrass



## 4 - Critical Success Factors

**Top 2-3 potential challenges (technical and non-technical) to be overcome for achieving successful project results.**

- 1. End use market to drive biofuels crop production**
- 2. Need to commercialize and deregulate transgenic biofuels crops (which will require the IP generated in this project for gene confinement)**

# Research and Technology Development for Genetic Improvement of Switchgrass



## 4 - Critical Success Factors

**Demonstrate that the successful project will advance the state of technology and positively impact the commercial viability of biomass and /or biofuels.**

- **Hybrid plant systems in this project will affect biofuels crop improvement and other agricultural crops generally**
- **Advanced genomics capabilities in this project will be broadly applied**
- **IP for gene confinement may well be used across many crop species for deregulation.**

# Research and Technology Development for Genetic Improvement of Switchgrass

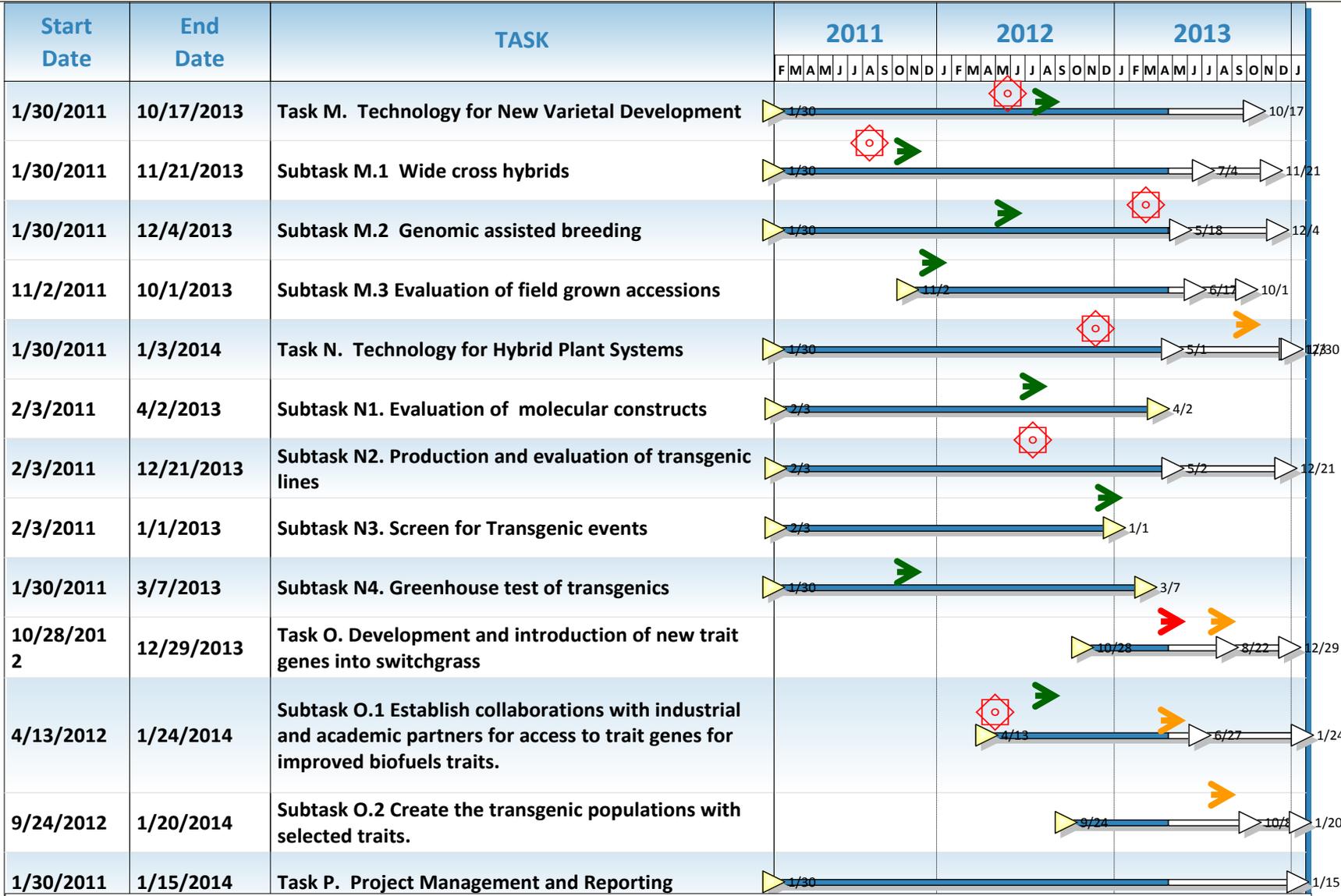


## 5. Future Work

- **Plans through the end of the project:**  
Complete and submit relevant publications  
Complete patent applications  
Progress toward commercialization with Plant Advancements LLC
- **Gantt Chart shows highlights of key milestones & Go/No go decision points.**

# Gantt Chart for UPDATED PMP Award # DE-FG-36-08GO88070

## Research and Technology Development for Genetic Improvement of Switchgrass



Key Milestones Go/No-Go Yes No Pending



# Summary



## 1) Approach

- **Hybrid Plant Systems, Non-GMO hybrids; Gene Confinement; Robust Genomics Platform**

## 1) Technical accomplishments

- **Demonstrated the technical success in all major goals**

## 1) Relevance **MYPP 2012 *Ft-A and Ft-C***

## 2) Critical Success factors and challenges

- **Dependant on future end use for cellulosic biofuels**

## 1) Future Work

- **Commercialization with Plant Advancements LLC**

## 1) Technology transfer

- **IP licensing; Publications and Presentations**

**Thank You**

# Additional Slides

# Publications, Presentations, and Commercialization

## Publications:

Heffelfinger, C Deresienski,..., K. Nelson, J. Hague, A Dellaporta, S. , Moreno, M and A.P. Kausch. **Genomic Characterization of Interspecific Hybrids and an Admixture Population Derived from *Panicum amarum* x *P. virgatum*. *The Plant Genome* (in press)**

1. Howard, TP, Tordillos, A, Fragoso, C., Moreno, MA, Mottinger, JP, Kausch, AP, Tohme, J, and Dellaporta, SL (2013) Identification of the maize gravitropism gene *lazy plant1* by a transposon-tagging genome resequencing strategy .PLoSOne.
2. Nelson, K., A. Deresienski, M. Tilelli, J. Hague, C. Longo and A.P. Kausch. (2013) In situ embryo rescue in Switchgrass (*Panicum virgatum* L.) and ‘Atlantic’ Coastal Panicgrass (*Panicum virgatum* Ell. var. *amarulum*) Plant Science (in preparation)
3. Kausch, AP, Deresienski A, Hague, J, Tilelli M, Dellaporta SD, Nelson, K and Li, Yi. (2013) Hybrid Plant Systems for Breeding and Gene Confinement in Bioenergy Crops. In: New and Future Developments in Catalysis – Catalytic Biomass Conversion. Steven Suib, Ed. Elsevier Press (in press)
4. Kausch, AP, Hague, J, Deresienski A, Tilelli M, Longo C, and Nelson, K (2013) Issues in Biotechnology: A Massive Open Online Course (MOOC) Covering in Simple Terms Basic Knowledge About DNA and Biotechnology INTED Proceedings
5. Joel P. Hague, Steven L. Dellaporta, Maria Moreno, Chip Longo, Kimberly Nelson, Albert P. Kausch (2012). Pollen Sterility - A Promising Approach to Gene Confinement and Breeding for Genetically Modified Bioenergy Crops. *Agriculture* 2:295-315
6. Kausch, A.P., J. Hague, A. Deresienski, Tilelli M and K. Nelson. (2012) Male Sterility and Hybrid Plant Systems for Gene Confinement. In Plant Gene Containment. M. Oliver and Yi Li. Eds. John Wiley & Sons,. Inc. New York, New York.
7. Albert P. Kausch, Joel P. Hague, Melvin J. Oliver, Yi Li, Henry Daniell, Peter Mascia, Lidia S. Watrud, and C. Neal Stewart Jr (2010). Transgenic perennial biofuel feedstocks and strategies for bioconfinement. *Biofuels* 1:163-176.
8. Albert P. Kausch, Joel Hague, Melvin Oliver, Yi Li, Henry Daniell, Peter Mascia, and C. Neal Stewart Jr. (2010). Genetic Modification in Dedicated Bioenergy Crops and Strategies for Gene Confinement. In: Plant Biotechnology for Sustainable Production of Energy and Co-products, Biotechnology in Agriculture and Forestry 66, P.N. Mascia et al. (eds.), Springer-Verlag Berlin Heidelberg, pp. 299-313.
9. Albert P. Kausch, Joel Hague, Melvin Oliver, Lidia S. Watrud, Carol Mallory-Smith, Virgil Meier, and C. Neal Stewart Jr. (2010). Gene Flow in Genetically Engineered Perennial Grasses: Lessons for Modification of Dedicated Bioenergy Crops. In: Plant Biotechnology for Sustainable Production of Energy and Co-products, Biotechnology in Agriculture and Forestry 66, P.N. Mascia et al. (eds.), Springer-Verlag Berlin Heidelberg. pp. 285-296.
10. Moon, H., J. Abercrombie, A. Kausch, and C. Stewart. 2010. Sustainable Use of Biotechnology for Bioenergy Feedstocks, pp. 1-8 Environmental Management. Springer New York.

## **Selected Recent Presentations in Meetings, Invited Lectures, Conferences and Symposia**

1. A. Kausch, J. Hague, L. Perretta and K. Nelson (2013) Agricultural Biotechnology: A Massive Open Online Course (MOOC) Module Covering in Simple Terms Basic Knowledge About DNA and Plant Biotechnology. Plant Biology 2013, Annual Meetings of the American Society of Plant Biologists, July 20-24, Providence, Rhode Island, USA.
2. J. Hague, M. Tilelli, D. Cunha, K. Nelson and A. Kausch (2013) In Situ Embryo Rescue as a Novel Method for Recovery of Non-GMO Hybrids from Wide Crosses. Plant Biology 2013, Annual Meetings of the American Society of Plant Biologists, July 20-24, Providence, Rhode Island, USA.
3. Kausch, Albert. Invited Speaker. (2012) The use of synthetic male and female sterility for recovery of Non-Genetically Modified Hybrids from Wide Crosses. Department of Horticultural Science, North Carolina State University, Mountain Horticultural Crops Research and Extension Center, October 19, 2012
4. Kausch, Albert (2012) Invited Speaker. Bioenergy: Genetic Improvement of Bioenergy Crops for Biofuels and Prospects for Artificial Photosynthesis. Department of Chemistry. Brown University, Providence Rhode Island. September 14, 2012
5. Kausch, Albert (2012) Invited Speaker. Bioenergy: Genetic Improvement of Bioenergy Crops for Biofuels Department of Botany connecticut College, new London CT. September 21, 2012
6. .A. Kausch, A. Deresienski, J. Hague, M.Tilelli, K. Nelson (2012) Issues in Biotechnology: An Online General Education Undergraduate Course Covering Simple Terms Basic Knowledge About DNA and Biotechnology. Plant Biology 2012, Annual Meetings of the American Society of Plant Biologists, July 20-24, Austin, TX, USA.
7. J. Hague, A. Deresienski, M.Tilelli, K. Nelson, A. Kausch (2012) The Analysis of Expression Characteristics of the Maize Pollen Specific Promoter MPSP Zm13 And A Strategy for Gene Confinement in Transgenic Bioenergy Crops. Plant Biology 2012, Annual Meetings of the American Society of Plant Biologists, July 20-24, Austin, TX, USA.
8. A. Deresienski, K. Nelson, M.Tilelli, J. Hague, A. Kausch (2012) Use of a Herbicide Resistance Selectable Marker for Recovery of Intraspecific and Interspecific Hybrids in Switchgrass. Plant Biology 2012, Annual Meetings of the American Society of Plant Biologists, July 20-24, Austin, TX, USA.
9. K. Nelson, A. Deresienski, M.Tilelli, J. Hague, A. Kausch (2012) A Project-based Undergraduate Internship Program in Agricultural Biotechnology. Plant Biology 2012, Annual Meetings of the American Society of Plant Biologists, July 20-24, Austin, TX, USA.
10. M.Tilelli, K. Nelson, A. Deresienski, J. Hague, A. Kausch (2012) Use of a Selectable Marker for In Situ Embryo Rescue using Transgenic Switchgrass for Recovery of Wide Crosses. Plant Biology 2012, Annual Meetings of the American Society of Plant Biologists, July 20-24, Austin, TX, USA.
11. A. Deresienski, K. Nelson, J. Hague, A.P. Kausch (2009) Male sterility as a method for constructing wide crosses and for gene confinement in switchgrass and other biofuels grasses. Plant Biology 2009, Annual Meetings of the American Society of Plant Biologists, July 18-22, Hawaii, USA.
12. K. Nelson, J. Hague, A. Deresienski and A.P.Kausch. (2009) Improved methods for tissue culture and genetic transformation of switchgrass. Plant Biology 2009, Annual Meetings of the American Society of Plant Biologists, July 18-22, Hawaii, USA.

# Publications, Presentations, and Commercialization

## Related Patents:

1. Kausch AP, Hague, J, Deresienski, A, Tilelli, M, and Nelson, K., Inventors. 2014 The Use of Genetically Modified Plants for Recovery of Non- genetically Modified Hybrids from Wide Crosses. United States Patent Application. US 2013/004769 Assignee; University of Rhode Island.
2. Kausch AP, Hague, J, Deresienski, A, Tilelli, M, and Nelson, K., Inventors. 20114 In Situ Embryo Rescue as a Method for Recovery of Wide Crosses. United States Patent Application. US 2013/005832 Assignee; University of Rhode Island.
3. Luo; Hong; Chandlee; Joel M.; Kausch; Albert P.; Oliver; Melvin J. , Inventors. 2011. Development of controlled total vegetative growth for prevention of transgene escape from genetically modified plants and for enhancing biomass production.. United States Patent Application Number 20100122366
4. Albert P. Kausch and Stephen Dellaporta , Inventors. 2011 Male and female sterility lines used to make hybrids in genetically modified plants. United States Patent Application. Assignee; University of Rhode Island.

## Status of commercialization efforts:

**Plant Advancements has acquired significant IP and licensing rights to relevant technologies**

**Plant Advancements LLC is recent recipient of USDA SBIR grant for commercialization of male and female sterility lines in switchgrass and related species.**

Area: Small Business Innovation Research Program (SBIR)

Program: Plant Production and Protection-Biology

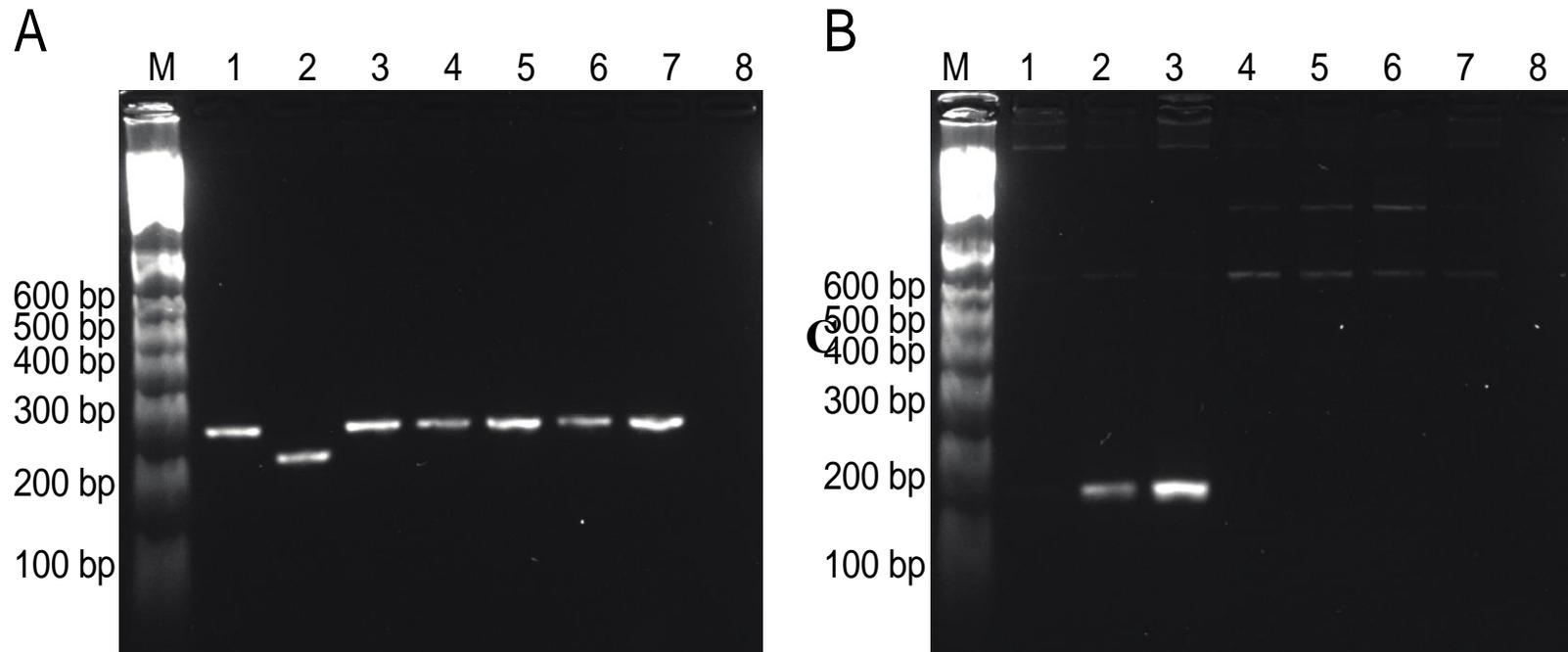
Proposal Number: 2013-00140

Project Director: Thomas K Hodges/Albert P Kausch

Proposal Title: Hybrid Systems for Gene Confinement and Breeding of Perennial Plants Used for Biofuels

# Research and Technology Development for Genetic Improvement of Switchgrass

## 2 - Technical Accomplishments/Progress/Results



**Chloroplast DNA and bar transgene** Chloroplasts are maternally inherited nuclear genes are paternally inherited. A chloroplast DNA tRNA-Leu (*trnL*) 49 bp deletion in Alamo (wild-type state present in ACP) served as a marker to confirm maternal contribution from ACP (Missaoui, Paterson, et al., 2006), whereas the presence of the *bar transgene* confirmed parental contribution from transgenic Alamo