Final Technical Report
DOE-URI-11111-1

Project Title: Research and Technology Development for Genetic Improvement of Switchgrass Albert P. Kausch and Richard Rhodes III
Award Number: Award # DE-FG-36-08GO88070

Recipient: University of Rhode Island
Project Locations: University of Rhode Island South Kingston RI
Yale University Center for Genomics, New Haven CT
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Ernst Conservation Seeds Inc. Meadville PA

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Executive Summary

This research adds to the understanding of switchgrass genetics and the increasing of biomass relevant to production of bioenergy. Switchgrass, *Panicum virgatum L.,* and its related species are well known as potential bioenergy crops since the early 1990s. There are global economic, political, US national security and environmental pressures to increase renewable biofuel production and utilization to offset gasoline and diesel fuel use and climate change, especially in the liquid fuel transportation sector. To realize the potential of bioenergy crops, rapid genetic improvement of the most promising perennial grass feedstocks, such as switchgrass, are anticipated by current genomics, association genetics, marker assisted breeding, hybrid plant development, advanced tissue culture, conventional genetics and other approaches to increase yield, processability, and regional adaptation. The technical effectiveness and economic feasibility of the methods or techniques investigated are demonstrated by several publications, presentations and patents produced as an outcome and deliverable of this research. This project is of a broad benefit to the public not only through the dissemination of this information but also to the development of new methods which will be applied to future bioenergy crop improvement as well as other crops.

Comparison of the goals and objectives of the project with the actual accomplishments

Goal and Objectives:

The overarching goal of this project was 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 publications, presentations and intellectual property that is widely applicable to bioenergy and agricultural crops generally. 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 through the USDA and eventually released for commercial production on a large scale. This project addressed the following goals and objectives of Biomass Program Multi-Year Program Plan (MYPP, updated November 2014): *2.1.3 Feedstock Technical Challenges and Barriers;* Ft-A Feedstock Availability and Cost Sustainable production and yield in Switchgrass and related species; and, 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.

The specific objectives of this project included:

- 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

The objectives of this project relate to the relevance of the Bioenergy Technologies Office, alignment with MYPP goals, and relevance for the overall bioenergy industry including:

- 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

Actual accomplishments

This project has demonstrated significant technical success relevant to all of our goals and objectives. The market, business and commercial viability is currently dependant on end use for cellulosic biofuels. The actual accomplishments from this project demonstrate that the successful applications that will advance the state of technology and positively impact the commercial viability of biomass and /or biofuels. During this project we have demonstrated: (1) the development of hybrid plant systems in this project will affect biofuels crop improvement and other agricultural crops generally (2) development of the ability to recover and breed wide cross outcomes in switchgrass and related species; (30 the use of advanced genomics capabilities which will be broadly applied to bioenergy crop improment as well as other crop systems; and, (4) IP for gene confinement which may well be used across many crop species for deregulation. Publications and patents generated during this project will be broadly applicable to the bioenergy crops field and agricultural biotechnology generally. The top potential challenges to this project going forward (technical and non-technical) for achieving successful results related to bioenergy

crops in the future, specifically; (1) include the end use market to drive biofuels crop production; and, (2) The need to commercialize and deregulate transgenic biofuels crops (which will require adequate gene confinement).

Summary of Project Activities

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 bioenergy crops. The high level objectives of this project, dubbed 'Project Golden Switchgrass' were to create hybrid systems, advanced genomics assisted breeding, and gene confinement platforms that are broadly applicable to bioenergy and agricultural crops. This enabling technology aimed to improve biofuels crop improvement.

The project activities we developed to address these objectives included:

I. The Development of 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. The Development of *Genomics Assisted Breeding for Switchgrass*
Genomic characterization of Non-GMO hybrids (F1BC1 population)
Wide crosses recovered through a novel embryo rescue techniques
Recovery of Non-GMO hybrids
***III.* Transgenic Trait Improvement**
Gene Confinement and Development of GM Traits for Biofuel Crop Improvement
Patent and other IP development
Seeking industry partnerships for collaboration and introduction of novel transgenic traits

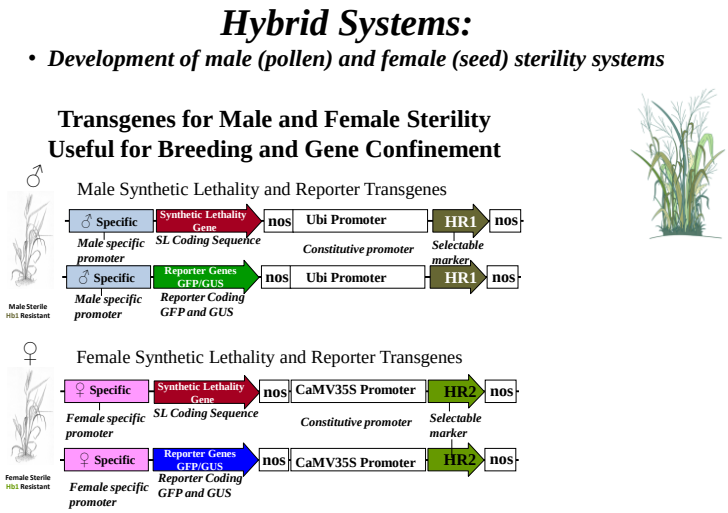


FIGURE 1. Male and female sterile lines useful for hybrid plant breeding or gene confinement. Male and female lines are created through the application of the promoters and/or the coding sequences

The development of hybrid systems was tested using constructs such as those show below in Figure 1. We first demonstrated successfully this approach in rice (see publications, Hague et al. 2011) Concerning problems and obstacles encountered and departure from planned methodology with this approach, We learned that while the promoters were tissue specific, the method for cell ablation (i.e. barnase) was not as effective as we would have hoped in switchgrass. Future studies should focus on a different system.

The development using a wide cross system is diagramed in Figure 2 (see Heffelinger et al. 2015)

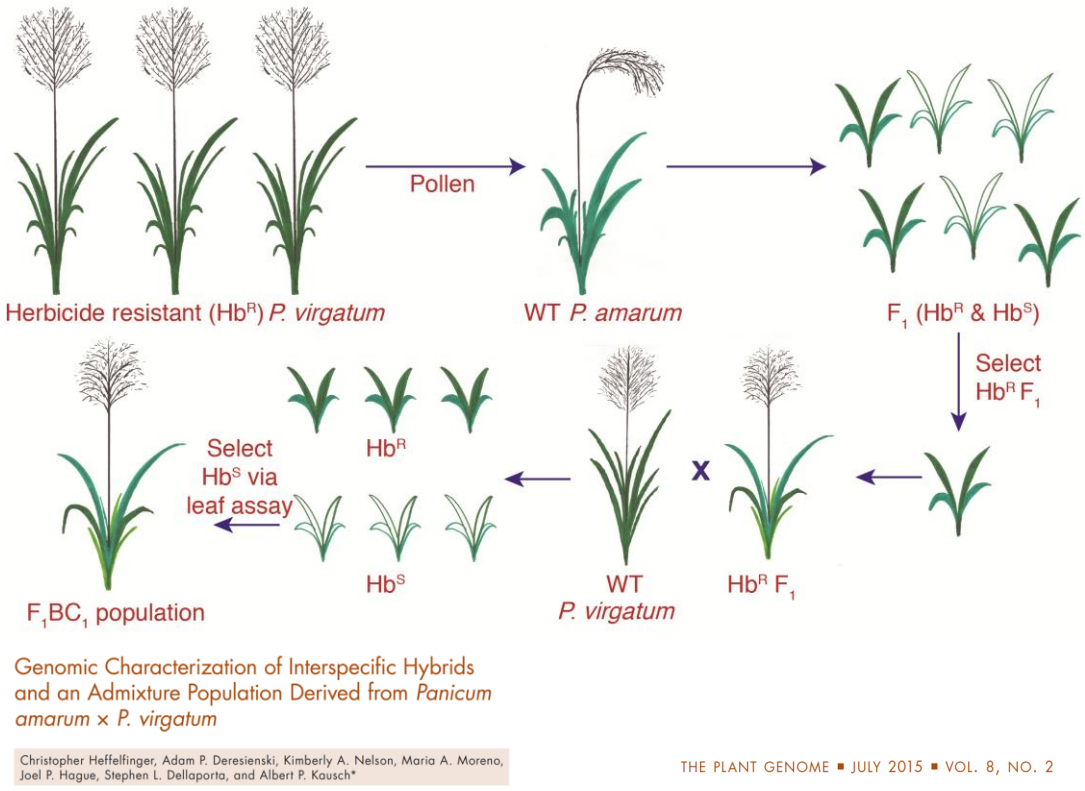
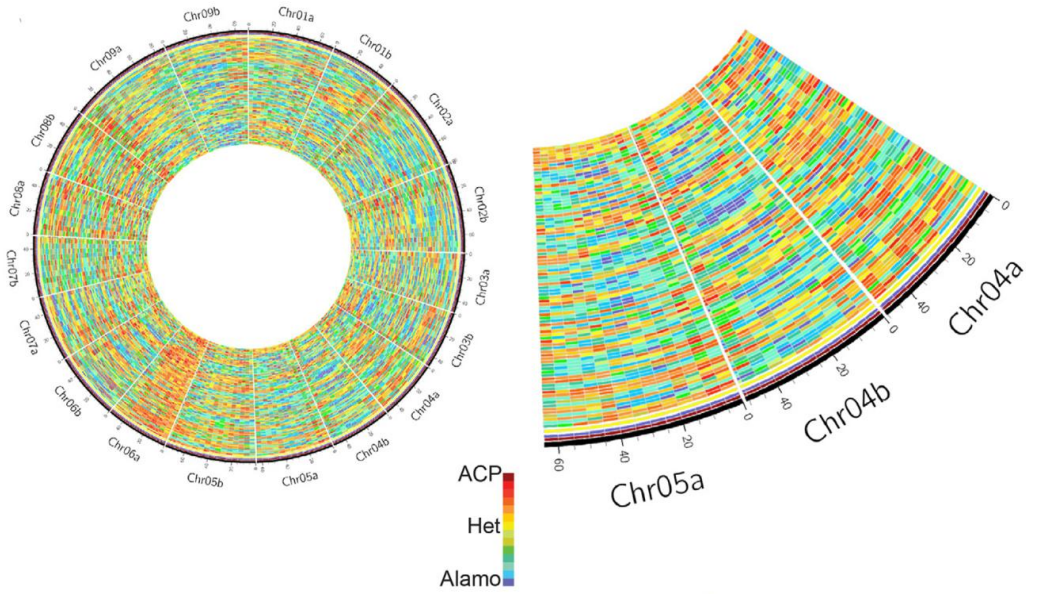


Figure 2

Using this approach we were able to show recovery of non-GMO hybrids and enabled us to establish our genomics platform (see Figure 3 below from Hefellfinger et al. 2015). This analysis helped us to establish our Genomics Assisted Breeding platform as according to our objectives. 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 F1 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 F1BC1 individuals was found to be 31.62% (± 6.35% (*SD)*



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*

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Figure 3

Products and Deliverables Developed Under This Award

Publications

1. 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 P.N. Mascia et al. (eds.), *in* Plant Biotechnology for Sustainable Production of Energy and Co-products, Biotechnology in Agriculture and Forestry 66, DOI 10.1007/978-3-642-13440-1_10 Springer-Verlag Berlin Heidelberg 2010

2. 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 P.N. Mascia et al. (eds.), *in* Plant Biotechnology for Sustainable Production of Energy and Co-products, Biotechnology in Agriculture and Forestry 66, DOI 10.1007/978-3-642-13440-1-10 Springer-Verlag Berlin Heidelberg 2010

3. Kausch, A. P*., J. Hague, M. Oliver, Y. Li, H. Daniell, P. Mascia, Watrud L. S., and C. Neal Stewart Jr. 2010. Transgenic perennial biofuel feedstocks and strategies for bioconfinement. Biofuels 1(1):163-176.

53. Moon, H., J. Abercrombie, A. P. Kausch, and C. Stewart. (2010) Sustainable Use of Biotechnology for Bioenergy Feedstocks, *in* Environmental Management. Springer New York. pp. 1-8

4. Albert P. Kausch*, Joel Hague, Adam Deresienski, Michael Tilelli, Chip Longo Jr. and Kimberly Nelson. (2012) Male Sterility and Hybrid Plant Systems for Gene Confinement (2012). *in* Plant Gene Confinement EDS Melvin Oliver and Yi Li. Wiley-Blackwell John Wiley & Sons MA . Chapter 6 pgs 85-100.

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, 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*. Vol. 13 pgs 97-102

7. 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. *Plos 1*

58. Heffelfinger, C., Deresienski, A.,Nelson, K., Moreno, M., Hague, J., Dellaporta, S. and Kausch, A.P. (2015) Genomic Characterization of Interspecific Hybrids and an Admixture Population Derived from *Panicum amarum* x *P. virgatum* *The Plant Genome Vol. 8. No.2, pgs 1-12*

9. Kausch, Albert; Tilelli, Michael; Hague, Joel; Heffelfinger, Christopher; Cuhna, David; Moreno, Maria; Dellaporta, Stephen; Nelson, Kimberly (2016) In Situ Embryo Rescue for Generation of Wide Intra- and Inter-specific Hybrids of *Panicum virgatum L.* *Plant Biotechnology Journal doi: 10. 1111/pbi. 12573 pgs. 1-8*

Patents

1. 2009. Male and female sterility lines used to make hybrids in genetically modified plants. Inventors: Albert Kausch and Stephen Dellaporta. United States Patent Application. Assignee; University of Rhode Island (pending)

2. 2010. Prevention of transgene escape in genetically modified perennial plants. Luo; Hong; Hu; Qian; Vasilchik; Kimberly Nelson; Longo, JR.; John P.; Kausch;Albert P.; Zilinskas;

Barbara; Lakkaraju; Subha Continuation in Part United States Patent Application Number 20100031387 Filed February 4, 2010

3. 2012 The use of genetically modified plants for recovery of non-genetically modified hybrids from wide crosses Inventors: Adam Deresienski; Kimberly Nelson; Michael Tilleli; Joel Hague, Stephen Dellaporta and Albert Kausch. United States Patent Application. Assignee; University of Rhode Island

4. 2012. In situ embryo rescue and recovery of non-genetically modified hybrids from wide crosses Inventors: Kimberly Nelson; Adam Deresienski; Michael Tilleli; Joel Hague and Albert Kausch. United States Patent Application. Assignee; University of Rhode Island

Presentations

1. **Albert Kausch** Invited Speaker (2013) In Situ Embryo Rescue as a Novel Method for Recovery of Non-GMO Hybrids from Wide Crosses. *Department of Cell and Molecular Biology, University of Rhode Island, Kingston, 530 Liberty Lane West Kingston Rhode Island USA 02892* Plant Biology 2013, Annual Meetings of the American Society of Plant Biologists (ASPB), July 20-24, Providence, Rhode Island, USA.

2. **A. Kausch**, Invited Speaker 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.

3. J. Hague, M. Tilelli, D. Cunha, K. Nelson and **A. Kausch** Invited Speaker (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.

4. **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

5. **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

6. **Kausch, Albert** (2012) Invited Speaker. Bioenergy: Genetic Improvement of Bioenergy Crops for Biofuels Department of Botany Connecticut College, New London CT. September 21, 2012

7. **A. P. Kausch**, Invited Speaker 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.

8. J. Hague, A. Deresienski, M.Tilelli, K. Nelson, **A. P. Kausch** Invited Speaker (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.

9. **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 Plant Science, University of Massachusetts September 9, 2012

10. A. Deresienski, K. Nelson, M.Tilelli, J. Hague, **A. P. 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.

11. K. Nelson, A. Deresienski, M.Tilelli, J. Hague, **A. P. Kausch** Invited Speaker (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.

12. M.Tilelli, K. Nelson, A. Deresienski, J. Hague, **A. P. Kausch** Invited Speaker (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.

13. A. Deresienski, K. Nelson, J. Hague, **A.P. Kausch** Invited Speaker (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.

14. K. Nelson, J. Hague, A. Deresienski and **A.P. Kausch**. Invited Speaker (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.

15. Transgenic biofuel feedstocks from perennial plants: requirements and strategies for biocontainment
    Albert P. Kausch Invited Speaker Plant Biotechnology seminar series University of Massachusetts, Amherst MA Sept 22, 2009.

16. Improved methods for tissue culture and genetic transformation of switchgrass **A.P Kausch**, K. Nelson, J. Hague, and A. Deresienski, Plant Biotechnology Laboratory,

Department of Cell and Molecular Biology, University of Rhode Island, West Kingston RI 02892 Amer. Soc. Plant Biology July 22-26 2009.

17. Strategies for Gene Confinement in Genetically Modified Perennial Plants Used for Biofuels **A.P. Kausch**, J. Hague, A. Deresienski, K. Nelson, and Melvin Oliver. Plant Biotechnology Laboratory, Department of Cell and Molecular Biology, University of Rhode Island, West Kingston RI 02892 Soc. In Vitro Biology June 6-9 2009