# STUDY OF THE DIVERSITY, INBREEDING POTENTIAL, AND SWARD HERITABILITY OF SWITCHGRASS

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# CHAPTER I

# INTRODUCTION

## THE RESEARCH PROBLEM

Switchgrass (*Panicum virgatum* L.) is in the Paniceae tribe in the subfamily Panicoideae of the Poaceae (Gramineae) family. Switchgrass was widespread throughout the United States east of the Rocky Mountains before European settlement (Hitchcock, 1935). Switchgrass is a C4 grass with characteristics similar to other C4 grasses (Waller and Lewis, 1979). Ecologically it is linked to the grass eating mammals of the Great Plains of the United States and was initially bred for forage (Anderson, 2000; Coppedge et al., 1998).

But recently, switchgrass has become popular as a biofuel crop. The U.S. Department of Energy Herbaceous Energy Crops Program was funding research in 1985 on non-woody species in search of potential biofuel feedstocks. More than 30 herbaceous crop species were screened and, a decision was made in 1991 to focus the future Bioenergy Feedstock Development Program on switchgrass (Parrish and Fike, 2005). It was selected as a model herbaceous species for several reasons including: its perennial growth habit, high yielding

potential on marginal lands, wide adaptation, excellent conservation attributes, and compatibility with conventional farming practices (McLaughlin et al., 1999). As a long-lived perennial (Hitchcock and Chase, 1950) switchgrass is favored by the Conservation Reserve Program (CRP), because perennials will reduce management, energy, and chemicals used for establishment and production. It is beneficial to wildlife for shelter and it reduces plowing and thus reduces soil erosion (Dunn et al., 1993; Wright, 1994; Vogel, 2004). It is also beneficial by sequestering carbon in the soil (Andress, 2002), and switchgrass has good biomass yield stability across different environments (Taliaferrro, 2002). Switchgrass has shown potential for breeding improvement because it has a broad genetic base (Eberhart and Newell, 1959, Tobias et al., 2005).

When converted to ethanol, Schmer et al. (2008) found that switchgrass produced 540% more renewable energy than the non renewable energy used to grow and process the grass while maize's net energy return was about 60-70%. As a biofuel it is either converted to ethanol or co-fired with coal (McLaughlin and Kszos, 2005). Switchgrasses' total carbohydrate (based on weight) is higher than alfalfa, but carbohydrate recovery is dependent on maturity and lignin content (Dien et al., 2006). The yield of cellulose, hemicelluloses and lignin (lignocellulosic yield) parallels dry weight, and increasing dry weight should be included in breeding goals (Cassida et al., 2005).

To fill the demand for cellulose in biofuel refineries new high yielding cultivars are being developed by traditional plant breeding methods. Traditional plant breeding has been effective in increasing maize yield (Crow, 1998);

however, progress can be slow and requires many years of selection. However research can facilitate this process. Knowledge of the genetic diversity of switchgrass will aid in superior plant selections for more rapid improvement. Traditionally, this has been accomplished by phenotypic markers, but these markers are unreliable because they are affected by the environment. Molecular markers are not affected by the environment and are more reliable (Collard and Mackill, 2008). For this reason amplified fragment length polymorphisms will be performed on 56 tetraploid germplasm accessions to understand more accurately their genetic diversity.

Understanding heritability is another way to accelerate the breeding process. Heritability is the amount of phenotypic variation due to genotypic variation. Knowledge of heritability will increase breeding efficiency by aiding in planning for each cycle of selection because if heritability is low and environmental variation high, then more testing in multiple environments is required. Most previous biomass heritability studies used spaced plants, but spaced plant yield may not accurately predicted sward yield in some forage crops (Waldron et al., 2008). Sward heritability could be a more accurate method and increase progress. To study heritability for biomass in switchgrass, sward progeny plots of two populations (SL93 cycle 2 and NL94 cycle 2) were planted in a randomized complete block design and evaluated for biomass yield to calculate the variance components for heritability. A clone replicate plot of their parents was planted in another environment to perform a parent offspring regression. The trait investigated in this study was biomass yield because it is the most important factor for biofuel production and should be the primary

trait for switchgrass improvement (McLaughlin and Kszos, 2005). Inbreeding and hybridization could greatly increase yields. Vogel and Mitchell (2008) found that yields could be increased by 38% though panmictic hybridization. If the crop is inbred, then possibly greater increases in yield are possible. Switchgrass is an out-crossing species that will set some seed if bagged, but the inbreeding bagging methods have not been studied and selfing not confirmed. To study bagging methods and inbreeding in switchgrass an $S_2$ population of switchgrass was evaluated for seed yield over three years with three bag types (paper, microfiber and cotton muslin bags) and the inbreeding confirmed by SSR markers.

## LITERATURE REVIEW

### AFLP

Past molecular marker studies on genetic diversity analysis in switchgrass include random amplified polymorphic DNA (RAPD) (Gunter et al., 1996; Casler et al., 2007), restriction fragment length polymorphism (RFLP) (Missaoui et al., 2006), and simple sequence repeats (SSRs) (Narasimhamoorthy et al., 2008; Cortese et al., 2010; Zalapa et al., 2010). Gunter et al. (1996) performed RAPDs to assess the genetic diversity among and within 14 populations of switchgrass and to find markers that are useful for population identification. Casler et al. (2007) performed RAPD on forty-six remnant populations and eleven cultivars to discover if there was population differentiation and to evaluate possible correlations between genotypes, ecotypes, and geographic forms. They found little differentiation correlated with geography but a small amount was associated with hardiness zones and ecotypes. Casler et al. (2007) also reported

plants from the same region could be highly unrelated to each other. They further indicated the markers could not distinguish between cultivars and remnant wild populations. Missaoui et al. (2006) performed RFLP to assess the genetic variation between 21 switchgrass genotypes that were randomly selected from three synthetic cultivars (`Alamo', `Kanlow' and `Summer'), and found that there was higher diversity between upland and lowland accessions compared to genotypes within each of the cultivars. Missaoui et al. (2006) also found a polymorphism within the *trn*L (UAA) chloroplastic marker associated with upland and lowland ecotypes. The *trn*L UAA intron region is in the chloroplasts of all plants (Pirie et al., 2007). It is part of the chloroplast DNA and transferred through the maternal parent (Martinez-Reyna et al., 2001). Missaoui et al. (2006) found that this marker has a 49 nucleotide segment of DNA deletion in the lowland germplasm when aligned with upland germplasm. Narasimhamoorthy et al. (2008) studied the diversity of the USDA Germplasm Resources Information Network (GRIN) germplasm bank, and found that there was higher variation within populations than among populations. Cortese et al. (2010) combined marker and morphological data among 12 populations of switchgrass and could distinguish between upland populations based on geography. Their results also indicated that morphological and adaptive traits could be identified by molecular markers. Zalapa et al. (2010) used 55 SSR markers and six chloroplast markers to study diversity within and between 18 switchgrass cultivars (7 lowland and 11 upland). The SSR markers could discriminate ecotype correctly, but chloroplast markers alone (like the *trn*L marker) could not always distinguish ecotype (Zalapa et al., 2010).

## Heritability

There have been several papers studying switchgrass heritability. Newell and Eberhart (1961) studied heritability in several populations endemic to Nebraska and they used their own terminology to describe switchgrass types including `small blue green', `medium-tall blue green', `large green'. The narrow sense heritability estimates calculated from analysis of variance showed 0.72 unadjusted and 0.57 adjusted heritability for `small blue green' and 0.74 unadjusted and 0.40 adjusted for `medium-tall blue green' types. The adjustment removed the clone×year interactions. Heritability estimates for total yield calculated from twice the regression of offspring on parents was 0.18±0.06 for `small blue-green' and 0.52±0.22 for `medium tall blue-green' and 0.05±0.36 for `tall green'. The realized heritability for `small blue-green' was 0.4 and 0.91 for `medium-tall blue-green'. The heritability on a single plant basis was 0.23 for `small blue-green' types and 0.19 for `medium tall blue-green'. The heritability on a clone mean basis was 0.42 for `small blue-green' types and 0.45 for `medium tall blue-green' (Newell and Eberhart, 1961). The expected percent progeny gains were 9% and the observed gain in `small blue green' was 8% and 11% in `medium tall blue green' (Newell and Eberhart, 1961). Talbert et al. (1983) measured heritability for dry mass weight and in vitro dry matter digestibility (IVDMD) in 11 lowland populations and found the narrow sense heritability for dry weight was 0.25 on an individual basis and 0.59 on a family basis (Talbert et al., 1983). Godshalk et al. (1986) studied variance components and heritability of IVDMD and dry mass yield in lowland switchgrass in initial and regrowth using a modified head and row procedure. These heritabilities were based on one

environment, so they could be biased by family×location and family×location×year interactions. The dry mass narrow sense heritability among half-sib families was 0.52±0.26 and the narrow sense heritability within half sib families was 0.20±0.10 (Godshalk et al., 1986). Hopkins et al. (1993) studied the effectiveness of recurrent restricted phenotypic selection (RRPS) in improving forage yield and IVDMD in upland populations planted in sward rows. The predicted and realized gains for IVDMD and yield were measured. Hopkins did not create a parent offspring regression heritability estimate for forage yield, because yield data were not collected from parent clones. The forage yield heritability based on family variance components was negative for open pollinated progenies and was 0.22 ± 0.001 for closed pollinated progeny. Missaoui et al. (2005) studied 30 genotypes of `Alamo' to study heritability of P concentration, P uptake and biomass production. The heritability for biomass of individual plants was 0.6, 0.69 for family means, and 0.76 for parent offspring regression in the same environment, and 0.45 in different environments. Genetic gains from selection on an individual plant basis were 0.51, for half sib family selection 0.27, and for half sib progeny test it was 0.55 (Missaoui et al., 2005).

Rose (2005) and Rose et al. (2007, 2008) studied the heritability of spaced plants in two lowland populations `Southern Lowland 93' (SL-93) and `Northern Lowland 94' (NL-94). The original SL-93 population was synthesized in 1993 from `Alamo' and `PMT-279'. From this material two cycles of Restricted Recurrent Phenotypic Selection (RRPS) for increased biomass were performed. The NL-94 population was the result of two cycles of RRPS for biomass yield within `Kanlow' (Rose 2005). Rose (2005) collected data to estimate the genetic parameters of

switchgrass such as the genetic variances, narrow sense heritability ($h^2_n$), and predicted genetic gain from selection (ΔG) for biomass yield. Rose et al. (2007) studied the variance component heritabilities of NL-94 grown in two environments, a high yielding environment with fertilizer and irrigation and a low yielding environment that received no inputs. The combined narrow sense heritability for both high and low yielding environments of both $C_0$ protocols was low (0.09), but the heritability was much higher when looking at the environments separately (0.73 for high yielding environment and 0.65 for low yielding environments) (Rose et al., 2007). Rose et al. (2008) studied the heritability of three populations, one lowland, Southern Lowland '93 (SL-93) and two upland populations, Southern Northern Upland Early Maturing and Southern Northern Upland Late Maturing (SNU-EM and SNU-LM). SNU-EM per family mean heritability was 0.45. SNU-EM individual plant heritability was 0.44. SNU-LM per family mean was 0.46 and the SNU-LM individual plant heritability was 0.47 (Rose et al., 2008). For SL-93 the $h^2_n$ estimates based on individual plant and phenotypic family mean from parent offspring regression were 0.13 and 0.12, respectively (Rose et al., 2008). Bhandari et al. (2010) evaluated 37 half sib families from 2007 to 2009 to measure variation components and heritabilities for dry mass and morphological traits in switchgrass. The dry mass heritability based on variance components per plant from 2007 and 2008 was 0.13 with a standard error of 0.07, and a mean parent offspring regression heritability of 0.29 with a standard error of 0.14. Boe and Lee (2007) found heritabilities among family means for two upland cultivars Summer (0.62) and Sunburst 0.60 when harvested over four years.

## Inbreeding

Since switchgrass is a self incompatible cross-pollinated species, it has been improved by traditional methods for forage and biofuel. The first switchgrass cultivars were bred using the ecotype selection method which uses among accession variability (Vogel and Gabrielson, 1986). The recurrent selection method created by Burton (1974) was used by Vogel et al. (1991) to develop the cultivar 'Trailblazer'. Recurrent restricted phenotypic selection (RRPS) did not improve forage yield in the upland populations that Hopkins et al. (1993) developed because there was not enough variation in his population (Hopkins et al., 1993). Taliaferro (2002) tried fast track restricted recurrent phenotypic selection which failed to produce substantial results because establishment year performance is not predictive of subsequent year performance and the breeding value of an individual plant cannot be judged by its phenotypic yield performance. But, genotypic recurrent selection using half sib performance did produce results; for example, the biomass yield of the synthetic SL 93-3 averaged in all regional tests 9% higher than the biomass yield of 'Alamo' over a three year period (Taliaferro, 2002; Taliaferro and Hopkins, 1996). Recurrent Selection for General Combining Ability (RSGCA), the current method being employed at Oklahoma State University evaluates the yield of half sib progeny over years, and then selects the parents of the best performing plants to create a new population. These half sib plants were created using parents in a randomized complete randomized block design (RCBD) (Taliaferro, 2002; Rose et al., 2008).

Population breeding systems like RRPS or GRS improve plants utilizing their additive genetic variability. To utilize non additive genes, hybridization is required (Martinz-Reyna, 1998). Hybridization of two distinct lines often results in heterosis, which is the increased vigor of the $F_1$ over the mean of the parents (Hays, 1952). Lamkey and Edwards (1999) defined heterosis as the positive difference of the hybrid to the mean of its parents. This definition is also referred to as mid parent heterosis. High parent heterosis is the positive difference between the mean of the hybrid and the mean of the best parent (Lamkey and Edwards, 1999). Population heterosis, also called panmictic heterosis, happens when two randomly mated populations are mated and the mean of the $F_1$ is higher than the mean of the two parent populations (midparent) or best parent (high-parent). Heterosis tends to occur and increase with the genetic distance of the parents (Moll et al., 1965).

Heterosis could greatly increase biomass yield of switchgrass. Taliaferro (2002) created hybrids of three populations NU 94, NU 93, and SL 93 and estimated the heterosis for three years and found mid parent and high parent heterosis values of 56% and 39%, respectively. Martinz-Reyna et al. (2008) performed crosses between upland and lowland populations of switchgrass, and found that the lowland tetraploids represented by the cultivar `Kanlow' and the upland tetraploids represented by the cultivar `Summer' were in two different heterotic groups. In swards, the hybrids of these groups had 30 to 38% heterosis (Vogel and Mitchell, 2008).

Inbreeding can greatly increase heterosis (Shull, 1908; Shull, 1909), but inbreeding allogamous crops can affect fitness in forage yield and severely lower seed production (Gallais A, 1984; Brummer, 1999). Hybrid maize development which started in the early portion of the twentieth century has increased yields by 500% by 1998 compared to pre-hybridization yields (Crow 1998). Swichgrass is a cross-pollinated plant that has a self incompatibility system similar to the gametophitic S and Z incompatibility found in other Poaceae (Martinez-Reyna and Vogel, 2002), but switchgrass will set some seed if selfed (Newell, 1936). Taliaferro and Hopkins, (1996) observed a selfing rate of about 1-2%. Taliaferro (2002) planted three first generation selfed families from Blackwell, Caddo, Cave-in-Rock, Kanlow, and Alamo in 2000. From each of the 15 $S_1$ families, three plants were selected based on visual assessment and were selfed and bagged in 2001. Only 13 of the 45 selfed $S_1$ plants produced 20 or more seeds. Seven $S_1$ plants had more than 100 $S_2$ seeds. If inbreeding research is continued to determine the extent to which switchgrass can be inbred, the research carries the risk of an inbreeding depression (Taliaferro, 2002). This may happen before the plants are fully homozygous. Inbreeding depression has hampered the development of hybrids in other out-crossing forage crops (Jones and Bingham, 1995). Utz and Oettler (1978) reported a 49% yield reduction in *Lolium perenne* L. for the $S_1$ compared to $S_0$. Inbred plants like maize had lower vigor and smaller seeds with reduced seed and dry matter yields. Inbreeding depression also severely affected other forage crops such as big blue stem (*Andropogon furcatus* Muhl.) which had a 40% reduction in the $S_1$ and 29% in the $S_2$, and smooth bromegrass (*Bromus inermis* Leyss.) which had a 62.6% reduction

of the $S_1$ to open pollinated progenies (McDonald et al., 1952; Law and Anderson, 1940; Jones and Bingham, 1995).

In a diploid population homozygosity increases 50% each self, but tetrasomic tetraploids homozygosity increases 17-21% depending on the segregation pattern (Husband and Schemske, 1997). Disomic inheritance occurs in allotetraploids and autotetraploids with bivalent chromosome pairing at meiosis, and is tetrasomic in autotetraploids without bivalent pairing (Husband et al., 2008). This study will assume disomic inheritance, because the results from the molecular map of Okada et al. (2010) indicated that switchgrass has disomic inheritance.

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# CHAPTER II

## GENETIC DIVERSITY IN TETRAPLOID SWITCHGRASS REVEALED BY AFLP MARKER POLYMORPHISMS

### ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a perennial warm-season grass native to North America, which has been identified as a dedicated cellulosic biofuel feedstock. To increase biomass yield traditional breeding methods are being used to increase its biomass yield. Long term breeding progress requires high germplasm genetic diversity for breeding programs. The objectives of this study were to measure genetic diversity within the Oklahoma State University tetraploid switchgrass germplasm collection, and to characterize genetic relatedness among the collections from distinct regions. Fifty six tetraploid accessions including seven upland and 49 lowland genotypes from throughout the US were used in the investigation. Genomic DNA samples were isolated for each clonal accession using Zymo Research Plant/Seed Kit™. DNA profiling patterns were generated by fluorescence-labeled amplified fragment length polymorphism (AFLP) procedure. Amplified fragments were visualized using a Li-Cor 4300 DNA Analyzer and scored visually. Sixteen selective AFLP primer combinations were

used to amplify 452 polymorphic bands. The accessions' genetic similarity coefficients, UPGMA (unweighted pair-group method with arithmetic averaging) cluster analysis, and principle coordinate analysis were performed using NTSYS-pc (ver. 2.02i) (Numerical Taxonomy System) software. The upland and lowland accessions clustered according to ecotypes, with one exception (TN104). Genetic similarity coefficients among the accessions ranged from 0.73 to 0.95. An analysis of molecular variance (AMOVA) was performed using GenAlEx (ver. 6.3) resulting in a significant difference ($a<0.5$) between the upland and lowland genotypes. The *trn*L marker confirmed that TN104 was a lowland genotype, but the *trn*L marker identification of upland and lowland genotypes was not consistent with the AFLP in two germplasms (Miami and AR4).

## INTRODUCTION

Genetic diversity and relationships are better evaluated using DNA markers than morphological traits because markers are unaffected by environmental factors (Singh et al., 1999). The first DNA-based markers were restriction fragment length polymorphisms (RFLPs). These are highly specific, but require high amounts of high quality DNA and the ability to use radioactivity. Randomly amplified polymorphic DNAs (RAPDs) is a polymerase chain reaction technique that does not require radioactivity and can use low quality DNA but lacks reproducibility and specificity. RAPD is highly sensitive to variations in concentrations of DNA, $Mg^{2+}$ ions, Taq enzyme, and thermocycler used (Davin-Regli et al., 1995; Singh et al., 1999). Amplified fragment length polymorphism (AFLP) overcomes the problems of other techniques. It does not require radiation and is highly

reproducible (Singh et al., 1999). The AFLP method has been used to analyze the diversity of many crops including rice (Zhu et al., 1998) barley (Russell et al., 1997) and Neem (Singh et al., 1999). The AFLP marker system can detect more point mutations per hundred nucleotides than RFLP, because, unlike RFLP, AFLP includes nucleotidesoutside of the restriction site (Becker et al., 1995; Singh et al., 1999). Amplified Fragment Length Polymorphism (AFLP) can be used in applications such as shallow phylogenetics, population genetics, linkage mapping and many other purposes (Meudt and Clarke, 2006).

Switchgrass breeding and genetic research has been performed at OSU since 1992 (McLaughlin and Kszos, 2005). A large switchgrass germplasm collection has been assembled as a result of this research. Objectives of the study were to characterize genetic diversity in the tetraploid switchgrass germplasm assembled at OSU from around the US, to analyze the genetic relatedness among the switchgrass germplasm and to clarify upland and lowland germplasm using the *trn*L marker.

## MATERIALS AND METHODS

A total of 56 respective tetraploid switchgrass plants maintained in an OSU switchgrass germplasm nursery were used in this study (Table 2.1) (Hopkins et al., 1996). Of the germplasm, 7 were classified as upland and 49 as lowland by previous morphological marker phenotyping (Taliaferro, 2006, personal communication). DNA extraction was performed using the Zymo ZR Plant/Seed Kit TM (Zymo Research Corperation, CA) according to the manufacturer's instructions. DNA quality of each sample was checked by 1% agarose gel

electrophoresis. The concentration was measured using a NanodropTM 1000 spectrophotometer (Nanodrop Products, DE).

The AFLP procedure was performed according to Vos et al. (1995) with modifications made according to Wu et al. (2005). The DNA was digested using EcoRI and MseI enzymes, and AFLP adapters were ligated to these DNA fragments. Fragments were pre-amplified by PCR using primer combinations based on the AFLP adaptors. Sixteen AFLP fluorescently labeled selective amplification primer combinations were used to characterize genetic diversity (Table 2.2). The quantity of primer combinations was selected because it was necessary to generate >400 polymorphic bands (loci), which were considered appropriate to estimate the diversity of a crop like switchgrass. All the PCR reactions were conducted using an Applied Biosystems 2720 thermocycler (Applied Biosystems Inc., IL). Banding patterns were visualized on a 0.25 mm thick 6.5% polyacrylamide gel with a 64 tooth comb in a Li-COR 4300 DNA Analyzer (Li-Cor Inc., NE, USA) and run at 1500 Volts with a scan speed 2 for 2.5 hours. A DNA size marker (50-700bp) was also loaded to determine the size of the fragments.

Polymorphic bands in each AFLP gel were scored visually as '1' for presence and '0' for absence for each of the 56 switchgrass accessions, while '9' was assigned to an ambiguous band. The bands were counted between ~75 bp and 204 bp. The collected data were analyzed using NTSYSpc version 2.02i program for calculating simple matching similarity coefficients, performing a cluster analysis, and principles coordinate analysis (Rohlf, 1993). An AMOVA was

performed using GenAlex 6 (Peakall and Smouse, 2006), which partitioned the data between upland and lowland including and excluding TN104. Genetic diversity of switchgrass was calculated by Shannon's information index (Brown and Weir, 1983) and expected heterozygosity (He) (Lynch and Milligan 1994) and unbiased expected heterozygosity (UHe) (Hartl and Clark, 1997) using Genalex (Peakall and Smouse, 2006).

To clarify upland and lowland identities of the germplasm, especially TN104, the DNA used in the AFLP procedure of all the germplasms was used with primers 'c' and 'd' as described by Taberlet et al. (1991) to amplify the *trn*L UAA intron region. 'Summer' and PI 421999 were used as controls because they were sequenced and confirmed as upland and lowland, respectively by Missaoui et al. (2006). The purified DNA was sequenced at OSU Recombinant DNA/Protein Resource Facility in both directions using primers from the PCR reactions and aligned using clustalw in the Mega (ver. 4.0) (Kumar et al., 2008) program to determine the presence or absence of 49 nucleotides that are deleted in lowland switchgrass (Missaoui et al., 2006).

## RESULTS AND DISCUSSION

The 16 AFLP selective primer combinations generated a total of 658 markers. There were 452 polymorphic bands counted resulting in a polymorphism percentage of 68.7%. The similarity coefficients ranged from to 0.73 (between pairs PI315727 and SWG005, PI76293 and SWG005, MO100 and SWG039) to 0.95 (between SWG031 and SWG024) with an average of 0.83. According to the UPGMA cluster tree (Figure 2.1) two major Clusters (I and II) had a similarity of

78%. All the lowland germplasm were in Cluster I and all upland germplasm were in Cluster II with the exception of TN104, which was previously classified as upland according to morphology. Cluster I was divided into two sub-clusters A and B with a similarity of 79.6%. Sub-cluster A had the most genotypes and could be divided into sections i and ii at 81.2% similarity. In the principle coordinate map (Figure 2.2) most of the lowland genotypes were clustered tightly together to the left, but the rest, including all the upland types were scattered across the field toward the right. The Principal Coordinate Analysis was mostly consistent with the Cluster Analysis (Figure 2.1). The clustering separation of upland and lowland plants supported the conclusion that lowland-tetraploid and upland-tetraploid switchgrasses represent different heterotic groups (Martinez-Reyna and Vogel, 2008).

There was cluster division among germplasm according to region, but there were some exceptions. Cluster IB contained germplasm from the east coast of the United States and TN104 from TN. Cluster IAi contained germplasm from Florida and Mississippi while Cluster IAii contained mostly germplasm from the central United States, but also a few from Mississippi and Florida. These differences were most likely caused by adaptation to geographic regions. Other studies Casler et al. (2007) and Narsimhamoorthy et al. (2008) found that most of the germplasm subclustered according to adaptive regions. The upland group of II and the lowland groups of IAi and IAii and IB were all possible sources of variation for breeding. To ensure adequate diversity, plants from each of the groups mentioned could be selected as germplasm when developing cultivars. Crosses between members of these groups could be made to test for heterosis in

field-based studies. These groups could be kept separate and improved and then crossed for heterosis in synthetics, hybrids or semi-hybrids (Brummer, 1999).

The upland and lowland germplasm mostly clustered separately, which is consistent with the result of Narasimhamoorthy et al. (2008), but TN104, an upland tetraploid segregated with the lowland types. It was interesting to note that TN103 and TN104 originated close together geographically, but were genetically distinct. The range of diversity of this study was less than some past studies; for example, Gunter et al. (1996) whose similarity coefficients ranged from 0.53 to 0.78 and Narasimhamoorthy et al. (2008) whose similarity coefficients ranged from 0.45-0.81 but were much lower in the study by Cortese et al. (2010) (0.03 to 0.24). The lower diversity in this study could be because it is limited to one plant per accession and switchgrass has higher within accession diversity (Narasimhamoorthy et al., 2008). This is an expected difference between this paper and others because the primary purpose of this study was to measure the diversity between different accessions and not within accessions. The difference could also be due to distinct germplasm materials, and indicates that the inclusion of some of the switchgrass germplasm from the National Plant Germplasm System into the working collection of the OSU breeding program will increase genetic diversity. Marker systems and marker numbers used in the above investigations could be important factors for genetic diversity investigation. Polymorphic dominant markers produced and used in this study were 452 while 91 were used by Gunter et al. (1996), 63 EST and genomic SSR loci by Narasimhamoorthy et al. (2008) and 16 EST SSR loci by Cortese et al. (2010). Mohammadi and Prasanna (2003) recommended a high degree of sampling

error could be related to rare alleles, which had 5% or lower in frequency and the percentage of polymorphic markers became reliable only when a large number of loci were generated. However, they did not indicate specific marker number for this kind of work. In this study > 400 polymorphic markers were used to make a good estimate of genetic diversity.

The Shannon's information index, expected heterozygosity (gene diversity) and unbiased expected heterozygosity and their respective standard errors can be found in Table 2.3. The expected heterozygosity, percent polymorphic bands and Shannon's index was higher in the lowland genotypes compared to the upland genotypes. This is not surprising because of the higher number of lowland samples, but it indicates that the germplasm used is diverse. The addition or subtraction of TN104 only slightly affects expected heterozygosity and Shannon's index, but it affects percent polymorphic bands more particularly in uplands. The total Shannon's index was higher but similar to that found in populations of big bluestem in Ohio that ranged from 0.22 to 0.27 created from RAPDs (Selbo and Snow, 2005). The total Shannon's index and expected heterozygosity were much higher in switchgrass (I=0.317, He=0.208) than in an AFLP study in Carpetgrass (I=0.24, He=0.16) from samples collected in the United States (Wang et al., 2010).

AMOVA

The results of the AMOVA were similar with TN104 included in either the upland or lowland ecotypes. There was more diversity within ecotypes than among them in both analyses, but the analysis with TN104 (21% among 79% within) as a lowland had a higher among ecotype variation than the analysis with TN104

(18% among 82% within) as an upland and a higher PhiPT score (0.18 and 0.21 respectively). The AMOVA PhiPT p value (0.01) (Table 2.4) was significant with and without TN104 and was significantly different between upland and lowland at (95%) but not at (99%) significance level. The AMOVA revealed that the amount of diversity within ecotypes was high (79%) and among ecotypes low (21%). High within ecotype diversity allows for breeding and selection within each ecotype.

### *Trn*L marker

The *trn*L PCR for the entire germplasm was performed and the gel analysis showed lowland germplasm including TN104 (~560.5 bp) and AR4 (~563.5 bp) migrated with the shorter lowland DNA of PI 421999 (~564.3 bp). Moreover, the upland accessions including the lowland accession Miami (~620 bp) migrated with the longer DNA of Summer (~612.5 bp). Once sequenced TN104 and AR4 lacked the 49 nucleotide segment that Summer and Miami possess (Figure 4). The Miami and AR4 *trn*L sequences were opposite of what was expected from the AFLP. Miami, which was considered lowland germplasm had the 49 nt and AR4 lacked this segment like the lowlands. The Miami results are similar to those found by Gunter et al. (1996) where the cpDNA type of Miami were of the upland type and the RAPD results were lowland. It was interesting to note that the *trn*L marker sequenced by Missaoui et al. (2006) from *Panicum amarum*, to which Miami was phenotypically similar, when aligned also had the 49 nt *trn*L sequence typical of uplands. Native hybrids of P. *amarum* var. amarulum and P. *virgatum* have been found (Palmer, 1975). It could be that Miami has chloroplast

DNA from *Panicum amarum* from a past interspecific cross. AR4 having the lowland marker was unexpected because it clustered with the uplands, which could be caused by gene flow between uplands and lowlands. The *trn*L marker was in the chloroplasts, so if there was hybridization between upland and lowland genotypes it will measure the contribution of the maternal parent (Martinez-Reyna et al., 2001). Chloroplast capture, the transfer of chloroplast from one population to another has been documented in other studies and was possible at the infraspecific level (Wolfe and Elisens, 1995). The *trn*L analysis confirmed the AFLP analysis that TN104 was a lowland cultivar, but the presence of the lowland *trn*L marker in AR4 and an upland marker in Miami when the AFLP clearly indicated otherwise demonstates that the marker may not always correlate with upland and lowland genotype or phenotype. Zalapa et al. (2010) found similar results when screening switchgrass cultivars with chloroplast markers. This marker probably is associated with ecotype, but gene flow may occur when upland and lowland ecotypes hybridize in hybrid zones (Modliszewski, 2006). The incongruence between the phenotypic markers, AFLP markers and the *trn*L marker indicated the possibility of gene flow between upland and lowland genotypes in some germplasm.

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Table 2.4. Analysis of molecular variance (AMOVA) from upland and lowland ecotypes of 56 switchgrass accessions based on 16 AFLP primer combinations generating 452 polymorphic markers and TN104 treated as lowland germplasm.

Table 2.1. Oklahoma State University switchgrass tetraploid germplasm collection used in the AFLP analysis.

| ID | Ecotype | Origin/reference | ID | Eco-type | Origin/reference |
|---|---|---|---|---|---|
| MIAMI | LOW L. | Miami, FL | SWG043 | LOW L. | Bristow, Oklahoma |
| MS4 | LOW L. | Yalobusha County, MS | SWG044 | LOW L. | Bristow, Oklahoma |
| MS6 | LOW L. | Mississippi | SWG045 | LOW L. | Bristow, Oklahoma |
| PANGBURN | LOW L. | Arkansas | SWG046 | LOW L. | Bristow, Oklahoma |
| PI 422016 | LOW L. | Florida | SWG047 | LOW L. | Bristow, Oklahoma |
| PMT 279 | LOW L. | From Matt Sanderson | SWG048 | LOW L. | Bristow, Oklahoma |
| STUART | LOW L. | Stuart, FL | SWG049 | LOW L. | Bristow, Oklahoma |
| SWG002 | LOW L. | A Pangburn selection | SWG051 | LOW L. | Shawnee, Oklahoma |

Table 2.1 Continued.

| ID | Ecotype | Origin/reference | ID | Eco-type | Origin/reference |
|---|---|---|---|---|---|
| SWG003 | LOW L. | Union County, AR | TX1 | LOW L. | Texas |
| SWG004 | LOW L. | Yalobusha County, MS | WABASSO | LOW L. | Wabasso, Florida |
| SWG005 | LOW L. | Coffeyville, MS | AR4 | UP L. | Arkansas |
| SWG020 | LOW L. | Labled as Pathfinder from NB AES | MO100 | UP L. | Butler Hollow Glades, MO |
| SWG021 | LOW L. | Stillwater, OK | MO101 | UP L. | Hercules Glades, Missouri |
| SWG022 | LOW L. | Stillwater, OK | NC1 | UP L. | Fort Bragg, North Carolina |
| SWG024 | LOW L. | Bristow, OK | SUMMER | UP L. | Nebraska |
| SWG029 | LOW L. | McAlester, OK | TN103 | UP L. | Shelby County, Tennesse |
| SWG030 | LOW L. | McAlester, OK | TN104 | UP L. | Tennessee |

Table 2.1 Continued.

| ID | Ecotype | Origin/reference | ID | Ecotype | Origin/reference |
|---|---|---|---|---|---|
| SWG031 | LOW L. | McAlester, OK | PI 315723 | LOW L. | North Carolina |
| SWG032 | LOW L. | Bristow, OK | PI 315727 | LOW L. | North Carolina |
| SWG033 | LOW L. | Bristow, OK | PI 315728 | LOW L. | Maryland |
| SWG034 | LOW L. | Slick, OK | PI 414065 | LOW L. | Arkansas |
| SWG035 | LOW L. | Slick, OK | PI 414070 | LOW L. | Kansas |
| SWG036 | LOW L. | Bristow, OK | PI 421999 | LOW L. | Arkansas |
| SWG037 | LOW L. | Bristow, OK | PI 476291 | LOW L. | Maryland |
| SWG038 | LOW L. | Drumright, OK | PI 476293 | LOW L. | New Jersey |
| SWG039 | LOW L. | Drumright, OK | PI 607837 | LOW L. | Texas |
| SWG040 | LOW L. | Bristow, OK | PI 607838 | LOW L. | Texas |
| SWG042 | LOW L. | Drumright, OK | PI 636468 | LOW L. | Texas |

Table 2.2. Selective AFLP primer combinations used to amplify PCR bands on 56 switchgrass accessions.

| Primer Combinations | TNB† | NPB‡ | PPB§ |
|---|---|---|---|
| M-CAC/E-ACC | 36 | 25 | 69.4 |
| M-CAC/E-ACT | 36 | 25 | 69.4 |
| M-CTG/E-ACA | 55 | 32 | 58.2 |
| M-CTG/E-ACT | 39 | 29 | 74.4 |
| M-CTG/E-ACC | 42 | 27 | 64.3 |
| M-CTG/E-AGC | 41 | 25 | 61.0 |
| M-CTA/E-AAG | 52 | 34 | 65.4 |
| M-CTA/E-AGC | 43 | 29 | 67.4 |
| M-CTC/E-AAC | 47 | 33 | 70.2 |
| M-CTC/E-ACG | 41 | 29 | 70.7 |
| M-CAT/E-ACC | 44 | 37 | 84.1 |
| M-CAT/E-ACG | 43 | 28 | 65.1 |
| M-CAA/E-AAG | 46 | 31 | 67.4 |
| M-CAA/E-AGG | 39 | 29 | 74.4 |
| M-CAG/E-ACA | 27 | 21 | 77.8 |
| M-CAG/E-ACG | 27 | 18 | 66.7 |
| Total | 658 | 452 | - |
| Mean | 41.1 | 28.2 | 68.7 |

†TNB, Total number of bands.
‡NPB, No. of polymorphic bands.
§PPB, percentage polymorphic bands.

Table 2.3. Band frequencies, Shannon’ information index and biased and unbiased estimated heterozygosity for binary AFLP data for the total number of samples and upland and lowland ecotypes with or without TN104.

| | Upland | | Upland - TN104 | | Lowland | | Lowland + TN104 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Ne† | 1.25 | 0.014 | 1.221 | 0.013 | 1.327 | 0.014 | 1.33 | 0.014 | 1.347 | 0.014 |
| I‡ | 0.223 | 0.011 | 0.195 | 0.01 | 0.295 | 0.011 | 0.298 | 0.011 | 0.317 | 0.011 |
| He§ | 0.147 | 0.007 | 0.129 | 0.007 | 0.194 | 0.008 | 0.196 | 0.008 | 0.208 | 0.007 |
| UHe¶ | 0.159 | 0.008 | 0.141 | 0.008 | 0.196 | 0.008 | 0.198 | 0.008 | 0.21 | 0.008 |
| %PPB# | 45.1 | | 38.0 | | 63.5 | | 64.1 | | 68.7 | |

†Ne, No. of Effective Alleles.

‡I, Shannon's Information Index.

§He, Expected Heterozygosity.

¶UHe, Unbiased Expected Heterozygosity.

#PPB, Percentage of Polymorphic Loci.

Table 2.4. Analysis of molecular variance (AMOVA) from upland and lowland ecotypes of 56 switchgrass accessions based on 16 AFLP primer combinations generating 452 polymorphic markers and TN104 treated as lowland germplasm.

| Source | df | SS† | MS‡ | Est. var. | % |
|---|---|---|---|---|---|
| Among ecotypes | 1 | 255.02 | 255.02 | 17.57 | 21% |
| Within ecotypes | 54 | 3602.99 | 66.72 | 66.72 | 79% |
| Total | 55 | 3858.02 | | 84.30 | 100% |

| Statistic | Value | P(rand >= data) |
|---|---|---|
| ΦPT | 0.208 | 0.010 |

†SS, Sum of Squares.

‡MS, Mean Square.

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Figure 2.1. UPGMA phenogram of genetic diversity based on simple match similarity coefficients derived from switchgrass AFLP markers. Roman numerals I and II represented the major groupings. A and B were subdivisions of I and the lower case Roman numerals i. and ii. were subdivisions of A.

Figure 2.2. Principle coordinate map based on switchgrass AFLP polymorphic markers. The numerals and letters were derived from the UPGMA phenogram. Roman numerals I and II represented the major groupings. The letters A and B were subdivisions of I and the lower case Roman numerals i and ii were subdivisions of A.

Figure 2.3. Example of 1% agarose gel analysis of PCR amplified switchgrass trnL (UAA) intron. Lowland germplasm DNA migrated farther than upland. The controls were Summer (upland) and PI421999 (lowland). TN104 showed a lowland band while it is considered upland germplasm on the basis of morphology.

Figure 2.4. Segment of aligned trnL switchgrass sequences showing the 49 nt deletion in lowland genotypes visualized using Mega (v. 4). The controls were `Summer' (upland) and PI421999 (Lowland). The AR4 and TN104 germplasm were considered upland but lacks the 49 nt segment and Miami was considered lowland and contains the segment.

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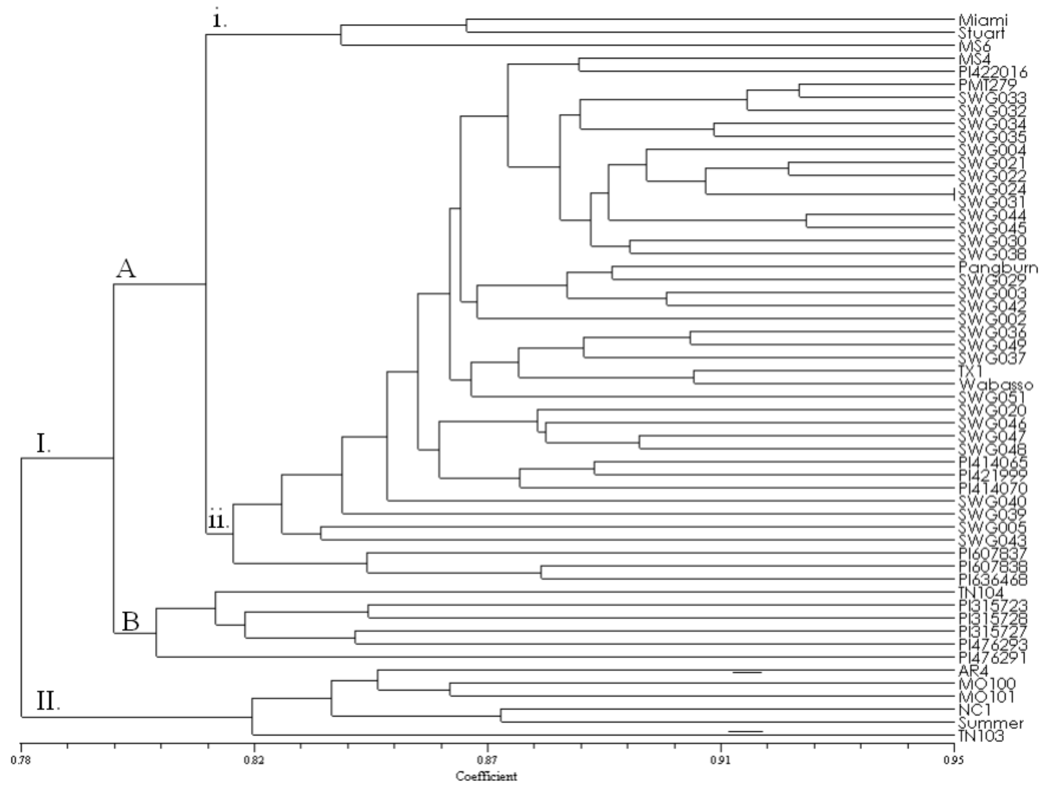

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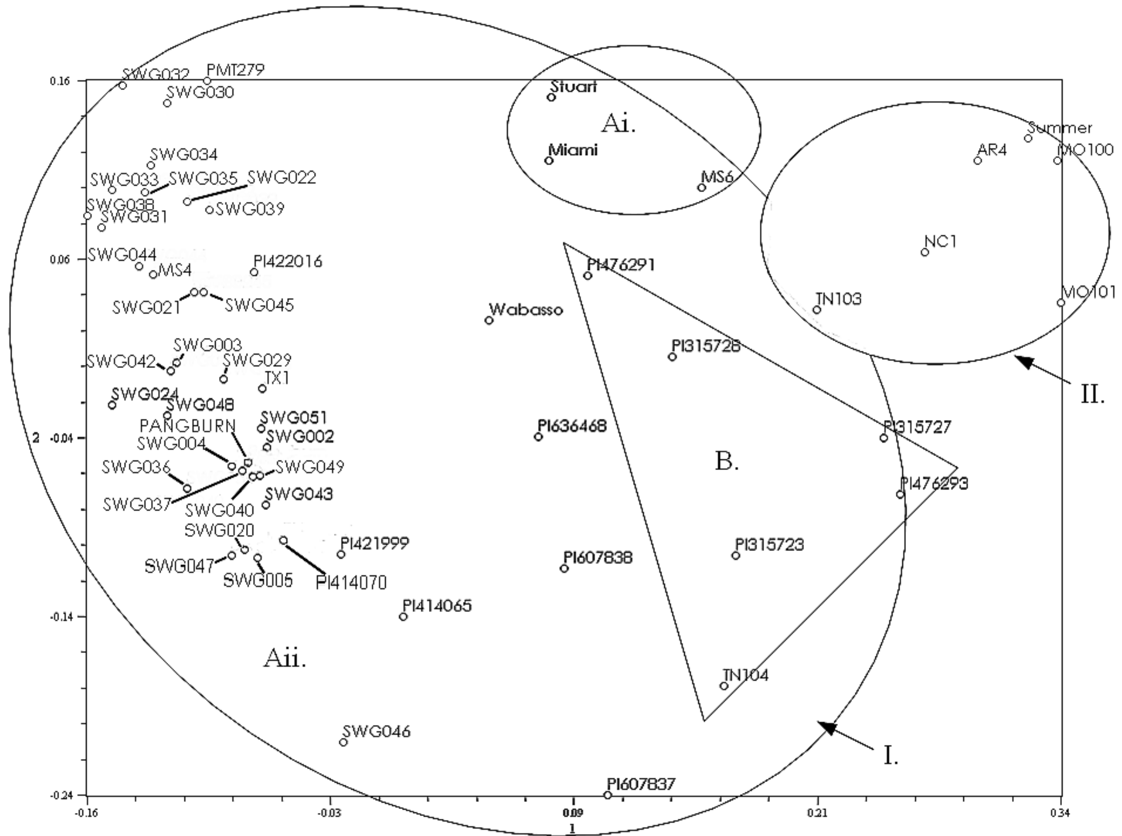

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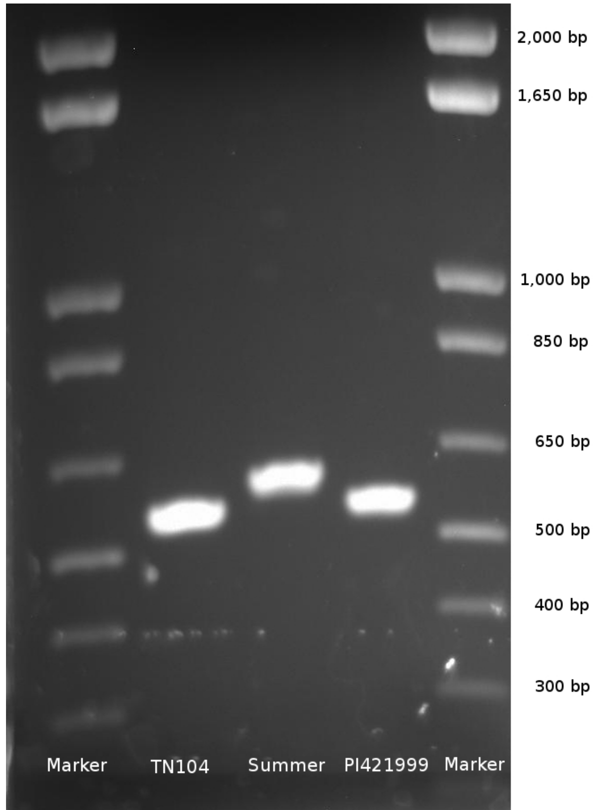

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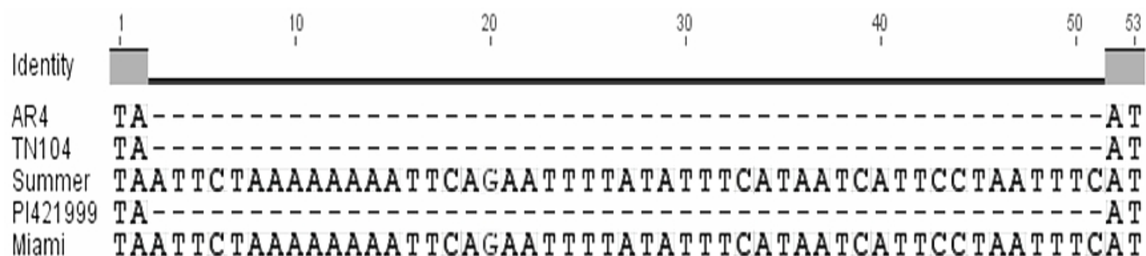

# CHAPTER III

## HERITABILITY OF SWITCHGRASS BIOMASS YIELD IN SWARDS

### ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a perennial warm-season grass native to North America, which has been identified as a dedicated cellulosic biofuel feedstock. Traditional breeding methods are being employed to increase switchgrass yield. Heritability is important in plant breeding for planning the number of years and locations suitable to make adequate selections. Heritability measures the amount of phenotypic variability that is due to genotype variability. Information on heritability and projected gains will aid planning, increase the efficiency of breeding, and assist in decisions of selection type and intensity. Most switchgrass heritability studies used spaced plants, which are not necessarily reliable predictors of sward yields. Sward rows have a different number of plants and different environmental effects than spaced plants, so the heritability estimates could be different. To measure the heritability of switchgrass a parent offspring regression was made with the offspring in one environment (Cimarron Research Station, Perkins OK) and a replicate parent plot in another environment (Agronomy Research Station, Stillwater OK). Both were planted in a randomized complete block design parents with three replications and progeny with four. The variance component method was also utilized to calculate

heritability for offspring data. Heritability by parent offspring regression and variance components was estimated by the REML method using the GLIMMIX procedure in the SAS software package. The drought of 2011 affected the heritability estimates. The variance component estimates per sward plot for 2008-2011 for NL-94 and SL-93 were 0.08 and 0.08, respectively, compared to 2008-2010 which were 0.16 and 0.07, respectively. The per family mean estimates for 2008-2011 for NL-94 and SL-93 were 0.58 and 0.59, respectively, compared to 2008-2010 estimates which were 0.70 and 0.46 respectively. The parent offspring regression for 2010-2011 for NL-94 and SL-93 were 0.10 and 0.31 respectively. The differences in estimates could be caused by drought, removing error or genotype×environment interactions.

## INTRODUCTION

Heritability is the amount of phenotypic variation due to genotypic variation. It can be measured in two ways, broad sense which includes all genetic effects or narrow sense heritability which measures the amount of additive genetic effects. The narrow sense is the most practical because additive effects can be selected for improvement of the next generation. Heritability is important to plant breeders because information on heritability and projected gains will aid planning and increase the efficiency of breeding.

Different agronomical practices affect selection and heritability estimates (Rose et al., 2008). These estimates could help determine which practices, such as planting techniques, optimize heritability and selection. Two common

methods for planting switchgrass are spaced plants and seeded swards. The spaced plant method involves germinating and growing plants in the greenhouse and then planting them equally spaced in the field. The seeded sward row method involves digging a shallow trench and planting seed in it. The spaced plant method is favored by researchers and sward plantings are favored by producers (Casler and Brummer, 2008).

Sward yields are not used as often for research as spaced plants (Casler and Brummer, 2008), but sward plantings have advantages including a more accurate measurement of forage yield than spaced plants (Casler and Brummer, 2008). Commercial plantings are in swards (Missaoui et al., 2005). The genetic composition of sward rows may be different than spaced plants because of selection pressure caused by competition and mortality in the sward row (Casler and Brummer, 2008). Spaced plant yield may not accurately predicted sward yield in some forage crops, and evaluation of spaced plants was ineffective for sward row improvement in fescue (Waldron et al., 2008). Spaced plants do not always correlate with sward yields (Wilkins and Humphreys, 2003; Casler and Brummer, 2008). The spaced plants yields and sward plot yield can be consistent or inconsistent according to species or population. Environmental selection pressures select for different genotypes than spaced plants. Selection within sward populations increases selection intensity due to competition and mortality. Plants that survive in long-term pasture swards are more prostrate, head later, have increased number of tillers and smaller tillers (Casler et al., 1996; Falkner and Casler, 2000; Casler and Brummer, 2008). This may affect yield because Redfearn et al. (1997) found that yield was primarily

affected by tiller growth and development. Vogel and Mitchell (2008) found higher heterosis expressed in the competitive sward environment (Vogel and Mitchell, 2008). In hybrids planted by Vogel and Mitchell (2008) the same plant grown under spaced conditions exhibited less heterosis than the ones planted in sward conditions. This indicates that sward conditions affect the expression of non additive genes. Sward rows affect important developmental modifications including leaf blades, leaf sheaths and stems. The survivorship in sward plots is a component of ground cover because the ability to survive in a sward relates to the ability to cover the ground and fill in gaps in the sward (Casler and Brummer, 2008). If swards have a different heritability than spaced plants heritability, then the information will be important for accurate planning, which could increase breeding progress. If heritability and ΔG estimates are higher in swards than spaced plants then sward selection would be more efficient.

Researchers use spaced plants because spaced plants have several advantages over swards. Using recurrent selection for general combining ability (RSGCA) would require a lot of additional seed, if swards and not spaced plants are used (Hopkins 2005; Rose et al., 2008). Traits can be assayed quicker using spaced plants in RSGCA (Poehlman and Sleper, 1995; Rose et al., 2008). Spaced planted nurseries allow for the calculation of total genetic, including additive and phenotypic variation, within a population (Humphreys 1989; Rose et al., 2008). The performance of spaced and sward rows can be similar depending on the plant, because Humphreys (1989) found similar performance of seeded sward and spaced planted arrangements (Humphreys, 1989; Rose et al., 2008). Burton (1974, 1982) found that selection in spaced plants improved Pensacola

bahiagrass (*Paspalum notatum* var. saure Parodi) for sward growth. Missaoui et al., (2005) found that the yield of switchgrass selected from spaced plants was greater than sward selected plants, but noted that progeny selection from swards would select for plants with tolerance to high densities. Lowland type switchgrass was used in this study because it has a higher biomass yield than upland types in the southern US (Fuentes and Taliaferro, 2002; Bhandari et al. 2010).

There have been several studies on switchgrass heritability. Newell and Eberhart (1961) studied heritability in several populations endemic to Nebraska. The narrow sense adjusted heritabilies estimates calculated from analysis of variance was 0.57 and 0.40 for two populations of switchgrass. Heritability estimates for total yield calculated by parent offspring regession was 0.18±0.06, 0.52±0.22 and 0.05±0.36 for three populations of switchgrass. The heritability on a clone mean basis was 0.42 and 0.45 (Newell and Eberhart, 1961).

Talbert et al. (1983) measured heritability for dry mass weight in 11 lowland populations and found the narrow sense heritability for dry weight was 0.25 on an individual basis and 0.59 on a family basis (Talbert et al., 1983).

Godshalk et al. (1986) studied variance components and heritability for dry mass yield in lowland switchgrass for initial and re-growth. The dry mass narrow sense heritability among half-sib families was 0.52±0.26 and the narrow sense heritability within half sib families was 0.20±0.10 (Godshalk et al., 1986).

Hopkins et al. (1993) studied the effectiveness of recurrent restricted phenotypic selection (RRPS) in improving forage yield and in vitro dry matter digestibility (IVDMD) in upland populations planted in sward rows. The forage yield heritability based on family variance components was negative for open pollinated progenies and was 0.22 for closed pollinated progeny.

Missaoui et al. (2005) studied 30 genotypes of `Alamo' for heritability. The heritability was 0.6 for biomass of individual plants, 0.69 for family means, 0.76 for parent offspring regression in same environment and 0.45 in different environments. Genetic gains from selection on an individual plant basis were 0.51, for half sib family selection it was 0.27, and for half sib progeny test it was 0.55 (Missaoui et al., 2005).

Rose (2005) and Rose et al. (2007, 2008) studied the heritability of spaced plants in two lowland populations `Southern Lowland 93' (SL-93) and `Northern Lowland 94' (NL-94). The original SL-93 population was synthesized in 1993 from `Alamo' and `PMT-279'. From this material two cycles of Restricted Recurrent Phenotypic Selection (RRPS) for increased biomass were performed. The NL-94 population was the result of two cycles of RRPS for biomass yield within `Kanlow' (Rose 2005). Rose et al. (2007) studied the variance component heritabilities of NL-94 grown in two environments; a high yielding environment with fertilizer and irrigation and a low yielding environment that received no inputs. The combined narrow sense heritability for both high and low yielding environments of both $C_0$ protocols was low (0.09), but the heritability was much higher when looking at the environments separately (0.73 for high yielding environment and 0.65 for low

yielding environments) (Rose et al., 2007). Rose et al. (2008) studied the heritability of three populations, one lowland (Southern Lowland '93 (SL-93)) and two upland populations (Southern Northern Upland Early Maturing and Southern Northern Upland Late Maturing (SNU-EM and SNU-LM)). SNU-EM heritability per family mean was 0.45. SNU-EM individual plant heritability was 0.44. SNU-LM per family mean heritability was 0.46 and the SNU-LM individual plant heritability was 0.47 (Rose et al., 2008). For SL-93 the $h^2_n$ estimates based on individual plant and phenotypic family mean from parent offspring regression were 0.13 and 0.12, respectively (Rose et al., 2008).

Bhandari et al. (2010) evaluated 37 half sib families from 2007 to 2009 to measure variation components and heritabilities for dry mass and morphological traits in switchgrass. The dry mass heritability based on variance components per plant from 2007 and 2008 was 0.13 with a standard error of 0.07, and a mean parent offspring regression heritability of 0.29 with a standard error of 0.14.

Boe and Lee (2007) found heritabilities among family means for two upland cultivars, Summer (0.62) and Sunburst 0.60 when harvested over four years.

This study will be similar to Rose et al. (2007-2008) in several ways. It will utilize the same environments (Agronomy Research Station and Perkins Research Station) and similar populations. Rose et al. (2007-2008) used NL-94 and SL-93 $C_1$ and this study uses the $C_2$ form of these populations. Rose used spaced plants for his studies, but in this study the heritability of half sib offspring in swards will be

estimated. If the heritability is much greater in swards then the sward method would be the preferable method to be used for breeding and selection.

## MATERIALS AND METHODS

From SL-93 and NL-94 populations the $C_2$ parents were selected and put in 30 by 34 crossing blocks. The half-sib progeny families were selected phenotypically by each row. There were 110 half sib families from SL-93 and 132 half sib families from NL-94 selected. The 1.0668 m × 3.6576 m seeded swards with ~1 g of seed each plot for NL-94 and SL-93 offspring were planted at Perkins Research Station (35.57°N, 97.01°W) in an RCBD design with four replications. The Perkins soil was Teller loam (fine, loamy, mixed, active, thermic Udic Argiustolls) (Rose et al. 2008). To create unbiased estimates of $h^2_n$, a replicated trial of clonal parents was transplanted into a field at Agronomy Research Station in an RCBD with three replications to remove bias in the calculation of narrow sense heritability (Casler, 1982). At the Agronomy Farm the soil is a Kirkland Silt Loam (fine, mixed, superactive, thermic Udertic Paleustolls)(Rose et al. 2008). Before fertilization, the soil was tested and found to have 6.7 Kg/ha N, 49.3 Kg/ha P, and 406.6 Kg/ha K. To achieve a goal of about 134 Kg of N per hectare and greater than 73 kg of P per hectare, we added 112 kg per hectare of 18-46-0 (Diammonium Phosphate) before planting and 224 Kg per hectare of 46-0-0 (urea) once the plants were established. The half-sib populations were evaluated for four years and the parent clones for two years. The 2008 year replicated trials of the parents were not planted. The parent clonal replicates were planted in 2009 but harvested in

2010 and 2011. Samples from each plot were taken and weighed then dried to measure % dry mass, so the per plot dry weight could be estimated for each replication. For the sward plots, the dry mass % was estimated from 2 replications from 2008-2010 but from only one plot in 2011. Each sward was harvested as one plot per replication so there was not a plant to plant within plot variance and the heritability per individual plant could not be estimated (Holland et al. 2003).

In June 2011 (6-15-2011, 6-16-2011) parent plants were evaluated for smut by the number of heads produced. A scale was made from 0-10 based off of the rough visible plant coverage of seed heads with 0 being no heads and 1 being 1-10% and 2 being 20% and so on.

## Analysis

The yield data were analyzed over years using pseudo-likelihood in the PROC GLIMMIX (SAS Institute, 2008) to calculate the standard error of the variance components with the 95% confidence bounds calculated using estimated likelihood with the estimated likelihood option in covtest (SAS Institute, 2008). The data were calculated using the random effects model. The offspring data were collected from years 2008-2011.

The progeny analysis variance component heritability method was performed using pseudo-likelihood according to following model:

$Y_{ijk} = \mu + \alpha_r + \beta_j + \tau_y + \beta\tau_{jk} + e$

where $\mu$ indicates overall mean of biomass yield;

$\alpha_r$, random effect of replication r;

where r = 1, 2, 3, 4;

$\beta_j$, random effect of plant family (genotype) j;

where for NL-94 j =1, 2, 3…….132 and for SL-93 j = 1, 2, 3,……..110;

$\tau_y$, random effect of year y;

where y = 1, 2, 3, 4;

$\beta\tau_{ry}$, random interaction effect of plant family j and year y

and e, experimental error, with mean 0, variance $\sigma_e{}^2$

(Rose et al. 2007);

The variance component data were log transformed. Two SL-93 numbers (Perkins 2008 rep 1, 15X21; Perkins 2008 rep 1, 15X5) were zero and converted to missing numbers by the SAS log function. These numbers were allowed to be replaced instead of adding 1 for the sake of consistency because adding 1 caused a poor fit in NL-94 offspring plants.

The variance components were calculated using a SAS program derived from Holland et al. (2003) and using formulas from Rose et al. (2007).

Narrow sense heritability via the variance component method was calculated using the following formula:

$$h^2 = \frac{\sigma_F^2}{\sigma_F^2 + \sigma_{FY}^2 + \sigma_e^2}$$

Where

$\sigma_F^2$ =Family variance component

$\sigma_{FY}^2$ = Family by year variance component

$\sigma_e^2$ = Experimental error variance

The narrow-sense heritability on a phenotypic mean basis averaged over replications and years were estimated

$$h_{PFM}^2 = \frac{\sigma_F^2}{\sigma_F^2 + \frac{\sigma_{FY}^2}{y} + \frac{\sigma_\varepsilon^2}{ry}}$$

where

$\sigma_F^2$ =Family variance component

$\sigma_{FY}^2$ = Family by year variance component

$\sigma_\varepsilon^2$ = The residual variance

y = year

r = replication

The formula for predicted gains from the variance component method per family mean was:

$$\Delta G = ckh_{PFM}^2 = ck\frac{\sigma_F^2}{\sigma_{PFM}}$$

Where $\sigma^2{}_{PFM} = \sigma_F^2 + \frac{\sigma_{FY}^2}{y} + \frac{\sigma_\varepsilon^2}{ry}$

Where c= 2

and k=0.736 for a 30% selection(Falconer, 1989)

The c= 2 because the plants were selected according to half-sib progeny selection and to make a new population. The best parents were selected on the

basis of the mean yield performance of their offspring and intermated in isolation (Nguyen and Sleper, 1983).

To calculate the ΔG in terms of percent dry mass, ΔG was divided by the offspring mean (Vogel et al. 1981) from each respective time frame then multiplied times 100.

The parent offspring regression model is the same as the model above with the addition of parent as a covariate where r = 1, 2, 3 and y = 1, 2.

To create a better fit the parent offspring regression data were transformed by exponent transformation plus 1.

The parent offspring regression was calculated using pseudo-likelihood using a program derived from Saxton (2004), and formulas from Rose et al. (2007).

The parent offspring regression was performed with plants from the same year and the regression was performed with parent and offspring data from different years (inverse years) to reduce upward bias caused by genotype×environment interactions (Casler, 1982).

The regression single locus covariance formula:

$$E\left[\hat{b}_{OP}\right] = E\left[\frac{Cov(P,O)}{Var(P)}\right] = \frac{\frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_{AA}^2}{\sigma_P^2} = \frac{1}{2}\ h_1^2$$

(Holland et al., 2003)

The estimates of $h^2_n$ were calculated by the following formula:

$$h_n^2 = 2 \times \beta_1$$

Where $\beta_1$ = the slope of the parent offspring regression

(Rose et al. 2008).

The predicted gains ΔG were calculated by using the formula from Nguyen and Sleeper (1983) for the parent offspring regression.

$$\Delta G = ckh_n^2\sigma_P = ck2\frac{\sigma_{PO}}{\sigma_P^2}$$

Where c= 2

and k=0.736 for a 30% selection(Falconer, 1989)

The parent offspring ΔG was divided by the offspring means of the appropriate time period without rep 4, and then each was multiplied times 100.

Proc Univariate (SAS Institute, 2008) was used to calculate the total variance of the parent to calculate ΔG.

Yield means were calculated using PROC means in SAS (SAS Institute, 2008).

## RESULTS

The mean yield in kg for each year at Stillwater and Perkins is in Table 3.1. The heritability estimates with their standard errors and genetic gain (ΔG) calculated by variance components for each year and combined for NL-94 and SL-93 are in tables 3.2 and 3.3, respectively. The heritability estimates with their standard errors, t and genetic gain (ΔG) values calculated by the parent offspring regression method for 2010 and 2011 individually and combined for NL-94 and SL-93 are in Table 3.4. The variance components and their standard errors and 95% estimated likelihood significance bounds are in Tables 3.5-3.28.

Temperature and precipitation were analyzed to search for patterns that affect switchgrass growth. Table 3.30 contains a list of the monthly average temperatures (◦C) for Stillwater (2011-2010) and table 3.31 has a list of monthly temperatures (◦C) for Perkins (2008-2011). The average min and max temperatures for the growing season of 2008 at Perkins were15.65 ◦C and 28.24 ◦C, respectively. In 2009 the min and max were 16.39 ◦C to 28.52 ◦C and 2010 the min and max 18.15 ◦C to 29.91 ◦C. The temperatures in 2011 were higher, and the min and max temperatures were 18.15◦C and 32.41◦C, respectively. The precipitation was also similar. It was 62.69 cm. in 2008, 60.38 cm. in 2009, and 60.53 cm. in 2010. The precipitation in 2011 was different and was only 25.09 cm. The min and max temperatures in Stillwater in 2010 were 18.06 and 29.72 and the min and max were 17.69 and 32.32 in 2011. The precipitation in Stillwater was also lower in 2011 (19.03 cm.) compared to in 2010 (65.81 cm.).

## DISCUSSION

The heritability calculated by the variance component method was only measured in one environment, so it could be influenced by family×location and family×location×environment interactions (Godshalk et al., 1986). Location is an important source of variation (Redfearn et al., 1997; Bhandari et al., 2010) and several other studies found that year, location and their interaction are highly significant (Newell and Eberhart, 1961; Redfearn et al., 1997; Fuentes and Taliaferro, 2002; Boe and Lee, 2007; Bhandari et al., 2010). Multiple year analysis removes bias caused by family×year interactions from the heritability estimate and is important because this interaction was highly significant in switchgrass in

several studies (Newell and Eberhart, 1961; Redfearn et al., 1997; Fuentes and Taliaferro, 2002; Boe and Lee, 2007; Bhandari et al., 2010).

There are other (than swards) differences between this study and Rose et al. (2007 and 2008). Rose et al. (2007) harvested for three years for his variance component analysis and this study used four years. Rose et al. (2008) used $C_1$ plants for his analysis (Rose, 2005) and this study $C_2$. Rose provided regression estimates based off inverse year and in this study none of the inverse year regression estimates were significant. The year conditions were also different. There is a difference between our method of this paper and that of Rose's. We measured plants on a per plot basis and Rose's estimate was based on individual plants, but both measured heritability based on a family mean basis.

The regression inverse year analysis for NL-94 was positive and sometimes more than the same year estimates (Tables 3.4), but none of inverse year estimates were significant (α=0.05). The SL-93 regression with inverse years estimates were negative and not significant (α=0.05). The difference between the same year and inverse year estimates could be due to large differences in yield due to drought conditions.

In June 2011 Smut (*Tilletia maclaganii*) was noticed on the parent plot on the Agronomy farm. Smut infects the switchgrass plants and causes them to flower early, so the seeds will be replaced by fungal sori (Thomsen et al., 2008). Thomsen et al. (2008) recorded a negative relationship between infection and biomass yield. This probably affected yield of the parent in 2011. This increases

the environmental variation due to environment between the parent and progeny plots, but did not affect the variance component methods.

There were drought conditions in 2011. The min and max growing season (April-September) temperatures for the years of 2008, 2009 and 2010 were similar, but temperatures in 2011 were higher. The precipitation was much lower in 2011 also. Biomass yield was lower in 2011 compared to the other years (Table 3.1). The climatic conditions and their soil interactions could affect dry mass yield (Bhandari et al. 2010). Bhandari et al. (2010) hypothesized that strong soil and precipitation differences could have a greater effect on year×family and year×location interactions (Bhandari et al., 2010). Rose et al. (2008) noted that weather differences among years can change the yield ranks of the Half-sib families. Within each switchgrass population in this study there is only one year×family variance component for NL-94 in 2010-2011 in the parent offspring regression. This indicates that year×family interactions in NL-94 were caused by drought conditions or smut. The drought conditions did affect the heritability estimates though. With the addition of the 2011 data the sward heritability of NL-94 was dramatically lower; almost half of what it was without the 2011 data. The NL-94 estimates from variance components indicated that the NL-94 had a higher heritability before 2011 than SL-93. The variance component heritability of 2011 alone was not calculable because the family variance component was zero. The heritability of NL-94 variance component heritability in 2011 was zero, but just the opposite occurred in SL-93 the heritability was almost double. This indicates that drought decreases heritability in NL-94 and increases it in SL93 using the variance component methods. This could be caused by the removal of

some family×location bias by the drought environment in 2011. It is interesting to note that the SL-93 variance heritabilities from 2008 and 2009 had higher standard errors than heritability estimates, and therefore these estimates alone are not reliable.

The different reactions between the SL-93 and NL-94 to the drought conditions in 2011 could be due to genotype by environment interactions between SL-93 and NL-94 populations and these differences might be worth investigating in the future.

The parent offspring regression was different. The heritability of NL-94 did increase in 2011, which could be because it did not have the bias that the variance component method had or it could be because of the influence of year×family interactions caused by the smut or drought. The average smut infection for SL-93 was higher (4.64) than NL-94 (2.36) and this difference was significant at α<.0001. There was a year×family variance component in the 2010-2011 NL-94 regression calculation. The SL-93 2010-2011did not have the year×family variance component, but the heritability of SL-93 in 2011 was lower. The parent offspring regression heritability was only significant for NL-94 in 2011 (α=0.1), but not for NL-94 in 2010 or the combined 2010-2011analysis. SL-93 was not significant in 2011, but significant in 2010 (α=0.1) alone or the combined 2010-2011 analysis (α=0.01) (Table 3.3), but the family variance component for the SL-93 2010-2011 analysis had a standard error larger than the estimate (Table 3.19). This was also true for the NL-94 2010-2011 family covariance estimate (Table 3.7) and the NL-94 2011 family covariance estimate (Table 3.6).

The heritability estimates for SL-93 from the parent offspring regression (0.31) was larger than all the per plot variance components estimates, but less than the variance component per family mean estimates. The estimates for NL-94 for the per plot variance method and the parent offspring regression were similar. Only the 2011 estimate (0.1619) was significant and was similar to the per plot 2008-2010 estimates, but not in 2011. The family mean estimates were greater than the parent offspring regression estimates. Since the 2011 regression estimate was only tested in one year it could be biased by genotype by environment interactions.

The parent offspring regression estimates for heritability of NL-94 were similar (0.1012) to the parent offspring regression results for SL-93 (0.13) for individual plants from Rose et al. 2007, but the SL-93 result in this study was twice as large (0.31).

The family mean variance components heritability for southern lowland and northern lowland across years (Tables 3.2, 3.3) are similar to family variance component heritability in Rose et al. (2007) in one environment (0.62-0.76), which makes sense because both studies are in one environment. This indicates that sward row heritability is not that much different from spaced plant heritability for NL-94 in one environment, but Rose et al. (2007) per family mean estimates from combined environments was much less (0.09) than the combined NL-94 2008-2011 result (0.58).

The individual plant heritability estimates of Rose et al. (2007 and 2008) from parent offspring regression and variance component method was based

on single plants, but the estimates in this study are based on per plot harvested. Individual plant analysis was not possible due to the nature of sward rows.

The per plot variance component heritability estimates of this study were less than Talbert et al. (1983) (0.5) and Godshalk et al. (1986)(0.52) who also created per plot heritability estimates like this study. Godshalk et al. (1986) and Talbert et al. (1983) evaluated the plants for two years. The family mean estimate for NL-94 2008-2010 (0.58) and SL-93 2008-2010 (0.59) were similar to per family mean heritability estimates (0.60, 0.62) in upland cultivars by Boe and Lee (2007). Hopkins et al. (1993) made a family mean heritability estimate (0.22) based off of sward forage yield and three years data. It was less than both the 2008-2011 year family mean estimates in this study (0.58, 0.59).

The parent offspring regression result for SL-93 (2010-2011)(0.31) is similar to parent offspring regression of Bandari et al. 2010 (0.29) in another lowland population.

The SL-93 and NL-94 family variance components, which represent the additive portion of the variance, from the parent offspring regression and variance component method fell within the 95% confidence bounds and were significant with the exception of NL-94 2010-2011 parent offspring regression estimate. This estimate (0.000396) with a standard error (0.00133) includes negative figures and the estimate also has a lower bound of 0 which is not significantly different than 0 (Chisq p = 0.6712). This throws the accuracy of this heritability estimate into question. Predicted gains based off of parent offspring regression are considered less reliable than those base on variance components

if the heritability estimates of the parent offspring regression are not significantly different from zero (Rose, 2005 ).

The year*family variance from NL-94 2010- 2011 fell between the upper and lower bounds for 95% confidence even though the lower bound (0) is off a little (chisq p=0.0511). This interaction could be caused by the smut that was in the parent population but not in the offspring.

There are morphological differences between spaced and sward plantings (Casler and Brummer, 2008), and these morphological traits could cause differences between heritability of swards and spaced plants (Redfearn et al., 1997). Bhandari et al. (2010) demonstrated that the biomass yield was positively correlated with tillering ability, and these traits are affected by the sward environment (Casler and Brummer, 2008).

It is difficult to discern if the differences in heritability are caused by the sward environment, different years, or genetic differences ($C_1$ vs. $C_2$). Even though most of the heritability estimates are higher than Rose's, it is difficult to discern if the sward environment has distinct advantage for heritability selection, because some of the estimates are similar to Rose's and most of the heritability estimates are similar to those of other space planted studies (Bandari et al. 2010).

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Table 3.1. Mean SL-93 and NL-94 switchgrass dry mass yield in kg for each year (2008-2011).

| | 2008 | | 2009 | | 2010 | | 2011 | | 2008-2011 | | 2008-2010 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Prog. | μ† | SD‡ | μ† | SD‡ | μ† | SD‡ | μ† | SD‡ | μ† | SD‡ | μ† | SD‡ |
| SL-93 | 0.91 | 0.27 | 1.48 | 0.36 | 1.92 | 0.43 | 0.82 | 0.27 | 1.28 | 0.56 | 1.43 | 0.55 |
| NL-94 | 1.05 | 0.24 | 1.92 | 0.57 | 2.49 | 0.58 | 1.12 | 0.35 | 1.64 | 0.75 | 1.82 | 0.77 |
| Prog. | | | | | | | | | 2010-2011 | | | |
| SL-93 | | | | | | | | | 1.37 | 0.66 | | |
| NL-94 | | | | | | | | | 1.80 | 0.84 | | |
| Parent SL-93 | - | - | - | - | 0.96 | 0.45 | 0.65 | 0.30 | 0.80 | 0.41 | | |
| Parent NL-94 | - | - | - | - | 0.90 | 0.42 | 0.66 | 0.30 | 0.78 | 0.38 | | |

†Mean

‡Standard deviation

Table 3.2. Variance component narrow sense heritability estimates of NL-94 Switchgrass planted in swards with standard errors and predicted gain estimates.

| Year | Per Plot | SE† | Family mean | SE† | $\Delta G_{PFM}$ | % gain |
|---|---|---|---|---|---|---|
| 2008 | 0.07 | 0.04 | 0.24 | 0.11 | 0.04 | 3.97 |
| 2009 | 0.13 | 0.04 | 0.38 | 0.09 | 0.06 | 3.28 |
| 2010 | 0.19 | 0.05 | 0.49 | 0.07 | 0.09 | 3.78 |
| 2011 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2008-2010 | 0.16 | 0.02 | 0.70 | 0.04 | 0.10 | 5.63 |
| 2008-2011 | 0.08 | 0.02 | 0.58 | 0.05 | 0.07 | 4.43 |

†Standard error

Table 3.3. Variance component narrow sense heritability estimates of SL-93 Switchgrass planted in swards with standard errors and predicted gain estimates.

| Year | Per Plot | SE† | Family mean | SE† | $\Delta G_{PFM}$ | % Gain |
|---|---|---|---|---|---|---|
| 2008 | 0.03 | 0.04 | 0.12 | 0.14 | 0.03 | 2.79 |
| 2009 | 0.03 | 0.04 | 0.11 | 0.14 | 0.02 | 1.39 |
| 2010 | 0.07 | 0.04 | 0.23 | 0.12 | 0.04 | 2.02 |
| 2011 | 0.14 | 0.05 | 0.40 | 0.09 | 0.11 | 13.42 |
| 2008-2010 | 0.07 | 0.02 | 0.46 | 0.08 | 0.06 | 4.49 |
| 2008-2011 | 0.08 | 0.02 | 0.59 | 0.06 | 0.09 | 6.71 |

†Standard error

Table 3.4. Parent offspring regression narrow sense heritability estimates for SL-93 and NL-94 Switchgrass planted in swards with standard errors and predicted gain estimates.

| Year | Heritability | SE† | t‡ | P>t§ | ΔG¶ | % Gain# |
|---|---|---|---|---|---|---|
| NL-94 | | | | | | |
| 2010 | 0.03 | 0.08 | 0.40 | 0.69 | 0.01 | 0.57 |
| 2011 | 0.16 | 0.09 | 1.81 | 0.07 | 0.05 | 4.49 |
| 2010-2011 | 0.10 | 0.06 | 1.59 | 0.11 | 0.04 | 2.15 |
| Parent 2010 Prog. 2011 | 0.180 | 0.40 | 0.44 | 0.66 | 0.07 | 6.66 |
| Parent 2011 Prog. 2010 | 0.02 | 0.53 | 0.04 | 0.97 | 0.01 | 0.29 |
| Inverse years | 0.06 | 0.33 | 0.18 | 0.86 | 0.02 | 1.27 |
| SL-93 | | | | | | |
| 2010 | 0.24 | 0.14 | 1.70 | 0.09 | 0.10 | 18.49 |
| 2011 | 0.07 | 0.18 | 0.37 | 0.71 | 0.02 | 11.98 |
| 2010-2011 | 0.31 | 0.10 | 3.17 | 0.002 | 0.12 | 32.95 |
| Parent 2010 Prog. 2011 | -0.03 | 0.44 | -0.07 | 0.95 | -0.01 | -1.54 |
| Parent 2011 Prog. 2010 | -0.02 | 0.65 | -0.04 | 0.97 | -0.01 | -0.35 |
| Inverse years | -0.03 | 0.37 | -0.08 | 0.94 | -0.01 | -0.85 |

†Standard error

‡t score for t distribution

§p-score for significance of t

¶Predicted gains estimate

# Percent predicted gain

Table 3.5. NL-94 switchgrass 2010 parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Rep | 0.000041 | 0.000351 | 0 | 0.8986 | 0.005831 | 0.05 |
| Family | 0.006308 | 0.002712 | 0.002202 | 0.05 | 0.01199 | 0.05 |
| Residual | 0.04028 | 0.003522 | 0.03463 | 0.05 | 0.04713 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.6. NL-94 switchgrass 2011 parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Rep | 0.01004 | 0.01028 | 0.002091 | 0.05 | 0.18 | 0.05 |
| Family | 0.000447 | 0.001572 | 0 | 0.7238 | 0.003474 | 0.05 |
| Residual | 0.0302 | 0.002641 | 0.02628 | 0.05 | 0.03492 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.7. NL-94 switchgrass 2010-2011 parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Standard Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.213 | 0.3014 | 0.03145 | 0.05 | 26.7822 | 0.05 |
| Rep | 0.002538 | 0.002694 | 0.000455 | 0.05 | 0.04706 | 0.05 |
| Family | 0.000396 | 0.00133 | 0 | 0.6712 | 0.002638 | 0.05 |
| Y*Family | 0.002323 | 0.001988 | 0 | 0.0511 | 0.005256 | 0.05 |
| Residual | 0.03748 | 0.002312 | 0.0338 | 0.05 | 0.04167 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.8. NL-94 switchgrass 2010 parent on to 2011 progeny parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Rep | 0.01117 | 0.01143 | 0.002335 | 0.05 | 0.2 | 0.05 |
| Family | 0.0004 | 0.001555 | 0 | 0.7497 | 0.00339 | 0.05 |
| Residual | 0.02999 | 0.002621 | 0.0261 | 0.05 | 0.03467 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.9. NL-94 switchgrass 2011 parent on to 2010 progeny parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower | | Upper | |
|---|---|---|---|---|---|---|
| | | | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Rep | 0.000031 | 0.00035 | 0 | 0.9255 | 0.005818 | 0.05 |
| Family | 0.006277 | 0.002711 | 0.002173 | 0.05 | 0.01195 | 0.05 |
| Residual | 0.04032 | 0.003525 | 0.03467 | 0.05 | 0.04718 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.10. NL-94 switchgrass inverse year 2010 and 2011combined parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.2125 | 0.2894 | 0.03262 | 0.05 | 27.8013 | 0.05 |
| Rep | 0.00236 | 0.002517 | 0.000415 | 0.05 | 0.04395 | 0.05 |
| Family | 0.000455 | 0.001325 | 0 | 0.6236 | 0.002698 | 0.05 |
| Y*Family | 0.002142 | 0.001975 | 0 | 0.0731 | 0.005064 | 0.05 |
| Residual | 0.03767 | 0.002323 | 0.03399 | 0.05 | 0.04188 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.11. NL-94 switchgrass 2008 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0 | . | 0 | 1 | 0.0001 | 1 |
| Family | 0.003319 | 0.001845 | 0.000478 | 0.05 | 0.007247 | 0.05 |
| Rep | 0.001862 | 0.001777 | 0.0003 | 0.05 | 0.01867 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.003927 | 0.05 |
| Residual | 0.04145 | 0.002957 | 0.03655 | 0.05 | 0.04723 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.12. NL-94 switchgrass 2009 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0 | . | 0 | 1 | . | . |
| Family | 0.004803 | 0.001659 | 0.002178 | 0.05 | 0.008432 | 0.05 |
| Rep | 0.01746 | 0.01445 | 0.004754 | 0.05 | 0.1541 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.003629 | 0.05 |
| Residual | 0.03135 | 0.002237 | 0.02755 | 0.05 | 0.03585 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.13. NL-94 switchgrass 2010 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0 | . | 0 | 1 | . | . |
| Family | 0.008418 | 0.002236 | 0.004816 | 0.05 | 0.0134 | 0.05 |
| Rep | 0.004429 | 0.003837 | 0.001055 | 0.05 | 0.04072 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.004979 | 0.05 |
| Residual | 0.03571 | 0.002548 | 0.03131 | 0.05 | 0.04094 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.14. NL-94 switchgrass 2011 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower | | Upper | |
|---|---|---|---|---|---|---|
| | | | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Year | 0 | . | 0 | 1 | . | . |
| Family | 0 | . | 0 | 1 | 0.005023 | 0.05 |
| Rep | 0.01372 | 0.01166 | 0.003466 | 0.05 | 0.124 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.005023 | 0.05 |
| Residual | 0.07424 | 0.004587 | 0.06593 | 0.05 | 0.08401 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.15. NL-94 switchgrass 2008-2011 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.1787 | 0.146 | 0.05031 | 0.05 | 1.559 | 0.05 |
| Family | 0.004233 | 0.000908 | 0.002716 | 0.05 | 0.00633 | 0.05 |
| Rep | 0.004655 | 0.003877 | 0.001246 | 0.05 | 0.04132 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.000872 | 0.05 |
| Residual | 0.04913 | 0.001564 | 0.04621 | 0.05 | 0.05231 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.16. NL-94 switchgrass 2008-2010 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.1933 | 0.1934 | 0.04386 | 0.0500 | 3.3886 | 0.05 |
| Family | 0.006959 | 0.001239 | 0.004885 | 0.05 | 0.009825 | 0.05 |
| Rep | 0.005808 | 0.004817 | 0.001572 | 0.05 | 0.05135 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.000513 | 0.05 |
| Residual | 0.03632 | 0.00135 | 0.03381 | 0.05 | 0.03909 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.17. SL-93 switchgrass 2010 parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Rep | 0.009135 | 0.009838 | 0.001532 | 0.05 | 0.1717 | 0.05 |
| Family | 0.07688 | 0.01301 | 0.05551 | 0.05 | 0.1074 | 0.05 |
| Residual | 0.05432 | 0.005234 | 0.04529 | 0.05 | 0.06586 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.18. SL-93 switchgrass 2011 parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower | | Upper | |
|---|---|---|---|---|---|---|
| | | | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Rep | 0.000758 | 0.001249 | 0 | 0.2686 | 0.0214 | 0.05 |
| Family | 0.06902 | 0.01181 | 0.04964 | 0.05 | 0.09666 | 0.05 |
| Residual | 0.05115 | 0.004918 | 0.04268 | 0.05 | 0.06198 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.19. SL-93 switchgrass 2010-2011 parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.000138 | 0.000496 | 0 | 0.6982 | 0.04366 | 0.05 |
| Rep | 0.003547 | 0.003837 | 0.000591 | 0.05 | 0.06673 | 0.05 |
| Family | 0.074 | 0.01124 | 0.0554 | 0.05 | 0.1005 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.004052 | 0.05 |
| Residual | 0.05252 | 0.003186 | 0.04675 | 0.05 | 0.05928 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.20. SL-93 switchgrass 2010 parent on to 2011 progeny parent offspring regression variance components with 95% confidence bounds.

| | | | Lower | | Upper | |
|---|---|---|---|---|---|---|
| Cov Parm† | Estimate | Error | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Rep | 0.001419 | 0.001703 | 0.000102 | 0.05 | 0.02957 | 0.05 |
| Family | 0 | . | 0 | 1 | 0.002995 | 0.05 |
| Residual | 0.02326 | 0.002115 | 0.01957 | 0.05 | 0.02795 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.21. SL-93 switchgrass 2011 parent on to 2010 progeny parent offspring regression variance components with 95% confidence bounds.

| | | | Lower | | Upper | |
|---|---|---|---|---|---|---|
| Cov Parm† | Estimate | Error | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Rep | 0.001119 | 0.001556 | 0 | 0.1075 | 0.02682 | 0.05 |
| Family | 0.000528 | 0.002426 | 0 | 0.7855 | 0.0054 | 0.05 |
| Residual | 0.036 | 0.004026 | 0.03021 | 0.05 | 0.04333 | 0.05 |

†Covariance parameter

‡Tests boundary = 0

Table 3.22. SL-93 switchgrass inverse year 2010 and 2011combined parent offspring regression variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.1807 | 0.2499 | 0.02727 | 0.05 | 23.2621 | 0.05 |
| Rep | 0.001304 | 0.001486 | 0.000156 | 0.05 | 0.0258 | 0.05 |
| Family | 0.000257 | 0.001393 | 0 | 0.8193 | 0.002858 | 0.05 |
| Y*Family | 6.24E-23 | . | 6.24E-23 | 1 | 0.002593 | 0.05 |
| Residual | 0.02957 | 0.002333 | 0.02612 | 0.05 | 0.03364 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.23. SL-93 switchgrass 2008 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0 | . | 0 | 1 | 7.08E+10 | . |
| Family | 0.002564 | 0.003406 | 0 | 0.3591 | 0.009731 | 0.05 |
| Rep | 0.02001 | 0.01693 | 0.005127 | 0.05 | 0.1801 | 0.05 |
| Y*Family | 0.000041 | . | 0 | 0.9893 | 0.007208 | 0.05 |
| Residual | 0.07858 | 0.006179 | 0.06869 | 0.05 | 0.0904 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.24. SL-93 switchgrass 2009 offspring heritability variance components with 95% confidence bounds.

| | | | Lower | | Upper | |
|---|---|---|---|---|---|---|
| Cov Parm† | Estimate | Error | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Year | 0 | . | 0 | 1 | . | . |
| Family | 0.001738 | 0.002348 | 0 | 0.3681 | 0.006676 | 0.05 |
| Rep | 0.00348 | 0.003248 | 0.000624 | 0.05 | 0.03419 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.004939 | 0.05 |
| Residual | 0.05476 | 0.004283 | 0.04791 | 0.05 | 0.06294 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.25. SL-93 switchgrass 2010 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower | | Upper | |
|---|---|---|---|---|---|---|
| | | | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Year | 0 | . | 0 | 1 | . | . |
| Family | 0.00303 | 0.001975 | 0.00004 | 0.05 | 0.007294 | 0.05 |
| Rep | 0.001495 | 0.001527 | 0.000153 | 0.05 | 0.01593 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.004264 | 0.05 |
| Residual | 0.04115 | 0.003218 | 0.03587 | 0.05 | 0.04746 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.26. SL-93 switchgrass 2011 offspring heritability variance components with 95% confidence bounds.

| | | | Lower | | Upper | |
|---|---|---|---|---|---|---|
| Cov Parm† | Estimate | Error | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Year | 0 | . | 0 | 1 | 7.31E+10 | . |
| Family | 0.01375 | 0.004885 | 0.006099 | 0.05 | 0.02466 | 0.05 |
| Rep | 0.004374 | 0.004175 | 0.000703 | 0.05 | 0.04386 | 0.05 |
| Y*Family | 0.000023 | . | 0 | 0.996 | 0.01093 | 0.05 |
| Residual | 0.08117 | 0.006353 | 0.07041 | 0.05 | 0.09411 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.27. SL-93 switchgrass 2008-2011 offspring heritability variance components with 95% confidence bounds.

| Cov Parm† | Estimate | Error | Lower Bound | Lower Chisq Pr >‡ | Upper Bound | Upper Chisq Pr >‡ |
|---|---|---|---|---|---|---|
| Year | 0.1721 | 0.1407 | 0.04840 | 0.0500 | 1.5029 | 0.05 |
| Family | 0.005803 | 0.001285 | 0.003624 | 0.05 | 0.008665 | 0.05 |
| Rep | 0.003996 | 0.003385 | 0.001019 | 0.05 | 0.03601 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.001473 | 0.05 |
| Residual | 0.06558 | 0.002335 | 0.06127 | 0.05 | 0.07031 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.28. SL-93 switchgrass 2008-2010 offspring heritability variance components with 95% confidence bounds.

| | | | Lower | | Upper | |
|---|---|---|---|---|---|---|
| Cov Parm† | Estimate | Error | Bound | Chisq Pr >‡ | Bound | Chisq Pr >‡ |
| Year | 0.1514 | 0.1516 | 0.03426 | 0.05 | 2.6564 | 0.05 |
| Family | 0.004166 | 0.001321 | 0.001968 | 0.05 | 0.007032 | 0.05 |
| Rep | 0.004948 | 0.004186 | 0.001267 | 0.05 | 0.04454 | 0.05 |
| Y*Family | 0 | . | 0 | 1 | 0.001154 | 0.05 |
| Residual | 0.05885 | 0.002485 | 0.05438 | 0.05 | 0.0638 | 0.05 |

† Covariance parameter

‡Tests boundary = 0

Table 3.29. Stillwater average monthly temperatures °C for 2010 and 2011.

| | 2010 | | 2011 | |
|---|---|---|---|---|
| Month | min | max | min | max |
| April | 11.11 | 22.78 | 8.89 | 25.00 |
| May | 13.33 | 25.56 | 13.89 | 26.11 |
| June | 22.22 | 31.67 | 21.67 | 35.56 |
| July | 22.78 | 33.33 | 25.00 | 39.44 |
| August | 21.67 | 35.00 | 23.33 | 38.89 |
| September | 17.22 | 30.00 | 13.33 | 28.89 |
| Average | 18.06 | 29.72 | 17.69 | 32.31 |

Table 3.30. Perkins average monthly temperatures °C for 2008-2011.

| | 2008 | | 2009 | | 2010 | | 2011 | |
|---|---|---|---|---|---|---|---|---|
| Month | min | max | min | max | min | max | min | max |
| April | 7.78 | 21.11 | 7.78 | 21.11 | 10.56 | 22.22 | 10.00 | 25.00 |
| May | 14.44 | 27.22 | 13.33 | 24.44 | 13.89 | 26.11 | 14.44 | 26.11 |
| June | 20.00 | 31.11 | 20.56 | 33.33 | 21.67 | 32.22 | 21.67 | 36.11 |
| July | 21.67 | 33.89 | 20.56 | 33.89 | 22.78 | 33.33 | 25.00 | 40.00 |
| August | 21.11 | 32.78 | 20.00 | 31.67 | 22.22 | 35.56 | 23.89 | 38.33 |
| September | 8.89 | 23.33 | 16.11 | 26.67 | 17.78 | 30.00 | 13.89 | 28.89 |
| Average | 15.65 | 28.24 | 16.39 | 28.52 | 18.15 | 29.91 | 18.15 | 32.41 |

CHAPTER IV

SWITCHGRASS SELFING CONFIRMED BY SSR MARKERS

ABSTRACT

Switchgrass is an allogamous, self-incompatible species currently being bred for biomass as a biofuel feedstock. Genotypic recurrent restricted selection for general combining ability has been used to improve switchgrass yield. This method capitalizes on additive gene effects but neglects non-additive genes. Inbreeding and hybridization have greatly improved the yield of major crops, such as maize (*Zea mays*), sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*). Hybridizing inbreds may have the potential to improve yield in switchgrass. The objective of this study was to investigate seed yield of selfing lowland switchgrass plants by bagging, and confirm selfed progeny by SSR markers. In 2008, 33 $S_1$ Alamo and 33 $S_1$ Kanlow switchgrass plants were bagged using two bag types: paper Lawson bags, and microfiber bags. In 2009 the same plants were bagged with larger cotton muslin bags. In 2010 the plants were bagged with all three bag types. Seeds were collected and counted. In the 2008 harvest, a total of 304 Alamo seeds were collected from which 100 progenies were grown and a total of 321 Kanlow seeds collected from which 91 progenies were grown. From the 2009 plants a total of 498 Alamo seeds were harvested from which 123 progenies

were grown, and a total of 1231 Kanlow seeds from which 267 progenies were grown. In 2010 a total of 301 Alamo seeds were harvested from which 26 progenies were grown and 263 Kanlow seeds were collected from which 17 progenies were grown. Bag by cultivar interaction was significant in 2008 (a=0.05) and bag type was significant in 2010 (a=0.1). DNA was extracted from the respective parents and the progeny of every year. Six SSR primer pairs were used to confirm selfed progeny identity by determining if all progeny alleles matched its maternal parent. Of the 2008 Kanlow offspring 23 were inbreds (25.3%) and of Alamo 33, were inbreds (33%). Of the 2009 Alamo plants, 24 (23.07%) were inbred and 133 (82.1%) Kanlow plants were inbred. Of the 2010 Alamo plants 10 were inbred (38.56%), and of the 2010 Kanlow plants, 5 plants were inbred (31.25%)

## INTRODUCTION

Switchgrass is a self incompatible cross-pollinated species (Martinez-Reyna and Vogel, 2002), but switchgrass will set some seed if selfed (Newell, 1936) and Taliaferro and Hopkins (1996) observed a selfing rate of about 1-2%. To determine switchgrasses' selfing potential Taliaferro (2002) planted the first generation selfed families from Blackwell, Caddo, Cave-in-Rock, Kanlow, and Alamo in 2000. Three plants from each of the 15 $S_1$ families were selected based on visual assessment and were selfed and bagged in 2001. Of these, 13 $S_1$ plants produced 20 or more seeds and 7 $S_1$ plants had more than 100 $S_1$ seeds. This high number of seed indicates high inbreeding potential of the $S_1$ (Taliaferro, 2002). These inbreeding studies did not confirm results using molecular markers. To

understand the effects of bagging and confirm selfing the inbreeding research will be continued by enforcing selfing using different bag types. Switchgrass has been improved by traditional methods for forage and biofuel including ecotype selection, recurrent restricted selection (RRPS) and recurrent selection for general combining ability (Vogel and Gabrielson, 1986; Burton, 1974; Vogel et al., 1991; Taliaferro, 2002). With the exception of recurrent selection for general combining ability these methods had limited success because environmental effects affected selection ability (Taliaferro, 2002), and all these methods select for additive genes. But hybridization can employ non additive genes which could speed up improvement (Martinz-Reyna, 1998); for instance, Vogel and Mitchell (2008) had 30-38% heterosis from sward hybrids of 'Summer' and 'Kanlow'.

Okada et al. (2010) indicated that switchgrass has near disomic inheritance, so for each inbred generation switchgrass's homozogosity will increase 50% (Husband and Schemske, 1997). For switchgrass to reach 98.44% homozygous would require 6 generations (Husband and Schemske, 1997).

Microsatellite markers also called Simple Sequence Repeats (SSRs) are good for homozygosity measurements because they are highly abundant, highly polymorphic, somatically stable, and inherited in a co-dominant manner (Morgante and Olivieri, 1993). In each selfed generation the SSR banding pattern will be compared to the maternal parent. The offspring should have fewer heterozygous bands than the parent. Null alleles could bias the data because heterozygotes could be mislabeled as homozygotes if there are null alleles in the

switchgrass population. A 'null' allele is found in the parents but not inherited in their offspring (Callen et al., 1993).

## Objectives

To quantify the production of $S_2$ progeny from two switchgrass $S_1$ populations, Alamo and Kanlow, using different bagging methods and confirm progeny selfing by comparing the progeny alleles to their maternal parent using SSR markers.

## MATERIALS AND METHODS

In 2008, 33 of the 'Alamo' and 33 of the 'Kanlow' $S_1$ populations planted in 2001 were selfed by bagging in August using a combination of microfiber and paper bags (Lawson 17.1 cm ×15.9 cm ×12.1 cm × 39.4 cm No. GB504) attached to poles with 2-3 heads per bag. The microfiber bags were inherited from another study and records about their dimensions, manufacturer and pore size etc. were lost, but the bags were measured to be 22 cm wide and 45 cm long. In 2009 the same $S_1$ populations were bagged using chicken wire covered with a pillow case (~2 heads per bag) attached to two t-posts. In 2010 the same $S_1$ genotype plants were bagged with pillowcase chicken wire cages, paper bags and fabric bags (1 head per bag) and one per plant (except microfiber which had fewer bags) to compare how bag type affects seed yield in the same year.

## Bag methods

The paper bag was attached to the pole by sticking a wire through the bottom near both of the two corners at the opening of the bag. The bag is placed over the head with the wire portion of the bag away from the stem (toward the pole) and the ends of the wire which pierce the bag are coming out away from the pole but then pulled back and tied around the pole. Then the bag internal space was enlarged by pulling the bag to inflate it. The top of the bag was tied to the pole for support during rain or wind. This can be accomplished by running a wire though the top flap in the paper but not below the paper seam which would allow air and water into the bag. The microfiber bags were attached by placing a wire around part of the bag that does not crush the switchgrass stem. Switchgrass cage creation involved wrapping a segment of chicken wire into a cylinder with a diameter of 69.85 cm, a length of 111.76 cm and a circumference of 219.44 cm. Then a pillowcase was placed over the cage. About 2-3 heads were placed in the cage. Wire pierced the pillowcase on both sides and attached it on either side to two poles or one side if only one pole or t-post was used. A wire secured the bag at the bottom.

The seed was removed from the panicle by rubbing and cleaned utilizing a South Dakota Seed Blower. The 2008 seeds were collected and counted for each plant. The seeds were placed on blotters soaked one time with 0.2% $KNO_3$ and benomyl solution (9.67g of 50% benomyl per 3.78 L of water) and placed in a Stults Scientific Engineering Corporation germination chamber set at a temperature control program composed of 8 hours of light at 30 °C and 16 hours

of dark at 15 °C (AOSA, 2007). The seeds from 2009 and 2010 were placed in a Perceval growth chamber. In 2008, germination was counted after 14 days, and the 2008 seedlings were transplanted into conetainers in Oklahoma State University's Controlled Environment Research Lab (CERL) and watered by floating the conetainers in water for a few minutes as needed. After two months in the CERL the plants were transplanted to 4'' pots and placed into a greenhouse at the OSU Agronomy Research Station. In 2009-2010 the germination was counted after 7 and 14 days. After germination of the 2009 seed, the seedlings were transplanted into flats and kept in the Percival growth chamber, then transferred to 4" pots in the greenhouse. The 2010 plants after germination were transplanted into conetainers and kept in a bright room for about a week for acclimation. Then the plants were maintained in the greenhouse.

SSR Homozygosity test

A total of 6 primer combinations were performed because if the bag is compromised and there is contamination the probability of misidentification is 50%. The formula for calculating SSR misidentification of selfing is (1/2)n where n is the number of SSR primers scored assuming high heterozygosity of polymorphisms within the population. Dje et al. (2004) used six SSR primers to estimate outcrossing and inbreeding in Sorghum.

DNA was extracted from putative $S_2$ plants and their parents using the CTAB method according to Doyle and Doyle (1990). Fluorescently labeled M13 SSR procedure was performed according to Tan et al., (2010). At least 6 primers were

used to confirm selfing in each sample and sometimes more primers were used when no reaction occurred. In 2008 the following primers (PVAAG -2861-2862, PVAAG-3017-3018, PVCAG-2167-2168, PVAAG -2895-2896, PVAAG-3365-3366, PVCAG-2209-2210, PVGA-1983-1984, PVGA-1703-1704, PVGA-1733-1734) were performed on all samples and PVCA-615-616 and PVCA-535-536 were performed on 68 samples. The extra primers were used to fill in spaces where the reaction was void. In 2009 the following primers were used (PVCA-615-616, PVCAG-2167-2168, PVAAG-3017-3018, PVCAG-2209-2210, PVGA-1703-1704, and PVAAG -2895-2896). In 2010 the same primers were used with the addition of PVCA-535-536 on all samples and PVGA-1733-1734, and PVCAG-2167-2168 on four samples. The SSR markers were performed using primers created by Wang et al. (2011) and one unpublished primer pair PVAAG-2895 (TTTGACCGTTCCAGTTTCGA), 2896 (CGTCGTCTCCTCTGGGTAAT) which is redundant to the PVAAG-3297-3298 primer that was published by (Wang et al., 2011). Inbreeding was determined by matching the alleles of the progeny plants with those of their maternal parent. Primers and their linkage group are listed in Table 4.1 from the linkage information of Liu et al. 2012 (in Press). The banding patterns were visualized on a 0.25 mm thick 6.5% polyacrylamide gel with a 64 tooth comb in a Li-COR 4300 DNA Analyzer (Li-Cor Inc., NE, USA) and run at 1500 Volts with a scan speed 2 for 1 hour 45 min. A DNA size marker (50-350 bp) was also loaded to determine the size of the fragments. Bands were visually scored. An example of a gel is in Figure 4.1.

## Seed Data Analysis

The seed data were count data and had many zeros and therefore non-normal, so a negative binomial regression ($\mu + k\,\mu^2$) (Lawless, 1987; SAS Institute, 2008) was performed with seed as dependent variable and cultivar as the independent variable using PROC GLIMMIX in SAS (SAS Institute, 2008). Family was used as a random variable for 2008 and 2010 but not in 2009 because it prevented the algorithm from converging. It was not possible to compare families to one another because there were no family replications. For the cultivar variable, each maternal plant or family was a replication. Each year was considered a random effect, so analysis was conducted separately. Within the years of 2008 and 2010 bag type was used as an independent variable. Head number was assumed random in all analyses.

# RESULTS

From 33 bagged Alamo plants, 27 plants produced 318 seeds and from 33 bagged Kanlow plants, 22 Kanlow plants produced 322 seeds in 2008 and the seed yield per parent ranged from 0 to 155. In 2009 of the 33 bagged Alamo plants, 32 produced 498 seed and of the 33 bagged Kanlow plants, 28 produced 1231 seeds and the seed yield per parent ranged from 0 to 461. In 2010 from 33 bagged plants, 29 Alamo Plants produced 303 seeds and from 33 Kanlow bagged plants, 20 plants produced 261 seeds and the seed yield per parent ranged from 0 to 100. From each year the seed count statistics were calculated. Cultivar was not significant in 2009, 2008 and 2010. In 2008 bag type was not significant but the cultivar×bag interaction was significant ($\alpha$=0.05). Bag

type was highly significant in 2010 (a=0.01) and the bag×cultivar interaction was not significant. If the microfiber bag data were not included in 2010, then bag is not significant. The least square mean analysis for 2008 bag type and cultivar combinations are in Table 4.2. The number of seeds for each bag type, germinated seed and surviving progeny for 2010 are listed in Table 4.3.

For the 2008 Alamo seed count, 235 germinated normally, 6 were abnormal, 33 were moldy, 17 were lost or dead and 14 did not germinate. From the Kanlow plants 275 germinated normally, 26 were moldy, 20 were lost or dead, 0 were abnormal and 12 did not germinate. The average germination was good; it was 72.9% for Alamo and 71% for Kanlow. Most of the loss was due to fungus, and not un-germinated seed. There was trash in the seed that molded and increased mold on seeds on the plate. Because of the mold it was difficult to distinguish between immature and mature seed. In 2009 on the 14$^{th}$ day of germination from a total of 504 Alamo seeds, 106 germinated normally, 3 were abnormal, 3 did not germinate, 41 died or were lost, and 353 were moldy. Of the 1213 Kanlow seeds 209 germinated normally, 140 did not germinate, 14 were loss or died, 850 were moldy. The germination totals for 2009 seeds were lower, 29.8% for Alamo, and 10.26% for Kanlow. In 2010 the number of Kanlow that germinated normally was 99. The number of un-germinated seed was 8. The number that was lost or dead was 2 and the number that was moldy was 252. Mold was also a problem for the 2010 germination. Of the 2010 Alamo seeds after 14 days of germination 160 germinated normally. The number of un-germinated seed was 16. The number of abnormal seedlings was 1 and the number of seed lost or dead was 6. The number of moldy Alamo was 105. The germination rates were 47.31% for

Alamo and 50.25% for Kanlow. The percent dormancy was on average very low 3.90% for Kanlow and 7.56% for Alamo in 2010, but mold affected the germination, so the low germination percentages were mostly due to moldy seeds. Table 4.4 contains germination data for each year.

There was high mortality after germination each year; for instance, after germination of the 2008 seeds some seedlings died when transplanted into conetainers in the CERL. There were 247 Alamo plants total and 97 died which left 150 live plants. There were 290 Kanlow total and 98 died, leaving 192 alive. More plants died when transplanted into the greenhouse, and later there was a nutrient deficiency in the greenhouse and after fertilization more plants perished. A total of 304 Alamo seeds were collected from which 100 progenies were grown and a total of 321 Kanlow seeds from which 91 progenies were grown from 2008 samples. Other years had similar mortality. There were more survivors in the 2009 generation than were recorded as germinated and 139 plants survived in addition to the seedlings counted as germinated. This could be due to some moldy seedlings surviving and or some seed germinating after the 14 day germination count. This led to a total survivability score of 100 which is not technically correct. The 2009 family average survivability and mortality were calculated with only the number of germinated seedlings and the number of surviving plants ignoring unexpected additional plants. There was some mortality in 2009 progeny mostly due to weak plants and fungus gnats in the growth chamber. From the 2009 plants a total of 498 Alamo seeds were harvested from which 123 progenies were grown and a total of 1231 Kanlow seeds from which 267 progenies were grown. 2010 had the highest mortality most of which

happened when the seedlings were transferred into conetainers. It could have been caused by lack of light because the seedlings were sheltered indoors to protect them from the heat, or it could have been due to higher inbreeding depression because a higher percentage of inbreds in 2010. The number of seeds, germinated seedlings and surviving plants for each family are in Table 4.5. The percentage survivability and mortality from total plants or averaged across family are in Table 4.6. Survivability was variable and ranged from 62.8% in 2009 to 15.3% in 2010.

## SSRs Identification of Selfed Progeny

Most of the SSR procedure was performed before the development of the linkage map by (Liu et al. 2012), so possible linkage was unknown between the SSR primers. The strongest linkage occurs between primers PVAAG-2895-2896 and PVAAG-3365-3366 both found at position 118.0 cM on chromosome 5b. The PVAAG-3365-3366 was only used on 2008 samples and additional primers were used in addition to PVAAG-3365-3366 so 6 primers were used on the samples even if PVAAG-3365-3366 or PVAAG-2895-2896 were thrown out. Other closely linked markers are PVGA-1703-1704 at 62.7 cM on 3b and PVCA-535-536 at 62.1 cM on 3b. The primer PVCA-535-536 was used in 2008 and 2010 in a few cases to clarify ambiguous bands. Other primers linked in 3b are PVGA-1983-1984 which was mapped at 107.5 cM, and PVGA-1733-1734 which was 92.6 cM. The primers PVCAG-2209-2210 and PVCA-615-616 are also part of the same linkage group with PVCAG-2209-2210 at 32.0 cM on 4a,b and PVCA-615-616 at 49.2 cM on

4a,b. This linkage is not a big concern as long as the linked markers are highly polymorphic.

In the 2008 plants, there were 267 Kanlow progenies and 23 were inbreds (25.3%) and of the 123 Alamo progenies, 33 were inbreds (33%) with a combined total of 56 inbreds (29.3%). Of the 2009 plants 330 survived and were transplanted into the field and from these plants DNA was extracted from 269 plants. Of the 269 plants, 104 were Alamo and 162 were Kanlow; 3 were mislabeled and excluded from the analysis to form at total of 266. These plants were derived from 21 Alamo parents and 15 Kanlow parents. From the 2009 plants 24 of the 104 Alamo plants were inbred (23.07%) and 133 of the 162 Kanlow plants were inbred (82.1%), for a total of 59.02% inbred. In 2010 there were 564 seeds total, 263 Kanlow from which 16 progeny were grown and 301 Alamo from which 26 progeny were grown. Of the 16 Kanlow progeny 5 were inbred (31.25%) and of the 26 Alamo 10 were inbred (38.56%). The number of progeny for each year and the percent inbred are in Table 4.7. The number of 2010 inbred and out-crossed progeny is listed in table 4.8.

## DISCUSSION

The paper bags are too small for most switchgrass seed heads. The average length of a switchgrass panicle is 46.32 cm and the width is 25.28 cm (Porter, 1966) while the bag has a length of 39.4 cm and width of 17.1 cm. If the bag is removed the head looks misshapen. The biggest problems with paper bags are the possibility that the switchgrass head will slip out and the bag falling over if not supported. The fabric bags go over the head but do not allow free movement of the head. The head also slips out of the bag easily if not attached tightly. If

attached tightly then sometimes the head will snap inside the bag. The bag can also slip out of the wire and fly away making it more difficult to find. The biggest problem with cotton muslin cage method is the cage can shift in the wind if not secured and heads can grow taller than the bag if not placed correctly. The pillow case starts to tear badly at the end of season. Heads can also slip out and should be marked with tape.

Contamination is difficult to control in all bag types under field conditions. Wind and heat are destructive to all bag types and water is damaging to paper. In a more controlled environment selfed seed yield would probably be increased and outcrossing yield minimized.

With the exception of one parental genotype in 2009, the number of inbred seeds is less than the average outcrossing switchgrass seed number per head for Alamo (476) and Kanlow, (587) (Das and Taliaferro, 2009). This is consistent with other inbreeding experiments which reported low seed yield in other allogamous grasses including *Andropogon gayanus* and smooth Brome grass (*Bromus inermis* Leyss.) (Casler et al. 2005; Foster 1962).

The cause of the unusually high seed number in 2009 is unknown. The bag used could have affected the yield; the same bag was used in 2010 but with only one head instead of multiple heads of 2009. The location and year environmental effects could have affected its seed yield; for instance, Das and Taliaferro (2009) found population×location interactions with seed yield per plant and various seed yield components including, panicle number per plant, seed number per panicle (Das and Taiaferro, 2009).

The 2008 cultivar×bag interaction could be caused by unequal numbers of microfiber bags. There were only 8 microfiber bags on Kanlow and 11 microfiber bags on Alamo. The least square means for seed yield in 2010 indicated that microfiber bags have a higher yield than the other bag types despite being less in number than the other bag types. According to the least square means the significance in bag in 2010 is due to the microfiber bags. If microfiber bags are ignored then there is not a significant difference between the paper bags and pillowcases bags. The difference in microfiber bags could also be caused by bag failure. The microfiber bags were inherited from another experiment and were older than the other bag types. Unnoticed holes could have increased seed number.

Mold affected seed germination. The germination rates from 2008 were 66.8% for Alamo and 70.8% for Kanlow were similar to those found by Aiken and Springer (1995) after 14 days, which were 69.2% for Alamo and 72.7% for Kanlow, but the rates found in 2009 and 2010 were much lower (Table 4.4). This could be due to environmental factors which affected the size of the seed since seed size affects germination (Aiken and Springer, 1995). The lower germination totals for 2009 seeds were probably due to the fact seeds were examined carefully for mold contamination and more seeds were excluded from the germination count. Since the seed yield varied according to year, environment played an important part in determining seed yield. Different year moisture levels could have affected seed yield. Das and Taliaferro (2009) found significant variation ($p<0.01$) between populations among and over locations for seed weight. There

could be variability within each of the cultivars that cannot be determined because there are not plant replicates within each cultivar.

The bag type with the highest number of inbreds in 2010 was paper (61.5%) and microfiber was the lowest (20%) (Table 4.8). The significance of these percentages could be affected by the size of the sample. The low percentage inbred in the microfiber bags is probably due to bag age. It is difficult to tell if bag type affected inbreds seed numbers since the inbreeding test is not done until adulthood and some inbred seed could have perished. Assuming that inbreds are more likely to die than outbreds due to inbreeding depression, it may be useful to look at mortality according to bag type in 2010. Microfiber had the lowest mortality (75%). The mortality for paper (85.4%) and pillowcase cages (86.4%) were similar. This could indicate that there were more inbred plants in paper and cages that died than in the microfiber bags.

The outcrossing rate as determined by a program such as MLTR (Ritland 2002) requires a progeny sample size of at least 200 (Ribeiro et al. 2004). Only one parent (K241) had close to that number (199) and only 109 survived to extract DNA.

The seedling mortality was high and varied across years (Table 4.6). This could be due to the number of inbreds, and the different seedling growth treatments. Different seedling growth treatments are the most likely reason for mortality differences because 2009 had higher survivability despite having more inbreds than 2008. High mortality was recorded in other species; Wu and Jain (1980) reported 47% seedling mortality in *Anthoxanthum odoratum* L. compared

to 7% in controls. Mortality was probably due to a combination of environmental factors (fungus, insects, too much water, soil level etc.) and inbreeding depression which is affected by the homozygosity of the populations studied.

Even if different growth treatments affected mortality, inbreeding depression was still likely a major factor of seedling mortality. If inbreeding depression is a problem, one method to reach full homozygosity is that the plants should be selfed until seed can no longer be produced or it is homozygous. If the plant no longer produces seed by selfing, but can produce seed by outcrossing then it can be used to make a hybrid, and also be crossed with a full sibling to increase homozygosity of the next inbred generation. This generation may be able to be selfed. If the plant is so depressed that it no longer produces seed from crosses or is too weak to live, then that generation is discarded and its parents are further inbred by full sib mating. If this process is repeated homozygosity should eventually be achieved. The perennial nature of switchgrass is beneficial for hybridization because inbred material can be preserved for several years. The inbred plants can be used for semi hybrids before complete homozygosity (Brummer, 1999). Since heterosis can be observed in crosses between individuals and populations, inbreds are not required for the expression of panmictic heterosis (Brummer, 1999) which could be improved by partial inbreeding.

This set of SSR primers could be less useful in future generations for determining inbreeding as the population becomes more homozygous and therefore different and more primers should be used. With the creation of a

molecular map SSRs could be selected from each chromosome or linkage group.

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Table 4.1. SSR primer pairs used to confirm inbreeding in $S_2$ families of Switchgrass created by Wang et al. 2011 and linkage information from Liu et al. 2012 (in press).

| cm† | LG‡ | Primers | SSR pattern | Primer pairs | Ex. size bp | Melting points |
|---|---|---|---|---|---|---|
| 47.0 | 5a | PVAAG 2861,2862 | (GT)36-(TA)5 | F: GCCAATCAGCCATAGAACAA<br>R: TCTCAATGCAACACTCCCTT | 223 | 58.723<br>58.287 |
| 58.8 | 6b | PVAAG 3017,3018 | (AAG)11 | F: ATTTTCCGGTCGATCGTTAG<br>R: AGCGAGGATGCTGTTGAAG | 142 | 59.043<br>59.128 |
| 0.0 | 7b | PVCAG 2167,2168 | (GAAGG)5-(AGC)6 | F: GGCCGATGCACATTACATAC<br>R: ACGCCTTCAAGAGACTCCTC | 312 | 58.874<br>58.619 |
| 118.0 | 5b | PVAAG 2895,2896 § | (GAA)10 | F:TTTGACCGTTCCAGTTTCGA<br>R:CGTCGTCTCCTCTGGGTAAT | 335 | 59.162 |
| 118.0 | 5b | PVAAG 3365,3366 | (GAA)10 | F: AATGCATGCTCAGGAGTCAA<br>R: CGGAATGATTGTCGTTCATC | 329 | 59.399<br>58.926 |
| 32.0 | 4a,b | PVCAG 2209,2210 | (GC)8-(AC)6 | F: GTAGCACTGCAACCGTTGAT<br>R: TGCTCAGGTTTGCTGATTTC | 263 | 58.804<br>59.002 |
| 107.5 | 3b | PVGA 1983,1984 | (AG)14 | F: AGTTCATCCAACTGCACGAG<br>R: TTACGTATGGGCCACATTTC | 188 | 58.882<br>58.361 |
| 62.7 | 3b | PVGA 1703,1704 | (AG)36 | F: CTCTCCCCTCTCCCTCTCTT<br>R: TGTGAAGGAAGGCCAAAGTA | 293 | 58.98<br>58.352 |
| 92.6 | 3b | PVGA 1733,1734 | (GA)16 | F: CGCTACATAGCACCTTGCAT<br>R: TATGGCATATTCTCCCCCTC | 267 | 58.97<br>58.81 |
| 49.2 | 4a,b | PVCA 615,616 | (AC)11 | F: GACGAATTACAGAGACTGTTGG<br>R: ATATAGTCCTGCGGAGGGTG | 219 | 56.483<br>59.031 |
| 62.1 | 3b | PVCA 535,536 | (TG)11 | F: TGCTGCCACTGCAGATAGAT<br>R: AAGAGAAGGGGGTTCCAACT | 273 | 59.577<br>59.042 |

†Centimorgans from end of chromosome.

‡Linkage group.

§Redundant to 3297-3298 (Wang et al., 2011).

Table 4.2. Least square mean comparisons of the means with their standard errors for the bag×cultivar combinations in 2008 switchgrass seed yield.

| | Alamo Paper | Alamo Microfiber | Kanlow Paper | Kanlow Microfiber |
|---|---|---|---|---|
| Alamo Paper | 0.84±0.30 | NS | NS | NS |
| Alamo Microfiber | S | 1.29±0.34 | S | * |
| Kanlow Paper | NS | NS | 0.79±0.30 | NS |
| Kanlow Microfiber | NS | * | NS | 0.23± 0.43 |

S significant at 0.1 level.

* significant at 0.05.

NS not significant.

Table 4.3. Switchgrass number of seeds, germinated plants, and surviving progeny for each bag type harvested in 2010.

| Bag Type | Alamo | | | Kanlow | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Seed | Germ. | Prog. | Seed | Germ. | Prog. | Seed | Germ. | Prog. |
| Microfiber | 78 | 37 | 12 | 107 | 23 | 3 | 185 | 60 | 15 |
| Paper | 122 | 58 | 7 | 56 | 31 | 6 | 179 | 89 | 13 |
| Cotton Muslin | 102 | 65 | 7 | 100 | 45 | 8 | 201 | 110 | 15 |
| Total | 302 | 160 | 26 | 263 | 99 | 17 | 565 | 259 | 43 |

Table 4.4. Bagged switchgrass seed germination rates measured after the 7th and 14th day for 2008-2010.

| Year | 2008 | 2009 | | 2010 | |
|---|---|---|---|---|---|
| Day | Day 14 | Day 7 | Day 14 | Day 7 | Day 14 |
| Alamo | 66.8% | 33.17% | 29.8% | 61.50% | 47.31% |
| Kanlow | 70.8% | 8.53% | 12.15% | 41.94% | 50.25% |

Table 4.5. The seed yield, germination and number of adult progeny of inbreed switchgrass for each family.

| Family | 2008 | | | 2009 | | | 2010 | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | S† | G‡ | P§ | S† | G‡ | P§ | S† | G‡ | P§ | S† | P§ |
| A111 | 0 | 0 | 0 | 2 | 0 | 1 | 4 | 3 | 0 | 6 | 1 |
| A114 | 8 | 8 | 7 | 4 | 3 | 1 | 22 | 10 | 9 | 34 | 17 |
| A116 | 24 | 15 | 11 | 21 | 2 | 4 | 40 | 20 | 1 | 85 | 16 |
| A122 | 0 | 10 | 6 | 5 | 2 | 0 | 23 | 14 | 1 | 28 | 7 |
| A123 | 103 | 84 | 37 | 56 | 10 | 12 | 28 | 15 | 7 | 187 | 56 |
| A125 | 5 | 5 | 4 | 11 | 2 | 0 | 3 | 2 | 0 | 19 | 4 |
| A126 | 1 | 0 | 0 | 4 | 0 | 2 | 3 | 2 | 1 | 8 | 3 |
| A131 | 1 | 1 | 0 | 6 | 3 | 0 | 3 | 1 | 0 | 10 | 0 |
| A135 | 36 | 31 | 11 | 3 | 2 | 0 | 1 | 0 | 1 | 40 | 12 |
| A136 | 3 | 0 | 0 | 3 | 0 | 0 | 9 | 6 | 1 | 15 | 1 |
| A143 | 3 | 3 | 3 | 1 | 0 | 1 | 6 | 4 | 0 | 10 | 4 |
| A145 | 5 | 2 | 2 | 19 | 0 | 3 | 26 | 4 | 0 | 50 | 5 |
| A146 | 2 | 1 | 0 | 6 | 3 | 2 | 11 | 7 | 0 | 19 | 2 |
| A151 | 4 | 4 | 2 | 63 | 19 | 8 | 5 | 11 | 1 | 72 | 11 |
| A152 | 0 | 0 | 0 | 161 | 30 | 60 | 10 | 0 | 0 | 171 | 60 |
| A154 | 13 | 8 | 5 | 5 | 3 | 2 | 11 | 5 | 0 | 29 | 7 |
| A216 | 3 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 5 | 0 |
| A221 | 4 | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 6 | 1 |
| A225 | 19 | 14 | 2 | 13 | 5 | 2 | 28 | 13 | 1 | 60 | 5 |
| A234 | 1 | 1 | 0 | 3 | 1 | 0 | 3 | 1 | 0 | 7 | 0 |
| A241 | 14 | 12 | 3 | 3 | 0 | 0 | 3 | 1 | 0 | 20 | 3 |
| A243 | 0 | 0 | 0 | 4 | 1 | 2 | 1 | 0 | 0 | 5 | 2 |
| A246 | 10 | 7 | 4 | 17 | 5 | 6 | 4 | 2 | 0 | 31 | 10 |
| A251 | 2 | 2 | 1 | 20 | 1 | 6 | 0 | 0 | 0 | 22 | 7 |
| A255 | 15 | 10 | 6 | 10 | 1 | 2 | 7 | 5 | 1 | 32 | 9 |
| A256 | 3 | 2 | 0 | 2 | 1 | 0 | 19 | 12 | 1 | 24 | 1 |
| A311 | 8 | 1 | 1 | 9 | 4 | 3 | 1 | 0 | 0 | 18 | 4 |
| A313 | 4 | 1 | 0 | 21 | 0 | 2 | 2 | 0 | 0 | 27 | 2 |
| A316 | 5 | 4 | 0 | 5 | 2 | 2 | 19 | 16 | 0 | 29 | 2 |
| A321 | 2 | 1 | 0 | 8 | 2 | 0 | 1 | 1 | 0 | 11 | 0 |
| A331 | 0 | 0 | 0 | 6 | 0 | 1 | 0 | 0 | 0 | 6 | 1 |
| A352 | 6 | 5 | 0 | 4 | 2 | 1 | 0 | 0 | 0 | 10 | 1 |
| A356 | 0 | 0 | 0 | 1 | 1 | 0 | 8 | 4 | 1 | 9 | 1 |
| Total | 304 | 235 | 106 | 498 | 106 | 123 | 303 | 160 | 26 | 1105 | 255 |

† S, Seeds; ‡G, Germinated seed. §P, Progeny

Table 4.5 Continued.

| Family | 2008 | | | 2009 | | | 2010 | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | S† | G‡ | P§ | S† | G‡ | P§ | S† | G‡ | P§ | S† | P§ |
| K115 | 11 | 8 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 12 | 6 |
| K116 | 23 | 17 | 12 | 2 | 0 | 1 | 15 | 11 | 2 | 40 | 15 |
| K121 | 11 | 10 | 8 | 2 | 0 | 1 | 0 | 0 | 0 | 13 | 9 |
| K123 | 14 | 12 | 7 | 1 | 0 | 0 | 2 | 1 | 1 | 17 | 8 |
| K126 | 1 | 1 | 0 | 0 | 0 | 0 | 26 | 7 | 0 | 27 | 0 |
| K131 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| K132 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| K133 | 1 | 1 | 1 | 7 | 1 | 1 | 4 | 2 | 0 | 12 | 2 |
| K134 | 4 | 3 | 3 | 1 | 0 | 0 | 20 | 18 | 2 | 25 | 6 |
| K135 | 3 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 4 | 2 |
| K141 | 5 | 3 | 2 | 0 | 0 | 0 | 12 | 10 | 3 | 17 | 6 |
| K146 | 29 | 22 | 2 | 126 | 27 | 8 | 100 | 31 | 0 | 255 | 10 |
| K152 | 1 | 0 | 0 | 10 | 0 | 4 | 1 | 1 | 0 | 12 | 4 |
| K153 | 12 | 11 | 0 | 23 | 3 | 0 | 1 | 1 | 0 | 36 | 0 |
| K154 | 22 | 14 | 7 | 2 | 0 | 0 | 31 | 4 | 0 | 55 | 7 |
| K155 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 3 | 2 | 16 | 2 |
| K156 | 0 | 0 | 0 | 4 | 1 | 1 | 0 | 0 | 0 | 4 | 1 |
| K216 | 2 | 1 | 1 | 111 | 2 | 2 | 2 | 0 | 0 | 115 | 3 |
| K221 | 1 | 1 | 0 | 95 | 1 | 11 | 0 | 0 | 0 | 96 | 11 |
| K223 | 2 | 1 | 1 | 35 | 1 | 2 | 2 | 0 | 0 | 39 | 3 |
| K226 | 0 | 16 | 8 | 7 | 0 | 1 | 3 | 1 | 0 | 10 | 9 |
| K235 | 0 | 0 | 0 | 85 | 0 | 0 | 0 | 0 | 0 | 85 | 0 |
| K236 | 2 | 1 | 0 | 31 | 5 | 1 | 0 | 0 | 0 | 33 | 1 |
| K241 | 0 | 0 | 0 | 461 | 155 | 199 | 4 | 1 | 0 | 465 | 199 |
| K243 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 0 |
| K256 | 155 | 132 | 28 | 11 | 0 | 4 | 2 | 1 | 0 | 168 | 32 |
| K311 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| K312 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K315 | 0 | 0 | 0 | 161 | 5 | 21 | 0 | 0 | 0 | 161 | 21 |
| K321 | 0 | 0 | 0 | 11 | 0 | 2 | 0 | 0 | 0 | 11 | 2 |
| K336 | 8 | 8 | 6 | 22 | 3 | 5 | 11 | 6 | 5 | 41 | 16 |
| K345 | 6 | 6 | 4 | 8 | 1 | 1 | 7 | 0 | 0 | 21 | 5 |
| K354 | 7 | 4 | 1 | 10 | 0 | 0 | 0 | 0 | 0 | 17 | 1 |
| Total | 321 | 275 | 99 | 1231 | 206 | 267 | 261 | 99 | 16 | 1813 | 383 |
| All Total | 625 | 510 | 205 | 1729 | 312 | 390 | 564 | 259 | 42 | 2918 | 638 |

†S, Seeds; ‡G, Germinated seed. §P, Progeny;

Table 4.6. The survivability and mortality percentages of switchgrass plants calculated from total number seedlings divided by the total germinated and mortality and survivability calculated within each family and averaged across families. The 2009 plants had plants that were not counted as germinated that survived, increasing the total seedling survivability.

| | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|
| | Survive | Mort. | Survive | Mort. | Survive | Mort. |
| Total Seed | 40.20 | 59.80 | 100† | None | 16.60 | 83.40 |
| Average Family‡ | 46.03 | 53.97 | 62.79§ | 37.21§ | 15.26 | 84.74 |

†based on the total progeny that survived not including plants in addition to germination total

‡the survivability or mortality for each family calculated and then averaged across families

§includes only the plants that were counted as germinated

Table 4.7. Seed yield, progeny number, and progeny percentage inbred for an $S_2$ population of switchgrass.

| Family | 2008 | | | 2009 | | | 2010 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Seeds | Prog. | % inbred | Seeds | Prog. | % inbred | Seeds | Prog. | % inbred |
| Alamo | 302 | 100 | 33.0% | 498 | 104 | 23.1% | 301 | 26 | 38.6% |
| Kanlow | 321 | 91 | 25.3% | 1231 | 162 | 82.1% | 263 | 16 | 31.3% |
| Total | 623 | 191 | 29.3% | 1729 | 266 | 59.0% | 564 | 42 | 35.7% |

Table 4.8. Number of inbred switchgrass plants in 2010 confirmed selfed according to bagging type: Paper (Lawson paper bags), Cage (chicken wire cylindrical frames covered with cotton pillowcase), and Microfiber (Microfiber bags).

| Bag type | Inbreds | Out-crossed | %Inbred |
|---|---|---|---|
| Paper | 8 | 5 | 61.5% |
| Cage | 6 | 8 | 42.9% |
| Microfiber | 3 | 12 | 20.0% |
| Total | 17 | 25 | 40.5% |

LIST OF FIGURES

Figure 4.1. Example of a SSR PCR polyacrylamide gel using primer PVAAG-2895-2896 used to compare parent and progeny of Switchgrass plant using 2010 samples.

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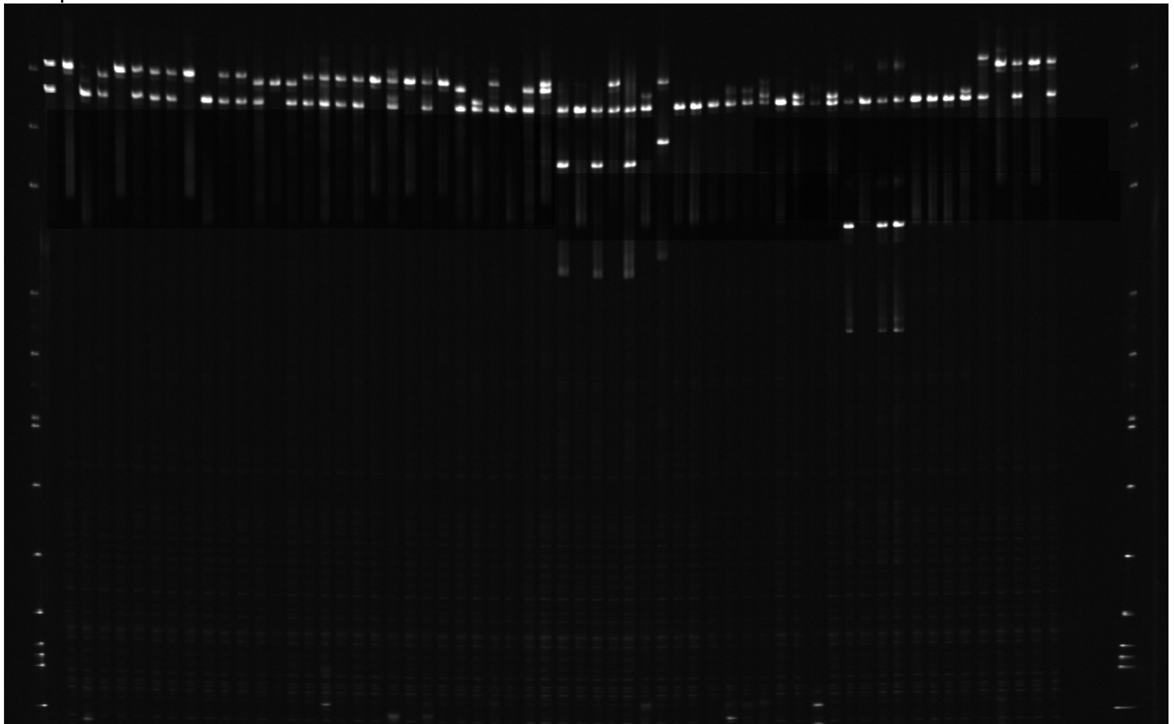

# VITA

James Ryan Todd

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDY OF THE DIVERSITY, INBREEDING POTENTIAL, AND SWARD HERITABILITY OF SWITCHGRASS

Major Field: Crop Science

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Crop Science at Oklahoma State University, Stillwater, Oklahoma in December, 2011.

Completed the requirements for the Master of Science in Biology at Midwestern State University, Wichita Falls, Texas in 2006.

Completed the requirements for the Bachelor of Science in Biology at Midwestern State University, Wichita Falls, Texas in 2003.

Experience: Teaching Biology labs, SAS programming, heritability statistics calculation, plot design, DNA extraction, SSR and AFLP techniques.

Professional Memberships: ASSA, CSSA, SSSA

Name: James Todd Date of Degree: December, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: STUDY OF THE DIVERSITY, INBREEDING POTENTIAL, AND SWARD HERITABILITY OF SWITCHGRASS

Pages in Study: 136 Candidate for the Degree of Doctor of Philosophy

Major Field: Crop Science

Scope and Method of Study: Study the diversity of 56 genotypes of switchgrass from different regions of the US using AFLP. Study the heritability of two populations of Switchgrass (SL-93 $C_2$ and NL-94 $C_2$) in swards. Investigate 3 different bag methods (paper, microfiber and cotton muslin) to evaluate inbreeding seed yield of two populations (Kanlow and Alamo) of $S_2$ switchgrass.

Findings and Conclusions: The upland and lowland accessions clustered according to ecotypes, with one exception (TN104). Genetic similarity coefficients among the accessions ranged from 0.73 to 0.95. An analysis of molecular variance (AMOVA) was performed using GenAlEx (ver. 6.3) resulting in a significant difference (α=0.05) between the upland and lowland genotypes. The *trn*L marker confirmed that TN104 was a lowland genotype, but the *trn*L marker identification of upland and lowland genotypes was not consistent with the AFLP in two germplasm (Miami and AR4). From the heritability study, the drought of 2011 affected the heritability estimates. The variance component estimates for 2008-2011 for NL-94 and SL-93 were 0.08 and 0.08 respectively compared to 2008-2010 which were 0.16 and 0.07 respectively per sward plot. The per family mean estimates for 2008-2011 for NL-94 and SL-93 were 0.58 and 0.59 respectively compared to 2008-2010 estimates which were 0.70 and 0.46 respectively. The parent offspring regression estimates for 2010-2011 for NL-94 and SL-93 were 0.10 and 0.31 respectively. Only one bag type in 2010 had a significant difference (microfiber) and it is difficult to tell if that difference is not caused by bag failure. Six SSR primer pairs were used to confirm selfed progeny identity by determining if all progeny alleles matched its maternal parent. Of the 2008 Kanlow offspring 23 were inbreds (25.3%) and of Alamo 33, were inbreds (33%). Of the 2009 Alamo plants, 24 (23.07%) were inbred and 133 (82.1%) Kanlow plants were inbred. Of the 2010 Alamo plants 10 were inbred (38.56%), and of the 2010 Kanlow plants, 5 plants were inbred (31.25%).

ADVISER'S APPROVAL: Dr. Yanqi Wu