

Introduction

A critical challenge of the 21st century is to discover large-scale sources of sustainable energy. While various alternatives to fossil fuels are currently being developed for electricity production (e.g. solar, nuclear, wind), there will also be a continuing need for combustible liquid fuels. The US government has mandated 36 billion gallons of biofuel production by 2022 (Baker & Galik 2009). Meeting those mandates will require large-scale production of cellulosic ethanol or other novel biofuels (Westcott 2009; Galik et al. 2009; Somerville et al. 2010). Corn-based ethanol poises a number of challenges including large water and fertilizer demands and potential conflicts that result in reductions in global food security (Groom et al. 2008; James 2008; Haye & Hardtke 2009). Cellulosic ethanol derived from perennial feedstocks (Figure 1), such as switchgrass (*Panicum virgatum*), are predicted to bring higher returns on energy inputs (>500%; Schmer et al. 2008), have greater potential in reducing greenhouse gases (Tilman et al. 2006; Tobias 2009), and be less damaging to ecosystems than corn-based ethanol (McLaren 2005; Baker & Galik 2009). Switchgrass requires less fertilizer and water inputs than corn, depletes soil moisture less than *Miscanthus* (McIsaac 2010), and can be grown on marginal lands (Evanylo et al. 2005; Schmer et al. 2008; Varvel et al. 2008; Barney et al. 2009). As only limited breeding has been completed in switchgrass, there is enormous potential for improvement in abiotic stress tolerance, yield, and biofuel capacity through conventional breeding and genetic engineering (James 2008). Elucidating the genetic mechanisms of abiotic stress tolerance in switchgrass will be crucial for these advances, especially in the face of predicted changes in precipitation under projected global climate change and a future of less water, fertilizer, and fossil fuels (Meehl et al. 2007; Stillwell et al. 2009; Eckardt et al. 2009).

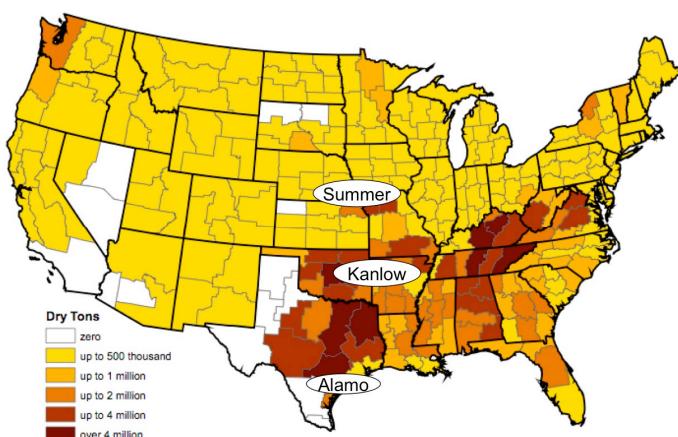


Figure 1: Projected distribution of all cellulosic feedstocks in 2020 as modeled by Ugarte et al. (2006). The locations of origin for the three focal cultivars: Alamo, Kanlow, and Summer are also shown on map.

Switchgrass occurs naturally across eastern North America and exhibits substantial phenotypic and genetic variation in many ecologically and agronomically important traits (McMillan 1959, 1961 1965, 1967; Sanderson et al. 2006; Cassler et al. 2004, 2007; Cassler 2005 Yang et al. 2009). Natural variation in traits including flowering time, timing of growth and senescence, biomass production, and winter hardiness are all distributed clinally across ecoregions and plant cold hardiness zones. For example, latitude from which populations are collected explains 72-83% of the variation in flowering time for switchgrass

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when grown in common gardens (Figure 2; McMillan 1959, 1965).

Southern varieties differ from northern varieties genetically in that they initiate growth earlier in the spring, senesce later in the fall, flower later, produce more biomass, and are less cold tolerant (McMillan 1959, 1965; Sanderson et al. 1999; Cassler 2005; Cassler et al. 2004, 2007). In addition to clinal variation in switchgrass, there are also distinct lowland and upland switchgrass ecotypes. Lowland varieties are typically tetraploid and found in mesic areas, while upland varieties are most often octoploid and occur in more xeric locations (Hultquist et al. 1996; Sanderson et al. 2006; Cassler et al. 2007). Lowland varieties typically have higher photosynthetic rates, water use efficiencies, biomass production, and nitrogen remobilization rates before fall senescence than upland varieties (Sanderson et al. 1996; Barney et al. 2009; Yang et al. 2009).

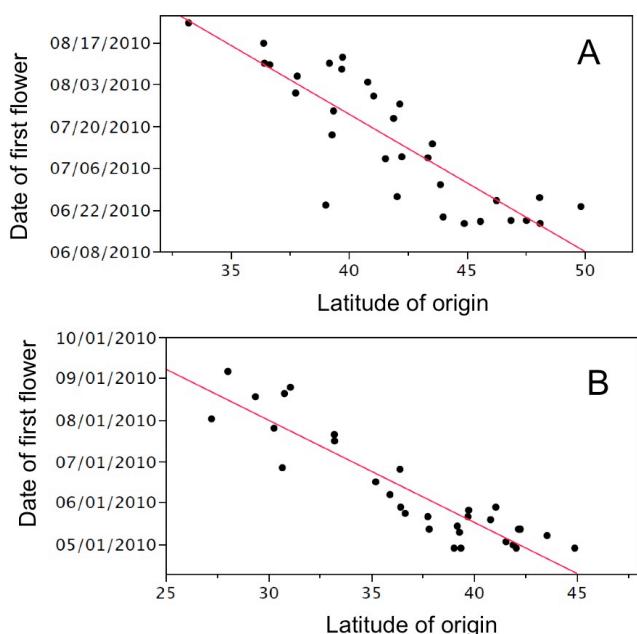


Figure 2: Latitude origin of switchgrass populations was highly correlated with flowering time in common gardens in both A) Lincoln, NE and B) Austin, TX.

breeding (Alamo is the only major cultivar released from Texas) and the abundance of tetraploids makes QTL mapping feasible (Sanderson et al. 2006; Cassler et al. 2007; Okada et al. 2010). This is not to mention that recent models project switchgrass-based biofuel production in 2020 to be centered in the southern US (Ugarte et al. 2006; Figure 1).

Here, I propose a set of three projects to better understand the genetics of drought tolerance, nutrient use, phenological/morphological trait variation, and gene x

Numerous laboratory and agronomic studies have made major advances in understanding the biology of switchgrass in the last three decades (reviewed in Sanderson et al. 2006). One of the most productive avenues moving forward will be to leverage the massive genetic and genomic resources (Tobias 2009) currently being generated for switchgrass (especially recent unpublished sequencing by the Joint Genome Institute (DOE)) to map loci involved in previously identified agronomically important traits. Once genomic regions of importance are discovered these loci can be used to initiate marker assisted selection for cultivar improvement (Sanderson et al. 2006; Bouton 2007; Okada et al. 2010). This is especially promising in the southern United States where only a small fraction of the natural genetic variation has been exploited for

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environment interactions in switchgrass. These studies will set the stage for future breeding efforts.

Objective 1: Conduct genetic mapping of drought response and other traits involved in biomass production as a launch point for marker assisted selection

Objective 2: Create outbred mapping populations for genetic mapping of adaptive regional variation

Objective 3: Conduct genetic mapping of nutrient assimilation and remobilization in response to municipal biosolid fertilization

This research builds directly from the aims of my dissertation research, which was focused on genetic mapping and marker-assisted selection of traits involved in drought avoidance, salt tolerance, and nutrient uptake in the yellow monkeyflower, *Mimulus guttatus* (Lowry et al. 2009; Hall et al. 2010; Lowry & Willis 2010; Lowry et al. *in prep*). We know surprisingly little about drought tolerance and nutrient use in switchgrass, despite the importance of these factors for successful biofuel production (Barney et al. 2009; Yang et al. 2009). Further, I am aware of no study that has mapped genetic loci controlling traits of agronomic importance in switchgrass, despite this being the first step to any marker-assisted breeding program. Additionally, the findings of the proposed studies may be applicable to closely related food crop species such as maize (Schnable et al. 2009), sorghum (Paterson et al. 2009), and foxtail millet (Doust et al. 2009) due to high collinearity of grass genomes (Okada et al. 2010).

Rationale and Significance

I propose here a two-year research plan to investigate the genetics of abiotic stress tolerance, regionally adaptive traits, and nutrient metabolism in switchgrass. In addition, I will conduct a study to examine the potential use of municipal biosolids for growing switchgrass. ***These applied research projects will address the Program Area Priority to secure America's energy future through renewable biofuels.***

The proposed research will utilize the infrastructure and methodology developed by Dr. Thomas Juenger's lab, funded by an NSF Plant Genome grant (IOS - 0922457). That NSF grant is focused on the physiological genomics of switchgrass responses to climate change. Much of the funding of that grant is allocated toward gene expression responses to drought across varieties of switchgrass. However, there is no allocation in the NSF grant for salary support or supplies for the genetic mapping in *Panicum virgatum*, which is the primary aim of the research proposed here.

A key component to this research will be to understand the gene x environment interactions for mapped QTLs. Theory predicts that locally adaptive QTLs may trade-off across habitat with opposite effects on yield and fitness in different environments (reviewed in Kawecki & Ebert 2004). However, most studies have found a lack of trade-offs across environments for individual loci. In these cases, adaptive alleles have

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beneficial effects in one environment but no effects in other environments (Verhoeven et al. 2004, 2008; Gardner & Latta 2006; Lowry et al. 2009; Lowry 2010; Figure 3). This result suggests that it should be possible to move some desired QTL alleles, but not others, between varieties through marker-assisted selection breeding without deleterious environmental dependent consequences. Therefore, understanding QTL x environment interactions is crucial to determining which loci to target for marker assisted selection in switchgrass.

The proposed research involves a multidisciplinary approach that will address many of the core goals laid out in a recent National Research Council report (NRC report 2009). In particular, the report calls for the biology in the 21st century to tackle four major societal issues: food, energy, environment, and health. The long-term goal of my research will be to improve yield in switchgrass while lessening the impact on water and fertilizer resources. This research thus will contribute to an energy future for an America less dependent on foreign fossil fuels. This research will also result in deeper insights into drought tolerance and nutrient

remobilization in plants across environmental conditions, which in general is critical for keeping pace with global demand for food, fuel, and fiber production (Eckardt et al. 2009). The support of this fellowship will help to meet these goals through additional training, empirical investigation, and immersion in applied science. Importantly, the training I will receive as a postdoctoral fellow will provide me with a diversified skill set that I can use to train basic research scientists to innovative crop breeders.

My career goal is to establish a research group dedicated to a synthetic understanding of the genetic, genomic, ecological, and physiological mechanisms of plant adaptations to complex environments and using that information to improve agricultural crops. Meeting this challenge will require an integration of the knowledge and skills developed during my graduate training (population genetics, quantitative genetics, ecophysiology, breeding, and field experimentation) with more sophisticated use of genomic data and agronomic practices. Future advances in biology will depend on this type of highly integrated approach, where laboratory research, bioinformatics, and rigorous field experimentation complement each other and iteratively impact discovery.

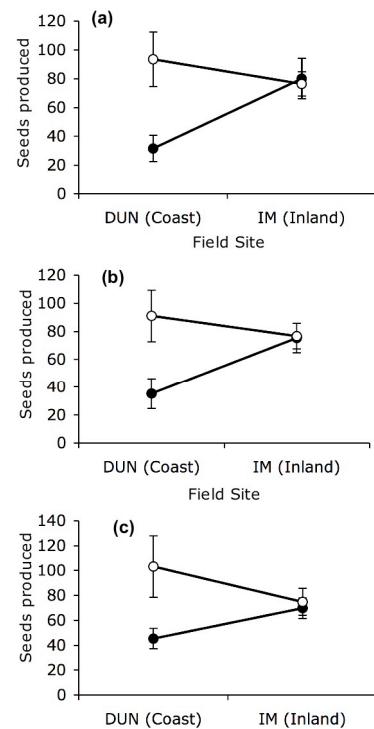


Figure 3: Salt tolerance QTLs have a strong effect on seed yield in coastal habitat, but no effect in inland habitat, indicating a lack of trade-off for this trait across environmental conditions (from Lowry et al. 2009).

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Approach

Project 1. Genetic mapping of drought response and other traits involved in biomass production as a launch point for marker assisted selection

The major goal of this aim is to determine the genetic architecture of drought tolerance physiological traits as well as phenological/morphological traits within two major agronomic cultivars, Kanlow and Alamo.

Background on varieties:

Alamo is a lowland agronomic cultivar collected originally from George West, TX (Figure 1, Average annual rainfall = 28 inches, cold hardiness zone 9). It is currently the most productive agronomic line in the southern regions of the United States (Sanderson 1996, 1999; Yang et al. 2009). Previous breeding has been directed toward improving the efficiency of germination and thus, field establishment, through selection of heterogeneous Alamo population seed (McLaughlin & Adams Kszos 2005; Sanderson et al. 2006). Alamo is currently the major target of whole genome sequencing by the Joint Genome Institute (DOE).

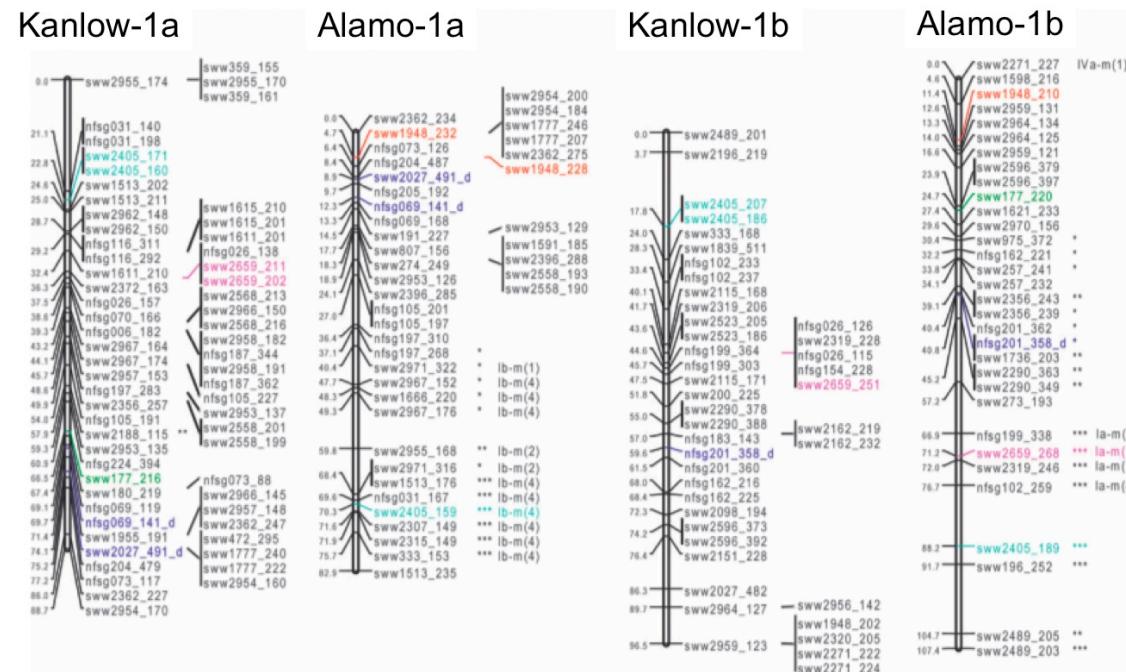


Figure 4: Example of linkage map density for Albany mapping population. The figure depicts the two linkage groups (1a and 1b) that are syntenic with chromosome 1 of sorghum. Since this is an outbred F1 mapping population, there are separate linkage maps for Alamo and Kanlow. (Image from Okada et al. 2010).

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Kanlow is a lowland agronomic cultivar collected originally Wetumka, OK (Figure 1, Average annual rainfall = 42 inches, cold hardiness zone 7). It was released in 1963 and has been used in the past for the stabilization of riparian areas subject to flooding. In field trials, Kanlow typically is the second highest yielding line behind Alamo in Texas (Sanderson 1999), but can be more productive than Alamo at more northern latitudes (Moser & Vogel 1995; Lemus et al. 2002).

The Albany mapping population:

Panicum virgatum exhibits considerable diversity in genome size and ploidy ($2n=36$, 54, 72, 90, and 108 (Missaoui et al. 2005)) in natural populations. In particular, ploidy series are common with tetraploid ($2n=36$) and octoploid ($2n=72$) genomes commonly grown in agronomic settings. Until recently, little was known about interfertility of the commonly studied agronomic varieties or whether their inheritance would be poly- or disomic. The results of two recent mapping efforts (Missaoui et al. 2005; Okada et al. 2010) discovered a predominance of preferential pairing and largely disomic inheritance, greatly simplifying the construction of genome wide linkage maps and opening the possibility of traditional QTL mapping approaches.

Recently, Dr. Christian Tobias' group at the USDA ARS in Albany, CA created a dense linkage map from an outbred cross between the cultivars Kanlow and Alamo (Figure 4). From this cross, Okada et al. (2010) selected 238 fullsibs that are henceforth referred to as the Albany mapping population. This full-sib family was used to assemble a linkage map composed of over 500 single dose amplicons.

The Juenger lab has developed an ongoing collaboration with Dr. Tobias centered on field trials with Albany population. Over the past year, the Albany population has been clonally increased by the Juenger lab through tiller cuttings and is available for greenhouse, growth chamber, and field experiments. As the population has been thoroughly genotyped any phenotypic data collected from the sibship can be easily evaluated for the occurrence of heterozygous QTL inherited from either the Alamo or Kanlow parents.

Experimental design

My experimental approach centers on growing replicate clones of the Albany population under an experimental rainout shelter at the Brackenridge Field Laboratory (BFL) in Austin, Texas (Figure 5a). The large rainout shelter will exclude all precipitation from the plantings, and afford the application of controlled water treatments to experimental rows. The establishment of rainout shelters was a major piece of infrastructure funded by the NSF Plant Genome grant to Dr. Thomas Juenger.

In the fall of 2010, I will plant two clonal replicates of each of 194 fullsibs of the Albany mapping population in rows (Figure 5b). Parent lines ($N=15$ per treatment) of Alamo and Kanlow will be planted with the Albany population. All plants will be fully randomized

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within treatments applied at the level of rows. Half of the plants in the experiment will receive 100% of water necessary to replace water lost by evapotranspiration. The other set of plants will receive a yet to be determined percentage of the water lost by evapotranspiration, to simulate a drought year. To establish the level of water supplied to the drought treatment, I will monitor soil and leaf water potential as suggested by Barney et al. (2009). I have also established a collaboration with Dr. Guy Fipps and Charles Swanson at Texas A&M, who designed a drip irrigation system for this project and will help determine a suitable drought treatment regime throughout the growing season.

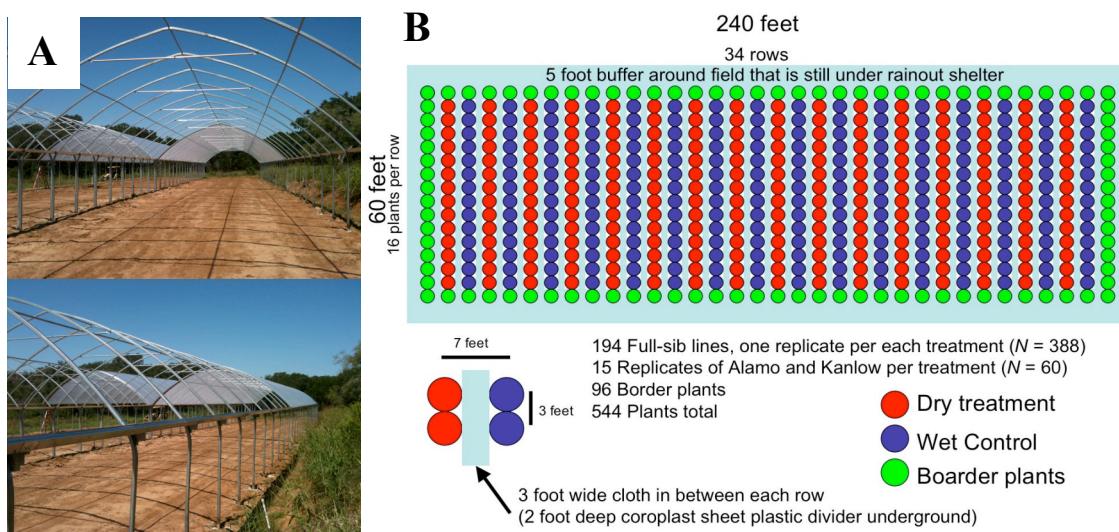


Figure 5: A) Photos of the rainout shelter under construction at the Brackenridge Field Laboratory (BFL). B) Experimental design for Albany fullsib mapping population at BFL.

Phenotypic measurements and genetic mapping

I will initiate phenotypic measurements of the mapping population in the spring of 2011. Various drought related traits will be assessed including instantaneous gas exchange (Licor 6400), soil water potential, chlorophyll content, proline content (Verslues 2010), total phenolic content (Ainsworth & Gillespie 2007), and total antioxidant content (Gillespie et al. 2007). Undergraduate students working in the Juenger lab and enrolled in the Freshman Research Initiative “Biology of Biofuels” program (see Mentoring Relationship section) will make large-scale phenotyping of labor-intensive physiological traits possible. In addition, multiple phenological and morphological traits associated with adaptation to different ecoregions will be measured: Date of first growth in spring, flowering time, date of fall senescence, leaf length/width, and plant height. Finally, we will harvest the above ground biomass each season after senescence to assess yield per plant. QTL mapping of all traits will be carried out using a combination of rQTL (Broman et al. 2003) and the cross pollinated module of MapQTL (Van Ooijen et al. 2002).

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Expected outcomes

The proposed study will result in the identification of genomic regions containing gene(s) impacting study traits related to plant performance under stress. Because the mapping population is composed of fullsibs derived from outbred parents, the progeny may segregate up to four alleles at a locus and the population will exhibit multiple forms of marker and QTL segregation. As such, our mapping strategy will be primarily effective at identifying QTLs that were heterozygous in either the Alamo or Kanlow parents and limited in its ability to detect genetic effects differing between Alamo and Kanlow. Nevertheless, it is likely that the parental lines carry considerable genetic variation (high heterozygosities) and our mapping approach will detect QTLs.

Project 2. Creation of outbred mapping populations for genetic mapping of adaptive regional variation

Understanding the genetic architecture of adaptive regional variation is crucial for future switchgrass breeding. Previous studies have found large genotype x environment interactions for yield and other traits (Hopkins et al. 1995; Sanderson et al. 1999; Cassler et al. 2004, 2007). Seed companies typically prefer as large a distribution as possible to market their product and thus any expansion in the range at which new cultivars can grow could potentially be very important. Future marker assisted selection breeding programs for traits such as drought tolerance and nitrogen use efficiency will also benefit from

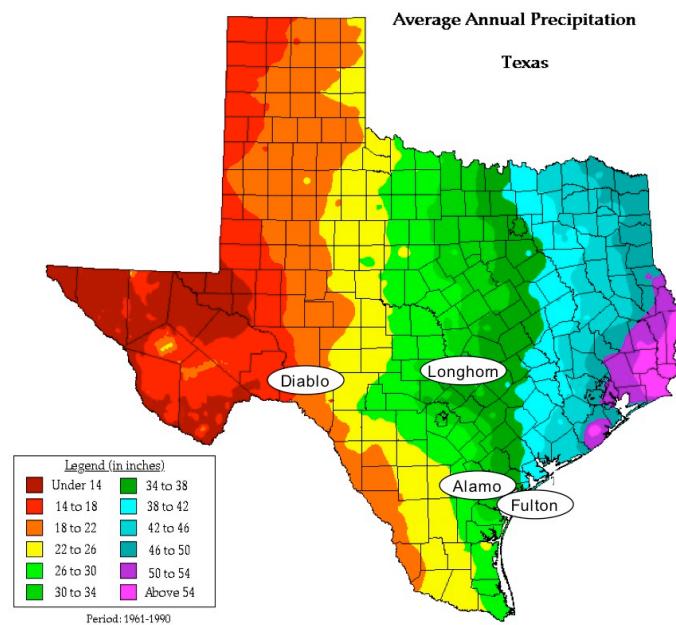


Figure 6: Thirty-year rainfall map for Texas with locations of populations for collections plotted on the map.

understanding which parts of the genome are crucial to retaining regional adaptations, when introgressing desired alleles between divergently adapted varieties.

Previous breeding efforts (e.g. Vogel & Mitchell 2008) have successfully created F2 and advance generation hybrids. However, none of these previous breeding programs have utilized these hybrid populations for the purposes of creating genetic mapping populations. I intend to create three major mapping populations that I will use to better understand the genetics of natural trait variation in switchgrass. These mapping populations will become a

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community resource that can be planted in many locations by different researchers to better assess the genetic mechanisms that differentiate the response of cultivars across ecoregions. The lines that I have selected to use for mapping will serve three key purposes: **1) determine the genetic basis of differences between upland and lowland varieties of switchgrass, 2) assess the genetic architecture within and among the two major southern cultivars, Alamo and Kanlow, and 3) assess genetic architecture of variation within Texas to facilitate breeding for future lines tailored to the southern United States.**

Background on varieties:

The research program will involve Alamo, Kanlow, and four additional varieties/cultivars of switchgrass (Figures 1, 6). All of these varieties have been confirmed as tetraploids by flow cytometry, making crossing and QTL mapping possible.

Summer is a tetraploid upland variety derived from near Nebraska City, NE (Figure 1, average annual rainfall = 34 inches, cold hardiness zone 5). It was commercially bred and released as a major cultivar by the South Dakota Agricultural Experiment Station.

Longhorn was collected from a large population just west of Bee Caves, Texas in 2010. Average annual rainfall = 32 inches, cold hardiness zone 8.

Fulton was collected from the edge of a salt marsh along the Gulf Coast of Southern Texas in 2010. Average annual rainfall = 30 inches, cold hardiness zone 9.

Diablo was collected from a dry creek bed in arid west central Texas in Devil's River State Natural Area in 2010. Average rainfall = 17 inches, cold hardiness zone 8.

Crossing design:

All genetic mapping in switchgrass must be conducted in outbred mapping populations because switchgrass is self-incompatible (Martinez-Reyna & Vogel 2002).

I have already conducted two independent paired crosses between Summer and Longhorn lines (Figure 7a). One progeny from each cross will be selected for intercrossing to create an outbred F2 mapping population. This mapping population is ideal for

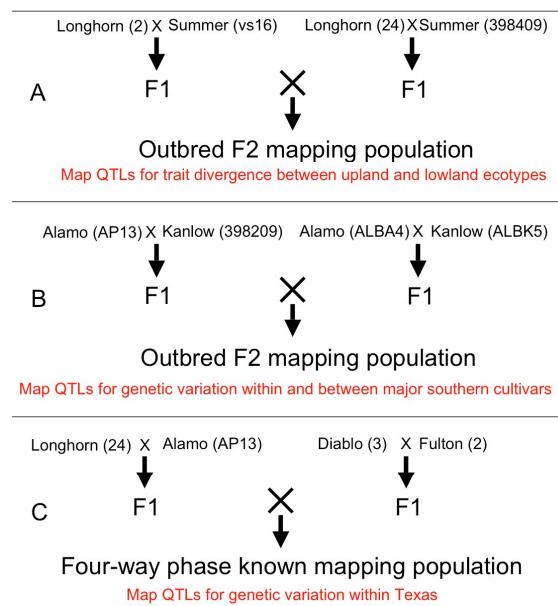


Figure 7: Crossing designs for creation of three novel outbred mapping populations.

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identifying loci underlying the highly divergent traits that differentiate upland and lowland ecotypes as well as uncover the causes of regional adaptations to abiotic stresses, such as heat, cold, and drought.

To map the genetic architecture of variation within and between Alamo and Kanlow (Figure 7b), I will cross the Alamo AP13 line to a Kanlow line (USDA 398209). AP13 is the major target of genome sequencing by the Joint Genome Institute (DOE). To create outbred F2s, a progeny from this cross will be crossed to a line from the Albany population (independent Alamo and Kanlow parents).

To create a mapping population for genetic variation within Texas, I will conduct a four-way phase known crossing design using Diablo, Fulton, Alamo, and Longhorn (Figure 7c) as grandparents. These varieties originated from two different hardiness zones and three plant adaptation regions (Vogel et al. 2005). The goal of this cross will be to assess genetic variation for drought tolerance and other traits within Texas. The populations are located across the moisture gradient of Texas (Figure 6). By introducing genetic variation from the coastal Fulton, along with Alamo (the only known salt tolerant variety (Alderson & Sharp 1995)), there is great potential to map salt tolerance QTL. Improving salt tolerance will be crucial for growing switchgrass cultivars on marginal saline lands.

RAD genotyping of outbred mapping populations

Genotyping of mapping populations has long been a time consuming and expensive endeavor and the major bottleneck for any QTL mapping study. Now, with the recent coupling of next generation sequencing with genome reduction techniques linkage map

assembly and QTL detection is far more efficient. I will utilize a restriction site assisted DNA (RAD) mapping scheme, which is a recently developed genome reduction technique whereby only regions directly adjacent to a selected restriction enzyme cut site are hybridized to a microarray (Miller et al. 2007a,b) or directly sequenced for genotyping purposes (Baird et al. 2008; Hohenlohe et al. 2010).



Figure 8: Example of SNP genotyping for a locus by high-throughput sequencing of short RAD-tags on the SOLiD platform. The figure depicts actual data from a switchgrass relative (*Panicum hallii*) using the modified technique developed in the Juenger lab at UT Austin.

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Dr. Eli Meyer (currently a postdoc in the Juenger lab) has recently developed a more robust and even sequencing method for RAD genotyping in switchgrass using an infrequent restriction enzyme cutter. The short RAD tags resulting from restriction digest are barcoded and sequenced on an ABI SOLiD sequencing platform. The estimated cost for this modified method is \$5-10 per individual plant, yielding over a thousand genetic loci (e.g. markers) sequenced at ~100X coverage. Dr. Meyer has also developed a bioinformatics pipeline to map sequenced RAD-tags to a reference genome (Figure 8). I will work with Dr. Meyer and Dr. Juenger to develop additional bioinformatics tools to deal with the challenge of outbred mapping populations, filter out paralogous tags, and identify single dose loci that can be used to create linkage maps. From this data I will assemble linkage maps for all three mapping populations. Genotype data across individuals will make subsequent QTL mapping feasible. I will genotype 200 individuals per each mapping population with RAD, for a total of 600 genotyped individuals. Linkage map assembly will then be conducted with RAD genotypes using the program JoinMAP (Stam 1993).

Phenotyping and QTL analysis

The outbred mapping populations will be grown in the Brackenridge Field Laboratory (BFL) in Austin, TX in one-gallon pots. BFL has served as an ideal location for growing switchgrass and dividing mapping populations for planting into the field. I will measure a suite of phenotypic traits (as described in Project 1) in both well watered and under water deficit conditions. QTL mapping will be carried out in rQTL (Broman et al. 2003).

Through divisions, these mapping populations will become a community resource available to collaborators to grow around the United States for genetic mapping under different environmental conditions. I plan to work with Dr. Jim Kiniry (USDA-ARS Temple, TX) to locate suitable sites within Texas to plant these mapping populations for future mapping efforts. I have already located one field site in Texas (Hornsby Bend) to conduct field based mapping as described in Project 3. In addition, I am actively looking for other collaborators outside of Texas to also grow these mapping populations.

Expected outcomes

This study will result in a better understanding of the genetic architecture of important traits across upland and lowland ecotypes, within and between major cultivars, and within the state of Texas. As there are currently no published QTL mapping studies in switchgrass and outbred F2 community resources with linkage maps have yet to be developed, this project would make significant progress toward marker assisted selection in switchgrass. Further, this project will serve as a confirmation of RAD mapping methods, currently being developed in the Juenger lab, to rapidly and cost effectively create linkage maps that can be used to identify functional loci in any tetraploid switchgrass cross. Finally, developing these outbred populations may serve as a key resource for mapping of assembled genome scaffolds to linkage maps as a part of the

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current *Panicum virgatum* whole genome sequencing effort by the Joint Genome Institute (DOE).

Project 3. Genetic mapping of nutrient assimilation and remobilization in response to municipal biosolid fertilization

Municipal wastewater and solid wastes are potential major resources for the production of alternative energy, including geothermal (Dellinger & Allen 1997; Lund 2006) and methane production (Stillwell et al. 2010). There is also a great potential to increase bioenergy crop yields using biosolids from solid waste treatment plants as fertilizer (Borjesson & Berndes 2006; Gopalakrishnan et al. 2009; Lai et al. 2010), especially in Texas (Figure 9; Stillwell et al. 2010)

Biosolids mostly consist of organic matter that is produced during wastewater treatment. Once biosolids are recovered, they are frequently used as a fertilizer source for agriculture. It is estimated that 4 million tons of biosolids (55% of the US total) are applied to agricultural lands annually (NACWA report 2008). Biosolids serve as cheap fertilizer source while helping to retain soil moisture and preventing these solid wastes from getting into the water supply. However, there are concerns about the use of

biosolids as a fertilizer for food crops since they contain heavy metals and a suite of industrial chemicals (Silveira et al. 2003; Lavado et al. 2005, 2007). There are also concerns that fertilizer pollution of riparian areas can result from run off of fields fertilized by biosolids (Peckenham et al. 2008). One solution is to shift the use of biosolids to the fertilization of bioenergy crops like switchgrass, which would prevent biosolid toxins from entering the food supply. Switchgrass also has a large capacity to uptake nitrogen and phosphorus, which can prevent runoff of fertilizer into streams and rivers (Sanderson et al. 2006; McIsaac et al. 2010).

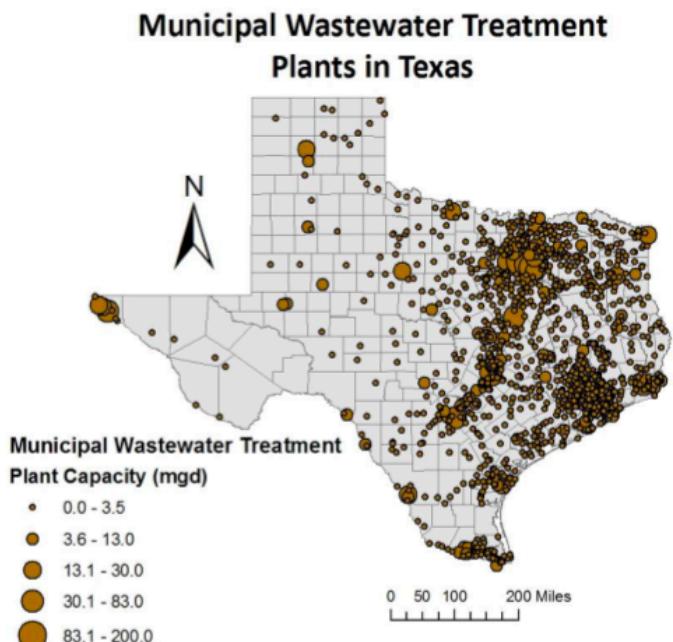


Figure 9: Locations and size of wastewater treatment plants in Texas (from Stillwell et al. 2010). Distribution of projected switchgrass agriculture (as seen in Figure 1) overlaps with the distribution of wastewater plants.

Nitrogen is considered the greatest limiting factor for biomass yield in switchgrass

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(Bransby et al. 1998; Vogel et al. 2002; Parrish & Fike 2005). Switchgrass remobilizes nitrogen and other nutrients from above ground biomass to the crown and roots each fall (Clark 1977; Parrish & Fike 2005; Lemus et al. 2009; Yang et al. 2009). This mechanism is thought to be key to the ability of switchgrass to grow with minimal fertilizer inputs (Lemus et al. 2008, 2009). However, 31 to 63 kilograms/ hectare of nitrogen is still lost during harvesting of senescent aboveground biomass each season (Lewandowski et al. 2003). With this loss of nitrogen and other macronutrients, fertilization is inevitably necessary.

Cultivars differ considerably in their nutrient remobilization during the fall (Yang et al. 2009). Most northern upland cultivars have significantly lower remobilization rates of nutrients than southern lowland populations during the fall (Figure 10; Yang et al. 2009). Understanding the genetic mechanism for nitrogen remobilization and recycling will thus be important for breeding sustainable lines that will require fewer nutrient inputs.

I am currently arranging a collaborative project at the Hornsby Bend Biosolids Management Plant (HBBMP) to study the response of switchgrass to biosolid application. HBBMP currently processes all of the sludge from Austin, TX and processes this into a biosolid. A portion of the total biosolid output is then combined with municipal yard waste to produce a heavily organic fertilizer that is sold back to the general public as compost fertilizer (DilloDirtTM). This Biosolids Management Plant is heavily focused on recovery of energy from solid wastes and wastewater. For example, they capture enough methane gas from biosolid processing to fully power the plant. As HBBMP is a major center focused on sustainability, especially on the water-energy nexus (Stillwell et al. 2009; Lund 2010), it is an ideal location for research exploring new sustainable energy resources.

Experimental Design

I propose an addition experiment using the outbred F2 mapping population from the cross between the southern lowland Longhorn and northern upland Summer varieties to explore the genetic basis of traits involved in nutrient utilization of municipal biosolid wastes. The significant differences in nutrient remobilization between upland and lowland varieties (Figure 10; Table 1; Yang et al. 2009) are a key reason for selecting this mapping population to be planted at Hornsby.

My experiment will have two

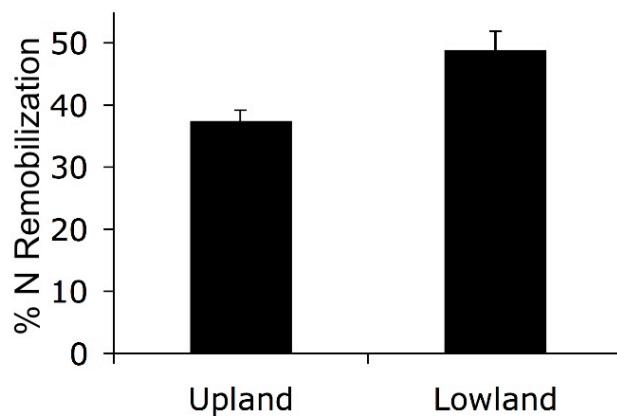


Figure 10: There are significant differences ($P < 0.05$) in percent of nitrogen remobilization during senescence between upland and lowland ecotypes (Data from Yang et al. 2009).

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treatment classes: 1) Control with no additions, 2) A fertilizer addition treatment using Dillo Dirt™ (a biosolid and municipal waste mixture) produced at Hornsby Bend. In the summer of 2011, I will plant 200 F2 lines in each of the two treatment classes, along with 20 Longhorn and 20 Summer parental lines, for a total of 480 experimental plants. A row design with the same distances between plants for project 1 will be used for this experiment. Every other row will receive a biosolid fertilizer addition at rates that will replace nitrogen loss by seasonal harvest. To prevent spread of nutrients between treatments, all rows will be separated by sheets of coroplast plastic that are inserted into the soil to a depth of 2 feet. All lines will be randomized within treatment. To prevent edge effects in our experiment, I will also plant a strip of plants around the border of the experiment that will not be part of the analysis. Supplemental irrigation will be conducted when necessary using drip lines run off a tap connected directly to a reclaimed wastewater source.

I will measure morphological and phenological traits as well as end of season biomass as described for the Albany population (Project 1). In addition, I will quantify the leaf concentration of 19 different elements (P, K, Li, B, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, and Cd; Baxter et al. 2007) and %N (Cornelissen et al. 2003) in both treatments across the mapping population, both during the peak of maturity and immediately after fall senescence in 2012. Many of these elements are already known to differ in leaf concentrations between upland and lowland ecotypes (Table 1).

All of my leaf samples will be from the upper most leaf blade on a tiller with an exposed ligule, since a previous study (Lemus et al. 2009) found that this leaf stage gives the best estimate of plant nitrogen status. Ion concentrations will be quantified at Dr. Ivan Baxter's lab at the Donald Danforth Plant Science Center in Saint Louis, MO (Baxter et al. 2007). I have experience with ionomic analysis from research conducted during my Ph.D. dissertation on *Mimulus guttatus* (Lowry et al. *in prep*). Ion analysis will be conducted mid-summer and after senescence in the fall to assess how nutrients are assimilated and remobilized in the fall, as in Yang et al. (2009). As with the Albany populations, QTL mapping will be implemented in rQTL (Broman et al. 2003).

Element	Lowland	Upland	P-value
B	16.70 (1.53)	24.79 (1.48)	0.0053
Na	1348.72 (289.55)	52.48(8.30)	<0.0001
K	10358.9 (701.55)	7173.2 (380.44)	0.0003
Ca	4381.61 (525.75)	6176.85 (310.07)	0.0064
Zn	20.58 (0.52)	17.19 (0.58)	0.0027
As	0.028 (0.013)	0.077 (0.009)	0.0083
Fe	58.36 (13.15)	120.30 (10.86)	0.0044
Co	0.05 (0.01)	0.15 (0.08)	0.0033

Table 1: Examples of significant differences in mature leaf ion concentrations ($\mu\text{g g}^{-1}$) between upland and lowland ecotypes (data from Yang et al. 2009). Means, with standard errors in parentheses, listed for each ecotype.

Project Narrative

Examining the scalability biosolids and wastewater for energy crops

To assess the potential utility and scalability of biosolid fertilization of switchgrass, I have initiated collaboration with Dr. Michael Webber's engineering group at the University of Texas (see attached letter of support). The main goal of this collaboration will be to comprehensively model the system properties and scalability of using biosolids for growing switchgrass and compare this to direct harvesting of energy from biosolids through incineration. Dr. Webber's group recently modeled (Stillwell et al. 2010) the potential energy returns from biosolids in Texas and thus is already aware of many of the issues associated with the uses of biosolids.

Expected outcomes

This study will result in the identification of QTLs involved in response to biosolid fertilizer addition, including morphological traits, nitrogen remobilization efficiency, leaf ion concentrations, and other physiological traits. Identification of loci involved in toxic heavy metal (Cd, Cu, Ni, Zn) uptake will also be extremely important to breeding varieties that uptake or exclude those metals based on downstream uses. Further, the results of the experiments conducted at Hornsby will serve as data points for modeling the utility of biosolid additions for growing switchgrass in Texas.

Timeline:

Activity	Su 2011	F 2011	W 2012	Sp 2012	Su 2012	F 2012	W 2013	Sp 2013
Project 1-Drought response QTL mapping								
Impose drought stress								
Measure phenotypes on Albany population								
Conduct QTL mapping analysis								
Write manuscripts								
Project 2-Creation of novel outbred mapping populations for QTL analysis								
Collect tissue from mapping populations								
Measure phenotypic traits								
Build linkage maps by RAD mapping								
Divide population and make available								
Write manuscripts								
Project 3-QTL mapping nutrient assimilation and remobilization in response to biosolid fertilization								
Plant mapping population								
Implement biosolid nutrient addition								
Measure phenotypic traits								
Collect tissue for ionomic analysis								
Conduct QTL mapping analysis								
Write manuscripts								

Documentation of progress:

In order to document my career progress I have established a profile on LinkedIn, a personal webpage (<http://web.me.com/davidbryanthlowry/Site>Welcome.html>), and wiki page (http://openwetware.org/wiki/Mimulus:David_Lowry).

Mentoring Relationship

Dr. Thomas Juenger, will serve as my primary mentor for the tenure of this fellowship. Dr. Juenger (University of Texas at Austin) has an excellent research background in plant quantitative genetics, genomics, and physiology. As mentioned above, Dr. Juenger is the lead-PI on a four year (2009-2013) National Science Foundation grant to study the genomics of drought tolerance and global change in switchgrass. This funding base has served to build the infrastructure and develop the methodologies that I will utilize as a NIFA fellow. The NIFA fellowship will primarily provide necessary salary support for me to pursue independent research on genetic mapping in switchgrass, as well as help cover expenses associated with RAD genotyping, ionomics, and other physiological assays, which are not allocated for in the NSF grant.

Dr. Juenger's group has recently conducted a series of studies on the ecophysiology, genetic architecture, and genome-wide gene expression of drought response in *Arabidopsis thaliana* and *Brachypodium distachyon* in collaboration with statisticians and biologists across multiple research institutions. Dr. Juenger also has developed collaborations with Dr. James Kiniry and Dr. Philip Fay at the USDA-ARS in Temple, TX to study drought tolerance in switchgrass. I have been working with both Dr. Kiniry and Dr. Fay this past summer to develop my research plans and they will serve to give advice for the duration of the project. My research with the Albany population is part of an ongoing collaborative project with Dr. Christian Tobias at the Albany, CA USDA-ARS.

Further collaborative mentoring will ensure that I receive the training I need to complete the research as proposed and establish an independent career. I will receive extensive mentoring interactions from other PIs involved in the switchgrass research collaborative (see our wiki: openwetware.org/wiki/Texas_Switchgrass_Collaborative) at UT, including ecologists Dr. Christine Hawkes and Dr. Tim Keitt. Dr. Eli Meyer, a senior postdoc in the Juenger lab also will help train me to create libraries with his modified RAD genotyping protocol. Additional researchers at UT are working on the practical aspects of biofuel production, such as Dr. Hal Alper, who is currently optimizing biomass conversion to cellulosic ethanol. As mentioned above, collaboration with Dr. Michael Webber's group will be vital to determining the future potential of biosolid fertilization of switchgrass. To learn more about energy production and energy policy I will also enroll in Dr. Webber's three-day short course "Energy Technology and Policy" in the summer of 2011.

UT Austin is also an ideal location for my fellowship project because of the diversity of researchers utilizing bioinformatics analyses, computation biology, and next generation sequencing applications to successfully conduct RAD mapping. Further, Dr. Juenger has extensive experience with mapping in outbred populations, which is crucial for genetic mapping in the obligate outcrosser *Panicum virgatum*. Many of the computational analyses of RAD mapping will be enhanced due to the availability of Texas Advanced Computer Center (<http://www.tacc.utexas.edu/>), which houses a number of powerful

Project Narrative

clusters for scientific computing. Finally, I am currently enrolled in a three-week intensive “Programming for Biology” course at Cold Spring Harbor Laboratory in the fall of 2010, where I will gain additional training to analyze the large datasets produced by this project.

This fellowship will expand my teaching skills to several new topics (plant physiology, bioinformatics, energy production, agricultural science) and to a broader set of students. During graduate school, I have mentored high school (1) and undergraduate (6) students from underrepresented and minority groups in evolutionary genetics and plant biology. I have already started mentoring two undergraduate students at UT Austin and plan to expand on this through the Freshman Research Initiative “Biology of Biofuels” program for undergraduates, starting the spring semester of 2011. The “Biology of Biofuels” education stream will organize recruited freshman students into collaborative groups to engage in projects related to plant physiology, genetics/genomics, environmental biology, and climate change. I plan to assist with developing coursework and projects related to my genetic mapping research that these students would become involved in during the spring semester of the program and over the summer and fall following their freshman year.

I have initiated multiple avenues for communicating science with the general public and policy makers through new media including the design of community wiki pages and have published guest blog posts for Discover Magazine. Postdoctoral training in a field directly related to agricultural and energy production will bolster these communication efforts. I will also present the results of this research at a minimum of two scientific meetings annually (ASA-CSSA-SSSA International Annual Meetings/Plant and Animal Genome Conference), serve on the AFRI peer review panel, publish in peer-reviewed journals, and travel to the annual investigators meeting.

Overall, the NIFA fellowship will provide me with the invaluable opportunity to pursue independent research and build my career as collaborative enterprise across fields of expertise. Funding this fellowship will help to create new connections for myself and others across departments at UT Austin, multiple USDA facilities, the Hornsby Bend Biosolids Management Plant, the Donald Danforth Plant Science Center, Texas A&M, UC Berkeley, the Joint Genome Institute (JGI), as well as the greater switchgrass and plant genomic communities.