MOLECULAR MARKER ANALYSIS OF PROGENY ORIGINS IN SIBLING-MATING AND CROSSING POPULATIONS OF LOWLAND SWITCHGRASS

By

JOHN BAKER

Bachelor of Science in Plant and Soil Science

Oklahoma State University

Stillwater, Oklahoma

2014

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2017

MOLECULAR MARKER ANALYSIS OF PROGENY ORIGINS IN SIBLING-MATING AND CROSSING POPULATIONS OF LOWLAND SWITCHGRASS

Thesis Approved:

Dr. Yanqi Wu

Thesis Adviser

Dr. Michael P. Anderson

Dr. Vijaya Gopal Kakani

Dr. Lan Zhu

ACKNOWLEDGEMENTS

I am very grateful to my advisor, Dr. Yanqi Wu, who through great effort helped to ensure my success in my MS studies. His patience, and guidance were essential to enable me to complete my research, and develop a coherent understanding of my results. I would also like to thank my committee members, Dr. Michael P. Anderson, Dr. Gopal Kakani, and Dr. Lan Zhu who provided essential feedback and suggestions for my thesis as committee members, and helped me to better understand and apply important concepts as instructors.

My work in the lab and field would not have been completed without the help of Mr. Gary Williams, Dr. Tilin Fang, and Mrs. Pu Feng who helped me in the field, lab, and greenhouse. My fellow graduate students and undergraduate employees Dr. Shiva Makaju, Dr. Yuanwen Guo, Dr. Dan Chang, Mr. Clint Lucas, Mr. Jake Buchanan, Ms. Paige Overton, Mr. Joe Buchanan, and Mr. Aaron Purkins, also provided vital help in conducting field operations. My research would not have been possible without funding from the Oklahoma Agricultural Experiment Station, the Department of PSS at OSU, and the Meibergen Family Professorship. Finally I wish to express my deepest appreciation to my parents: Mr. T.K. Baker, and Mrs. Sara Baker, as well as my wife Mrs. Brenna Baker, and my sister Mrs. Madalyn Taylor without their support and encouragement I would not have been able to make it this far.

Name: JOHN BAKER

Date of Degree: MAY, 2017

Title of Study: MOLECULAR MARKER ANALYSIS OF PROGENY ORIGINS IN SIBLING-MATING AND CROSSING POPULATIONS OF LOWLAND SWITCHGRASS

Abstract:

Switchgrass (*Panicum virgatum* L.) is a C4 perennial grass which has been used for soil and water conservation and as forage for decades. In recent years it has been targeted for development as a bioenergy crop. However, breeding methods for developing economically viable hybrid cultivars are not available to improve switchgrass. Accordingly, the objective of this study was to assess genetic origins and seed yields of S1, S2, and S3 inbreds when grown in isolated sibling-mating, and crossing-plots of two conditionally self-compatible lowland switchgrass genotypes. Inbred progeny of two conditionally self-compatible switchgrass plants 'NL94 LYE 16'x13' (NL94) and 'SL93 7x15' (SL93) were established in 6 sibling-mating and 4 crossing plots at the Agronomy Research Station, Oklahoma State University in 2013. Seed yields for each plot were measured in 2014 and 2015. Seeds of 5 selected plants in each sibling-mating plot and of 5 plants of each genotype in the crossing plots were harvested separately for genotyping to determine parental origins. Significant effects on seed yield due to genotype, parental selection, and inbreeding depression were noted. Progeny origins were determined using 6 or 10 simple sequence repeat (SSR) markers. In sibling-mating plots, a high preference for sibling-mating over selfing was observed in both SL93 and NL94 S1 parents which set 68% and 96% sibling-mated seed respectively. At more advanced inbreeding levels distinguishing between selfed and sibling-mated seed became more difficult due to low marker polymorphisms. In the crossing plots, hybrid production related to genotype and inbreeding level. In an S1 crossing plot the NL94 and SL93 parents set a high percentage of hybrid seed averaging 73% and 94%, respectively in 2014 and 2015. In two of the S3 crossing plots NL94 parents set 100% hybrid seed. In these same S3 crossing populations, SL93 parents set only 4% and 19% hybrid seed. Information from this study gives insight into how multiple generations of inbreeding effect seed origin and yield, and will help breeders assess the viability of producing hybrids using inbred lines grown from seed under field conditions.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial grass species native to much of North America. Historically switchgrass was mainly used as forage, and for soil and water conservation. However, in 1991 the United States Department of Energy (USDOE) identified it as the "model" cellulosic bioenergy species following extensive evaluation in the previous decade (Parrish and Fike, 2005; Wright and Turhollow, 2010). One feature that suited switchgrass to this role is its genetic diversity. Switchgrass genotypes have a wide range of ploidy levels from diploid (2n=2x=18) to duodecaploid (2n=12x=108) with tetraploid (2n=4x=36) being common in lowland ecotypes, and octaploid (2n=8x=72) in upland ecotypes (Brunken and Estes, 1975).

In the past many switchgrass cultivars were natural track cultivars (Casler, 2012). These cultivars were often simple seed increases of native switchgrass collections with no or only a few generations of phenotypic selection for agronomic traits (Casler, 2012; Wu, 2014). Most of these natural track cultivars were adapted for the specific environmental conditions from which they were sourced, however as interest in switchgrass for biofuels increased some cultivars were found to have wider ranges of adaptation (Casler, 2012).

Notable for wide ranges of adaptation are ‘Alamo’ which was collected in central Texas, and is productive throughout the southeastern US, and ‘Cave-in-Rock’ which is adapted to hardiness zones four through seven (Casler, 2012).

Switchgrass has been characterized as exclusively outcrossing due to the strong self-incompatibility (SI) mechanism that exists in the species (Talbert et al., 1983; Martinez-Reyna and Vogel, 2002). The SI systems in switchgrass have led to the use of recurrent selection to develop synthetic cultivars (Vogel, 2004). Through the use of recurrent genotypic selection, and half-sib progeny testing ‘BoMaster’ and ‘Colony’ switchgrass cultivars with improved biomass yields have been developed, and ‘Performer’ switchgrass a cultivar with improved forage quality was released (Burns et al., 2008a, 2008b, 2010). Other recurrent selection methods have also been used to improve switchgrass. Restricted recurrent phenotypic selection (RRPS) followed by a cycle of recurrent selection for general combining ability (RSGCA) were utilized at Oklahoma State University to develop ‘Cimarron’ from a polycrossing of seven selected parents (Wu and Taliaferro, 2012).

While the gains from these breeding methods have been significant they do not take full advantage of heterosis. Heterosis, the degree to which hybrid offspring of two genetically different parents out perform these parents for a given trait, has been exploited in major crops including maize (*Zea mays* L.), rice (*Oryza sativa* L.) and sorghum [*Sorghum bicolor* (L.) Moench], and has been integral to large and continuous gains in yield in these species over the last century. There has been some success in harnessing heterosis in switchgrass through population hybrids developed by crossing ‘Summer’ an upland ecotype and ‘Kanlow’ a lowland ecotype (Martinez-Reyna, and

Vogel, 2008; Vogel and Mitchell, 2008). These two populations were identified as members of different heterotic groups, and their hybrid offspring showed high parent heterosis of 30-38% when grown in swards (Vogel and Mitchell, 2008). It has also been proposed that hybrids could be produced via the crossing of vegetative propagates from two heterozygous parents (Martinez-Reyna and Vogel, 2008; Casler, 2012). However, the aforementioned hybridization method comes with the high costs of propagating and transplanting clonal parental plants (Liu et al., 2014).

A potential alternative to this method was made possible by the recent identification of conditionally self-compatible switchgrass plants using SSR markers (Liu, Wu, 2011; Liu et al., 2014). Molecular technologies such as SSR markers are an invaluable tool for understanding switchgrass mating systems. The SSR marker system is based on the polymerase chain reaction (PCR) technology, which means they can consistently produce polymorphic bands using a small amount of DNA as a reaction template. SSRs are also a codominant molecular marker system which analyzes 1 locus at a time which makes them ideal for identifying the parental origins of progeny. A protocol for this purpose was developed in the Grass Breeding Lab at Oklahoma State University, and has proven useful in the identification of selfed progeny and cross pollinated progeny (Liu and Wu, 2011; Liu et al., 2013, 2014; Adhikari et al., 2015). Using SSR markers, 2 switchgrass plants 'NL94 LYE 16'x13' (NL94) and 'SL93 7x15' (SL93) were found to be self-compatible when grown in a growth chamber, but almost 100% outcrossing when grown in field conditions (Liu and Wu, 2011; Liu et al., 2014). Another study revealed that plants grown in the field with bagged inflorescences would reliably produce 100% self-pollinated seed (Adhikari et al., 2015). Conditionally self-

compatible genotypes such as these could be forcibly selfed or sibling-mated in isolation to create inbred lines. Parental plants could be inexpensively grown from seed, and crossed in the field exploiting the SI mechanisms to produce F_1 hybrids in switchgrass (Aguirre et al., 2011).

While previous studies have addressed mating behavior of switchgrass grown in a growth chamber, in heterogeneous populations, and with bagged inflorescences, no studies have yet investigated the mating behavior of switchgrass inbreds grown in sibling-mating and crossing plots under field conditions (Liu and Wu, 2011; Liu et al., 2014; Adhikari et al., 2015). Further investigation is needed to address certain important questions such as, (a) will inbred plants that are isolated from outcrossing pollen produce adequate amounts of viable selfed or sibling-mated seed, (b) will inbred parents reliably set a high percentage of hybrid seed when grown in crossing plots, and (c) will the hybrid seed be produced in the requisite amounts needed for commercial hybrid production? Thus, this study seeks to identify the parental origins of seed collected from S1, S2, and S3 lowland switchgrass grown in both sibling-mating plots and crossing plots, and to evaluate the seed production of these plants.

CHAPTER II

LITERATURE REVIEW

Switchgrass for bioenergy production

In recent decades as the environmental consequences and economic cost of fossil fuels have become a major concern, alternative energy sources have become an important research focus. One promising alternative to fossil fuels is ethanol produced from plant biomass. This fuel source is renewable, comparable in energy density to petroleum based fuels, and should result in net negative greenhouse gas emissions (Demirbas, 2007). In 2007, bioethanol made up 4% of global gasoline supplies (Balat and Balat, 2009). Two feedstocks occur for the majority of bioethanol produced, sugarcane and maize, in fact, in 2007, 45% of global bioethanol was produced from sugarcane in Brazil, while 47% was produced from maize in the US (Goldemberg, 2007; Balat and Balat, 2009). These feedstocks have shortcomings. While sugarcane conversion to ethanol is a highly efficient, sugarcane production is limited to tropical and subtropical environments (Balat and Balat, 2009; Ribera and Bryant, 2016). Maize is adapted to a wider range of climates, but it is less efficient in its conversion to ethanol, and requires the use of prime crop land which may be better utilized to produce food or feed (Pimentel and Patzek, 2005).

Cellulosic bioethanol may address these shortcomings. Bioethanol from cellulosic plant matter can be produced from a wide range of woody plants and grass species which are adapted to many environments, and productive on marginal land (Demirbas, 2007). Beginning in the 1980's, the USDOE began screening 34 woody and grass species as potential cellulosic bioethanol feedstocks (Wright and Turhollow, 2010). In 1991, the USDOE selected switchgrass from the 34 species screened as the model herbaceous cellulosic bioenergy crop (Wright and Turhollow, 2010). The impetus for selecting switchgrass was its reliable yields on marginal land, wide range of adaptation, and genetic diversity (Wright and Turhollow, 2010). Since its selection in 1991 much research and investment has gone towards the development and improvement of switchgrass for bioethanol production.

Biology of switchgrass

Switchgrass is a perennial C4 grass species (Parrish and Fike, 2005). As such switchgrass has high water use efficiency, and is of great value in soil and water conservation (Parrish and Fike, 2005). Its extensive root system helps to protect the soil from erosion, and can improve soil organic matter (Parrish and Fike, 2005). Along with its value in conservation, switchgrass has also been improved and used as a spring and summer forage and hay crop (Mitchell et al., 2012).

Switchgrass is adapted to most areas east of the Rocky Mountains in North America. With this wide area of adaptation comes a great deal of genetic diversity. Switchgrass is divided into two main ecotypes with a further division by latitude. The major ecotypes are Upland and Lowland, while the division within ecotypes distinguishes

Northern or Southern genotypes based on latitude (Casler et al, 2007). Upland ecotypes grow from 3 to 5 ft. in height and are normally found in upland prairies (Porter, 1966). Lowland ecotypes are found in flood plains and are usually much more vigorous, growing as tall as 10 ft. (Porter, 1966). There is also much variation of ploidy level ranging from diploid (2n=2x=18) to duodecaploid (2n=12x=108) (Sanderson et al, 1996). Most lowland switchgrass is tetraploid (2n=4x=36) while upland types are mostly tetraploid or octaploid (2n=8x=72) (Sanderson et al, 1996).

Switchgrass is characterized as an allogamous species which can be reproduced sexually from seed or asexually via cloning, and until recently was thought to be a completely self-incompatible species (Talbert, et al., 1983; Taliaferro, et al., 1999; Martinez-Reyna and Vogel, 2002). A previous study found that only 0.35 and 1.39% of seed set from tetraploid and octaploid switchgrass plants respectively resulted from selfing (Martinez-Reyna and Vogel, 2002). The mechanism of self-incompatibility (SI) in switchgrass is not exactly defined although it appears to be a gametophytic mechanism similar to the S and Z system found in many grass species (Martinez-Reyna and Vogel, 2002). The S-Z scheme is a pre-fertilization incompatibility system which is defined by the alleles of the S and Z loci of the pollen grain and the recipient flower's style (Martinez-Reyna and Vogel, 2002). If the style and pollen grain share S and Z alleles an incompatible reaction should result (Martinez-Reyna and Vogel, 2002). However, despite this SI mechanism 2 lowland switchgrass plants were recently identified as conditionally self-compatible (Liu and Wu, 2011). These plants were able to produce selfed seed both in the growth chamber, and in the field with bagged panicles (Liu and Wu, 2011, Adhikari et al, 2015). Further studies showed that in field conditions, where

open pollination was possible, these plants still set complete outcrossed seed (Liu et al., 2014). These results indicate that rather than incompatible pollen being incapable of fertilizing these genotypes, compatible pollen from other plants simply has a strong competitive advantage in fertilization (Liu et al., 2014). Similar phenomenon have been observed in bermudagrass in which pollen tubes have a much higher growth rate in crossed pollination than in selfing (Taliaferro and Lamle, 1997).

Switchgrass breeding for bioenergy

Prior to its selection as a model bioenergy species significant research and breeding had been directed at improving switchgrass as a forage and conservation crop. Initial breeding methods focused on increasing the seed of native switchgrass accessions with little selection (Casler, 2012). As switchgrass breeding progressed phenotypic recurrent selection methods came into use in programs for regionally adapted cultivars (Casler, 2012). Prior to switchgrasses selection as the model cellulosic bioenergy species, a recurrent restricted phenotypic selection (RRPS) method pioneered by Burton (1974) was successfully applied to switchgrass to improve *in vitro* dry matter digestibility (IVDMD) (Vogel et al., 1981; Casler, 2012). Further, phenotypic methods were used to improve seedling vigor in 'Sunburst,' and numerous other cultivars were developed with improvements to seed dormancy, increased seed size, and other traits (Boe and Ross, 1998; Wu, 2014).

Since the DOE identified switchgrass as a model bioenergy crop, improving biomass yield became a priority for new and existing switchgrass breeding programs (Wright and Turhollow, 2010). Because biomass yield is a complex trait that is

controlled by the additive effects of numerous genes and has a low narrow sense heritability, phenotypic selection methods are not reliable for improving biomass yields (Hopkins et al., 1993; Rose et al., 2008; Bhandari et al, 2011; Bhandari et al., 2010; Wu, 2014). To address the difficulties of breeding for increased biomass, genotypic selection methods have been used with some success. One notable cultivar developed utilizing these methods is 'BoMaster' which was developed by the USDA-Agricultural Research Service and the North Carolina Agricultural Research Service (Burns et al., 2008a). To develop 'BoMaster,' four selection cycles were used (Burns et al., 2008a). The initial population (Cycle 0) consisted of one hundred and sixty one lowland switchgrass plants (Burns et al., 2008a). These plants were evaluated for dry matter yield and IVDMD, and thirty one plants were selected. These thirty one plants and two additional plants were random mated and their seed was bulked to produce the Cycle 1 population (Burns et al., 2008a). The resulting population consisted of 660 half-sib progeny which were evaluated for dry matter yield, IVDMD, and N concentration (Burns et al., 2008a). Thirty three plants were selected from this population to form six synthetic populations based on three indices that were made up of differently weighted combinations of initial growth yield, IVDMD, and N concentration (Burns et al., 2008a). The six synthetic populations were transplanted into isolated crossing blocks, and the progeny was bulk harvested by clone to form Cycle 2 (Burns et al., 2008a). The Cycle 2 progeny were evaluated in progeny rows in a randomized complete block design with four replicates (Burns et al., 2008a). The progeny rows were evaluated for dry matter yield and IVDMD and the top eight plants were selected for Cycle 3 (Burns et al., 2008a). The top eight plants from the half-sib progeny test were then randomly mated to form the cultivar 'BoMaster' (Burns et al.,

2008a). These breeding methods including the half-sibling progeny selection resulted in a cultivar that produced 27% and 8.6% more dry matter than commercial cultivars Cave-in-Rock and Alamo, respectively (Burns et al., 2008a). Similarly 'Cimarron' switchgrass released by Oklahoma State University was developed utilizing two cycles of Restricted Recurrent Phenotypic Selection (RRPS) followed by a cycle of Recurrent Selection for General Combining Ability (RSGCA) which utilized a half-sib progeny test. 'Cimarron' switchgrass outperformed Alamo for biomass yield by an average of 7.5% over three years at two locations (Wu and Taliaferro, 2012).

These breeding methods have helped to produce higher yielding switchgrass cultivars, however, they have not fully captured the yield potential possible by exploiting heterosis. Hybrid breeding methods have been key in harnessing heterosis and increasing productivity in maize, sorghum, and other crops over the past century. The success seen in these species has led to substantial interest in developing hybrid breeding methods in switchgrass.

Potential for hybrid switchgrass breeding

Switchgrass is a very genetically diverse species with much potential for identifying heterotic groups. Using hybrid populations of 'Summer' genotypes and 'Kanlow' genotypes Vogel and Mitchell (2008) observed 30 to 38% high parent heterosis for biomass yield when grown in simulated swards (Vogel and Mitchell, 2008). While this hybrid vigor was seen in a cross between upland and lowland genotypes it does provide promise for heterosis within ecotype.

Alternatives to population hybrids like the one developed by Martinez-Reyna and Vogel (2008) have been proposed. Due to the strong SI mechanism within switchgrass the first hybrid breeding method proposed relied on the clonal propagation of two heterotic parents to produce a heterogenous hybrid population similar to a double cross hybrid in maize (Aguirre et al, 2011). Clonal propagates would be planted in alternating rows in which switchgrasses SI mechanism would be exploited guaranteeing 100% F_1 hybrid production (Aguirre et al, 2011). However, with this method, hybrid production would likely be cost and time prohibitive, as the clonal propagation of switchgrass is labor intensive, time consuming, and expensive. The recent discovery of conditionally self-compatible switchgrass genotypes has given rise to another hybrid breeding method. In 2011, Liu and Wu discovered that NL94 and SL93 produced self-pollinated progeny when attempting to generate a mapping population in a growth chamber (Liu and Wu, 2011). In a further study it was found that these same genotypes set completely outcrossed seed when grown in the field (Liu et al, 2013). These studies indicate that these switchgrass plants when isolated from outcrossing pollen will set selfed seed, but when outcrossing pollen is present they will still set 100% cross pollinated seed (Liu and Wu, 2011; Liu et al, 2013). Using conditionally self-compatible genotypes such as these it could be possible to produce inbred lines either through self-pollinating with bagged seed heads, or approaching homozygosity more slowly by sibling mating populations in isolation (Liu and Wu, 2011; Liu et al, 2013). If the inbreds produced through this method maintain the preference for outcrossing pollens after inbreeding then two inbred lines could be planted in the field and expected to reliably produce hybrid seed (Liu and Wu, 2011; Liu et al, 2013). With this method it would be possible to produce a uniform

F_1 hybrid using parents grown from seed avoiding the high cost of clonal propagation. To determine the viability of this method, the production of selfed and sibling-mated seed in isolation and the mating preferences of inbred plants must be evaluated.

To distinguish between self-pollinated or cross pollinated progeny, tools like molecular markers, especially codominant markers are invaluable to breeders. Molecular markers can also be used to identify the genetic sources of desirable traits, determine their inheritance, and can be used to investigate genetic variation in plants. These technologies can greatly accelerate plant breeding, and will be especially useful in the development of a hybrid breeding method for switchgrass.

Molecular markers as plant breeding tools

DNA molecular markers are known DNA sequences which can be used to identify a particular genotype or trait. With DNA markers plant breeders, can identify desirable traits at any growth stage, without dependence on environmental factors for expression (Liu and Wu, 2014). This offers breeders the opportunity to screen a large amount of plants without the time and labor requirements needed using only traditional phenotypic and morphological data. Multiple marker systems have evolved over the years, with restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), simple sequence repeat (SSR), and single nucleotide polymorphisms (SNP) having the most impact on switchgrass breeding.

RFLP markers were the earliest among marker systems to be used with switchgrass (Wu, 2014). RFLP markers were used in analyzing genetic diversity, and construction of the first linkage map in switchgrass (Hultquist et al. 1996; Missaoui et al. 2005, 2006; Liu and Wu, 2014). RAPD technology was the next marker system used in switchgrass, but RAPD technology is not easily reproducible making it difficult to verify results between labs, so this technology is less reliable than others (Liu and Wu, 2014). AFLP technology provided a more reliable albeit more labor intensive marker system than RAPD (Garcia et al, 2004). AFLP technology is a dominant marker system which analyzes multiple loci for a single primer pair (Mueller and Wolfenbarger, 1999). The capability to score multiple loci for a single primer pair has made AFLP's highly useful for genetic diversity analysis (Mueller and Wolfenbarger, 1999). SSR markers have also seen extensive use with switchgrass and other species (Liu and Wu, 2014). SSR markers are relatively short nucleotide sequences, and are codominant, polymorphic, and examine a single loci at a time (Hayden, and Sharp, 2001). SSR markers have been used extensively in switchgrass and have been particularly useful for identifying parental origin of progeny (Liu and Wu, 2011; Liu et al., 2014; Adhikari et al., 2015). The first report of effective SSR primer sequences in switchgrass occurred in 2005 when Tobias et al (2005, 2006) reported 32 SSR markers had been developed. In following years an additional 1753 SSR primer pairs were developed in two labs (Okada et al. 2010; Wang et al. 2011; Liu et al. 2013b). The most recent of these technologies is SNP technology. SNP technology enables efficient high

through put genotyping and can be used to identify changes to a single nucleotide (Kwok, 2001; Liu and Wu, 2014). This technology would offer greatly increased efficiency of molecular breeding in switchgrass and other species.

Much progress has been made in switchgrass breeding thanks to molecular technologies. One exciting development has been the identification of conditionally self-compatible genotypes using SSRs (Liu and Wu, 2011). This development gives breeders the potential to develop hybrid cultivars using inbred lines propagated inexpensively through seed. With switchgrass hybrids, it will be possible to rapidly increase biomass yields making switchgrass a more economically viable bioenergy crop. Previous studies have identified self-compatible genotypes, and established that under open pollinated conditions these genotypes will set 100% outcrossed seed (Liu and Wu, 2011; Liu et al. 2013). However, no research has been done to ascertain the level of seed production that inbreds are capable of, and the mating behavior of S1, S2, and S3 inbreds when grown in isolated sibling-mating, and crossing-plots of two self-compatible genotypes.

CHAPTER III

METHODOLOGY

Plant materials and experimental design

Selfed progeny of NL94 and SL93 derived from seed produced on bagged inflorescences and verified with SSR markers were transplanted into ten plots at the Oklahoma State University Agronomy Farm in July 2013 (Table 3.1, Planting Plans 1-10). Six individual sibling-mating plots, contained either S1, S2, or S3 selfed progeny of NL94 and SL93 genotypes, respectively. The remaining four plots were crossing plots planted in alternating rows of SL93 and NL94 inbred progeny. Three of these crossing plots were established with S3 plants of NL94 and SL93, and one was composed of S1 progeny. Transplants were spaced at 105 cm (3.5 ft) between two neighboring rows and between two neighboring plants within a row. The spacing between plots varied, as did the plot size, and number of transplants per plot. GPS coordinates for the Southeast corner of each plot are given in Table 3.1.

Field management

Prior to transplanting, a base fertilizer (18-46-0) was applied at a rate of 80.5 kg N ha^{-1} and 206 kg P (P_2O_5) ha^{-1}, and a clean seedbed was prepared. Immediately after transplanting, plants were watered and plots were treated with Dual® herbicide (s-

metolachlor ((s)-2-chloro-n-(2-ethyl-6-methylphenyl)-n-(2-methoxy-1-methylethyl) acetamide) at a rate of 3.36 kg ha^{-1} a.i. During winter 2013, dormant plots were trimmed to 10.16 cm in height. In March of 2014 a pre-emergence herbicide Atrazine® (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) was applied at 2.24 kg ha^{-1}, post emergent herbicides 2,4-D (2,4-Dichlorophenoxyacetic acid) 1.17 L ha^{-1}, and glyphosate (N-(Phosponomethyl) glycine at 4.67 L ha^{-1} were also applied. Urea (46-0-0) was also applied at 67 kg N ha^{-1} during April 2014. Following sample collection and harvest in the fall of 2014, dormant plots were again trimmed to 10.16 cm using a Cibus S plot harvester (Wintersteiger AG, Reid im Innkreis, Austria). During March 2015, a pre-emergent application of Atrazine and Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) at a rate of 1.1 kg ha^{-1} and 560 g ha^{-1} respectively were applied as recommended in Mitchell et al. (2010). Urea (46-0-0) was again applied at 101 kg N ha^{-1} during April 2015. In addition to herbicide applications manual weeding of the plots was undertaken during the 2014 and 2015 growing seasons.

Seed sample collection and bulk seed harvest

After the switchgrass plants had matured, panicles were harvested separately from selected plants. In sibling mating plots five plants were selected while ten were selected in crossing plots, resulting in 70 samples. The samples were transferred individually into labeled paper bags, and kept at room temperature for two weeks to dehydrate. Seed heads were then threshed by rubbing each sample in a pan lined with ridged rubber matting and cleaned with a Model B South Dakota seed blower (Seedburo Equipment Co., IL). The clean seed was then weighed for individual plant yield. Following the harvest of selected plants the remaining plants in each plot were bulk harvested and

threshed with an Almaco LPR thresher (Almaco, Nevada, Iowa) on October 22nd and 27th, 2014, and October 8th and 12th, 2015. After bulk harvest plot seed was cleaned using a C.S. Bell Co. hammer mill (C.S. Bell co., Tiffin, Ohio), and weighed for whole plot seed yield.

Germination and tissue collection

After cleaning, seed from selected plants was placed on blotter paper in petri dishes, and treated with 0.2% KNO_3 solution and a fungicide solution of 9.67g of 50% benomypl / 3.78 L H_2O. The petri dishes were placed in a 4° C refrigerator for two weeks, and occasionally treated with the KNO_3 solution to keep the blotter paper moist. After pre-chilling for two weeks, the seed samples were then planted in Metro Mix 250 growth medium (Sun Gro Horticulture, Bellevue, WA) in cells (9.5 cm by 9 cm per cell), and covered with a clear plastic growth chamber for germination in a greenhouse at the OSU Agronomy Research Station. After germination, up to ten randomly selected seedlings of each parental plant were transplanted into Redi-earth growth medium (Sun Gro Horticulture, Bellevue, WA) in Cone-tainers (3.9 cm in diameter and 29.96 cm in depth). They were watered daily, and fertilized periodically to encourage the growth of healthy tissue for sampling. Healthy tissue samples each weighing approximately 100 mg were individually sampled from the selected seedlings and placed in a -80° C freezer for DNA isolation. Similarly, maternal parent leaf samples of approximately 10 cm in length were individually hand collected from plots at the OSU Agronomy Farm in August 2014 and placed in a -80° C freezer in preparation for DNA isolation.

DNA isolation and SSR marker selection

Maternal leaf tissue samples were ground individually using a pestle with additional freezing in liquid nitrogen. Progeny tissue samples were ground using a SPEX SamplePrep Geno/Grinder (SPEX SamplePrep LLC., NJ). Genomic DNA was then extracted from each crushed sample using Phenol-chloroform by the method described by Dubcovsky et al. (1994). Leaf tissue DNA concentrations were quantified using a NanoDrop DN-1000 Spectrophotometer (NanoDrop products, DE). Each sample was adjusted to a working solution concentration of 10ng/µl as the template for polymerase chain reaction (PCR).

DNA testing panels were formed for SSR genotyping. Panels consisted of maternal families in which a maternal parent sample bookended samples of up to ten progeny samples, or if less than five progeny samples were available only one maternal sample was used. Families were grouped onto panels by plot, and genotype. Sixty four SSR primer pairs were selected using a linkage map published by Liu et al. (2012). These SSRs were tested using DNA template extracted from SL93/16/1/75 and NL94/85/3. From this test ten SSR primer pairs were originally selected (Table 3.2). These ten markers were used for PCR analysis, however one marker was replaced by one additional SSR primer pair which more efficiently underwent PCR with all samples. In total eleven primer pairs were selected from seven different linkage groups. After markers were selected six SSR primer combinations were used to genotype each maternal parent and its progeny. After initial genotyping the progeny from crossing plots that were not identified as hybrid progeny, and the progeny from sibling mating plots that

were not identified as sibling-mating progeny were consolidated to new panels with their maternal parents, and genotyped using four additional SSR PPs.

PCR amplification

Simple sequence repeat-polymerase chain reaction amplifications were performed in a 96-well PCR plate using Applied Biosystems 2720 thermal cyclers (Applied Biosystems INC., CA). Each reaction contained 3.55 µl of nuclease free water, 1.00 µl of 10X PCR Buffer, 0.20 µl 10 mM deoxynucleoside triphosphate (dNTPs), 0.05µl (5U/µl) Taq enzyme, 0.20 µl uM IR-M13 forward primer labeled with either 700- or 800-nm florescent dye (LI-COR, Lincoln, NE), 2.00 µl of 1 pmol/µl forward primer, 2.00 µl of 1 pmol/µl reverse primer, and 1.5 µl of 10 ng/µl genomic DNA resulting in 10.5 µl of a total volume. Thermal cycler settings were programmed according to Wu and Huang (2008). After the PCR reaction was complete 5 µl of blue stop solution were added to each PCR reaction well, spun down, and denatured for 3 min at 94^{o} C in the 2720 thermal cyclers (Applied Biosystems, IL). The PCR products from the plate labeled with 800 nm florescence dye, were transferred into the plate labeled 700 nm florescence dye (LI-COR Inc., NE), and spun down. The amplified PCR products were then placed on ice until gel loading.

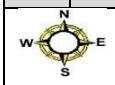
Gel electrophoresis and data analysis

To score the amplified target bands, the mixed PCR products were loaded into wells of 6.5% KB^{plus} LI-COR gels (LI-COR Inc., NE), and ran at 1500 V for 1 hour and 45 minutes in a LI-COR 4300 DNA Analyzer (LI-COR Inc., NE). 50 to 350 bp or 50 to 700 bp standard markers (LI-COR Biosciences, Lincoln, NE) were loaded into wells on

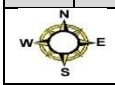
both sides of the gel to measure the size of the amplified fragments. Target bands were then visually scored, and band sizes were determined using Saga Generation 2 software, version 3.3 (LI-COR Biosciences, Lincoln, NE, USA). The progeny array method was used to identify the selfed progenies if all targeted bands were the same as those of the seed parents by comparing the SSR band patterns of open-pollinated progeny to their maternal parents. Progeny not showing foreign bands after analysis with ten SSR markers were considered to have originated from selfing, progeny showing foreign bands which belonged to a sibling of their maternal parent were consider the result of sibling-mating, and progeny showing foreign bands from another genotype were considered cross pollinated. Microsoft Excel was used to record data, and the outcrossing, sibling-mating, and selfing rates in the families were then calculated.

Table 3.1. Switchgrass planting plans 1 to 10 are given below. GPS coordinates mark the Southeast corner of each plot.

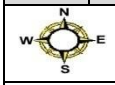
Planting plan 1. SL93 S3 sibling mating

Rows	9	/84	/85	/86	/87	/88					
	8	/74	/75	/76	/77	/78	/79	/80	/81	/82	/83
	7	/64	/65	/66	/67	/68	/69	/70	/71	/72	/73
	6	/54	/55	/56	/57	/58	/59	/60	/61	/62	/63
	5	/43	/44	/45	/46	/47	/48	/49	/50	/51	/52
	4	/32	/33	/34	/35	/36	/37	/39	/40	/41	/42
	3	/22	/23	/24	/25	/26	/27	/28	/29	/30	/31
	2	/12	/13	/14	/15	/16	/17	/18	/19	/20	/21
	1	SL93/16/1/1	/2	/3	/4	/5	/6	/7	/9	/10	/11
		1	2	3	4	5	6	7	8	9	10
		Columns									
GPS coordinates: N36.1195^{o}, W97.0934^{o}											

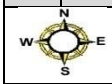
Planting plan 2. SL93 S1sibling mating

Rows	5	SL93/42	SL93/44					
	4	SL93/30	SL93/31	SL93/33	SL93/34	SL93/38	SL93/40	SL93/41
	3	SL93/18	SL93/19	SL93/23	SL93/25	SL93/26	SL93/27	SL93/29
	2	SL93/11	SL93/12	SL93/13	SL93/14	SL93/15	SL93/16	SL93/17
	1	SL93/01	SL93/04	SL93/05	SL93/06	SL93/07	SL93/08	SL93/09
		1	2	3	4	5	6	7
		Columns						
GPS coordinates: N36.1211^{o}, W97.0934^{o}								

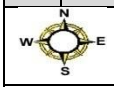
Planting plan 3. SL93 S2 sibling mating

Rows	6	SL93/18/16	SL93/18/17	SL93/18/18	SL93/18/19
	5	SL93/18/12	SL93/18/13	SL93/18/14	SL93/18/15
	4	SL93/18/8	SL93/18/9	SL93/18/10	SL93/18/11
	3	SL93/18/4	SL93/18/5	SL93/18/6	SL93/18/7
	2	SL93/4/5	SL93/18/1	SL93/18/2	SL93/18/3
	1	SL93/16/1	SL93/16/2	SL93/17/1	SL93/44/1
		1	2	3	4
		Columns			
GPS coordinates: N36.1210º, W97.0933º					

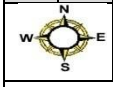
Planting plan 4. Mating among SL93 S3 plants and NL94 S3 plants

Rows	8	NL94/85/1/36	NL94/85/1/37	NL94/85/1/38	NL94/85/1/39	NL94/85/1/40	NL94/85/1/41	NL94/85/1/42	NL94/85/1/43		
	7	SL93/16/1/121	SL93/16/1/122	SL93/16/1/123	SL93/16/1/124	SL93/16/1/125	SL93/16/1/126	SL93/16/1/127	SL93/16/1/128		
	6	NL94/85/1/23	NL94/85/1/24	NL94/85/1/25	NL94/85/1/26	NL94/85/1/30	NL94/85/1/31	NL94/85/1/32	NL94/85/1/33	NL94/85/1/34	NL94/85/1/35
	5	SL93/16/1/110	SL93/16/1/111	SL93/16/1/112	SL93/16/1/114	SL93/16/1/115	SL93/16/1/116	SL93/16/1/117	SL93/16/1/118	SL93/16/1/119	SL93/16/1/120
	4	NL94/85/1/13	NL94/85/1/14	NL94/85/1/15	NL94/85/1/16	NL94/85/1/17	NL94/85/1/18	NL94/85/1/19	NL94/85/1/20	NL94/85/1/21	NL94/85/1/22
	3	SL93/16/1/100	SL93/16/1/101	SL93/16/1/102	SL93/16/1/103	SL93/16/1/104	SL93/16/1/105	SL93/16/1/106	SL93/16/1/107	SL93/16/1/108	SL93/16/1/109
	2	NL94/85/1/1	NL94/85/1/2	NL94/85/1/3	NL94/85/1/4	NL94/85/1/7	NL94/85/1/8	NL94/85/1/9	NL94/85/1/10	NL94/85/1/11	NL94/85/1/12
	1	SL93/16/1/90	SL93/16/1/91	SL93/16/1/92	SL93/16/1/93	SL93/16/1/94	SL93/16/1/95	SL93/16/1/96	SL93/16/1/97	SL93/16/1/98	SL93/16/1/99
		1	2	3	4	5	6	7	8	9	10
		Columns									
GPS coordinates: N36.1196º, W97.0914º											

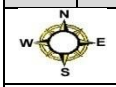
Planting plan 5. Mating among SL93 S3 plants and NL94 S3 plants

Rows											
	6	NL94/85/3/24	NL94/85/3/25	NL94/85/3/27	NL94/85/3/28	NL94/85/3/29	NL94/85/3/30	NL94/85/3/31	NL94/85/3/32	NL94/85/3/33	NL94/85/3/34
	5	SL93/16/1/149	SL93/16/1/150	SL93/16/1/151	SL93/16/1/152	SL93/16/1/153	SL93/16/1/154	SL93/16/1/155	SL93/16/1/156	SL93/16/1/157	SL93/16/1/158
	4	NL94/85/3/13	NL94/85/3/14	NL94/85/3/15	NL94/85/3/16	NL94/85/3/17	NL94/85/3/18	NL94/85/3/19	NL94/85/3/20	NL94/85/3/22	NL94/85/3/23
	3	SL93/16/1/139	SL93/16/1/140	SL93/16/1/141	SL93/16/1/142	SL93/16/1/143	SL93/16/1/144	SL93/16/1/145	SL93/16/1/146	SL93/16/1/147	SL93/16/1/148
	2	NL94/85/3/1	NL94/85/3/2	NL94/85/3/3	NL94/85/3/4	NL94/85/3/5	NL94/85/3/6	NL94/85/3/7	NL94/85/3/8	NL94/85/3/9	NL94/85/3/12
	1	SL93/16/1/129	SL93/16/1/130	SL93/16/1/131	SL93/16/1/132	SL93/16/1/133	SL93/16/1/134	SL93/16/1/135	SL93/16/1/136	SL93/16/1/137	SL93/16/1/138
		1	2	3	4	5	6	7	8	9	10
		Columns									
GPS coordinates: N36.1194º, W97.0912º											

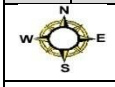
Planting plan 6. Mating among SL93 S3 plants and NL94 S3 plants

Rows						
	6	NL94/85/5/12	NL94/85/5/14	NL94/85/5/15	NL94/85/5/16	
	5	SL93/16/1/169	SL93/16/1/170	SL93/16/1/171	SL93/16/1/172	
	4	NL94/85/5/6	NL94/85/5/7	NL94/85/5/8	NL94/85/5/9	NL94/85/5/10
	3	SL93/16/1/164	SL93/16/1/165	SL93/16/1/166	SL93/16/1/167	SL93/16/1/168
	2	NL94/85/5/1	NL94/85/5/2	NL94/85/5/3	NL94/85/5/4	NL94/85/5/5
	1	SL93/16/1/159	SL93/16/1/160	SL93/16/1/161	SL93/16/1/162	SL93/16/1/163
		1	2	3	4	5
		Columns				
GPS coordinates: N36.1211, W97.0914						

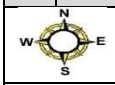
Planting plan 7. Mating among SL93 S1 plants and NL94 S1 plants

Row	6	NL94/158	NL94/176	NL94/177	NL94/185	NL94/190	NL94/206	NL94/210	NL94/248	NL94/289	NL94/300
	5	SL93/29	SL93/30	SL93/31	SL93/33	SL93/34	SL93/38	SL93/40	SL93/41	SL93/42	SL93/44
	4	NL94/66	NL94/67	NL94/69	NL94/83	NL94/85	NL94/88	NL94/98	NL94/102	NL94/114	NL94/145
	3	SL93/14	SL93/15	SL93/16	SL93/17	SL93/18	SL93/19	SL93/23	SL93/25	SL93/26	SL93/27
	2	NL94/18	NL94/33	NL94/34	NL94/35	NL94/48	NL94/51	NL94/57	NL94/58	NL94/62	NL94/63
	1	SL93/01	SL93/04	SL93/05	SL93/06	SL93/07	SL93/08	SL93/09	SL93/11	SL93/12	SL93/13
		1	2	3	4	5	6	7	8	9	10
		Columns									
GPS coordinates: N36.1210^{o}, W97.0912^{o}											

Planting plan 8. NL94 S1 sibling mating

Rows	5	NL94/289	NL94/300					
	4	NL94/176	NL94/177	NL94/185	NL94/190	NL94/206	NL94/210	NL94/248
	3	NL94/85	NL94/88	NL94/98	NL94/102	NL94/114	NL94/145	NL94/158
	2	NL94/58	NL94/62	NL94/63	NL94/66	NL94/67	NL94/69	NL94/83
	1	NL94/18	NL94/33	NL94/34	NL94/35	NL94/48	NL94/51	NL94/57
		1	2	3	4	5	6	7
		Columns						
GPS coordinates: N36.1330, W97.1060								

Planting plan 9. NL94 S2 sibling mating

Rows	5	NL94/85/503	NL94/85/504
	4	NL94/85/501	NL94/85/502
	3	NL94/85/6	NL94/85/7
	2	NL94/85/3	NL94/85/5
	1	NL94/85/1	NL94/85/2
		1	2
		Columns	
GPS coordinates: N36.1329, W97.1068			

Planting plan 10. NL94 S3 sibling mating

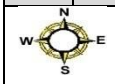
Rows	6	NL94/85/3/70	NL94/85/3/71	NL94/85/3/74	NL94/85/3/75	NL94/85/3/77
	5	NL94/85/3/63	NL94/85/3/66	NL94/85/3/67	NL94/85/3/68	NL94/85/3/69
	4	NL94/85/3/57	NL94/85/3/58	NL94/85/3/59	NL94/85/3/60	NL94/85/3/61
	3	NL94/85/3/52	NL94/85/3/53	NL94/85/3/54	NL94/85/3/55	NL94/85/3/56
	2	NL94/85/3/40	NL94/85/3/44	NL94/85/3/45	NL94/85/3/47	NL94/85/3/48
	1	NL94/85/3/35	NL94/85/3/36	NL94/85/3/37	NL94/85/3/38	NL94/85/3/39
		1	2	3	4	5
		Columns				
GPS coordinates: N36.1320, W97.1071						

Table 3.2. SSR marker ID, primer sequences, repeat motifs for genotyping.

No.	Marker ID	Primer sequences (5'-3')	Repeat motif	Expected product size
1	PVAAG 2939-40	F:CACGACGTTGTAAAACGACTT ATTACCCCTTGCTCCTGC R:CTGGAGTTTGGCCTTGATTT	(AAG)16	285
2	PVAAG 3051-52	F:CACGACGTTGTAAAACGACA CGGCGAGCATCAATGTAG R:GCGCCGTTAAGTGGTTTATT	(GAA)29	248
3	PVCAG 1 2207-08	F:CACGACGTTGTAAAACGACT GAAGTGCTTGAGGAACTGG R:GTAGTCATAGCCCAAGCCGT	(GCT)8(CTG)5	215
4	PVCAG 5 2503-04	F:CACGACGTTGTAAAACGACC CCAATGTCGAGGTCCTATT R:TAGGTACCCTCTGCTGCCTT	(TCC)7(TGC)8	278
5	PVCAG 5 2517-18	F:CACGACGTTGTAAAACGACTC CTCGTAAGCAACCAATCC R:TAGGCAATGCAAGTGAAAGG	(GCT)8	217
6	SWW 1394	F:CACGACGTTGTAAAACGACT ATGATAACCCAAAGGGCAA R:ACACCCTCGTCATCATCCTC	(GGT)n	
7	SWW 2561	F:CACGACGTTGTAAAACGACC GCCCTACGAGCACTACTTC R:GTTTGTCCCCCTCATTCTCCTT CTT	(GGA)n	
8	PVCA3 341-42	F:CACGACGTTGTAAAACGACTC TTCTT R:CCCACACTCACTCACACACA	(GT)18	231
9	PVCA5 687-88	F:CACGACGTTGTAAAACGACG TTTATTACGGCGGGAACAT R:TTAAACTGTTTGGGTGAGCG	(AC)16(AC)6	276
10	PVCAG 4 2473-74	F:CACGACGTTGTAAAACGACG TTTTGCCCGTAACTTTGGT R:GGTTGAACAATCGTGTCGTT	(GCA)12	145
11	PVCAG 5 2397-98	F:CACGACGTTGTAAAACGACA TTTCTGGAGTCTGTTGCCC R:AAATGGCATGTCTACCGTGA	(CAG)12	172

CHAPTER IV

FINDINGS

Seed production and germination

Whole plot seed yields for 2014 and 2015 are shown in Table 4.1. It should be noted that plots 4 and 5 contended with significant grassy weed pressure which likely suppressed yields particularly in 2014. In NL94 sibling-mating plots, seed yields declined with each generation of selfing with the S1, S2, and S3 plots averaging 89.8 kg ha^{-1}, 42.6 kg ha^{-1}, and 18.9 kg ha^{-1} respectively (Table 4.1). In their SL93 counterparts this trend was less visible as the S3 parents were much more prolific than their S2 counter parts in both years, and even produced more than the S1 plot in the second post establishment year (Table 4.1). Low seed production proved particularly problematic for getting useable progeny samples from plots 3, 6, and 10. Seedling emergence rates 14 days after planting also indicate that low seed vigor was also an obstacle for many selected parent plants (Table 4.2). Despite pretreatment that included treatment with 0.2% KNO_3 solution, a fungicide solution of 9.67g of 50% benomypl / 3.78 L H_2O, and prechilling to protect against pathogens and break dormancy, many seeds of selected plants did not germinate or emerged over a month after planting.

Progeny analysis

A total of 695 progenies from 57 maternal parents were used for genotyping in both years. This is only 49% of the number of progenies and 81% of the number of families which were planned for analysis. Poor seed production, germination, and DNA isolation failure accounted for the missing progenies. Additionally one physical contaminant was identified as it shared no bands with its maternal parent (NL94/85/1/16) when genotyped with SSR primer pair PVCAG5 2517-18 (Figure 4.1). The number of progeny tested from each of the 57 maternal parents ranged from 1 to 20 with an average of 12 progeny per family (Table 4.3).

In total 64 primer pairs were tested for polymorphisms using SL93/16/1/75 and NL94/85/3 DNA templates with the objective that each DNA template would be polymorphic with at least five of the ten selected primer pairs. Unfortunately due to the initial misidentification of stutter bands as informative, and the high level of homozygosity in S2 and S3 inbreds only two markers were polymorphic for SL93/16/1/75 and three for NL94/85/3, respectively. Due to the low number of polymorphic markers for S3 SL93 and NL94 inbreds, we may have misclassified sibling-mated progeny to be selfed (Tables 4.3 and 4.4). However, the selected markers were highly polymorphic in the SL93 and NL94 S1 inbreds. One of the initial ten selected SSR primer pairs, SWW 2561 was eventually replaced by PVCAG5 2503-04 as it had poor amplification with many sample templates, but SWW 2561 was utilized for panels with which it had adequate amplification before the decision to replace it was made (Table 3.2). Progeny were arrayed in families with maternal samples to determine if they originated from selfing, sibling-mating, or crossing. Selfed progeny were expected to

show bands only from their maternal parents, sibling-mated and hybrid progeny were expected to show recombinant banding types containing a maternal band, and a sibling band or a band from a hybridizing parent. Four gel examples showing banding patterns are given in Figure 4.1. Initial screening with six SSR PPs identified 288 hybrid progeny from crossing plots, and 148 sibling mated progeny in both sibling-mating and crossing plots. Further analysis with four additional SSR PPs revealed that three of the progeny which the first six markers identified as selfed and three that had been putatively sibling-mated were in fact hybrid progeny. Additional 41 progeny which had been considered selfed were identified as sibling-mated in analysis with the four additional markers. The 212 progeny which were not identified as sibling-mated or hybrids were putatively considered to have resulted from self-pollination (Tables 4.3 & 4.4).

The percentages of selfed, sibling-mated, and hybrid progeny varied according to pedigree, and plot type (Table 4.4). In S1 sibling-mating plots for both SL93 and NL94 inbreds a high proportion of progeny resulted from sibling mating (Table 4.3 & 4.4). In the S2 NL94 sibling-mating plot the proportion of putatively selfed progeny for both years had increased to 46%, and this proportion reached 92% with progeny collected from the S3 NL94 sibling-mating plot (Table 4.4). Although no viable samples were were collected from the S2 SL93 sibling mating plot a similar trend is visible with only 68% of progeny collected from the S1 sibling mating plot being putatively selfed while 100% collected from the S3 plot were considered selfed (Table 4.4). It is likely that due to the low number of polymorphic markers for both NL94 and SL93 S2 and S3 inbreds many of the putatively selfed progeny are actually mischaracterized sibling-mated progeny. Differences of progeny origins between genotypes were most notable in S3

crossing plots 4 and 5. In both plots 100% of the progeny of NL94 parents were identified as hybrids, while only 4% and 19% SL93 progeny were identified as hybrids in plots 4 and 5 respectively (Table 4.4). However in the S1 crossing plot 7 both genotypes produced a high percentage of crossed progeny with 94% of SL93 progeny and 73% of NL94 progeny being identified as hybrids (Table 4.4).

These results indicate that conditionally self-compatible lowland switchgrass plants produced self-pollinated or sibling-mated seed when grown in isolation under field conditions, and that inbred parents may reliably set hybrid seed if out crossing pollen is available. However, parental selection is essential to ensure adequate seed production. The importance of parental selection is best illustrated by comparing plots 1 and 3. Despite being S3 inbreds the full sibling progeny of SL93/16/1 in plot 1 produced higher quantities of viable seed than the S2 progeny of SL93/18 in plot 3, as shown by seed yields, 14 day emergence data, and the ultimate number of seedlings used in genotyping (Tables 4.1, 4.2, 4.3). Further study of plant vigor in inbreds may prove useful to identify potential inbreeding tolerant genotypes, unfortunately due to the isolation requirements for this study it was impossible to control for many environmental factors which affect phenotype.

This study also indicated that robust marker selection methods are needed to distinguish between selfed and sibling mated progeny at the S3 inbreeding level. S3 inbreds of SL93 and NL94 are expected to be more than 90% homozygous (Wu, 2014). With over 90% homozygosity in the inbreds, most SSR primers will be monomorphic requiring testing of large numbers of primers to find primers that are polymorphic and meet the other needs of a given study. The miss identification of stutter bands as

informative during marker selection in this study also indicates that in order to ensure adequate selection of polymorphic markers, multiple samples of siblings from an inbred line may be helpful. Another important consideration is potential genotype by environment (GxE) interaction in the ability of different inbred genotypes to "nick" for hybrid production. Nicking here refers to the overlap of anthesis periods which allows photoperiod sensitive genotypes to successfully cross pollinate. NL94 is a selection from the OSU Northern Lowland breeding population and is adapted for more northern latitudes, than SL93, because of this NL94 genotypes entered their reproductive phase earlier. In non-inbred plants and in S1 inbreds the differences in anthesis timing do not seem to prevent cross pollination between NL94 and SL93 (Tables 4.3, 4.4). However, in S3 crossing plots the low hybrid production of SL93 inbreds indicates that the differences in photoperiod had an effect on crosspollination (Tables 4.3, 4.4). In 2015 NL94 parents in plots 4 and 5 were observed to have exposed anthers prior to July, 27th while their SL93 counterparts were not observed to have begun pollen shed until as late as the August 14th. These observations indicate that it is likely that S3 NL94 plants had ceased viable pollen shed before the majority of SL93 S3 inbreds were receptive to pollen. In spite of this SL93 pollen was available to pollinate the sampled NL94 S3 inbreds which indicates that through segregation some SL93 S3 inbreds retained an early anthesis period while most SL93 S3 inbreds flowered later. This theory corresponds with observations in a study by Dong et al., (2015) in which significant variation of anthesis timing between S1 NL94 inbreds was observed.

Liu et al (2014) reported that lowland switchgrass plants which had been previously identified as conditionally self-compatible set completely outcrossed seed

under field conditions. However, we found that inbreds of these genotypes when grown under sufficient isolation in the field will self-pollinate and sibling-mate allowing a possible method to economically generate seed from inbred lines for hybrid production. Furthermore we confirmed that after three generations of selfing one genotype (NL94) set nearly 100% hybrid seed.

Table 4.1. Seed production of sibling-mating and crossing plots in 2014 and 2015.

Plot	Pedigree	2014		2015		2014 & 2015 combined average yields	
		Seed (g/plot)	Yield (kg/ha)	Seed (g/plot)	Yield (kg/ha)	Seed (g/plot)	Yield (kg/ha)
1	SL93/16/1/_	42.81	4.85	103.00	11.68	72.91	8.27
2	SL93/_	75.80	24.06	4.00	1.27	39.90	12.67
3	SL93/18/_	2.00	0.79	2.00	0.79	2.00	0.79
4	NL94/85/1/_ x SL93/16/1/_	15.43	1.93	90.00	11.28	52.72	6.61
5	NL94/85/3/_ x SL93/16/1/_	18.03	2.86	404.00	64.13	211.02	33.49
6	NL94/85/5/_ x SL93/16/1/_	23.25	7.91	71.00	24.15	47.13	16.03
7	NL94/_ x SL93/_	240.42	38.16	232.00	36.83	236.21	37.49
8	NL94/_	17.54	5.57	548.00	173.97	282.77	89.77
9	NL94/85/_	5.44	5.18	84.00	80.00	44.72	42.59
10	NL94/85/3/_	2.01	0.64	117.00	37.14	59.51	18.89

*indicates that not all plants in the plot were siblings (Table 1 Planting plan 3).

Table 4.2. Emergence rates 14 days after planting for seed produced of selected parent plants.

Plot	Parent	2014		2015	
		Seed planted	Germination (%)	Seed planted	Germination (%)
1	SL93/16/1/16	50	0	100	0
	SL93/16/1/28	50	4	100	5
	SL93/16/1/34	50	6	89	7
	SL93/16/1/71	50	2	100	4
	SL93/16/1/75	50	4	100	5
2	SL93/06	50	44	0	N/A
	SL93/11	50	48	100	2
	SL93/26	50	28	100	7
	SL93/31	50	4	21	0
	SL93/41	50	72	100	32
3	SL93/16/2	7	0	1	0
	SL93/18/3	1	0	41	0
	SL93/18/5	0	N/A	0	N/A
	SL93/18/10	0	N/A	30	0
	SL93/18/12	0	N/A	4	0
4	NL94/85/1/2	50	2	100	3
	NL94/85/1/9	6	0	93	8
	NL94/85/1/16	50	20	100	4
	NL94/85/1/30	9	22	100	11
	NL94/85/1/31	50	10	100	6
	SL93/16/1/101	50	0	100	3
	SL93/16/1/105	50	2	100	2
	SL93/16/1/107	50	0	100	4
	SL93/16/1/111	0	N/A	70	1
	SL93/16/1/115	50	6	100	3
5	NL94/85/3/2	50	30	100	6
	NL94/85/3/5	50	14	100	27
	NL94/85/3/14	50	20	100	22
	NL94/85/3/16	0	N/A	35	0
	NL94/85/3/19	50	12	100	23
	SL93/16/1/140	50	14	33	3
	SL93/16/1/142	50	4	100	1
	SL93/16/1/144	40	13	28	0
	SL93/16/1/153	50	2	100	1
	SL93/16/1/157	50	4	47	4

Plot	Parent	2014		2015	
		Seed planted	Germination (%)	Seed planted	Germination (%)
6	NL94/85/5/3	50	4	104	0
	NL94/85/5/4	9	0	2	0
	NL94/85/5/7	50	20	75	0
	NL94/85/5/8	50	2	3	0
	SL93/16/1/165	2	0	28	0
	SL93/16/1/166	50	2	47	0
	SL93/16/1/167	2	50	50	0
	SL93/16/1/170	4	0	72	0
	SL93/16/1/171	50	0	100	1
7	NL94/33	50	72	100	27
	NL94/48	50	56	100	26
	NL94/57	50	38	100	8
	NL94/69	50	64	100	20
	NL94/85	50	44	100	26
	SL93/15	50	50	100	0
	SL93/17	50	38	100	8
	SL93/19	50	36	103	21
	SL94/38	17	6	0	N/A
	SL93/41	50	66	100	12
8	NL94/62	50	30	100	44
	NL94/66	50	18	100	26
	NL94/69	50	10	100	24
	NL94/98	50	44	100	25
	NL94/114	50	38	100	46
9	NL94/85/3	50	4	100	38
	NL94/85/5	50	30	100	19
	NL94/85/6	2	0	100	12
	NL94/85/7	16	13	100	17
	NL94/85/501	50	8	100	24
10	NL94/85/3/44	11	0	100	10
	NL94/85/3/54	11	9	100	3
	NL94/85/3/58	0	N/A	100	1
	NL94/85/3/59	2	0	100	0
	NL94/85/3/67	8	0	42	0
	Total	2500	21	5426	12

Table 4.3. Origins of progeny of selected parents.

Plot	Parent	2014				2015			
		Progeny	Progeny Origins			Progeny	Progeny Origins		
			Selfed	Sibling-mated	Crossed		Selfed	Sibling-mated	Crossed
1	SL93/16/1/16	3	3	0	0	2	2	0	0
	SL93/16/1/28	5	5	0	0	7	7	0	0
	SL93/16/1/34	10	10	0	0	10	10	0	0
	SL93/16/1/71	10	10	0	0	9	9	0	0
	SL93/16/1/75	6	6	0	0	7	7	0	0
2	SL93/06	8	0	8	0	0	N/A	N/A	N/A
	SL93/11	10	8	2	0	5	2	3	0
	SL93/26	10	2	8	0	10	8	2	0
	SL93/31	9	1	8	0	2	N/A	2	0
	SL93/41	4	0	4	0	10	1	9	0
4	SL93/16/1/101	2	1	0	1	4	4	0	0
	SL93/16/1/105	10	9	0	1	10	10	0	0
	SL93/16/1/107	2	2	0	0	10	10	0	0
	SL93/16/1/111	0	N/A	N/A	N/A	1	1	0	0
	SL93/16/1/115	4	4	0	0	10	10	0	0
	NL94/85/1/2	10	0	0	10	4	0	0	4
	NL94/85/1/9	0	N/A	N/A	N/A	10	0	0	10
	NL94/85/1/16	8	0	0	8	10	0	0	10
	NL94/85/1/30	3	0	0	3	9	0	0	9
	NL94/85/1/31	10	0	0	10	10	0	0	10
5	NL94/85/3/2	8	0	0	8	10	0	0	10
	NL94/85/3/5	8	0	0	8	10	0	0	10
	NL94/85/3/14	8	0	0	8	10	0	0	10

Plot	Parent	2014				2015			
		Progeny	Progeny Origins			Progeny	Progeny Origins		
			Selfed	Sibling-mated	Crossed		Selfed	Sibling-mated	Crossed
5	NL94/85/3/19	9	0	0	9	10	0	0	10
	SL93/16/1/140	8	8	0	0	1	1	0	0
	SL93/16/1/142	5	4	0	1	1	1	0	0
	SL93/16/1/144	4	2	1	1	0	N/A	N/A	N/A
	SL93/16/1/153	0	N/A	N/A	N/A	7	5	0	2
	SL93/16/1/157	3	3	0	0	2	0	0	2
6	NL94/85/5/1	1	0	0	1	0	N/A	N/A	N/A
	NL94/85/5/3	1	0	0	1	0	N/A	N/A	N/A
	NL94/85/5/4	1	0	0	1	0	N/A	N/A	N/A
	NL94/85/5/7	10	0	0	10	0	N/A	N/A	N/A
	NL94/85/5/8	2	1	0	1	0	N/A	N/A	N/A
	SL93/16/1/171	1	0	0	1	0	N/A	N/A	N/A
7	NL94/33	8	0	2	6	8	0	0	8
	NL94/48	10	0	0	10	9	0	1	8
	NL94/57	8	0	0	8	9	1	5	3
	NL94/69	6	0	0	6	7	1	3	3
	NL94/85	10	1	1	8	10	0	8	2
	SL93/15	9	0	0	9	3	0	2	1
	SL93/17	9	0	0	9	8	0	2	6
	SL93/19	9	0	0	9	10	0	0	10
	SL93/41	9	0	0	9	9	0	0	9

Plot	Parent	2014				2015			
		Progeny	Progeny Origins			Progeny	Progeny Origins		
			Selfed	Sibling-mated	Crossed		Selfed	Sibling-mated	Crossed
8	NL94/62	8	1	7	0	9	0	9	0
	NL94/66	9	0	9	0	10	0	10	0
	NL94/69	8	0	8	0	9	0	9	0
	NL94/98	10	2	8	0	10	1	9	0
	NL94/114	9	0	9	0	9	0	9	0
9	NL94/85/3	3	3	0	0	10	2	8	0
	NL94/85/5	7	6	1	0	5	3	2	0
	NL94/85/6	0	N/A	N/A	0	9	0	9	0
	NL94/85/7	0	N/A	N/A	N/A	9	3	6	0
	NL94/85/501	9	7	2	0	4	2	2	0
10	NL94/85/3/44	0	N/A	N/A	N/A	9	8	1	0
	NL94/85/3/54	1	1	0	0	2	2	0	0
	NL94/85/3/58	0	N/A	N/A	N/A	1	1	0	0

Table 4.4. Genetic origins of progeny of selected parents by plot.

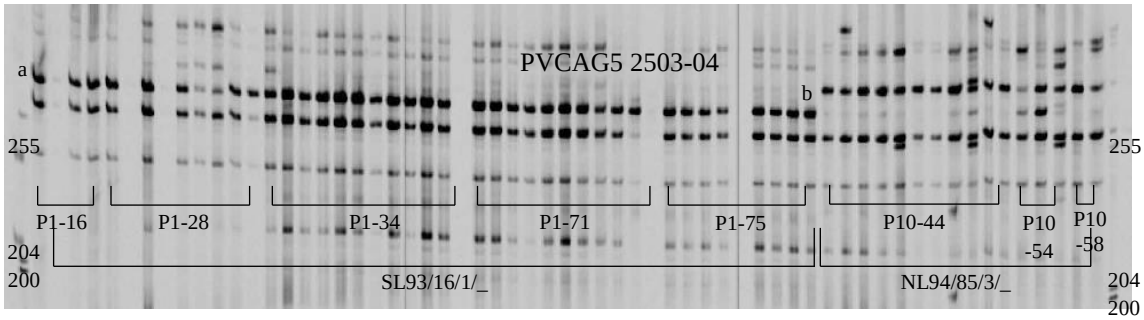
Plot	Parent	2014 Progeny	2014 Progeny Origins (%) Selfed	Sibling-mated	Crossed	2015 Progeny	2015 Progeny Origins (%) Selfed	Sibling-mated	Crossed
1	SL93/16/1/_	34	100	0	N/A	35	100	0	N/A
2	SL93/_	41	27	73	N/A	27	41	59	N/A
4	SL93/16/1/_	18	89	0	11	35	100	0	0
	NL94/85/1/_	31	0	0	100	43	0	0	100
5	SL93/16/1/_	20	85	5	10	11	64	0	36
	NL94/85/3/_	33	0	0	100	40	0	0	100
6	SL93/16/1/_	1	0	0	100	0	N/A	N/A	N/A
	NL94/85/5/_	15	7	0	93	0	N/A	N/A	N/A
7	SL93/_	36	0	0	100	30	0	13	87
	NL94/_	42	2	7	90	43	5	40	56
8	NL94/_	44	7	93	N/A	47	2	98	0
9	NL94/85/_	19	84	16	N/A	37	29	71	0
10	NL94/85/3/_	1	100	0	N/A	12	92	8	0

2014 & 2015 Average

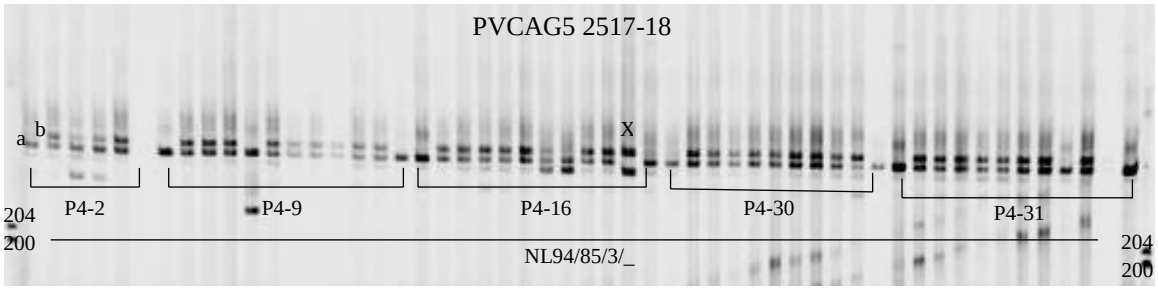
Plot	Parent	Progeny	Progeny Origins (%) Selfed	Sibling-mated	Crossed	Plot	Parent	Progeny	Progeny Origins (%) Selfed	Sibling-mated	Crossed
1	SL93/16/1/_	69	100	0	N/A	5	SL93/16/1/_	31	78	3	19
2	SL93/_	68	32	68	N/A		NL94/85/3/_	73	0	0	100
8	NL94/_	91	4	96	N/A	6	SL93/16/1/_	1	0	0	100
9	NL94/85/_	56	46	54	N/A		NL94/85/5/_	15	7	0	93
10	NL94/85/3/_	13	92	8	N/A	7	SL93/_	66	0	6	94
4	SL93/16/1/_	53	96	0	4		NL94/_	85	4	24	73
	NL94/85/1/_	74	0	0	100						

Figure 4.1. Screening gel images of progeny and their maternal parent DNA samples. Each image is labeled with the SSR marker ID on the top center. Progeny and their parent samples are grouped by braces, with progeny groups of 5 or more bookended by parent samples on either end of the braces, and progeny groups of less than 5 having only one parent sample on the left of the brace. Lower braces indicate the pedigree of the parent samples. Parental bands are marked with an "a", "b", or "c". Standard size marker (bp) are labeled on the left and right sides of the gel.

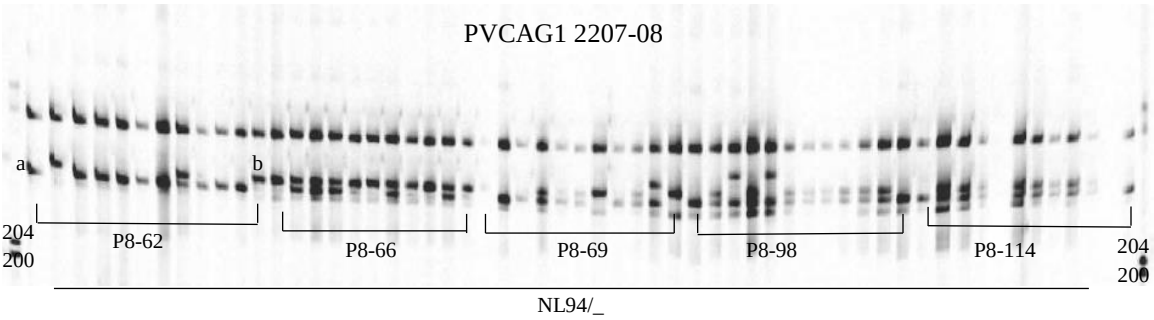
Gel Image 1. Progeny and their SL93 S3 parents from plot 1, and progeny and their NL94 S3 parents from plot 10, genotyped using SSR primer PVCAG5 2503-04. The parental band for SL93 parents with PVCAG5 2503-04 is identified as "a." The parental band for NL94 S3 parents is identified as "b."



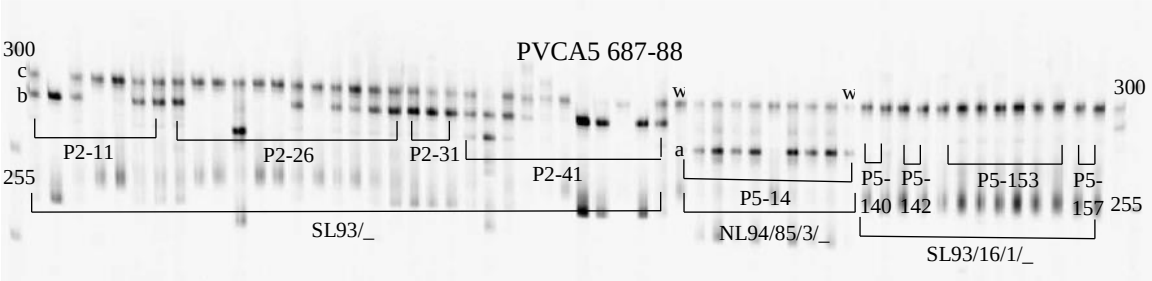
Gel Image 2. Progeny and their NL94 S3 parents from plot 4, genotyped using SSR primer PVCAG5 2517-18. The single parental band identified for NL94 S3 parents with PVCAG5 2517-18 is labeled as "a" while the parental band of SL93 S3 parents in this same is labeled as "b." A contaminant is also identified by an "X."



Gel Image 3. Progeny and their NL94 S1 parents from plot 8, genotyped using SSR primer PVCAG1 2207-08. Two parental bands are identified as “a” and “b.”



Gel Image 4. A consolidated panel containing SL93 S1 parents and progeny samples from plot 2, an NL94 S3 parent and progeny samples from plot 5, and SL93 S3 parents and progeny samples from plot 5. Samples were genotyped using PVCA5 687-88. The bands seen for SL93 S1 parents in plot 2 are “b” and “c.” In plot 5 SL93 S3 parents were monomorphic showing a single “c” band. This consolidated panel was only to include samples which were not identified as hybrid progeny from genotyping with the initial 6 markers. Due to a labeling error parental samples for SL93/16/1/140 (P5-140) were miss labeled as NL94/85/3/14 (P5-14) causing this family to be misidentified and included in the consolidated panel. As a result the parental bands shown here for P5-14 are also incorrect. Incorrect bands are marked with “w,” the true parental band for P5-12 is marked with a “a.”



CHAPTER V

CONCLUSIONS

This study reports the seed yields and origins of progeny from S1, S2, and S3 parents grown in sibling-mating and crossing plots under field conditions. Seed yields varied by genotype, plot type, inbreeding level, and environmental conditions. Hybrid production depended upon inbreeding level of parent and genotype with a high percentage of hybridization in S1 crossing plots, and near 100% hybrid production of S3 NL94 inbreds, however S3 SL93 set a low percentage of hybrid seed. The results indicate that care must be taken in parental selection to ensure adequate seed production and overlap in flowering time for reliable hybrid production.

REFERENCES

Adhikari, L., M. Anderson, A. Klatt, and Y.Q. Wu. 2015. Testing the efficacy of a polyester bagging method for selfing switchgrass. BioEnergy Res. 8: 380- 387. DOI10.1007/s12155-014-9528-3

Aguirre, A.A., B. Studer, U, Frei, and T. Lubberstedt. 2011. Prospects for hybrid breeding in bioenergy grasses. Bioenergy Research 5: 10-19. DOI:10.1007/s12155-011-9166-y

Balat, M., and H. Balat. 2009. Recent trends in global production and utilization of bio-ethanol fuel. Applied Energy 86: 2273-2282.

Bhandari, H.S., M.C. Saha, P.N. Mascia, V.A. Fasoula, and J.H. Bouton. 2010. Variation among half-sib families and heritability for biomass yield and other traits in lowland switchgrass (*Panicum virgatum* L.). Crop Sci. 50: 2355-2363.

Bhandari, H.S., M.C. Saha, P.N. Mascia, V.A. Fasoula, and J.H. Bouton. 2011. Estimation of genetic parameters for biomass yield in lowland switchgrass (*Panicum virgatum* L.). Crop Sci. 51: 1525-1533.

Boe, A., and J.G. Ross. 1998. Registration of 'Sunburst' switchgrass. Crop Sci.38: 540.

Brunken, J.N., and J. Estes. 1975. Cytological and morphological variation in (*Panicum virgatum* L.). The southwestern naturalist 19:379-985. DOI:10.2307/1935646

Burns, J.C., E.B. Godshalk, and D.H. Timothy. 2008a. Registration of 'BoMaster' switchgrass. Journal of Plant Registrations 2: 31-32.

Burns, J.C., E.B. Godshalk, and D.H. Timothy. 2008b. Registration of 'Performer'switchgrass. Journal of Plant Registrations 2: 29-30.

Burns, J.C., E.B. Godshalk, and D.H. Timothy. 2010. Registration of 'Colony' switchgrass.Journal of Plant Registrations 4: 189-194.

Burton, G.W. 1974. Recurrent restricted phenotypic selection increases forage yield of Pensacola bahiagrass. Crop Sci. 14:831-835.

Casler, M.D., K.P. Vogel, C.M. Taliaferro, N.J. Ehlke, J.D. Berdahl, E.C. Brummer, R.L. Kallenbach, C.P. West, and R.B. Mitchell. 2007. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Science 47: 2249-2260.

Casler, M.D. 2012. Switchgrass breeding, genetics, and genomics. *In:* A. Monti (ed.). Switchgrass: A Valuable Biomass Crop for Energy. Springer, New York, pp 29-53.

Demirbas, A. 2007. Producing and using bioethanol as an automotive fuel. Energy Sources Part B-Economics Planning and Policy 2: 391-401.

Dong, H., S. Thames, L. Liu, M.W. Smithe, L. Yan, and Y.Q., Wu. 2015 QTL Mapping for Reproductive Maturity in Lowland Switchgrass Populations. Bioenerg. Res. 8: 1925-1937.

Dubcovsky, J., A.F. Galvez, and J. Dvorak. 1994. Comparison of the genetic organization of the early salt stress response gene system in salt-tolerant *Lophopyrum elongatum* and salt-sensitive wheat. Theor. Appl. Genetics 87: 957-964.

Garcia, A.A.F., L.L. Benchimol, A.M.M. Barbosa, I.O. Geraldi, C.L. Souza jr., and A.P. deSouza. 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genetics and Molecular Biology 27: 579-588.

Goldemberg, J. 2007. Ethanol for a sustainable energy future. Science 315: 808-810.

Hayden, M.J., and P.J. Sharp. 2001. Targeted development of informative microsatellite (SSR) markers. Nucleic Acids Research 29: e44-e44.

Hopkins, A.A., K.P. Vogel, and K.J. Moore. 1993. Predicted and realized gains from selection for *in vitro* dry matter digestibility and forage yield in switchgrass. Crop Sci. 33: 253-258.

Hulquist, S.J., K.P. Vogel, D.J. Lee, K. Arumuganathan, and S. Kaeppler. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. Crop Sci. 36: 1046-1052.

Kwok, P.Y. 2001. Methods for genotyping single nucleotide polymorphisms. Annu. Rev. Genomics Hum. Genetic. 2: 235-258.

Liu, L.L., and Y.Q. Wu. 2011. Identification of a self-compatible genotype and mode of inheritance in switchgrass. BioEnergy Res. 5: 662-668.

Liu, L.L., and Y.Q. Wu. 2014. Switchgrass molecular genetics and molecular breeding for bioenergy traits. *In* Luo, H., Y.Q. Wu and C. Kole (ed.) Compendium of Bioenergy Plants: Switchgrass. CRC Press of Taylor and Francis Group, Boca Raton, FL. Pp 189-213.

Liu, L.L. and Y.Q. Wu, Y.W. Wang, and T. Samuels. 2012 A high-density simple sequence repeat-based genetic linkage map of switchgrass. G3: Genes Genomes Genetics 2: 357-370.

Liu, L.L., Y. Huang, S. Punnuri, T. Samuels, Y. Wu, and R. Mahalingam. 2013a. Development and integration of EST-SSR markers into an established linkage map in switchgrass. Mol. Breed. 32: 923-931.

Liu, L.L., S.Y. Lu-Thames (co-first author), and Y.Q. Wu. 2013b. Lowland Switchgrass plants in populations set completely outcrossed seeds under field conditions as assessed with SSR markers. BioEnergy Res. 7: 253- 259. DOI:10.1007/s12155 -013-9367-7

Martinez-Reyna, J.M., and Vogel, K.P. 2002. Incompatibility systems in switchgrass. Crop Sci. 42: 1800-1805.

Martinez- Reyna, J.M., and K.P. Vogel. 2008. Heterosis in switchgrass: spaced plants. Crop Sci. 48: 1312-1320.

Mitchell, R.B., K.P. Vogel, J. Berdahl, and R.A. Masters. 2010. Herbicides for establishing switchgrass in the central and northern Great Plains. Bioenergy Res. 3: 321-327.

Mitchell, R.B., K.P. Vogel, and M. Schmer. 2012. Switchgrass (Panicum virgatum) for

biofuel production. Sustainable Ag Energy Community of Practice, eXtension. http://articles.extension.org/pages/Switchgrass_for_Biofuel_Production.
Missaoui, A.M., A.H. Paterson, and J.H. Bouton. 2005. Investigation of genomic organization in switchgrass (*Panicum virgatum* L.) using DNA markers. Theor. Appl. Genet. 110: 1372-1383.
Missaoui, A.M., A.H. Paterson, and J.H. Bouton. 2006. Molecular markers for the classification of switchgrass (*Panicum virgatum* L.) germplasm and to assess genetic diversity in three synthetic switchgrass populations. Genet. Res. Crop Evol. 53: 1291-1302.
Mueller, U.G., and L.L. Wolfenbarger. 1999. AFLP genotyping and fingerprinting. Trends in Ecology & Evolution 14: 389-394.
Okada, M., C. Lanzatella, M.C. Saha, J. Bouton, R. Wu, and C.M. Tobias. 2010. Complete switchgrass genetic maps reveal subgenome collinearity, preferential pairing and multilocus interactions. Genetics 185: 745-760.
Parrish, D.J., and J.H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Critical Reviews in Plant Sciences 24: 423-459.
Pimentel, D., and T.W. Patzek. 2005. Ethanol production using corn, switchgrass, and wood; biodiesel production using soybean and sunflower. Natural Resources Res. 14: 65-76.
Porter, C.L. 1966. An analysis of variation between upland and lowland switchgrass, *Panicum virgatum L.*, in central Oklahoma. Ecology 47: 980-992.
Ribera, L.A., and H. Bryant. 2016. Economic issues for sugarcane as a biofuel feedstock. *In* Lam, E., Carrer, H., and da Silva, J.A., (ed.) Compendium of Bioenergy Plants: Sugarcane. CRC Press, Boca Raton, FL. Pp 51-62
Rose, L.W., M.K. Das, and C.M. Taliaferro. 2008. Estimation of genetic variability and heritability for biofuel feedstock yield in several populations of switchgrass. Ann. Appl. Biol. 152: 11-17.
Sanderson, M.A., R.L. Reed, S.B. McLaughlin, S.D. Wullschleger, B.V. Conger, D.J. Parrish, C. Taliaferro, A.A. Hopkins, and W.R. Ocumpaugh. 1996. Switchgrass as a sustainable bioenergy crop. Bioresource Technology 56: 83-93.
Talbert, L.E., D.H. Timothy, J.C. Rawlings, and R.H. Moll. 1983. Estimates of genetic parameters in switchgrass. Crop Sci. 23: 725-728.
Taliaferro, C.M., and J.T. Lamle. 1997. Cytological analysis of self-incompatibility in *Cynodon dactylon* (L.) Pers. International Turfgrass Society Res. J 8: 393-400.
Taliaferro, C.M., K.P. Vogel, J.H. Bouton, S.B. McLaughlin, and G.A. Tuskan. 1999. Reproductive characteristics and breeding improvement potential of switchgrass. *In*: Biomass, a growth opportunity in green energy and value-added products- Proceedings of the 4th Biomass Conference of the Americas. Oakland, California, pp. 147-153.
Tobias, C.M., P. Twigg, D.M. Hayden, K.P. Vogel, R.M. Mitchell, G.R. Lazo, E.K. Chow, and G. Sarath. 2005. Analysis of expressed sequence tags and the identification of associated short tandem repeats in switchgrass. Theor. Appl. Genet. 111: 956-964.
Tobias, C.M., D.M. Hayden, P. Twigg, and G. Sarath. 2006. Genic microsatellite markers derived from EST sequences of switchgrass (*Panicum virgatum* L.). Mol. Ecol. Notes 1: 185-187.

Vogel, K.P., F.A. Haskins, and H.J. Gorz. 1981. Divergent selection for in vitro dry matter digestibility in switchgrass. Crop Sci. 21: 39-41.
Vogel, K.P. 2004. Switchgrass. *In:* L.E. Moser, B.L. Burson and L.E. Sollenberger (eds.). Warm-Season (C4) Grasses. No. 45 in the series Agronomy. ASA-CSSA-SSSA, Madison, Wisconsin, pp. 561-588.
Vogel, K.P., and R.B. Mitchell. 2008 Heterosis in switchgrass: biomass yield in swards. Crop Sci. 48: 2159-2164.
Wang, Y.W., T.D. Samuels, and Y.Q. Wu. 2011. Development of 1,030 genomic SSR markers in switchgrass. Theor. Appl. Genet. 122: 677-686.
Wright, L., and A. Turhollow. 2010. Switchgrass selection as a "model" bioenergy crop: a history of the process. Biomass & Bioenergy 34: 851-868.
Wu, Y.Q. 2014. Classic genetics and breeding for bioenergy traits. *In* Luo, H., Y.Q. Wu and C. Kole (ed.) Compendium of Bioenergy Plants: Switchgrass. CRC Press of Taylor and Francis Group, Boca Raton, FL. Pp 170-188.
Wu, Y.Q., and Y. Huang. 2008. QTL mapping of sorghum resistance to greenbugs by molecular markers. Theor. Appl. Genet. 117: 117-124.
Wu, Y.Q., and C.M. Taliaferro. 2012. Switchgrass plant 'Cimarron'. US 8278500 B2. Date issued 2 October.

VITA

John Baker

Candidate for the Degree of

Master of Science

Thesis: MOLECULAR MARKER ANALYSIS OF PROGENY ORIGINS IN SIBLING-MATING AND CROSSING POPULATIONS OF LOWLAND SWITCHGRASS

Major Field: Plant Science

Biographical:

Education:

Completed the requirements for the Master of Science in Plant Science at Oklahoma State University, Stillwater, Oklahoma in May, 2017.

Completed the requirements for the Bachelor of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in December, 2014.

Professional Memberships: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, 2017.