



GENOTYPIC VARIATIONS AND GENOTYPE x ENVIRONMENT (G x E)  
INTERACTIONS AMONG NEW SWITCHGRASS (*PANICUM VIRGATUM* L.)  
POPULATIONS IN OKLAHOMA

By

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## TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
Review of Literature .....	2
Objectives .....	12
II. Genotypic Variability for Morphological and Physiological Traits among new Switchgrass Populations .....	14
Abstract .....	14
Introduction.....	15
Materials and Methods.....	17
Results and Discussion .....	22
III. Genotype x Environment (G x E) Interactions among new Switchgrass Populations in Oklahoma.....	47
Abstract .....	47
Introduction.....	48
Materials and Methods.....	51
Results and Discussion .....	53
IV. CONCLUSION.....	76
REFERENCES .....	79

## LIST OF TABLES

Table	Page
Table 1. Effect of genotype x environment interaction on biomass yield of switchgrass across the southern United States.....	13
Table 2. Genetic basis of 22 switchgrass populations.....	31
Table 3. Genetic variation for morphological traits among 22 switchgrass populations..	32
Table 4. Correlation coefficients between biomass and seven morphological traits of 22 switchgrass populations .....	33
Table 5. Eigenvectors of first three principle components from biomass and seven morphological traits.....	34
Table 6. Genetic variation for physiological traits among 22 switchgrass populations...	35
Table 7. Correlation coefficients between $P_n$ and nine physiological traits of 22 switchgrass populations .....	36
Table 8. Eigenvectors of first three principle components from $P_n$ and physiological traits.....	37
Table 9. Genetic variation for tiller and phytomer morphology among 21 switchgrass populations in Stillwater (2011) .....	38
Table 10. Correlation coefficients of tiller and phytomer traits among 21 switchgrass populations in Stillwater (2011).....	39
Table 11. Eigenvectors of first three principle components from tiller and phytomer morphology in Stillwater (2011).....	40

Table	Page
Table 12. Site description information of multilocation experiment .....	61
Table 13. Results of ANOVA for dry biomass and plant height among 21 switchgrass populations across the years and locations .....	62
Table 14. Mean dry biomass of 21 switchgrass populations during the establishment year (2010).....	63
Table 15. Mean dry biomass of 21 switchgrass populations during the post establishment year (2011).....	64
Table 16. Results of ANOVA for tiller density among switchgrass populations across the years and locations .....	65
Table 17. Correlation coefficients between biomass and tiller density, plant height across the genotypes for four locations (2010 and 2011).....	66
Table 18. Tiller density of switchgrass populations during establishment year (2010)...	67
Table 19. Tiller density of switchgrass populations during establishment year (2011)...	68
Table 20. Correlation coefficients between biomass and tiller density at four locations during the establishment year (2010).....	69
Table 21. Correlation coefficients between biomass and tiller density at four locations during the post establishment year (2011).....	70
Table 22. Plant height among switchgrass populations during the establishment year (2010).....	71
Table 23. Plant height among switchgrass populations during the post establishment year (2011).....	72
Table 24. Regression equation slopes from stability analysis of 21 switchgrass populations at four locations in 2010 and 2011.....	73

## LIST OF FIGURES

Figure	Page
Fig. 1. Mean dry biomass of 22 switchgrass populations.....	41
Fig. 2. Stem elongation rate, leaf addition rate, and tillering rate among 22 switchgrass populations .....	42
Fig. 3. Eigen values and eigenvectors of PCA from biomass and morphological traits of 22 switchgrass populations .....	43
Fig. 4. Eigen values and eigenvectors of PCA from $P_n$ and physiological traits of 22 switchgrass populations .....	44
Fig. 5. Eigen values and eigenvectors of PCA from tiller and phytomer morphological traits of 21 switchgrass populations .....	45
Fig. 6. Mean photosynthesis variations among switchgrass population groups from May to August (2011) in Stillwater .....	46
Fig. 7. Distribution of rainfall from March to September in Lane and Stillwater .....	74
Fig. 8. Distribution of rainfall from March to September in Chickasha and Woodward.....	75

## CHAPTER I

### INTRODUCTION

Switchgrass is a native perennial grass that is widely distributed in the North America continent. The Biofuels Feedstock Development Program (BFDP) and U.S. Department of Energy (DOE) selected switchgrass (*Panicum virgatum* L.) as the model herbaceous feedstock species in 1991. Biomass yield is the most important and economically viable trait of switchgrass (Casler 2010). The Energy Independence and Security Act (EISA) in 2007 mandated 21 billion gallons from advanced biofuel resources to meet the nation's target of 36 billion gallons of ethanol by 2022. These mandates could be achieved by substantial advancement in plant breeding, crop management and conversion technologies (Ragauskas et al. 2006).

Evaluation of genetically advanced materials over the existing cultivars is essential for further enhancement of economically viable traits. The switchgrass plant breeding and genetics program at Oklahoma State University was initiated 1992 to develop new switchgrass cultivars with increased biomass. Recurrent selection for general combining ability (RSGCA) has been used in the breeding program. The selection of progeny is based on phenotypic and genotypic performance, with final selection based on genotypic performance measured by biomass yield in half-sib (HS)



progeny. The RSGCA procedure was used in four switchgrass breeding populations: Southern Lowland (SL), Northern Lowland (NL), Southern and Northern Late Maturing Upland (SNLMU), and Southern and Northern Early Maturing Uplands (SNEMU) (Taliaferro 2002). Studies were very limited for genetic evaluation of these breeding populations and no study has been conducted for morphological and physiological variation.

## Review of Literature

### Biomass and bioenergy

Increasing concern of energy security, escalating oil prices, and climate change impacts are the prime considerations that led to the exploration of renewable energy sources to reduce the over dependence on conventional fossil fuels. The Biomass Research and Development Advisory Committee, a panel established by Congress, has set a vision to replace 30 percent of current United States (U.S.) petroleum consumption with biofuels by 2030. However, according to the U.S. Energy Information Administration (2009), currently only 8 percent of total energy consumption in the U.S. is from biobased renewable resources. To displace 30 percent of the country's present petroleum consumption, 1 billion dry matter tons of biomass feedstock will be required each year (Perlack et al. 2005). Annual biomass potential of US from forestland and agricultural land, (the two largest biomass sources), is over 1.3 billion tons; 368 million tons from forestlands and 998 million tons from agricultural lands (Perlack et al. 2005). The Energy Independence and Security Act (EISA) in 2007 mandates 21 billion gallons from advanced biofuel resources, 16 billion gallons from cellulosic feedstocks, to meet

the nation's target of 36 billion gallons of ethanol by 2022. Development of biomass based large scale biofuel industries requires an enhanced supply of cellulosic feedstock through advancement in plant breeding, crop management, and conversion technologies (Ragauskas et al. 2006).

### Switchgrass as a bioenergy feedstock

After extensive evaluation of thirty four herbaceous species, in trials across thirty-one sites in seven states, the Biofuels Feedstock Development Program (BFDP) and U.S. Department of Energy (DOE) selected switchgrass (*Panicum virgatum* L.) as the model feedstock species in 1991. Switchgrass has unique traits, including reliable productivity across a wide geographical range for approximately ten years, suitability for marginal lands, low water and fertilizer requirements, along with other beneficial environmental attributes (Fike et al. 2006; Wright and Turhollow 2010). Since the 1990's, switchgrass has been extensively studied for its potential deployment for conversion to energy by fermentation, gasification, or combustion processes (Bouton et al. 1998). Switchgrass demonstrates important traits to be considered as an ideal herbaceous bioenergy crop defined by Gonzalez-Hernandez et al. (2009), including reliable yields, broad adaptation in marginal lands under limited inputs, perennial nature, potential to enhance the biomass yield through breeding, and it does not compete with food crops for land and resources.

### Switchgrass biology

Switchgrass is perennial grass native to North America, adapted widely across the U.S., ranging from 20°N to 60°N latitude and east of 100°W longitude (Moser and Vogel 1995). Switchgrass is a dominant component of the North American native grass prairie

system (Weaver 1954) and considered among the “big three” grasses along with indiagrass (*Sorghastrum nutans* (L.) Nash) and big blue stem (*Andropogon gerardii* Vitman). Switchgrass dominates native grass prairies and its wide adaptation led to switchgrass use on conservation reserve program (CRP) lands. In the past, switchgrass was grown and managed as a soil conservator, a fodder crop and an ornamental grass along with other tall-grasses (Mitchell et al. 1997) and is actively being evaluated as a potential bioenergy feedstock.

Switchgrass stems are erect and smooth with an inflorescence on the top. Switchgrass inflorescence is a diffused panicle and produces spikelets at the end of long branches and its florets are staminate and fertile. Lemma and palea are firmly attached to the caryopsis (Bouton 2007). Switchgrass has a fibrous root system that can reach a depth of 2.7 to 3.3 m from the soil surface with huge below ground biomass (Weaver and Darland 1949). Switchgrass propagates reproductively through seeds and vegetatively through rhizomes (Moser and Vogel 1995). Switchgrass can be classified into lowland (L) and upland (U) based on morphology and habitat. Lowland ecotypes are thick stemmed, large and more robust, commonly found in wet regions, whereas upland ecotypes are thin stemmed and found in moderately dry regions (Porter 1966). The two ecotypes can also be further classified into two cytotypes, U (upland) and L (lowland), based on chloroplast DNA (cpDNA) polymorphism in upland and lowland populations, respectively (Hultquist et al., 1996; Martinez-Reyna et al. 2001).

## Switchgrass breeding

Switchgrass breeding in the USA started in the 1930's primarily to increase fodder production, seedling establishment, and digestibility (Moser 2004). High biomass and better digestibility with increasing neutral detergent fiber (NDF) and reducing acid detergent fiber (ADF) are the important breeding considerations for using switchgrass as biofuel feedstock (Casler and Boe 2003). Breeding for increased biomass and quality have been achieved in newer cultivars (Burns et al. 2008; Vogel et al. 1996), however switchgrass has been relatively less studied and not yet removed from native germplasm (Joseph 2007) as released cultivars closely resemble natural populations (Casler et al. 2007a).

Switchgrass is a highly heterozygous, self-incompatible species with different ploidy levels ranging from  $2n = 2x = 18$  to  $2n = 12x = 108$  (Burton 1942; Church 1940; Nielson 1944). Generally, lowland types are tetraploids and upland types are either tetraploid or, hexaploid or octaploids (Taliaferro et al. 1999). Due to the self incompatibility between the ecotypes, viable seed can be produced only from types within the same ploidy levels and by inter crossing between similar ecotypes within the same ploidy level (Martinez-Reyna and Vogel 2002). Heritability in switchgrass has been documented and heritability for biomass and digestibility facilitates breeders to improve targeted traits through the half-sib family selection method (Bhandari et al. 2011; Missaoui et al. 2005; Overend 1999; Talbert et al. 1983). Magnitude of heritability among the populations suggests that potential exists to increase biomass through breeding.

Molecular progress for switchgrass biomass and quality were reported. Transfer of maize *Corngrass1 (Cg1)* gene into switchgrass resulted in 250% more starch and complete inhibition of flowering (Chuck et al. 2011). Fu et al. (2011) reported that down-regulation of the switchgrass caffeic acid O-methyltransferase gene reduced recalcitrance of switchgrass and increased the ethanol yield up to 38% with conventional biomass fermentation processes.

Self incompatibility and pre and post-fertilization incompatibility issues impose restrictions for conventional selection and breeding among the ecotypes and to improve traits with lower heritability. However, restricted selection for general combining ability has been developed as a potential breeding method in improving switchgrass biomass yield (Taliaferro 2002). The RSGCA breeding method is commonly employed for complex quantitatively inherited traits like biomass, and especially for out crossing species like switchgrass (Taliaferro 2002).

#### Genetic variation of switchgrass

Genetic variation for biomass yield ranged from 1 Mg ha<sup>-1</sup> to 39.1 Mg ha<sup>-1</sup> and this variation was attributed to many factors, especially ecotype, temperature, precipitation and nitrogen management (Wullschleger et al. 2010). Therefore, selecting genotypes that have high biomass potential and greater response to best management practices is critical to optimize the switchgrass biomass production. Evaluation of different morphological traits among new breeding lines is important for breeders to identify the specific traits that can enhance biomass production. Moreover, an understanding of morphological development facilitates the adoption of the best

management practices (Sanderson and Moore 1999; Sanderson 1992). Switchgrass morphological development is influenced by both temperature and photoperiod, whereas vegetative growth is mostly influenced by temperature and reproductive growth is controlled by photoperiod (Sanderson and Wolf 1995). Strong photoperiodic response and cold hardiness differences between the ecotypes determine switchgrass adaptation. Lowland switchgrass populations moved north from their original adaptation areas are unable to survive winters. Similarly, reduced yields are reported in uplands when grown in southern latitudes and this is due to the early maturity induced by shorter photoperiods. Moreover, the latitudinal affect on biomass was greater in lowland compared to upland populations (Casler et al. 2004).

Morphological traits including tiller number, leaf number, leaf appearance rate, and leaf elongation rate facilitate the understanding of the canopy development rate (VanEsbroeck et al.1997). Several studies have reported switchgrass morphological development under diverse climatic conditions (Madakadze et al. 1998b; Mitchell et al. 1997; Redfearn et al. 1997; Sanderson and Wolf 1995). Relationships between switchgrass phenological traits and their relationship with biomass were also reported (Boe and Beck 2008; Casler et al. 2004; Das et al. 2004). Generally, lowland populations are taller compared to upland populations. Plant height is considered as the most important trait to estimate biomass (Schmer et al. 2010), is highly correlated with final biomass (Bhandari et al. 2011; Casler et al. 2004; Lemus et al. 2002a) and sensitive to latitude of origin (Casler et al. 2004). Tiller density is the major trait for switchgrass sward morphology. Tillers arise from the axil buds of the lower internodes of primary stem and later from other tillers. The perennial nature of grasses is explained by winter

survival of these axillary buds (Moore and Moser 1995). Under field conditions, switchgrass biomass yield was positively correlated with tiller density in both spaced planting (Das et al. 2004) and swards (Boe 2007). A strong linear relationship was found between biomass yield and tiller components, tiller density per plant, and mass per tiller (Boe 2007). Similarly, Das et al. (2004) indicated that high tiller density per plant was an effective indirect selection trait to improve biomass in switchgrass breeding. However, yield predictors were not consistent over locations and ecotypes. A study on Cave-In-Rock by Redfearn et al. (1997), found that tiller density was not a significant indicator of biomass, whereas leaf blade dry weight per tiller and mean stage dry weights were correlated with biomass. Selection studies on ryegrass (*Lolium perenne* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) showed that selection based on large leaf size decreased the tillering rate and increased tiller weight, and the results were opposite when selected for high leaf appearance rate (Edwards and Cooper 1963). A study on reed canarygrass (*Phalaris arundinacea* L.) reported specific leaf weight was negatively correlated with tiller density (Toparkngarm et al. 1977). Switchgrass photoperiod studies reported that the total leaf number varied based on the growing season (VanEsbroeck et al. 1997), location and year (Madakadze et al. 1998a; Redfearn et al. 1997). Biomass variations in the northern Great Plains of the United States were attributed to phenology and phytomer number. High yielding cultivars were late maturing and produced more phytomers than early maturing cultivars with lower phytomer number (Boe and Casler 2005).

## Variation in switchgrass for physiological traits

High productivity of C<sub>4</sub> plants is attributed to high water and nitrogen use efficiencies compared to C<sub>3</sub> plants. Higher water use efficiency in C<sub>4</sub> plants is explained by their unique carbon dioxide uptake mechanism that mitigates water losses through transpiration by regulating stomata operation (Stout et al. 1988). However, the biochemical mechanism of C<sub>4</sub> photosynthesis is not always the same among C<sub>4</sub> plants. Three biochemical photosynthesis mechanisms have been reported, including nicotinamide adenine dinucleotidemalic enzyme (NAD-ME), nicotinamide adenine dinucleotide phosphate -malic enzyme (NADP-ME) and phosphoenolpyruvate carboxykinase (PEP-CK). The three mechanisms are differentiated based on the mode of carbon dioxide (CO<sub>2</sub>) transportation from bundle sheath cells and regeneration of phosphoenol pyruvate (PEP). Switchgrass has NADP-ME type of C<sub>4</sub> pathway (Taub and Lerdau 2000).

Few studies have reported physiology differences between and within each ecotype. Interpretations on switchgrass photosynthesis differed among locations and ecotypes. Among native switchgrass populations, photosynthetic rate, DNA concentration, soluble protein and chlorophyll concentrations were higher in octaploid switchgrass populations compared to tetraploid populations (Warner et al. 1987a). Later, Wullschleger et al. (1996) demonstrated that photosynthesis of lowland and upland ecotypes were similar under common non-stressful greenhouse conditions, whereas lowland performed better than upland populations under field conditions. In contrast, genetic variations for major physiological traits in switchgrass were attributable to the



phenology and environmental stress rather than ploidy (McLaughlin and Adams Kszos 2005).

Studies also reported switchgrass photosynthesis variations between the ecotypes under abiotic stress conditions. Photosynthesis was not significantly different among lowland and upland populations under different nitrogen and water treatments; however, lowland yields were higher than uplands (Stroup et al. 2003). Lowland photosynthesis was greater than upland populations across different moisture levels (Barney et al. 2009). Photosynthesis acclimation was not significant among the ecotypes with respect to temperature shift from near optimal (32/24°C) to suboptimal temperature (22/14°C) (Gesch and Johnson 2010). Stomatal conductance ( $g_s$ ) is an essential physiological trait in regulating plant–water relations. Lower  $g_s$  mitigates transpiration water losses, which leads to better maintenance of high leaf water potential and high soil water content over the growing season, thereby, minimizing the drought affect on photosynthesis. Developing cultivars with such superior physiological traits provide tolerance to abiotic stresses. An understanding of the genetic variation among new breeding populations for important physiological traits such as net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ), internal carbon dioxide concentration ( $C_i$ ) and electron transport rate ( $ETR$ ) would be beneficial to develop cultivars for enhanced stress adaptation.

#### Leaf anatomy and morphology

Stomata are directly and indirectly involved in controlling plant physiological mechanisms, but mainly in diffusion of CO<sub>2</sub> for photosynthesis from the atmosphere to mesophyll cells and by regulating water lost through latent heat exchange. Stomatal

architecture is different in C3 and C4 plants. In grasses, stomata are distributed in parallel rows and each stoma is covered by a dumbbell shaped guard cell. Stomatal frequency between adaxial to abaxial surfaces is high in grasses; however, stomatal variations occur among species and within the same photosynthetic pathway (Anderson and Briske 1990). Stomatal response has been studied extensively for different abiotic stresses in both C3 and C4 plants, but few studies have been documented on native grasses like switchgrass.

#### Genetic Diversity and Genotype (G) x Environment (E) Interaction in Switchgrass

Phenotypic expression of the genotype is unique to the growing environment. Biomass production has been and continues to be a viable economic trait for grasses (Casler 2010), especially for potential biofuel crops such as switchgrass. Evaluation of genotypes for biomass potential under different climatic and edaphic conditions within the targeted region is important for decision making about future breeding advancements. These evaluations could be possible through G x E interaction studies on the traits of interest. Understanding genetic diversity has relevance to conserve and characterize the desirable traits. The G x E interaction for biomass is very obvious in multi-location trial evaluations of switchgrass and varied across locations, including the southern United States (Table 1). Although, switchgrass occurs widely across the United States, the biomass and adaptation of switchgrass populations are affected by original adaptation between lowland and upland ecotypes. Genetic diversity has been reported in switchgrass (Casler 2005; Casler et al. 2007a; Hultquist et al. 1996; Missaoui et al. 2006; Narasimhamoorthy et al. 2008). The results from different trials showed significant effect of environment on dry matter yield, agronomic, and biofuel traits (Casler and Boe 2003; Casler et al. 2004; Cassida et al. 2005a; Hopkins et al. 1995b; Hopkins et al. 1995c;

Koshi et al. 1982). Switchgrass germplasm adaptation differed across locations, ranging from wider (Hopkins et al. 1995c) to specific adaptation (Sanderson et al. 1999). A switchgrass latitudinal adaptation study concluded that switchgrass was sensitive to the region of its origin and cultivar performance decreased if cultivars were planted more than 500 km (or) one USDA hardiness zone away from its origin (Casler et al. 2007b). Latitudinal affect on biomass was greater in lowland than upland populations. Southern lowland population yields increased with latitude within south central United States (Cassida et al. 2005a) , whereas SL biomass yields decreased with increasing latitudes from 36°N to 39°N latitude (Casler et al. 2004). These contradictory results indicate the importance of switchgrass adaptation when developing new cultivars for high biomass within the targeted region. Thus, breeding efforts and testing of new cultivars over the existing cultivars within the targeted region is critically important to attract emerging cellulosic based biofuel industries to the southern United States.

### Objectives

The objective of this research were to

1. To evaluate genetic variation for morphological and physiological traits among new switchgrass populations
2. To study the genotype x environment (G x E) interactions among new switchgrass populations in Oklahoma

Table 1. Effect of genotype x environment interaction on biomass yield of switchgrass across the southern United States.

Location	Biomass range ( Mg ha <sup>-1</sup> )	Reference
Haskell and Chickasha (OK)	Chickasha- 13.5 (Alamo+ Summer) - 7.6 (Cave-In-Rock) Haskell- 19.0 (Alamo+ Summer) - 9.3 ( Summer)	Fuentes and Taliaferro 2002
Stillwater (OK)	Stillwater- 15.13(Southern Lowland) - 10.45(Northern Uplands)	Casler et al. 2004
Stephenville, Dallas and College Station (TX), Clinton (LA), Hope (AR)	Stephenville- 13.65(SL931) - 5.00(Caddo) Dallas- 19.48 (Alamo) - 6.05 (Caddo) College Station – 21.40 (SL 93) - 5.42(Caddo) Hope – 19.96 (SL 931) - 7.41 (Caddo) Clinton- 11.59(SL 941) - 1.12(Caddo)	Cassida et al. 2005
Beeville, Dallas, Temple, College Station, Stephenville(TX)	Beeville- 15.1 (Alamo) - 8.85(Cave-In-Rock) College Station- 16.9 (Alamo) - 8.69 (Cave-In-Rock) Dallas- 8.1(Alamo) - 4.36 (Cave-In-Rock) Stephenville- 15.2(Alamo) - 2.57 (Cave-In-Rock) Temple- 14.3 (Alamo) - 3.8(Cave-In-Rock)	Sanderson et al. 1999b

## CHAPTER II

### Genotypic variability for morphological and physiological traits among new Switchgrass populations

#### Abstract

Developing cultivars for high biomass production is a principle goal for grass breeders. Evaluation of advanced breeding populations in transferring potential viable traits and understanding the effect of different morpho-physiological traits on biomass among the breeding populations is important. Therefore, the objective of this research was to assess the biomass, morphological, and physiological variations among 19 new Switchgrass (*Panicum virginatum* L.) breeding lines and three check cultivars (Alamo, Kanlow, and Cave-In-Rock). Simultaneous experiments were established under both greenhouse and field conditions at Stillwater, OK. The principle component analysis (PCA) was carried out to screen the genotypes for morphological and physiological performance. Under greenhouse conditions, northern lowland (NL) genotype, NL 94 C2-4 produced 40.2% more biomass plant<sup>-1</sup> than the check (Alamo). The southern lowland (SL) genotype SL 93 C2-2 had 7% more photosynthesis capacity than Alamo and seasonal photosynthesis was higher in SL populations. The first two principle components (PRIN1 and PRIN2) accounted for 74% and 82% of total variability for

morphological traits under greenhouse and field conditions, respectively. Under greenhouse conditions PRIN1 and PRIN2 explained 64% of total variability for physiological traits. The PCA was able to discriminate lowland and upland populations for morphological traits, but was unclear for physiological traits. The results showed that the morphological traits including plant height, days to panicle initiation and tiller morphology (mass tiller<sup>-1</sup> and phytomer weight) would be the potential selection traits to enhance biomass potential of switchgrass.

### Introduction

Finite energy supplies, energy security, escalating oil prices and climate change impacts are accelerating the need for alternate renewable energy resources. A renewable biofuel economy is projected as a way to reduce reliance on fossil fuels, green house gas emissions, and enhance rural economies (Schmer et al. 2008). The United States of Department of Agriculture (USDA), Farm Service Agency (FSA) initiated the Biomass Crop Assistance Program (BCAP) to promote herbaceous bioenergy crops in Farm Bill 2008. The USDA renewable energy program included Oklahoma and Kansas states in BCAP and expected planting of native grasses and herbaceous species such as switchgrass over 20,000 acres. High biomass producing switchgrass cultivars would be required in the southern US region to meet these mandates.

Tiller density and tiller mass are important traits and correlate with final biomass within uplands (Boe 2007) and spaced planted lowland populations (Das et al. 2004). Path analysis of biomass yield and different morphological traits suggested that tiller density per plant is an effective indirect selection trait to improve biomass production

(Das et al. 2004). The Redfearn et al. (1997) biomass predictability study at Ames, IA and Mead, NE on Cave-In-Rock reported that leaf blade dry weight was more significant than tiller density to estimate the final biomass. Studies at Wisconsin and South Dakota on four upland populations reported that high biomass of cultivars were attributable to late maturity and larger phytomers than low yielding cultivars (Boe and Casler 2005). In switchgrass study with spaced planted populations Smart et al. (2004) reported that selection for tiller weight resulted in 25% more biomass than tiller density. Similarly, selection for shoot yield resulted greater yields than number of shoots per plants in alfalfa (*Medicago sativa* L.) (Volencic et al. 1987). Switchgrass genetic variation for leaf morphology including final leaf number and leaf appearance rate are closely associated with maturity of the plant. High biomass producing cultivars are late maturing with slow leaf appearance rate (VanEsbroeck et al. 1997). Leaf developmental events in switchgrass were reported to be based on length of phyllochron (phyllochron is a growing degree days (GDD) between the appearance of two successive leaves). The phyllochron was less in early flowering cultivars such as Cave-in-Rock (79 GDD) compared to late flowering Alamo (152 GDD) (VanEsbroeck et al. 1997). Similar results were also reported in short-growing season areas in southwestern Quebec (Madakadze et al. 1998). Biomass distribution among tillers and phytomer components were reported on switchgrass upland populations and other warm season grasses such as Cordgrass (*Spartina pectinata* Link.) (Boe and Casler 2005). Biomass variations in northern Great Plains of United States were attributed to phenology and phytomer number. High yielding cultivars were late maturing and produced more phytomers than early maturing cultivars with lower phytomer number

(Boe and Casler 2005). However, no study has reported tiller and phytomer morphology of lowland populations.

An understanding of genetic diversity is important to characterize and improve desirable traits. Evaluation of potential breeding methods in transferring needed biomass traits into new genotypes is important. Only a few studies reported genetic variation among new breeding lines including southern lowland (SL) and northern lowland (NL) populations (Casler et al. 2004; Cassida et al. 2005; Fuentes and Taliaferro 2002; Kiniry et al. 2008), however most of these studies were limited to biomass potential. Therefore, the objectives of this research were to (1) evaluate genetic variability for morphological and physiological traits under both controlled and field environmental conditions and (2) identify morpho-physiological traits contributing to biomass production.

## Materials and Methods

### Plant material

Seeds of twenty two switchgrass populations including three standard check cultivars and nineteen experimental lines (Table 1) were sown in small pots filled with Metro-Mix 250 (Scotts-Sierra Horticultural Products Co., Marysville, OH) growing medium. Pots were watered immediately after sowing and daily care was taken until the transplanting. Greenhouse grown seedlings were transplanted into the greenhouse and the agronomy research station facility of Oklahoma State University in June, 2010, in Stillwater, OK.

Greenhouse facility at Oklahoma State University (36°08'N, 97°05'W), Oklahoma, USA, was used to evaluate morphological and physiological variations.



Healthy greenhouse grown seedlings of 22 switchgrass populations were transplanted into 12L pots (0.75m length and 0.15m width) filled with pure fine sand. Pots were arranged in rows, five pots for each genotype and each pot contained a single healthy seedling. Greenhouse temperature was set at 30/22°C (day/night) throughout the experiment. The light in greenhouse was 10% lower than ambient environment. Optimum growth conditions were maintained throughout the experiment. Experimental design was completely randomized design (CRD). Plants were irrigated three times a day at 800, 1200 and 1700 with full- strength Hoagland's nutrient solution through drip irrigation system. Nutrients were provided through an automated timing device to ensure the favorable nutrient and water conditions throughout the experiment.

#### Measurements

Main stem was tagged in each pot immediately after transplanting to measure the growth events at three day intervals starting from 42 to 99 DAT (days after transplanting). Plant height (PH), leaf number (LN), tiller density (TD) and leaf length (LL) were measured at every three day interval. During harvest, plant height, tiller density, leaf number and leaf length were recorded. Leaf area was measured using LI-3100 leaf area meter (LI-cor., Lincoln, NE, USA). Reproductive traits including days to panicle initiation, seed weight per plant, and panicle number per plant were also recorded. Plants were harvested at 10cm height from surface of sand and plant components were oven dried for three days at 70 °C. Total dry matter was recorded. Seed weight and panicle number among the genotypes were also evaluated.

Photosynthesis, stomatal index and pigment concentration assessment

Forty days after transplanting, important physiological traits including net photosynthesis ( $P_n$ ), electron transport rate ( $ETR$ ), stomatal conductance ( $g_s$ ) and internal carbon dioxide ( $C_i$ ) were measured between 1000 to 1500 on clear sunny days from uppermost fully expanded leaves using an infrared gas analyzer built into a leaf cuvette in an open gas exchange system (LICOR-6400). The carbon dioxide ( $CO_2$ ) cylinder was used in  $CO_2$  Injection System for constant supply of  $CO_2$  ( $400 \mu L L^{-1}$ ) in the chamber throughout the measurements. The 6400-02 LED light source was used for photosynthetically active radiation (PAR) of  $1500 \mu mol m^{-2} s^{-1}$  and the temperature inside the leaf cuvette chamber was set to  $30^\circ C$ . The flow rate of  $500 \mu mol m^{-2} s^{-1}$  was fixed and relative humidity was adjusted to ambient level.

Methods described by Kakani et al. (2003) were adapted to estimate stomatal index (SI) and pigment concentrations among the populations. A thin layer of colorless nail polish was coated on both leaf surfaces of uppermost leaves of three plants from each genotype, then allowed to dry for 30 minutes to obtain clear impressions from both adaxial and abaxial surfaces. Leaf peels were carefully removed with forceps and placed under the light microscope at 400x magnification to measure stomata number and epidermal cell numbers from three random fields. Epidermal cells and stomatal density was calculated per unit leaf area ( $mm^2$ ). Final epidermal cells and stomatal density per unit area is represented by Stomata Index.

Leaf discs from fully expanded leaves were collected for pigment analysis. Five leaf discs ( $35 mm^2$ ) were punched randomly from upper most leaves and placed in 4ml vial of dimethyl sulfoxide (DMSO). The vials were placed at room temperature in dark immediately after sampling for 24 h to allow for complete extraction of chlorophyll

pigments. The absorbance of chlorophyll extracts was measured using Spectrophotometer (Spectronic Genesys 10 Bio) at 470, 648 and 664nm. The absorbance values from three different wavelengths were used to calculate the Chlorophyll a (*Chl a*), Chlorophyll b (*Chl b*) and carotenoid (*Car*) concentrations (Gitelson and Merzlyak 1994).

#### Field study

Field study was established at Agronomy Research Station facility of Oklahoma State University (36°07'N, 97°05'W), Stillwater, Oklahoma, USA in 2010. Experimental design was randomized complete block design with four replications. Each replication consisted of 21 test plots including 19 experimental lines and two check cultivars, Alamo and Cave-In-Rock. All test plots were bordered with check cultivar, Kanlow. Each plot was divided into six columns (south to north direction), and spacing between two neighboring columns was 03 m. Ten plants were included in each column (10 rows). Spacing between two neighboring plants in a column was 0.3 m. Border rows and border columns were not included in measurements. Each plot was 3.0 x 1.8 m area.

Greenhouse grown seedlings from each container were transplanted into a prepared seedbed. Field was irrigated with sprinklers immediately after transplanting. Soil type was port silt loam and soil samples were collected before transplanting to estimate fertility status. During establishment year, no fertilizer was applied to avoid weed competition and 85 kg ha<sup>-1</sup> of urea was applied early in the growing season of the post establishment year (2011). During post establishment year in 2011, five random tillers were sampled to evaluate ten traits including tiller height, phytomer number, leaf blade length, internode length, leaf blade width, leaf sheath length, phytomer weight and tiller weight among 21 switchgrass populations. Pruning tool was used to separate the

phytomers, and all the phytomers were oven dried separately for three days at 70°C. Dry weights were recorded for all phytomers and tillers. Seasonal leaf photosynthesis trend was measured using LI-6400 photosynthesis system during post establishment year starting from May to August in 2011 among 21 switchgrass populations. The LI6400 XT settings were similar to the greenhouse measurements.

#### Statistical analysis

Raw data were subjected to one way ANOVA with PROC GLM statistical procedure of SAS statistical software program (SAS Institute) to identify the significance differences among the 21 switchgrass populations for different traits. Pearson correlations coefficients were developed separately from morphological and physiological traits using PROC CORR procedure of SAS. Further, data were analyzed with principle component analysis to discriminate different genotypes for morphological traits and physiological traits both under greenhouse and field conditions.

#### Principle Component Analysis (PCA)

The PCA is an exploratory multivariate technique extensively used to understand the relationships among different quantitative variables. Data were analyzed with PRINCOMP procedure of SAS statistical software program. The standardized principle components scores (PRIN's) that represent high percentage of variation were used to group the genotypes for different morphological and physiological traits. Eigenvectors generated by PCA were used to identify variables that can best describe the performance of differentiated genotypes for different traits. Generally genotypes grouped for +PC1 and +PC2 scores were classified as best performing genotypes followed by +PC1 and –PC2 scores, –PC1 and +PC2, and finally –PC1 and –PC2. This standardized technique

was commonly used in genotype screening for temperature stress (Kakani et al. 2002; Kakani et al. 2005) and agronomic traits of switchgrass (Casler 2005; Casler et al. 2004).

## Results and Discussion

### Greenhouse study

#### Genotypic variation for biomass and morphological traits

The ANOVA was significantly ( $P < 0.01$ ) different among the genotypes for biomass. Fig. 1 shows the mean biomass differences among 22 switchgrass populations. Lowland populations mean biomass was 67% greater than upland populations. Among lowland populations genotype NL 94 C2-2 and SL 93 C2-3 produced 50.4 g (40.2%) and 38.0 g plant<sup>-1</sup>(30.3%), more biomass than Alamo (125.10 g plant<sup>-1</sup>), respectively. Longer vegetative growth and greater stem elongation rates of lowland populations resulted in more biomass compared to upland populations (Table 3 and Fig. 2). Moreover, other morphological traits including plant height, leaf traits (leaf number and leaf length), and days to panicle initiation were significantly correlated with biomass. These traits were higher in lowland populations and these findings also agreed with PCA analysis and vectors magnitude was greater for these two traits (Table 3 and Fig. 3). Selection for these traits may be a potential option to increase biomass through breeding and selection. Under common greenhouse controlled environmental conditions the biomass variations were attributable to the genetic potential of the genotypes. However, the relative performance of the genotypes for biomass and other traits were comparable to field conditions. In field trials on similar ecotypes at five locations in the southern US including Texas, Arkansas, and Louisiana, higher biomass recorded for NL and SL

ecotypes compared to the check Alamo, but the yields were not consistent across locations and years (Cassida et al. 2005). However, under field conditions biomass was determined by edaphic, environmental (Sanderson et al. 1999) and other agronomic traits such as stand density and row spacing (Muir et al. 2001).

The morphological traits such as tiller density, plant height, leaf number were highly correlated (data not shown) between main stem and tillers. Therefore, the main stem traits were presented for morphological variations among the populations. Table 3 shows mean variations of different morphological traits among 22 switchgrass populations. The ANOVA for plant height, leaf length and days to panicle initiation were highly significant at  $P < 0.001$ , leaf number, seed weight and panicle number at  $P < 0.01$  and tiller density at  $P < 0.05$ . Mean plant height for lowland populations was greater (139.4 cm) than upland populations (79.4cm). Plant height ranged from 143.6 (NL 94 C2-3) to 75.3 (SNU 98 EMBP C1-1) (Table 3). Results agreed with previous findings for plant height and correlation with biomass (Bhandari et al. 2011; Casler et al. 2004; Lemus et al. 2002b). Mean leaf number plant<sup>-1</sup> was higher in lowland than in upland populations, and ranged from 10.8 (NSL 2009-3) to 6.8 leaves plant<sup>-1</sup> (SNU 98 EMBP C1-1) (Table 3). Higher leaf appearance rate and lower number of leaves are more common in early maturing cultivars than late maturing cultivars (VanEsbroeck et al. 1997). Mean leaf blade lengths were greater in lowland populations compared to upland populations. Leaf lengths ranged from 58.8cm (NSL 2009-3) to 40.3cm (SNU 98 EMBP C1-1) (Table 3). Mean tiller density per plant ranged from 70 (SNU 98 EMBP C1-1) to 29 (SL 93 C2-2) (Table 3). Upland populations produced more thin and short tillers (56 tillers plant<sup>-1</sup>) than lowland populations (45 tillers plant<sup>-1</sup>). Barney and Mann (2009)

reported similar results with uplands populations (Cave-In-Rock and Caddo). Tiller density was greater in upland than in lowland populations (Alamo and Kanlow) at field capacity moisture levels under greenhouse conditions.

Pearson's correlation coefficients between biomass and seven morphological traits are presented (Table 4). Biomass was positively correlated with plant height, leaf number, leaf length, seed weight plant<sup>-1</sup>, panicle number plant<sup>-1</sup> and days to panicle initiation, but not with tiller density (Table 4). Biomass was highly significant with plant height at  $P < 0.001$ , leaf length and days to panicle initiation at  $P < 0.01$ , and leaf number at  $P < 0.05$ . These findings demonstrate the importance of plant height, leaf length, and days to panicle initiation among the evaluated traits (Fig. 4). The results of correlations were consistent with previous findings for plant height (Casler et al. 2004; Bhandari et al. 2011; Lemus et al. 2002) and days to panicle initiation (Bhandari et al. 2011). Most of the previous studies on tiller morphology were limited to either lowland or upland populations and interpretation were also varied based on growing environment and cultivars. The correlation between biomass and tiller density among 22 populations does not concur with previous findings, but data pooled by ecotype showed strong correlations for lowland (0.67) and upland populations (0.94) (data not shown) and these correlations were similar to previous findings for upland (Boe and Casler 2005) and lowland populations (Das et al. 2004). Under greenhouse conditions upland populations tend to produce more thin tillers than lowland populations, but their elongations were less than lowland populations, which led to negative correlations between biomass and tiller density. Since upland populations originated from cooler and drier regions, the

temperature regime of 30/22°C in the greenhouse may have inhibited the vegetative growth of upland populations.

The biomass and seven morphological traits including plant height, tiller density, leaf number, leaf length, days to panicle initiation, seed weight and panicle number of 22 switchgrass populations were subjected to PCA analysis. First three principle components (PRIN1, PRIN2, and PRIN3) accounted for 85% of total variability, with most contributed by PRIN1 (53%) and PRIN2 (20%) (Table 5). Therefore, the PRIN1 and PRIN2 were used for genotype discrimination for morphological traits. The eigenvectors from PRIN1 contrasted genotypes towards the right side of biplot for five variables including biomass, plant height, leaf number, leaf length and days to panicle initiation. Among the five variables, high positive loading was reported for plant height and days to panicle initiation (Table 5). The PRIN2 vector magnitude was greater for seed weight, biomass, panicle number, and tiller density (Table 5). The eigenvectors of PRIN1 and PRIN2 from eight variables classified the performance of 22 genotypes (Fig. 3). First principle component was positive for 14 populations including two check cultivars. However, six new genotypes were weighted for greater morphological performance with +PRIN1 and +PRIN2. First two principle components were able to segregate lowland and upland populations and these findings were similar with Casler et al (2004) under field conditions at Stillwater.

#### Physiological variations

Table 6 shows mean variations of different physiological traits including photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ), electron transport rate ( $ETR$ ), internal carbon dioxide ( $C_i$ ), leaf area (LA), stomatal index (abaxial (SIL) and adaxial (SIU)



surfaces), and pigment concentrations of chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), and carotenoids(*car*) among 22 switchgrass populations. The ANOVA was significantly different for  $P_n$ ,  $g_s$ ,  $C_i$ ,  $ETR$ ,  $Chl a$  at  $P < 0.001$ , and  $Chl b$  and  $SIU$  at  $P < 0.01$ , and  $car$  at  $P < 0.05$  among the populations. The  $SIL$  was not significant among the populations. Except for leaf area, most of the physiological traits were quite similar between lowland and upland populations. However, mean pigment concentrations and stomatal index were greater in lowland compared to upland populations, thus explaining the higher photosynthesis of lowland over upland populations. Mean photosynthesis ranged from 30.4 to 23.4  $\mu \text{mol/m}^2 \text{sec}^{-1}$  with mean of 26.2  $\mu \text{mol/m}^2 \text{sec}^{-1}$  (Table 6). Therefore, the photosynthesis results were not correlated with biomass. Results for photosynthesis were in congruence with finding by Wullschleger et al. (1996) and they also reported significant variations under field conditions rather than in greenhouse conditions. Pearson correlation coefficients between photosynthesis and nine physiological traits were positively correlated for eight traits and negatively correlated with leaf area (Table 7). Significant correlations were reported for stomatal conductance ( $P < 0.001$ ), internal carbon dioxide and electron transport rate ( $P < 0.05$ ). Since the measurements were at leaf level, the photosynthesis mostly related to amount of photosynthesis apparatus per unit area, rather than leaf area. Therefore, most of the correlations were reported for  $g_s$ ,  $C_i$ , and  $ETR$ . The PCA was able to classify the 22 populations for physiological traits. The first three principle components (PRIN1, PRIN2, and PRIN3) accounted for 77.8% of total variability (Table 8). First two principle components (PRIN1 and PRIN2) explained almost 65% of total variation. The positive eigenvectors of PRIN1 and PRIN2 mostly weighted for  $P_n$  and pigment concentrations (*Chl a*, *Chl b*, and *Car*) for four genotypes

including three lowlands (SL 93 C2-2, NL 94 C2-4, and SWG 2009-2) and one upland population (SNU 98 LMBP C1-2). The segregation between lowland and upland populations for physiological traits were not clear (Fig. 4). However, the results from PCA showed that *Pn* and pigment concentrations were most appropriate to screen switchgrass genotypes for physiological traits. Earlier studies also reported the importance of *Chl* concentration in the plants and they also noted that *Chl* concentrations were accurate and indirect indicators for nutrient levels in the plants (Moran et al. 2000).

#### Field study

##### Tiller morphology

Means for tiller weight, tiller height, internodal length, leaf number, leaf width, leaf length, leaf area, phytomer number, phytomer weight and leaf sheath lengths of 22 switchgrass populations are presented in Table 9. The mean tiller height was highly significantly ( $P < 0.001$ ) different among the populations. Lowland populations were taller (111.5cm) than upland populations (84.8cm), ranging from 136.5 (NL 94 C2-3) to 76cm (SWG 2007-3). Internode length among the populations was highly significant ( $P < 0.001$ ) and longer internodes were reported in lowland (17.3cm) than in upland populations (11.3cm) ranging from 23.3 (NL 94 C2-4) to 8.4cm (SWG 2007-4). Total leaf number/ tiller was highly significant ( $P < 0.001$ ) ranging from 9 (NL 94 C2-3) to 5 leaves/ tiller (SWG 2007-3). Leaf sheath lengths were greater in lowland (19cm) than in upland (15.2cm) populations and significantly ( $P < 0.05$ ) different among the populations. Among the populations mean leaf sheath lengths ranged from 27.6 (NL 94 C2-4) to 12.5cm (SNU 98 LMBP C1-2). Although the phytomer number/ tiller ranged from 5 to 7, it was significantly different among the populations ( $P < 0.01$ ). Mean phytomer weight was

highly significant ( $P < 0.001$ ) among the populations. Phytomer weight of lowland (1.3g) was almost twice than that of upland (0.7g) populations. Highest phytomer weights were recorded for genotypes NSL 2009-3 (1.82g) followed by NL 94 C2-3 (1.78g). Leaf lengths and leaf area were significant ( $P < 0.01$ ) among the populations. Highest leaf lengths were recorded in NL 94 C2-3 and least in SNU 98 EMBP C1-1. Leaf width among the populations was significant ( $P < 0.001$ ), ranging from 1.67 (NSL 2009-3) to 1.15cm (SWG 2007-3). Tiller weight was highly significant ( $P < 0.001$ ) among the genotypes. High tiller weights were recorded for NSL 2009-3(9.1g) followed by NL 94 C2-3(8.9g). Overall, lowland populations performed better than upland populations for most of the evaluated morphological traits. Within lowland populations, the NL populations, NL 94 C2-3 and NL 94 C2-4 were superior for the majority of these traits. Mean tiller weight was highly correlated with phytomer weight, leaf traits (width, length, and area) and tiller height (Table 10). Earlier study reported that mass tiller<sup>-1</sup> was better linear predictor of final biomass yield compared to tiller density m<sup>-2</sup>(Boe. 2007). Variations in tiller morphological traits including height and leaf area determine the number and size of phytomers (Briske and Derner 1998). Phytomer morphology is also suggested as a potential trait to increase biomass in Alamo (Van Esbroeck et al. 1998).

The PCA analysis of ten tiller morphological traits discriminated 22 switchgrass populations (Fig. 5). First two principle components, PRIN1 and PRIN2 accounted for 82.2% of total variability. Phytomer number, tiller height and leaf number were reported as the traits that led to high performance for six lowland populations. Except for genotype SWG 2007-1, all lowland populations were reported on the right side of biplot, thus explaining the exceptional performance of lowland populations for morphological traits.

## Seasonal photosynthesis

Temporal variation of photosynthesis was observed among the populations (Fig. 6). However, photosynthesis variations among the genotypes within each ecotype were not significantly different, which allowed to group the genotypes for six ecopopulations and two check cultivars (Alamo and Cave-In-Rock). Significant differences were recorded among the ecotypes and for different months ( $P < 0.001$ ). Across the populations, mean net photosynthesis gradually decreased from May (23.3) to August ( $10.4 \mu \text{ mol/m}^2 \text{ sec}^{-1}$ ) across the ecotypes. Over the months, net photosynthesis was higher in SL populations followed by Alamo. Net photosynthesis was significant ( $P < 0.001$ ) among the populations during May, June and July, and was not significant at August. Severe drought conditions and variations in crop maturity among the populations might be the reason for photosynthesis variations. During May, high photosynthesis was recorded for SWG upland group followed by Alamo. The southern lowland (SL) group had the highest photosynthesis capacity during June and July months. Alamo and SL populations had high photosynthesis during August. Check cultivar, Cave-in-Rock had the least net photosynthesis across the months. Physiological expression of switchgrass depends on growing environment and crop phenology (McLaughlin and Adams Kszos 2005). Due to exceptional drought conditions, results were different than expected. However, the temporal trend among the ecotypes showed that SL populations had higher photosynthesis than other populations. This might be attributable to high water use efficiency, and which was also reported at southern US locations (Kiniry et al. 2008).

In conclusion, ample genetic variations were evident among the new breeding populations. Results from greenhouse and the field study indicated that selection for

morphological traits would be more beneficial than physiological traits to enhance biomass. Our results for physiological evaluations showed that southern lowland populations have more photosynthesis capacity than other cultivars. However, research for multiple abiotic stresses will be required for a better understanding of different physiological responses among the populations. The PCA demonstrated that plant height, phytomer weight and days to panicle initiation would be the best traits among different morphological traits to enhance biomass. Northern lowland populations, especially NL 94 C2-4 performed best for biomass, morphological and physiological traits. The SL populations had high photosynthesis both under greenhouse and field conditions. Evaluation of these lines for different environments would be recommended for further understanding of genotype x environment interactions.

Table 2. Genetic basis of 19 breeding populations and three check cultivars.

Entry No	Genotype	ECOTYPE	Genetic basis
1	NSL 2009-1	L	Advanced from SL and NL
2	NSL 2009-2	L	Advanced from SL and NL
3	NSL 2009-3	L	Advanced from SL and NL
4	NSL 2009-4	L	Advanced from SL and NL
5	SL 93 C2-1	L	Synthesized from Alamo and PMT 279
6	SL 93 C2-2	L	Synthesized from Alamo and PMT 279
7	SL 93 C2-3	L	Synthesized from Alamo and PMT 279
8	SL 93 C2-4	L	Synthesized from Alamo and PMT 279
9	NL 94 C2-1	L	Synthesized from Kanlow and Pangburn
10	NL 94 C2-2	L	Synthesized from Kanlow and Pangburn
11	NL 94 C2-3	L	Synthesized from Kanlow and Pangburn
12	NL 94 C2-4	L	Synthesized from Kanlow and Pangburn
13	SNU 98 LMBP C1-1	U	Synthesized from late maturing SU and NU
14	SNU 98 LMBP C1-2	U	Synthesized from late maturing SU and NU
15	SNU 98 EMBP C1-1	U	Synthesized from early maturing SU and NU
16	SWG 2007-1	L	Selection from SL 93 C2 and NL 94 C2(HYE)
17	SWG 2007-2	L	Selection from NL 94 C2(LYE)
18	SWG 2007-3	U	Selection from 98 EMBP and SNU98LMBP
19	SWG 2007-4	U	Selection from SNU 98EMBP and SNU98LMBP
20	Alamo	L	Collected in south Texas and released by Natural Resource Conservation Service (NRCS), Texas
21	Kanlow	L	Collected in central Oklahoma and released by NRCS, Kansas
22	Cave-In-Rock	U	Collected from southern Illinois

SL- southern lowland, NL- northern lowland, NSL- northern southern lowland, SWGL- swg lowland type, SWGU- swg upland type, SNU- southern northern upland, C1- cycle 1 and C1-2 cycle1 synthesize 2

Table 3. Morphological traits including plant height (PH), tiller density (TD), leaf number (LN), leaf length (LL), days to panicle initiation (DP), seed weight (SW) and panicle number (PN) among 22 switchgrass populations.

Genotype	Plant height (cm)	Tiller density plant <sup>-1</sup>	Leaf number plant <sup>-1</sup>	Leaf length (cm)	Days to panicle initiation (DAT)	Seed weight (g plant <sup>-1</sup> )	Panicle number plant <sup>-1</sup>
Alamo	121.9	45	9	45.4	83	6.5	24
Cave-In-Rock	77.6	50	8	47.1	59	4.7	22
Kanlow	137.1	45	9	52.0	82	7.9	16
NL 94 C2-1	122.3	41	9	55.1	80	5.1	16
NL 94 C2-2	135.1	34	9.5	50.3	82	2.6	13
NL 94 C2-3	143.6	44	9	57.4	79	6.7	18
NL 94 C2-4	136.0	48	9	54.6	81	7.6	20
NSL 2009-1	137.8	39	10	51.8	81	4.5	13
NSL 2009-2	138.4	58	9	58.8	82	6.7	18
NSL 2009-3	142.5	36	11	52.0	84	2.2	11
NSL 2009-4	135.6	49	9	52.0	81	3.2	18
SL 93 C2-1	131.8	49	10	49.9	82	3.0	20
SL 93 C2-2	113.8	29	10	47.4	80	3.2	14
SL 93 C2-3	130.6	50	9	48.4	84	4.1	22
SL 93 C2-4	142.1	54	9	52.0	86	3.2	13
SNU 98 EMBP C1-1	75.3	70	9	40.3	47	6.1	26
SNU 98 LMBP C1-1	85.5	64	8	42.4	62	2.2	17
SNU 98 LMBP C1-2	79.0	51	7	46.2	53	10.6	20
SWG 2007-1	112.1	47	8	43.4	84	3.4	17
SWG 2007-2	131.5	50	10	49.8	85	5.4	26
SWG 2007-3	78.9	59	8	44.8	69	5.9	24
SWG 2007-4	80.5	43	7	42.7	63	3.0	19
Mean	117.7 <sup>***</sup>	48.0 <sup>*</sup>	8.9 <sup>**</sup>	49.3 <sup>***</sup>	75.9 <sup>***</sup>	4.9 <sup>**</sup>	18.4 <sup>**</sup>
LSD (5%)	19.4	18.9	1.5	4.7	4.8	3.9	8.6

\*\*\*, \*\*, and \* are significant at probability of 0.001, 0.01, and 0.05, respectively.

Table 4. Correlation coefficients between biomass and seven morphological traits of 22 switchgrass populations.

Traits	Biomass	Plant height	Tiller density	Leaf number	Leaf lengths	Days to panicle initiation	Seed weight	Panicle number
BM	-	0.76 <sup>***</sup>	-0.02	0.46 <sup>*</sup>	0.62 <sup>**</sup>	0.66 <sup>**</sup>	0.09	0.02
PH	0.76 <sup>***</sup>	-	-0.45 <sup>*</sup>	0.75 <sup>***</sup>	0.79 <sup>***</sup>	0.89 <sup>***</sup>	-0.12	-0.49 <sup>*</sup>
TD	-0.02	-0.45 <sup>*</sup>	-	-0.41	-0.33	-0.52 <sup>*</sup>	0.24	0.58 <sup>*</sup>
LN	0.46 <sup>*</sup>	0.75 <sup>***</sup>	-0.41	-	0.45 <sup>*</sup>	0.71 <sup>**</sup>	-0.39	-0.37
LL	0.62 <sup>**</sup>	0.79 <sup>***</sup>	-0.33	0.45 <sup>*</sup>	-	0.61 <sup>**</sup>	0.18	-0.42
DP	0.66 <sup>**</sup>	0.89 <sup>***</sup>	-0.52 <sup>*</sup>	0.71 <sup>**</sup>	0.61 <sup>**</sup>	-	-0.27	-0.43
SW	0.09	-0.12	0.24	-0.39	0.18	-0.27	-	0.42
PN	0.02	-0.49 <sup>*</sup>	0.58 <sup>*</sup>	-0.37	-0.42	-0.43	0.42	-

\*\*\*, \*\*, and \* are significant at probability of 0.001, 0.01, and 0.05, respectively.



Table 5. Firth three principle component analysis (PCA) eigenvectors (PRIN1, PRIN2 and PRIN3) of 22 switchgrass populations for biomass and seven morphological traits and variation accounted for each principle component vector.

Trait	Principle component eigenvectors		
	PRIN1	PRIN2	PRIN3
Biomass	0.32	0.48	0.27
Plant height	0.47	0.15	0.01
Tiller density	-0.29	0.37	0.43
Leaf number	0.39	-0.09	0.36
Leaf length	0.37	0.28	-0.37
Days to panicle initiation	0.45	0.03	0.13
Seed weight	-0.13	0.57	-0.57
Panicle number	-0.29	0.44	0.37
Variation (%)	53.0	20.7	11.7

Table 6. Means of ten physiological traits including net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ), electron transport rate ( $ETR$ ), internal carbon dioxide concentration ( $C_i$ ), pigment concentrations chlorophyll a (chl a), chlorophyll b, (chl b) and carotenoids (car), leaf area and stomatal index (lower (SIL) and upper (SIU) surfaces) among 22switchgrass populations.

Entry	$P_n$	$g_s$	$C_i$	$ETR$	Chla	SI(L)	LA	Chlb	Car	SI(U)
Alamo	28.4	0.169	100.8	139.8	21.5	19.7	41.8	1.42	6.73	25.3
Cave-In-Rock	24.8	0.155	113.9	151.0	20.7	17.6	44.1	1.31	6.76	21.0
Kanlow	28.9	0.182	111.9	148.7	25.2	18.4	52.0	2.42	7.18	25.1
NL 94 C2-1	27.0	0.148	82.9	154.9	25.5	20.7	57.8	5.47	6.38	26.3
NL 94 C2-2	25.0	0.133	75.3	142.0	23.7	17.6	49.3	4.43	5.56	21.5
NL 94 C2-3	25.0	0.133	73.8	134.9	25.4	18.8	62.5	5.82	6.33	26.7
NL 94 C2-4	27.0	0.165	104.0	152.8	29.7	18.0	56.9	8.39	7.94	25.6
NSL 2009 -4	24.6	0.129	66.0	125.1	20.2	20.8	51.8	5.30	6.41	25.4
NSL 2009-1	25.2	0.132	65.2	117.4	21.5	22.3	65.6	4.22	5.64	24.6
NSL 2009-2	23.4	0.115	49.1	118.3	23.5	17.8	52.2	4.91	6.43	24.9
NSL 2009-3	28.6	0.146	58.5	127.1	23.2	22.1	52.0	5.27	7.08	20.9
SL 93 C2-1	23.5	0.147	112.6	114.3	24.5	19.2	48.5	5.65	6.32	23.6
SL 93 C2-2	30.4	0.219	137.7	142.3	29.8	23.5	44.6	8.49	7.59	30.0
SL 93 C2-3	24.3	0.123	61.8	132.2	26.8	21.4	46.1	7.04	7.08	27.5
SL 93 C2-4	24.7	0.141	89.9	136.9	26.1	20.0	52.1	6.93	7.14	24.3
SNU 98 EMBP C1-1	27.5	0.176	118.0	139.4	24.4	18.5	35.0	6.24	6.19	21.2
SNU 98 LMBP C1-1	24.5	0.149	109.0	131.4	27.1	17.1	37.6	5.80	7.44	21.7
SNU 98 LMBP C1-2	26.6	0.180	128.2	130.4	29.0	18.3	42.9	7.80	8.11	21.6
SWG 2007-1	26.1	0.139	71.2	137.9	25.4	20.9	39.0	5.28	6.55	26.1
SWG 2007-2	26.2	0.166	107.6	149.2	27.8	22.2	48.4	5.53	7.29	20.4
SWG 2007-3	28.2	0.209	152.3	148.4	19.5	18.2	40.9	5.84	4.38	19.0
SWG 2007-4	26.7	0.169	112.0	147.2	22.8	19.8	38.1	2.87	7.12	20.9
Mean	26.2***	0.156***	95.5***	137.3***	24.7***	19.7**	48.15***	5.29**	6.71*	23.8 <sup>NS</sup>
LSD (5%)	3.15	0.040	41.4	18.5	4.54	3.34	7.49	3.51	1.74	7.17

\*\*\*, \*\*, \* and NS are significant at probability of 0.001, 0.01, 0.05 and non significant, respectively

Table 7. Pearson's correlation coefficients between net photosynthesis (Pn) and nine physiological traits including stomatal conductance (gs), electron transport rate (ETR), internal carbon dioxide concentration, pigment concentrations chlorophyll a (chl a), chlorophyll b, (chl b) and carotenoids (car), leaf area and stomatal index ( lower (SIL) and upper (SIU) surfaces) among 22 switchgrass populations.

Traits	<i>Pn</i>	<i>g<sub>s</sub></i>	<i>Ci</i>	<i>ETR</i>	Chla	SIL	LA	Chlb	Car	SIU
<i>Pn</i>	-	0.80***	0.49*	0.51*	0.14	0.31	-0.20	0.03	0.14	0.07
<i>g<sub>s</sub></i>	0.80***	-	0.90***	0.51*	0.19	0.05	-0.43	0.13	0.11	-0.15
<i>Ci</i>	0.49*	0.90***	-	0.46*	0.16	-0.22	-0.52*	0.10	0.08	-0.31
<i>ETR</i>	0.51*	0.51*	0.46*	-	0.16	-0.12	-0.19	-0.12	0.11	-0.10
Chla	0.14	0.19	0.16	0.16	-	0.13	0.02	0.69**	0.73**	0.37
SIL	0.31	0.05	-0.22	-0.12	0.13	-	0.22	0.20	0.12	0.40
LA	-0.20	-0.43	-0.52*	-0.19	0.02	0.22	-	0.08	-0.09	0.38
Chlb	0.03	0.13	0.10	-0.12	0.69**	0.20	0.08	-	0.27	0.27
Car	0.14	0.11	0.08	0.11	0.73**	0.12	-0.09	0.27	-	0.25
SIU	0.07	-0.15	-0.31	-0.10	0.37	0.40	0.38	0.27	0.25	-

\*\*\*, \*\*, and \* are significant at probability of 0.001, 0.01, and 0.05, respectively

Table 8. Principle component analysis eigenvectors (PRIN1, PRIN2 and PRIN3) of 22 switchgrass populations for photosynthesis (Pn) and nine physiological traits and percent of variation accounted by each principle component vector of 22 switchgrass populations.

Physiological trait	Principle Component eigenvectors		
	PRIN1	PRIN2	PRIN3
Photosynthesis	0.38	-0.26	-0.02
Stomatal conductance	0.36	0.06	0.45
Internal CO <sub>2</sub> concentration	0.45	0.06	0.23
Electron transport rate	0.44	0.00	-0.04
Chlorophyll a concentration	0.38	-0.03	0.14
Stomatal index (lower surface)	-0.17	0.31	0.54
Leaf area	-0.15	0.40	0.45
Chlorophyll b concentration	0.21	0.51	-0.29
Carotenoids concentration	-0.05	0.56	-0.28
Stomatal index (upper surface)	0.31	0.31	-0.28
Variation (%)	39.7	25.2	12.8

Table 9. Means of tiller morphological traits among 21 switchgrass populations.

Genotype	Tiller height (cm)	Internode length (cm)	Leaf number tiller <sup>-1</sup>	Leaf sheath length (cm)	Phytomer number tiller <sup>-1</sup>	Phytomer weight (g)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm <sup>2</sup> )	Tiller weight (g)
NSL 2009-1	107.8	14.2	8	18.3	6	1.332	47.4	1.54	48.9	6.66
NSL 2009-2	129.3	16.3	8	17.8	6	1.077	46.2	1.55	49.7	5.39
NSL 2009-3	117.5	15.0	8	16.7	6	1.821	50.3	1.67	47.6	9.11
NSL 2009-4	109.5	19.5	8	18.8	5	1.525	50.9	1.54	53.8	7.63
SL 93 C2-1	113.8	13.1	8	14.8	6	1.512	44.4	1.64	43.1	7.56
SL 93 C2-2	120.4	13.5	8	15.6	6	1.063	46.4	1.55	45.4	5.31
SL 93 C2-3	102.1	14.7	7	19.0	5	1.113	45.5	1.63	44.9	5.56
SL 93 C2-4	117.9	17.5	7	18.1	6	0.870	45.0	1.48	45.6	4.35
NL 94 C2-1	100.2	16.8	7	19.0	5	1.322	47.8	1.60	47.5	6.61
NL 94 C2-2	115.7	15.7	8	17.5	6	1.394	50.3	1.54	48.5	6.97
NL 94 C2-3	136.5	16.1	9	20.0	7	1.788	52.5	1.62	54.8	8.94
NL 94 C2-4	109.3	23.3	8	27.6	5	1.652	47.7	1.59	45.7	8.26
SNU 98 LMBP C1-1	81.6	11.1	7	14.2	5	0.694	46.8	1.27	37.4	3.47
SNU 98 LMBP C1-2	99.8	10.7	7	12.5	6	0.790	39.1	1.31	37.3	3.95
SNU 98 EMBP C1-1	78.8	13.5	7	18.7	5	0.849	38.9	1.25	34.0	4.25
SWG 2007-1	100.8	21.1	7	21.0	5	0.848	44.9	1.35	39.4	4.24
SWG 2007-2	91.7	21.2	6	21.3	5	1.108	51.1	1.64	53.7	5.54
SWG 2007-3	76.0	14.7	5	19.4	5	0.778	40.8	1.15	35.1	3.89
SWG 2007-4	89.4	8.4	7	12.7	6	0.362	40.5	1.21	36.0	1.81
Alamo	100.3	21.7	7	18.9	5	1.435	45.6	1.54	42.0	7.17
Cave-In-Rock	83.2	9.3	7	13.7	6	0.581	41.3	1.40	40.2	2.91
Mean	103.9 <sup>***</sup>	15.6 <sup>***</sup>	7 <sup>***</sup>	17.9 <sup>*</sup>	6 <sup>**</sup>	1.139 <sup>***</sup>	45.9 <sup>**</sup>	1.5 <sup>***</sup>	44.3 <sup>**</sup>	5.7 <sup>***</sup>
LSD (5%)	14.6	2.3	1	2.5	1	0.191	7.8	0.19	11.8	0.196

\*\*\*, \*\*, and \* are significant at probability of 0.001, 0.01, and 0.05, respectively.

Table 10. Correlations coefficients of 21 switchgrass populations for ten morphological traits and their significant levels among the traits.

Trait	Tiller weight	Tiller height	Internode length	Leaf number Tiller <sup>-1</sup>	Leaf sheath length	Phytomer number tiller <sup>-1</sup>	Phytomer weight	Leaf length	Leaf width	Leaf area
Tiller weight	-	0.65**	0.54*	0.55*	0.49*	0.20	0.99***	0.75***	0.80***	0.70***
Tiller height	0.65**	-	0.28	0.75***	0.16	0.65**	0.65**	0.60**	0.70***	0.72***
Internode length	0.54*	0.28	-	-0.08	0.86***	-0.41	0.54*	0.53*	0.46*	0.48*
Leaf number	0.55*	0.75***	-0.08	-	-0.06	0.75***	0.55*	0.40	0.54*	0.46*
Leaf sheath length	0.49*	0.16	0.86***	-0.06	-	-0.35	0.49*	0.42	0.35	0.38
Phytomer number	0.20	0.65**	-0.41	0.75***	-0.35	-	0.20	0.14	0.21	0.21
Phytomer weight	0.99***	0.65**	0.54*	0.55*	0.49*	0.20	-	0.75***	0.80***	0.70***
Leaf length	0.75***	0.60**	0.53*	0.40	0.42	0.14	0.75***	-	0.75***	0.89***
Leaf width	0.80***	0.70***	0.46*	0.54*	0.35	0.21	0.80***	0.75***	-	0.83***
Leaf area	0.70***	0.72***	0.48*	0.46*	0.38	0.21	0.70***	0.89***	0.83***	-

\*\*\*, \*\*, and \* are significant at probability of 0.001, 0.01, and 0.05, respectively.

Table 11. Principle component analysis (PCA) eigenvectors (PRIN1, PRIN2 and PRIN3) of 21 switchgrass populations for tiller and phytomer morphological traits and percent of variation accounted for each principle component vector.

Trait	Principle component eigenvector		
	PRIN1	PRIN2	PRIN3
Tiller height	0.34	0.26	0.12
Internode length	0.24	-0.47	0.21
Leaf number	0.26	0.42	0.30
Leaf sheath length	0.21	-0.46	0.46
Phytomer number	0.13	0.56	0.24
Phytomer weight	0.38	-0.04	0.19
Leaf length	0.36	-0.07	-0.45
Leaf width	0.37	0.01	-0.26
Leaf area	0.37	-0.01	-0.50
Tiller weight	0.38	-0.04	0.19
Variation (%)	57.9	24.4	5.9

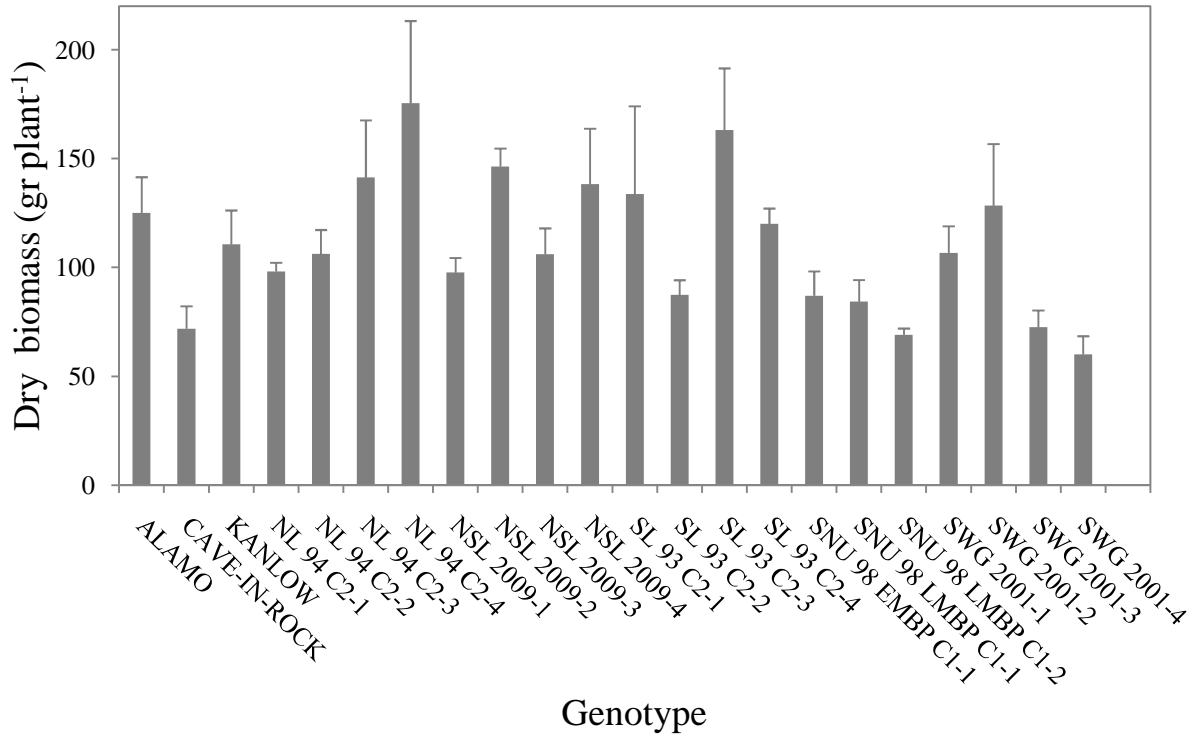


Fig.1 Mean biomass of 22 switchgrass populations grown in greenhouse. The error bars are standard errors.



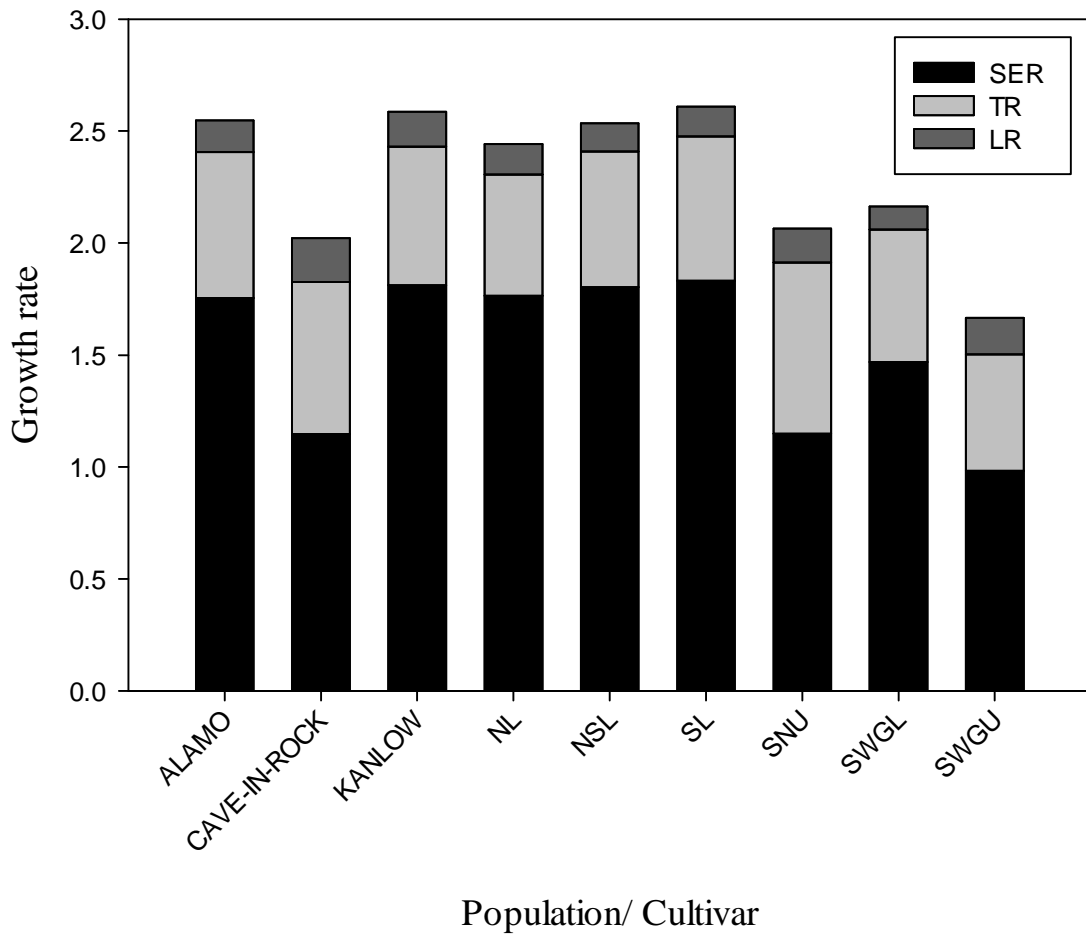


Fig.2 Growth events including leaf addition rate (LR), tillering rate (TR), and stem elongation rate (SER) among six parental populations including northern lowland (NL), southern lowland(SL), northern southern lowland (NSL), SWG lowland(SWGL), upland (SWGU) and southern northern upland (SNU) and three check cultivars (Alamo, Kanlow and Cave-In-Rock)

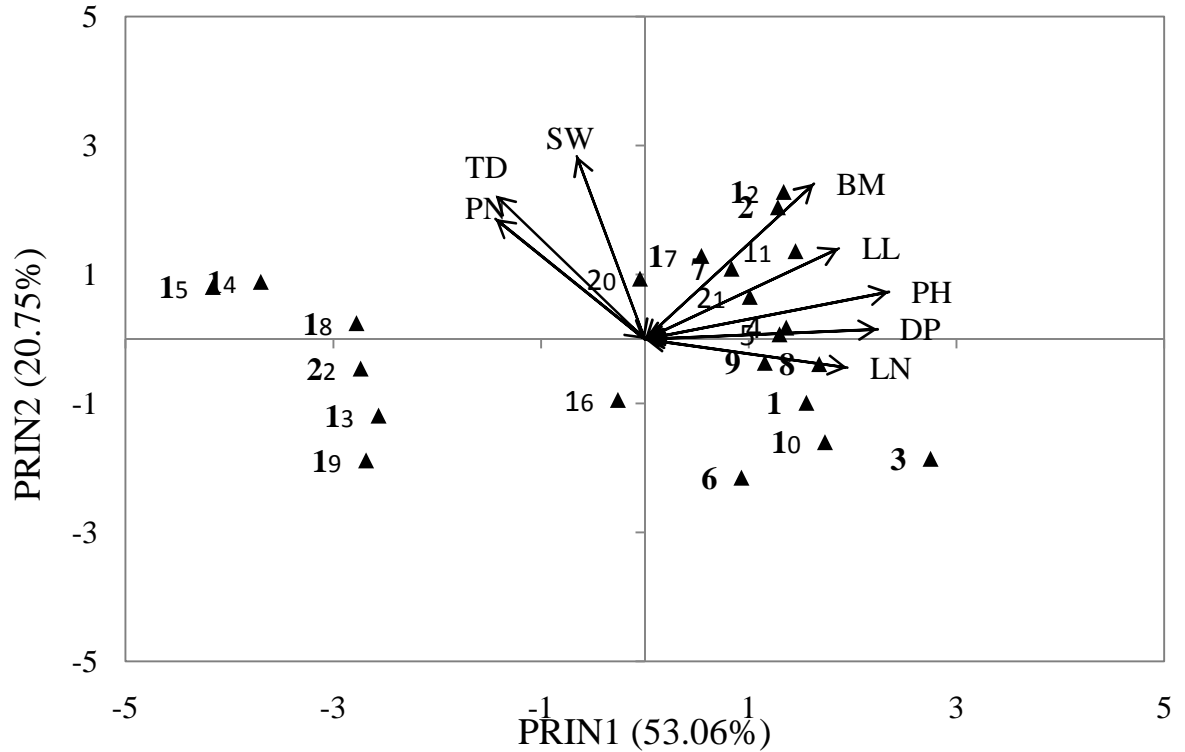


Fig.3 The PCA analysis of biomass (BM), plant height(PH), tiller density (TD), leaf length(LL), leaf number (LN), panicle number (PN), seed weight (SW) and days to panicle initiation(DP) of 22 switchgrass populations and actual eigenvectors are magnified for better illustration. Below is the list of twenty two populations and the respective ID's from the figure

ID	Genotype	ID	Genotype	ID	Genotype
1	NSL 2009-1	8	SL 93 C2-4	15	SNU 98 EMBP C1-1
2	NSL 2009-2	9	NL 94 C2-1	16	SWG 2007-1
3	NSL 2009-3	10	NL 94 C2-2	17	SWG 2007-2
4	NSL 2009-4	11	NL 94 C2-3	18	SWG 2007-3
5	SL 93 C2-1	12	NL 94 C2-4	19	SWG 2007-4
6	SL 93 C2-2	13	SNU 98 LMBP C1-1	20	ALAMO
7	SL 93 C2-3	14	SNU 98 LMBP C1-2	21	KANLOW
				22	CAVE-IN-ROCK

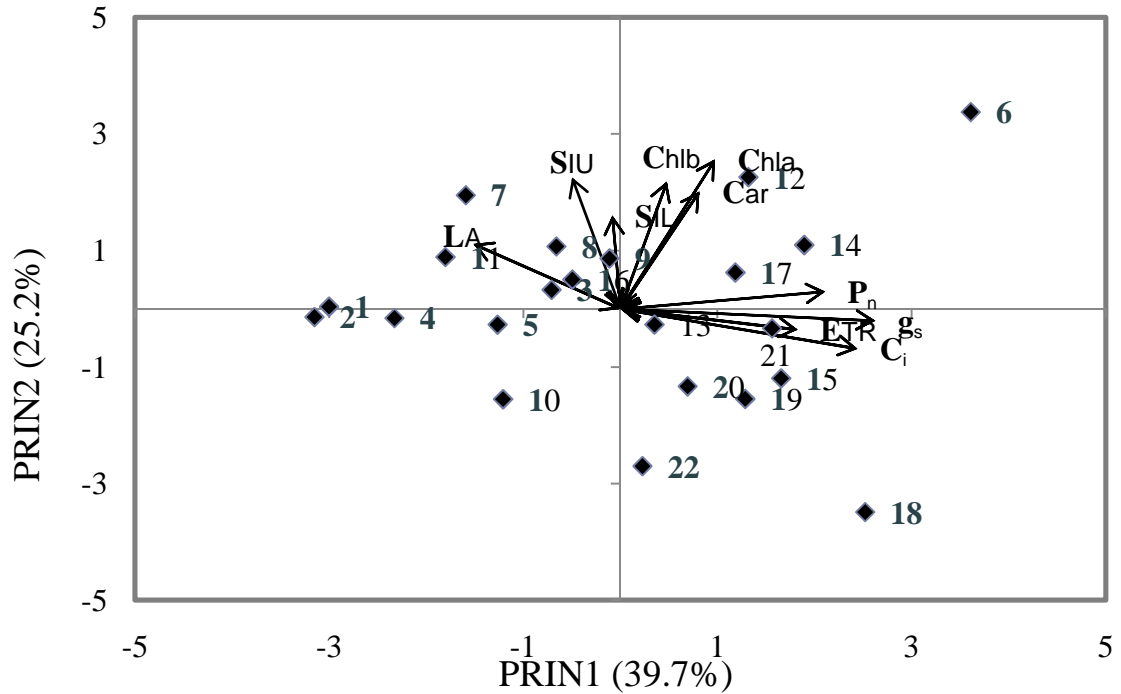


Fig.4 The PCA analysis of net photosynthesis (Pn), stomatal conductance ( $g_s$ ), electron transport rate (ETR), internal carbon dioxide ( $C_i$ ), leaf area, stomatal index on both leaf surfaces (abaxial(SIL) and adaxial (SIU)) and pigment concentrations of 22 switchgrass populations. The eigenvectors for ten traits are magnified for better illustration. Below is the list of twenty two populations and the respective ID's from the figure

ID	Genotype	ID	Genotype	ID	Genotype
1	NSL 2009-1	8	SL 93 C2-4	15	SNU 98 EMBP C1-1
2	NSL 2009-2	9	NL 94 C2-1	16	SWG 2007-1
3	NSL 2009-3	10	NL 94 C2-2	17	SWG 2007-2
4	NSL 2009-4	11	NL 94 C2-3	18	SWG 2007-3
5	SL 93 C2-1	12	NL 94 C2-4	19	SWG 2007-4
6	SL 93 C2-2	13	SNU 98 LMBP C1-1	20	ALAMO
7	SL 93 C2-3	14	SNU 98 LMBP C1-2	21	KANLOW
				22	CAVE-IN-ROCK

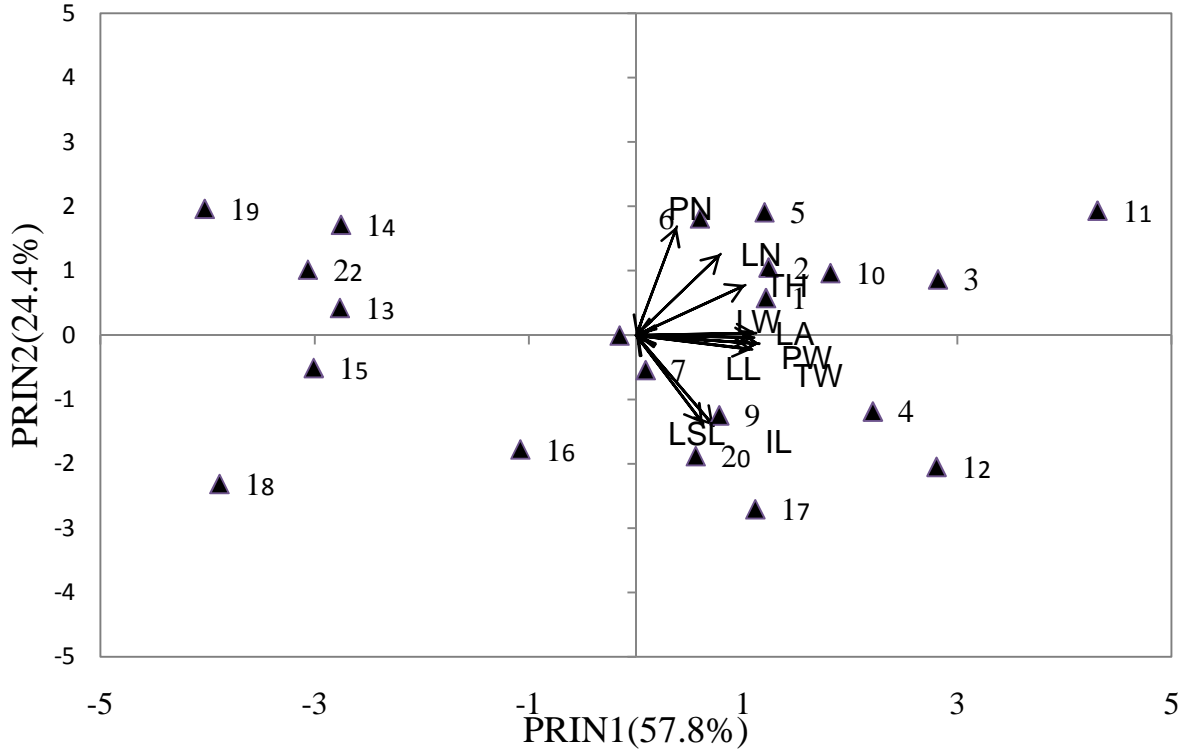


Fig.5 The principle component analysis (PCA) of tiller weight (TW), tiller height (TH), internodal length (IL), leaf number (LN), leaf width (LW), leaf length (LL), leaf area (LA), phytomer number (PN), phytomer weight (PW) and leaf sheath length (LSL) among the populations.

Respective ID's and genotypes of 21 populations are listed in the table

ID	Genotype	ID	Genotype	ID	Genotype
1	NSL 2009-1	8	SL 93 C2-4	15	SNU 98 EMBP C1-1
2	NSL 2009-2	9	NL 94 C2-1	16	SWG 2007-1
3	NSL 2009-3	10	NL 94 C2-2	17	SWG 2007-2
4	NSL 2009-4	11	NL 94 C2-3	18	SWG 2007-3
5	SL 93 C2-1	12	NL 94 C2-4	19	SWG 2007-4
6	SL 93 C2-2	13	SNU 98 LMBP C1-1	20	ALAMO
7	SL 93 C2-3	14	SNU 98 LMBP C1-2	22	CAVE-IN-ROCK

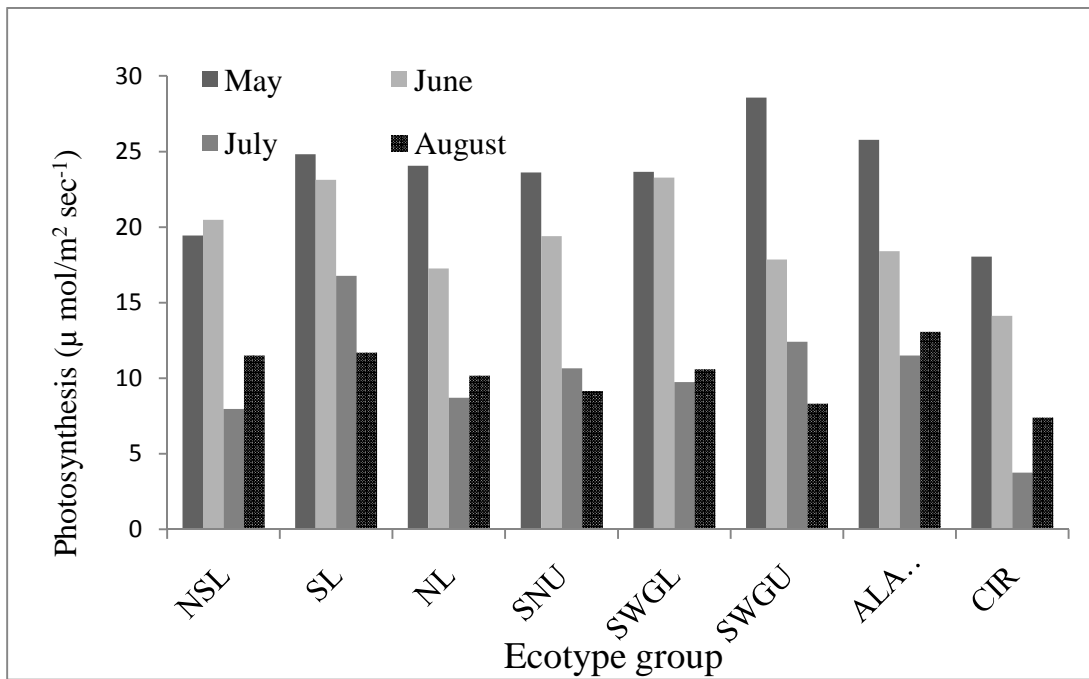


Fig.6 Mean photosynthesis variations among northern lowland (NL), southern lowland(SL), northern southern lowland (NSL), southern norther upland (SNU), SWG populations for upland(SWGU) and lowlands(SWGL) and check cultivars (Alamo and Cave-In-Rock(CIR))

## CHAPTER III

### Genotype x Environment (G X E) Interactions among new Switchgrass Populations in Oklahoma

#### Abstract

Evaluation of advanced breeding populations in transferring economically viable traits within the targeted region is important to develop high biomass Switchgrass (*Panicum virginatum L.*) cultivars. The objective of this study was to evaluate genotype x environment (G x E) interaction for agronomic and biofuel traits among 19 new switchgrass populations and two check cultivars (Alamo and Cave-In-Rock) at four strategically selected locations (Chickasha, Lane, Stillwater and Woodward) in Oklahoma. Genotypes were evaluated for biomass, plant height and tiller density in 2010 and 2011. Significant G x E interaction was found for biomass and plant height. The Northern Southern Lowland (NSL) genotype, NSL 2009-1 produced significantly more biomass ( $15.2 \text{ Mg ha}^{-1}$ ) than the check cultivars in 2011. The Northern lowland (NL) and NSL populations were taller, but not significantly different from Alamo. The Southern Lowland (SL) and SWG lowland type populations tiller density was very similar to Alamo. Stability analysis demonstrated higher stability of lowland populations for dry

biomass with greater slopes compared to upland populations. The genotypes, NL 94 C2-3 (b=1.37) and NSL 2009-2 (b=1.32) ranked highest for biomass with greater stability compared to Alamo (b=1.25). Due to G x E interactions, the correlations between biomass, plant height and tiller number were different from establishment to post establishment year. However, multi-year evaluations would be required to assess the biomass stability of these new breeding populations.

## Introduction

Biomass has been and continues to be an economically viable trait for the grasses, especially those deployed as potential biofuel feedstocks like switchgrass. Though switchgrass is a native species, adaptation between ecotypes varies widely (Hopkins et al., 1995c; Sanderson et al., 1999). Therefore, switchgrass biomass production varied due to original adaptation, strong photoperiodic nature, and cold hardiness between ecotypes. Switchgrass population trials were reported across the USA, mostly in the midwest (Vogel et al. 2002; Lemus et al. 2002; Casler and Boe 2003) the southern (Sanderson et al. 1999; Cassida et al. 2005; Fuentes and Taliaferro 2002; Das et al. 2004; Fike et al. 2006; Bhandari et al. 2011) and the northern Great Plains of USA (Lee and Boe 2005; Lee et al. 2009). The genotype x environment (G x E) interactions for biomass is very obvious in multi-location trial evaluations of switchgrass and the results showed significant effect of environment on dry matter yield (Casler and Boe, 2003; Casler et al., 2004; Cassida et al., 2005a; Hopkins et al., 1995b; Hopkins et al., 1995c; Koshi et al., 1982) and magnitude of G x E interactions for biomass was greater than other traits

(Hopkins et al., 1995b; Hopkins et al., 1995c). However, results and interpretations were different among different geographic regions. In southern USA trials, lowland populations produced higher biomass than upland populations (Table 1), but upland populations were superior in northern USA. Results in Midwestern states such as Iowa also showed high biomass potential of lowland populations (Lemus et al. 2002). Specific biomass yield responses were also found between ecotypes and new breeding ecopopulations under different latitudes ranging from southern (36°N) to northern (46°N) latitudes of the United States. Lowland population biomass decreased with increasing latitude, whereas upland biomass increased from southern to northern US environments. However, lowland populations are more sensitive to latitudinal change than upland populations (Casler et al. 2004). Switchgrass latitudinal adaptation study concluded that switchgrass was sensitive to the region of its origin, and cultivar performance decreased if cultivars were planted more than 500 km or one USDA hardiness zone away from its origin (Casler et al. 2004). These findings indicate the importance of growing environment to achieve high biomass potentials of switchgrass.

A understanding of genetic diversity has relevance in order to conserve the germplasm resources and characterize desirable traits through conventional breeding methods. Phenotypic expression of the plant is unique to the growing environment. Though biomass is primary goal for grass breeders, understanding and evaluation of biomass influencing traits is important. Plant height was highly correlated with final biomass (Schmer et al. 2010 Bhandari et al. 2011; Casler et al. 2004; Lemus et al. 2002b) and the results also infer that plant height is a potential trait for selection to increase biomass. Similarly, tiller density is an important trait and correlated positively with final



biomass (Das et al. 2004; Bhandari et al. 2011). Results for tiller density varied among the populations and across the locations. However, lowland populations had higher tiller density than upland populations (Cassida et al. 2005). Tiller density is also influenced by row spacing (Muir et al. 2001). However, under field conditions biomass variations were attributable to variations in soil, environment (Sanderson et al. 1999) and other agronomic traits such as stand density and row spacing (Muir et al. 2001).

Breeding for switchgrass biomass was reported in new breeding populations. Biomass evaluations of switchgrass breeding lines at Texas, Arkansas and Louisiana demonstrated high biomass potential of southern lowland populations (Cassida et al., 2005). Similarly, high biomass yields of SL populations were also reported in Oklahoma (Fuentes and Taliaferro 2002; Casler et al., 2004). However, the performance of these new populations was not consistent SL population yields were greater at Stillwater, OK (36°N), whereas northern lowland populations produced higher biomass at Manhattan, KS (42°N). Similarly, mixed results were also reported in a multi-state evaluation study by Cassida et al. (2005). These contradictory results demonstrate the importance of switchgrass adaptation when developing new cultivars for high biomass yield within the southern USA.

Phenotypic expression of the genotype is unique to the growing environment. Evaluation of advanced breeding populations for biomass potential under different climatic and edaphic conditions within the targeted region is critical for decision making especially for future breeding deployment. Breeding efforts and testing of new cultivars against existing cultivars will be critically important to attract emerging cellulose-based biofuel industries in the southern US region. However, yield responses of new breeding

populations under diverse climatic conditions in southern US is limited, especially in Oklahoma. These evaluations could be possible through G x E interaction studies. Thus, the objective of this study was to evaluate the effect of genotype x environment interaction on biomass production of new breeding populations in Oklahoma.

## Materials and Methods

### Multi-location description

Multi-location trials were established at five locations in Oklahoma, including Stillwater, Lane, Woodward, Chickasha and Tipton in 2010. At Tipton during post establishment year, all plants were killed due to exceptional drought and restricted root growth caused by hard plough pan. Therefore, present study was restricted to four locations. Table 12 shows the weather data of four locations including mean rainfall year<sup>-1</sup>, solar radiation, and average temperature of past 15 years (1994-2010) obtained from Oklahoma Climatological Survey (Table 12). Soil samples were collected prior to transplanting to assay the soil fertility status. However, no fertilizers were applied during the establishment year to minimize the weed competition. Urea (85 kg ha<sup>-1</sup>) was applied during early growing season of post establishment year.

### Plant material and stand establishment

Nineteen advanced breeding populations derived from recurrent selection for general combining ability (RSGCA) procedure and two check cultivars (Alamo and Cave-In-Rock) were included in this study. Seed material was developed and obtained

from Oklahoma State University Switchgrass Breeding and Genetics program. Seeds of 22 switchgrass populations were sown in small pots filled with Metro-Mix 250 growing medium (Scotts-Sierra Horticultural Products Co., Marysville, OH). Seeds were placed on the top of growing medium, and then covered with thin layer (0.5cm). Healthy seedlings were transplanted into 10cm deep containers and then filled with growth medium. Adequate water was given immediately after transplanting and optimum conditions were maintained until seedlings were transplanted into field plots.

The experimental design was a randomized complete block (RCB) with four replications and each replication consisting of 19 experimental lines and two check cultivars, Alamo and Cave-In-Rock. All the test plots were bordered with Kanlow. The test plot was divided into six (6) columns (south to north direction), and spacing between two neighboring columns was 30 cm. Ten (10) plants were included in each column (10 rows). Spacing between two neighboring plants in a column was 30 cm. Greenhouse grown seedlings from container were transplanted manually into field plots across the five locations. Fields were irrigated using sprinkler system immediately after transplanting at Stillwater, Lane, and Woodward. At Tipton, plots were manually irrigated by-plant. Chickasha had enough moisture during transplanting and no irrigation was provided.

Biomass, plant height, and tiller density were evaluated after killing frosts in years 2010 and 2011. Plant height was taken from base to topmost node of leaf. Five random representative plants from each plot were harvested approximately at 10cm above soil surface for biomass estimation. Tiller density (tiller number plant<sup>-1</sup>) of each plant was measured from harvested plants. All samples were dried at 60°C in a forced-air oven for

three days. Data were analyzed using ANOVA procedure of SAS statistical analysis (SAS 9.2). Data were arranged as split-plot in time as described by Steel and Torrie (1980). Appropriate error terms were used to account for genotype, year, and location interactions. Fisher's protected least significant difference (LSD) procedure was used for mean separations at 5% level. Biomass yield stability was calculated and regression equations were developed between biomass and environment index (location biomass mean- grand biomass mean) described by Eberhart and Russell (1966).

## Results and Discussion

### Dry biomass

Exceptional drought conditions and hardpan within the uppermost soil surface at Tipton restricted the growth of root growth, and subsequent drought conditions affected the biomass of all plots resulting in a total stand loss. Therefore, Tipton was not included in the data analysis. The ANOVA for dry biomass was significantly different for main factors (genotype, location, and year), interaction effects (genotype x environment, year x environment, year x genotype) and genotype x environment x year (Table 13). However, most of these significant variations were due to variations in date of stand establishment and severe drought conditions during the post establishment year. Therefore, locations and years were presented separately for dry biomass.

Mean biomass yields in 2010 varied across the locations. Stillwater location had dry biomass of 5.4 Mg ha<sup>-1</sup> and was significantly ( $P < 0.05$ ) greater than biomass at Woodward (3.6 Mg ha<sup>-1</sup>), Chickasha (3.8 Mg ha<sup>-1</sup>) and Lane (1.1 Mg ha<sup>-1</sup>) (Table 14).

Most of these yield variations were associated with time of transplanting. The highest and lowest yields were reported at early and late translating locations. However, the very low yields at Lane were possibly due to highly acidic soils ( $P^H = 4.8$ ). Significant yield reductions in non-limed ( $P^H=4.9$ ) and limed soils ( $P^H=5.9$ ) were also reported for switchgrass by Bona and Belesky (1992). Stillwater had 4% higher rainfall compared to a mean rainfall of the past 15 years (Fig. 7) and it allowed the production of more biomass compared to the other locations. Though Lane had 2% more rainfall compared to past 15years, late planting and soil acidity lowered biomass yields (Fig. 7). Biomass variations with translating date also demonstrates the importance of proper date of stand establishment for biomass production. Across the locations, lowland population's dry biomass ( $4.13 \text{ Mg ha}^{-1}$ ) was significantly higher than that of upland ( $1.17 \text{ Mg ha}^{-1}$ ) population's (Table 14).

Northern lowland genotypes, NL 94 C2-4 and NL 94 C2-3 produced the highest biomass at Lane and Woodward. Alamo had high biomass both at Stillwater and Chickasha. Phenotypic expression of genotypes within common environment is unique, from vegetative growth to seed maturity. Different dates of translating might have inhibited complete phenotypic expression of the genotypes within each location. Therefore, there was no significant difference were found among the lowland populations for dry biomass. This also extended to genotype groups within each population and across the locations, NL type biomass ( $4.3 \text{ Mg ha}^{-1}$ ) produced high biomass among the new populations, but it was not significantly different from the check cultivar, Alamo ( $4.7 \text{ Mg ha}^{-1}$ ). High biomass of Alamo in south most locations including Chickasha and Lane during an establishment year may be due to broader and well established adaptation

within the southern US environments. Northern lowland population, NL 94 C2-3 produced high biomass at Woodward. Casler et al. (2004) reported variations in biomass ranking from 36°N to 39°N from SL to NL populations. Our results did not follow any trend with latitude during the establishment year.

Mean dry biomass across locations during the post establishment year was almost three times greater than in the establishment year. Since switchgrass is a perennial grass, the increase in biomass compared to the establishment year is obvious. The biomass increase varied across the locations, ranging from 7.2 (Lane) to 0.3 (Woodward) times greater compared to the establishment year. Across the locations, highest yield improvements were observed at Lane and Stillwater, which were 7.2 and 3.1 times greater than in the establishment year. At Chickasha and Woodward yields were 1.5 and 0.36 times higher than in the establishment year. Mean biomass yields for locations were significantly different, ranging from 22.3 Mg ha<sup>-1</sup> (Stillwater) to 4.9 Mg ha<sup>-1</sup> (Woodward) (Table 15). Chickasha and Lane biomass yields were very similar, 9.8 and 9.3 Mg ha<sup>-1</sup> (Table 15). These biomass variations were mostly attributable to environment conditions, fertility status, and especially the amount and distribution of precipitation across the locations. Sanderson et al. (1999) reported that rainfall during the early growing season increases biomass production. Exceptional drought conditions, especially at Woodward, severely affected biomass yield compared to the other locations. Season rainfall was well below normal compared with mean seasonal rainfall of the past 15 years at these locations (Fig. 7 and Fig. 8). The 2011 seasonal rainfall was 51.9%, 47.75%, 62.4% and 59.11% less than mean rainfall of the past 15 years at Chickasha, Lane, Stillwater, and Woodward, respectively. Even though the quantity of precipitation was

low in 2011 at Stillwater, the rainfall was well distributed with more than 3cm precipitation during early growing season (Fig. 7). Switchgrass germplasm evaluation by Sanderson et al. (1999) also noted that early rainfall is critical for high biomass. Chickasha and Lane had high rainfall during early season but the distribution was poor, whereas rainfall distribution was good at Woodward but the quantity of rainfall was very limited (Fig. 8). Since fertilization was applied during early growing season (last week of May), Stillwater, Lane and Chickasha locations used subsequent rainfall and nutrients effectively compared to that of Woodward.

Across the locations, biomass yields for genotypes, NSL 2009-1(15.2 Mg ha<sup>-1</sup>) and NSL 2009-2 (15.0 Mg ha<sup>-1</sup>) were significantly higher than Alamo (13.4 Mg ha<sup>-1</sup>) (Table 15). The NSL populations NSL 2009-1 and NSL 2009-2, NL populations NL 94 C2-3 ranked among first five high biomass producing genotypes across the locations. Among these three populations NL 94 C2-3 had high stability followed by NSL 2009-2 (Table 24). High stability for dry biomass of these three breeding populations over the check cultivar was mostly attributable to their genetic advancement. Three NL populations reported high biomass in north most location at Woodward, this possibly due to the specific adaptation of NL types towards north western region of Oklahoma. However, present results did not show any significant trend with latitude. Switchgrass latitudinal evaluations by Casler et.al (2004) also found that SL populations were best at south most location (36°7' N) and with increasing latitude, at 39°25' N the NL populations yields were greater than SL populations. Multiyear evaluations possibly could reveal the specific adaptation of these new breeding populations.

## Tiller density

Tiller density was significantly different for main effects, but was not significant for interaction effect and this allowed to present tiller density across the locations and years (Table 16). Tiller density among the genotypes within each population was not significantly different during 2010 and 2011. Therefore, genotypes within each population were grouped. Among the locations, tiller density was significantly different and ranged from 21 tillers plant<sup>-1</sup> (Stillwater) to 10 tillers plant<sup>-1</sup> (Lane) (Table 18). Lowland populations had greater tiller density than upland populations and within lowland populations, Alamo and SL populations produced more tillers. Subsequent significant variations among the populations were also evident only at Lane. Alamo produced more tillers at Chickasha and Stillwater. The SWG lowland type populations produced more tillers at Lane and Woodward.

Over the locations, biomass was significantly ( $P < 0.01$ ) correlated with tiller density with a correlation coefficient of 0.72 and significant correlations were also reported at each location. High correlation coefficients were reported at Lane ( $r = 0.81$ ), followed by Chickasha ( $r = 0.63$ ), Woodward ( $r = 0.43$ ), and Stillwater ( $r = 0.42$ ) (Table 17). Correlations for upland and lowland populations were also different across locations (Table 18). However, except at Stillwater, lowland populations tiller density was highly and significantly correlated with biomass. Generally in grasses, tiller ontogeny can be divided into four phases: vegetative, elongation, reproductive and seed maturity (Moore et al. 1991). Environmental factors including temperature, photoperiod and radiation have the most impact on elongation phase compared to the developmental phase in grasses (Moore et al. 1991). The significant correlations between biomass and tiller density



during establishment year were mostly due to variations in date of transplanting. Late transplanting locations possibly had limited elongation phase compared to early transplanting locations. Therefore, the tiller density was highly correlated at most of the late transplanting locations compared to early transplanting location, Stillwater. With long growing season at Stillwater, the plants interacted with the environment for long duration; therefore, biomass was probably less dependent on tiller density.

Tiller density across locations in 2011 was 55% greater than in 2010 and ranged from 35plant<sup>-1</sup> (Stillwater) to 23 plant<sup>-1</sup> (Lane) (Table 19). At Stillwater, tiller density was significantly different ( $P<0.05$ ) among the populations. Across the locations, SL and SWG populations had more tiller density, but not significantly different from Alamo (Table 20). During post-establishment year, correlations coefficients between biomass and tiller density were non-significant or less significant compared to establishment year. These variations were attributable to environment interactions across the locations and populations within locations. Significant correlations between biomass and tiller density in lowland populations were observed at high yielding and low yielding environments, Stillwater and Woodward, respectively. Previous studies also reported the importance of tiller mass compared to tiller density (Boe 2007). Under high yielding environments, tiller elongation was relatively less influenced by environment compared to low yielding environments. Mean plant height was high at Stillwater (140.5cm) compared to Woodward (62.2cm) (Table 23). Therefore, based on the present results we interpret that under both high yielding and low yielding environmental conditions selection for tiller density would be a potential option to increase biomass. However, further research on

tiller morphology across a wide geographic region might be required to prove this interpretation.

#### Plant height

Plant height was significantly different for main effects and interactions effects. Most of these significant differences were due to lowland and upland height variations, establishment and post establishment and location x year (Table 23). However, there was no significant difference among the genotypes within each population, which allowed for general comparison among the populations. During the establishment year, height was significantly different among the populations (Table 22). Across the locations and populations, the NL populations were taller, but were not significantly different from the check cultivar Alamo. Plant height was significantly correlated with biomass across the locations. Highest correlations coefficients were reported at Stillwater ( $r=0.75$ ), followed by Chickasha ( $r=0.73$ ), Woodward ( $r=0.68$ ) and Lane ( $r=0.59$ ) (Table 17). Highest correlations during post establishment were reported for Stillwater ( $r=0.56$ ), Chickasha ( $r=0.54$ ), Lane ( $r=0.49$ ) and least at Woodward ( $r=0.34$ ). Correlations among the biomass, tiller density, and plant height were mostly significant with high positive correlations, whereas weak correlations during post establishment are more likely attributable to environmental conditions (exceptional drought in Oklahoma).

#### Stability analysis

Regression slopes between environmental index (location mean biomass- grand mean biomass) were greater for lowland compared to upland populations. This implies that higher biomass production potential of lowland populations across the environments

compared to upland populations. During establishment and post establishment years, lowland population slopes were significantly higher than upland populations (Table 24). Similar results were also reported in multi-location and multi-year study by Sanderson et al. (1999). Highest slope was recorded for Alamo during the establishment year. During post establishment year, high stability was reported for SL 93 C2-4, NL 94 C2-3, SL 93 C2-2, and NSL 2009-2, which were higher than Alamo. Among the high stability populations, NL 94 C2-3 and NSL 2009-2 were high yielding genotypes. Selecting genotypes that can produce high biomass with greater stability across the targeted regions is an important consideration in developing cultivars.

In conclusion, genotypic variations and genotype by environment interactions were evident for dry biomass. Tiller density variations were not significantly different between the populations under different environment conditions. High biomass and stability were reported for NSL 2009-2 and NL 94 C2-3 populations. Correlations analysis showed that biomass under low yielding environments was explained by tiller density due to limited stem elongations caused by high temperatures in these environments (Kandel and Kakani 2010). Whereas under high yielding environmental conditions plant height and tiller density were major potential traits for selection to enhance biomass. However, multiyear evaluations would be needed to assess the stability of different genotypes for the biomass potential.

Table 12. Experiment sites description on soil series and weather parameters including mean annual rainfall (cm), temperature (°C) (minimum, maximum and average) and solar radiation of past 15 years data from Oklahoma Climatological Survey, OK.

Location	Coordinates	Transplanting date (2010)	Soil series	Mean daily temperature(°C)			Mean rainfall (cm/year)	Daily Avg solar radiation (MJ/m <sup>2</sup> )
				Max	Min	Avg		
Stillwater	36°07'N 97°05'W	25 <sup>th</sup> June	Port silt loam	22	9	16	93	16.1
Lane	34°17' N 95°59' W	26 <sup>th</sup> July	Bernow fine-loam	23	11	17	110	16.1
Chickasha	35°1' N 97°54' W	10 <sup>th</sup> July	Mc clain silt loam	23	9	16	81	16.7
Woodward	36°25' N 99°24' W	12 <sup>th</sup> July	Woodward loam	22	8	15	64	17.4

Table 13. The ANOVA across the two years and four locations of 21 switchgrass populations for dry biomass and plant height.

Source of variation	df	Mean squares	
		Biomass	Plant height
Location (L)	3	2710.80 <sup>***</sup>	64962.26 <sup>***</sup>
Reps/Location (R/L)	12	46.12 <sup>***</sup>	308.64 <sup>***</sup>
Genotype (G)	20	85.67 <sup>***</sup>	4918.06 <sup>***</sup>
L x G	60	11.29 <sup>***</sup>	340.79 <sup>***</sup>
Error A	232	4.90 <sup>NS</sup>	87.50 <sup>*</sup>
Year (Y)	1	9574.95 <sup>***</sup>	390638.61 <sup>***</sup>
Y x L	3	1564.81 <sup>***</sup>	39999.19 <sup>***</sup>
Y x G	20	17.0 <sup>***</sup>	174.68 <sup>**</sup>
Error B	12	25.64 <sup>***</sup>	277.38 <sup>***</sup>
G x Y x L	60	7.32 <sup>***</sup>	132.85 <sup>**</sup>
Error C	216	4.6 <sup>**</sup>	67.4 <sup>***</sup>

\*\*\*, \*\* and NS are significant at the probability levels of 0.001, 0.01 and non significant at p=0.05

Table 14. Mean dry biomass of 21 switchgrass populations at four locations and grand mean across the four locations in 2010. Dry biomass mean and least significant difference at 5% levels for each location and across the locations.

Dry biomass (Mg ha <sup>-1</sup> )					
Genotype	Chickasha	Lane	Stillwater	Woodward	Grand mean
NSL 2009-1	4.88	1.21	5.50	3.68	3.82
NSL 2009-2	4.24	1.65	5.39	3.85	3.78
NSL 2009-3	4.65	1.64	7.18	4.11	4.40
NSL 2009-4	5.12	1.26	6.54	4.10	4.26
SL 93 C2-1	4.31	1.31	6.30	3.90	3.96
SL 93 C2-2	4.50	1.02	6.68	3.97	4.04
SL 93 C2-3	4.63	1.17	5.55	4.47	3.96
SL 93 C2-4	4.65	1.48	6.46	4.84	4.36
NL 94 C2-1	4.33	1.59	7.54	4.23	4.42
NL 94 C2-2	4.38	1.38	6.47	3.59	3.96
NL 94 C2-3	4.13	1.32	6.82	5.53	4.45
NL 94 C2-4	4.71	1.70	7.91	3.72	4.51
SNU 98 LMBP C1-1	2.28	0.60	2.02	2.98	1.97
SNU 98 LMBP C1-2	2.22	0.26	1.63	1.66	1.44
SNU 98 EMBP C1-1	1.93	0.54	2.10	1.97	1.64
SWG 2007-1	3.64	1.34	4.97	4.63	3.65
SWG 2007-2	4.30	1.24	5.67	3.03	3.56
SWG 2007-3	2.16	0.28	2.61	2.06	1.78
SWG 2007-4	2.96	0.34	2.83	2.15	2.07
ALAMO	5.27	1.70	8.58	4.20	4.94
CAVE-IN-ROCK	1.68	0.40	2.76	2.18	1.76
Location Mean	3.86***	1.12***	5.31***	3.56***	3.46***
LSD ( <i>P</i> <0.05)	2.78	0.66	2.46	1.52	0.94

\*\*\* Significant at the 0.001 probability level

Table 15. Mean dry biomass of 21 switchgrass populations at four locations and grand mean across the four locations in 2011. Dry biomass mean and least significant difference at 5% levels for each location and across the locations.

Genotype	Dry biomass yield (Mg ha <sup>-1</sup> ) in 2011				Grand mean
	Chickasha	Lane	Stillwater	Woodward	
NSL 2009-1	14.2	11.2	27.8	7.6	15.2
NSL 2009-2	12.4	11.9	28.8	6.2	14.8
NSL 2009-3	11.5	9.6	21.7	4.7	11.8
NSL 2009-4	11.0	12.7	20.5	5.1	12.3
SL 93 C2-1	11.7	11.7	23.7	4.5	12.9
SL 93 C2-2	9.8	8.3	26.8	4.1	12.2
SL 93 C2-3	7.8	10.1	21.7	4.6	11.0
SL 93 C2-4	11.2	9.2	29.8	3.8	13.5
NL 94 C2-1	9.8	10.8	23.0	6.4	12.5
NL 94 C2-2	9.6	12.2	22.4	6.2	12.6
NL 94 C2-3	11.7	10.3	29.2	6.5	14.4
NL 94 C2-4	9.9	10.5	21.9	5.8	12.0
SNU 98 LMBP C1-1	7.6	6.7	14.2	3.4	8.0
SNU 98 LMBP C1-2	6.2	4.4	16.9	3.6	7.8
SNU 98 EMBP C1-1	7.5	7.0	15.9	3.2	8.4
SWG 2007-1	10.5	7.9	21.9	5.4	11.4
SWG 2007-2	10.8	10.0	20.8	5.8	11.9
SWG 2007-3	8.4	6.6	18.6	3.3	9.2
SWG 2007-4	7.2	6.4	17.0	3.4	8.5
ALAMO	11.3	10.8	26.5	5.0	13.4
CAVE-IN-ROCK	5.9	5.2	12.8	4.0	6.9
Location mean	9.8 <sup>***</sup>	9.2 <sup>***</sup>	22.0 <sup>***</sup>	4.9 <sup>***</sup>	11.5 <sup>***</sup>
LSD ( <i>P</i> <0.05)	2.73	5.19	8.50	2.49	2.42

\*\*\* Significant at the 0.001 probability level

Table 16. The ANOVA across the two years and four locations of 21 switchgrass populations for dry biomass and plant height.

Source of variation	df	Mean square
Location (L)	3	3487.0***
Reps/Location (R/L)	8	96.5***
Genotype (G)	20	95.3***
L x G	60	31.5 <sup>NS</sup>
Error A	160	37.2**
Year (Y)	1	11856.9***
Y x L	3	355.9***
Y x G	20	50.9***
Error B	12	41.5 <sup>NS</sup>
G x Y x L	60	21.5 <sup>NS</sup>
Error C	154	24.1***

\*\*\*, \*\* and NS are significant at the probability levels of 0.001, 0.01 and non significant at p=0.05



Table 17. Correlation coefficients between biomass and tiller density and plant height across the genotypes for four locations in 2010 and 2011

Location	Tiller density		Plant height	
	2010	2011	2010	2011
Chickasha	0.63 <sup>***</sup>	NS	0.73 <sup>***</sup>	0.54 <sup>***</sup>
Lane	0.81 <sup>***</sup>	NS	0.59 <sup>***</sup>	0.49 <sup>***</sup>
Stillwater	0.42 <sup>***</sup>	0.27 <sup>*</sup>	0.75 <sup>***</sup>	0.56 <sup>***</sup>
Woodward	0.43 <sup>***</sup>	NS	0.68 <sup>***</sup>	NS

\*\*\*, \*\* and NS are significant at the probability levels of 0.001, 0.01 and non significant at p=0.05

Table 18. Tiller density among six breeding population groups and two check cultivars (Alamo and Cave-In-Rock) in 2010. Location and grand mean across the locations and least significant difference at 5%.

Population group	Chickasha	Lane	Stillwater	Woodward	Grand mean
ALAMO	21.9	11.6	28.8	21.1	20.9
CIR	14.9	5.9	20.9	18.9	15.2
Northern Lowland	18.9	11.9	24.2	21.9	19.2
Northern Southern Lowland	19.7	11.4	22.7	19.9	18.4
Southern Lowland	19.8	12.9	25.4	22.1	20.1
Southern Northern Upland	17.7	6.9	22.9	21.8	17.3
SWG- Lowland	19.5	13.8	23.8	24.2	20.3
SWG-Upland	16.5	4.9	23.7	21.4	16.6
Mean	18.6 <sup>NS</sup>	9.9 <sup>**</sup>	24.0 <sup>NS</sup>	21.4 <sup>NS</sup>	18.4 <sup>**</sup>
LSD	4.1	3.7	6.1	5.2	2.3

<sup>\*\*</sup>, NS significant at the probability levels of 0.01 and non significant at p=0.05

Table 19. Tiller density among six breeding population groups and two check cultivars (Alamo and Cave-In-Rock) in 2011. Location and grand mean across the locations and least significant difference at 5%.

Populations group	Chickasha	Lane	Stillwater	Woodward	Grand mean
ALAMO	33.6	25.0	36.0	28.0	30.6
Cave-In-Rock	24.7	18.7	29.7	27.0	25.0
Northern Lowland	27.6	20.9	29.2	25.5	25.8
Northern Southern Lowland	29.5	20.6	33.8	25.6	27.3
Southern Lowland	32.4	25.6	37.3	25.2	30.1
Southern Northern Upland	30.6	21.8	34.8	31.8	29.7
SWG- Lowland	32.3	25.0	39.8	28.5	31.4
SWG-Upland	28.5	23.3	43.5	24.8	30.0
Mean	29.9 <sup>NS</sup>	22.6 <sup>NS</sup>	35.5 <sup>*</sup>	27.0 <sup>NS</sup>	28.7 <sup>**</sup>
LSD	6.6	8.5	8.7	8.3	3.84

\*\* , \* , and NS are significant at the probability levels of 0.01,0.05 and non significant at p=0.05

Table 20. Pearson's correlation coefficients between biomass and tiller density of different switchgrass population groups at four locations in 2010.

Population	Chickasha	Lane	Stillwater	Woodward
Lowlands	0.58 <sup>***</sup>	0.70 <sup>**</sup>	0.36 <sup>*</sup>	0.77 <sup>**</sup>
Uplands	0.58 <sup>*</sup>	0.95 <sup>**</sup>	0.61 <sup>*</sup>	0.76 <sup>**</sup>
Northern Southern Lowland(NSL)	0.68 <sup>*</sup>	0.85 <sup>***</sup>	0.56 <sup>*</sup>	0.64 <sup>*</sup>
Southern Lowland (SL)	0.64 <sup>*</sup>	0.81 <sup>***</sup>	NS	0.66 <sup>*</sup>
Northern Lowland(NL)	0.78 <sup>**</sup>	0.83 <sup>***</sup>	NS	0.80 <sup>***</sup>
Southern Northern Upland(SNU)	0.75 <sup>*</sup>	0.97 <sup>***</sup>	NS	0.81 <sup>*</sup>
SWG lowland type(SWGL)	NS	NS	NS	0.90 <sup>*</sup>
SWG upland type (SWGU)	NS	NS	NS	NS
Alamo	NS	NS	NS	NS
Cave-In-Rock	NS	NS	NS	0.99 <sup>*</sup>

\*\*\*, \*\*, \* and NS are significant at the probability levels of 0.001, 0.01, 0.05 and non significant at p=0.05

Table 21. Pearson correlation coefficients between biomass and tiller density of different switchgrass population groups at four locations in 2011.

Population	Chickasha	Lane	Stillwater	Woodward
Lowlands	NS	NS	0.42 <sup>**</sup>	0.42 <sup>**</sup>
Uplands	0.58 <sup>*</sup>	NS	0.57 <sup>*</sup>	NS
Northern Southern Lowland(NSL)	NS	NS	0.78 <sup>**</sup>	0.81 <sup>**</sup>
Southern Lowland (SL)	NS	NS	0.64 <sup>*</sup>	NS
Northern Lowland(NL)	NS	NS	NS	NS
Southern Northern Upland(SNU)	NS	NS	NS	NS
SWG lowland type(SWGL)	NS	NS	NS	NS
SWG upland type (SWGU)	NS	NS	NS	NS
Alamo	NS	NS	NS	NS
Cave-In-Rock	NS	NS	NS	NS

\*\*\*, \*\*, \* and NS are significant at the probability levels of 0.001, 0.01, 0.05 and non significant at p=0.05

Table 22. Plant height among six breeding population groups and two check cultivars (Alamo and Cave-In-Rock) in 2010. Location and grand mean across the locations and least significant difference at 5%.

Populations	Chickasha	Lane	Stillwater	Woodward	Grand mean
Alamo	85.3	45.0	79.7	57.3	66.8
Cave-In-Rock	48.7	36.0	49.7	37.5	43.0
Northern Lowland(NL)	85.8	49.4	85.2	59.2	69.9
Northern Southern Lowland(NSL)	82.2	43.4	85.6	57.3	67.1
Southern Lowland (SL)	81.2	43.7	80.6	58.1	65.9
Southern Northern Upland(SNU)	52.7	30.5	42.6	40.6	41.6
SWG lowland type(SWGL)	79.8	40.2	64.8	56.1	60.2
SWG upland type (SWGU)	56.1	29.5	40.5	44.2	42.6
Mean	71.5 <sup>***</sup>	39.7 <sup>***</sup>	66.1 <sup>***</sup>	51.3 <sup>***</sup>	57.1 <sup>***</sup>
LSD (5%)	5.6	8.0	9.4	5.6	8.2

\*\*\* Significant at 0.001 probability level

Table 23. Plant height among six breeding population groups and two check cultivars (Alamo and Cave-In-Rock) in 2011. Location and grand mean across the locations and least significant difference at 5%.

Populations	Chickasha	Lane	Stillwater	Woodward	Grand mean
Alamo	123.3	132.0	146.7	64.7	116.7
Cave-In-Rock	70.3	101.3	107.3	46.3	81.3
Northern Lowland(NL)	118.0	129.3	156.7	75.4	119.8
Northern Southern Lowland(NSL)	119.0	128.9	164.8	74.5	121.8
Southern Lowland (SL)	109.7	121.4	163.6	68.3	115.7
Southern Northern Upland(SNU)	84.6	93.6	114.6	48.8	85.4
SWG lowland type(SWGL)	113.7	112.2	145.0	72.3	110.8
SWG upland type (SWGU)	95.0	94.8	125.0	47.3	90.5
Mean	104.2 <sup>***</sup>	114.2 <sup>***</sup>	140.5 <sup>***</sup>	62.2 <sup>***</sup>	105.3 <sup>***</sup>
LSD (5%)	16.6	14.4	15.8	14.7	16.5

<sup>\*\*\*</sup> Significant at 0.001 probability level

Table 24. Regression equation slopes of stability analysis of 21 switchgrass populations across the four locations during 2010 and 2011

Genotype	Slope of regression equation	
	Year 2010	Year 2011
NSL 2009-1	1.06 <sup>*</sup>	1.19 <sup>**</sup>
NSL 2009-2	0.90 <sup>**</sup>	1.32 <sup>***</sup>
NSL 2009-3	1.29 <sup>*</sup>	0.97 <sup>**</sup>
NSL 2009-4	1.27 <sup>**</sup>	0.83 <sup>*</sup>
SL 93 C2-1	1.17 <sup>**</sup>	1.07 <sup>*</sup>
SL 93 C2-2	1.33 <sup>**</sup>	1.36 <sup>**</sup>
SL 93 C2-3	1.08 <sup>*</sup>	1.00 <sup>*</sup>
SL 93 C2-4	1.19 <sup>**</sup>	1.54 <sup>**</sup>
NL 94 C2-1	1.36 <sup>*</sup>	0.98 <sup>**</sup>
NL 94 C2-2	1.19 <sup>*</sup>	0.93 <sup>*</sup>
NL 94 C2-3	1.29 <sup>*</sup>	1.37 <sup>**</sup>
NL 94 C2-4	1.41 <sup>*</sup>	0.94 <sup>**</sup>
SNU 98 LMBP C1-1	0.39 <sup>NS</sup>	0.62 <sup>**</sup>
SNU 98 LMBP C1-2	0.38 <sup>NS</sup>	0.83 <sup>*</sup>
SNU 98 EMBP C1-1	0.40 <sup>NS</sup>	0.73 <sup>**</sup>
SWG 2007-1	0.87 <sup>NS</sup>	0.98 <sup>**</sup>
SWG 2007-2	1.05 <sup>*</sup>	0.86 <sup>**</sup>
SWG 2007-3	0.57 <sup>*</sup>	0.89 <sup>**</sup>
SWG 2007-4	0.64 <sup>NS</sup>	0.80 <sup>***</sup>
Alamo	1.58 <sup>*</sup>	1.25 <sup>***</sup>
Cave-In-Rock	0.55 <sup>*</sup>	0.53 <sup>**</sup>

\*\*\*, \*\*, \* and NS are significant at the probability levels of 0.001, 0.01, 0.05 and non significant at p=0.05



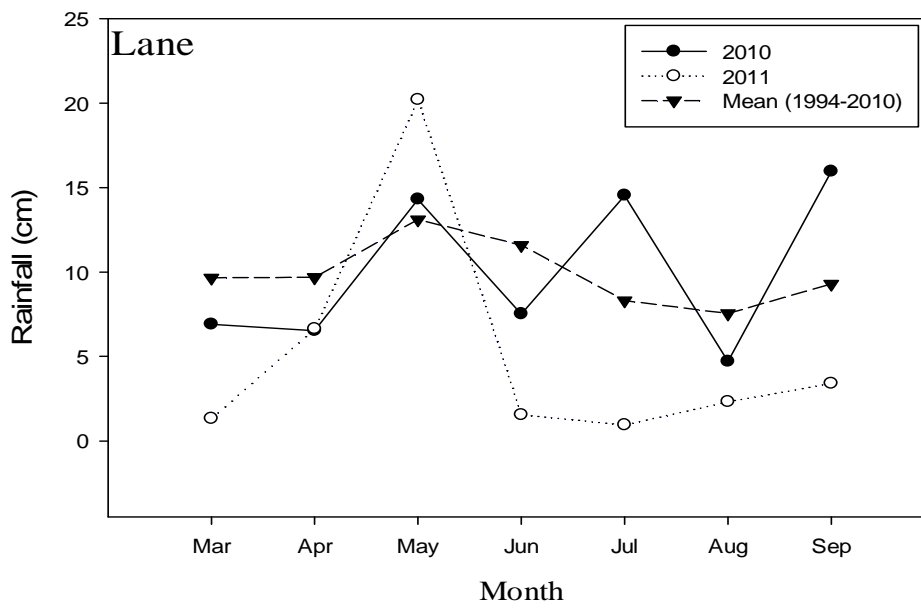
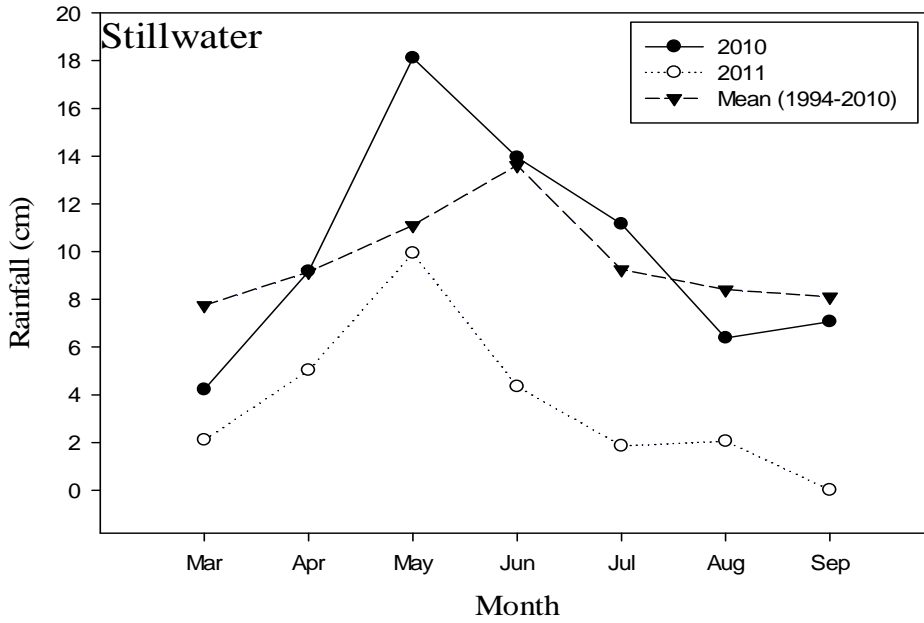


Fig.7 Rainfall distribution from March to September in Lane and Stillwater

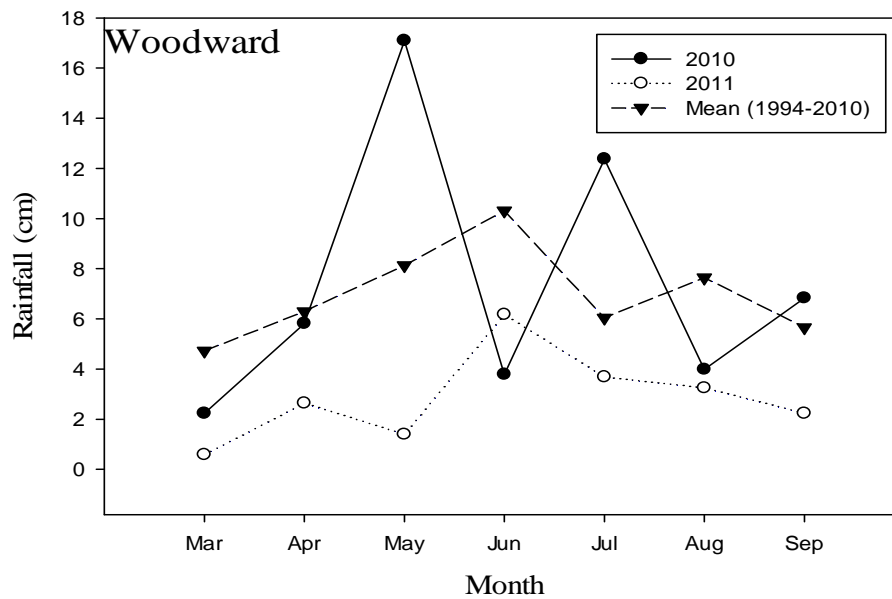
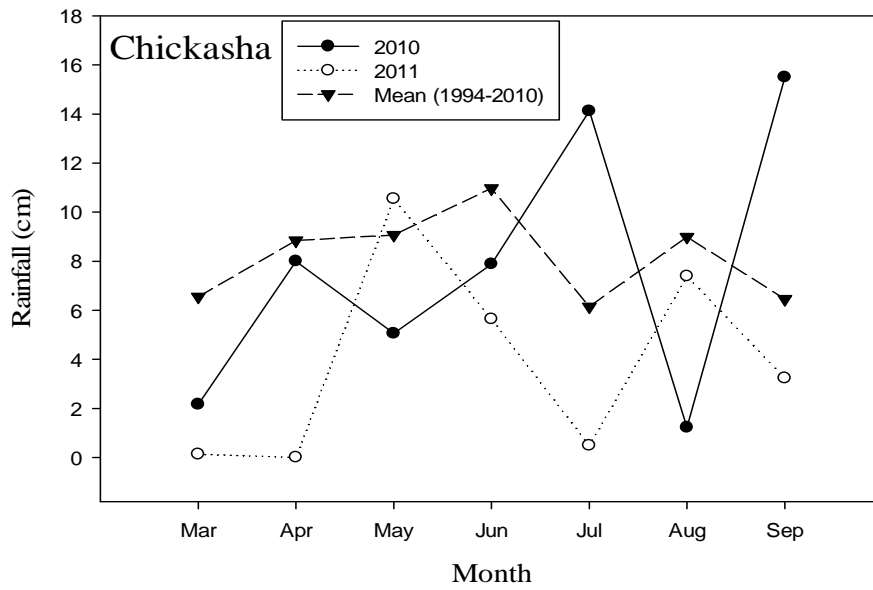


Fig.8 Rainfall distribution from March to September in Chickasha and Woodward

## CHAPTER IV

### CONCLUSION

The objective of this research project was to investigate genetic variations among advanced breeding populations developed by the Switchgrass Breeding and Genetic Program at Oklahoma State University. Simultaneous experiments of controlled and multiple environmental conditions were conducted to assess the genetic variations and genotype by environment (G x E) interactions of new breeding populations. Greenhouse facility at Oklahoma State University was used to study morpho-physiological variations and mutli-location trial study was to assess the genotype x environment (G x E) interaction and stability of dry biomass.

Greenhouse and field experiments at Stillwater showed morphological and physiological variations among the populations. Under greenhouse conditions, Northern lowland (NL) and Northern Southern Lowland (NSL) populations were superior for most of the morphological traits. The SL genotype, SL 93 C2-2 had the highest photosynthesis. Genotype NL 94 C2-4 produced higher biomass than check cultivars. The eigenvectors from Principle Component Analysis (PCA) on different morphological traits among 22 switchgrass populations weighted most for biomass, plant height and days to panicle to describe the superior performance of most NSL and NL populations. The eigenvalues from two principle components (PRIN1 and PRIN2) were able to separate lowland and

upland populations clearly. Highest eigenvalue from PRIN1 was reported for genotype NL 94 C2-3. The PCA separation between lowland and upland populations for ten physiological traits were not clear under greenhouse conditions. However, eigenvectors from first two principle components (PRIN1 and PRIN2) reported high values for photosynthesis and pigment concentrations. Genotype SL 93 C2-2 had highest eigenvalues from PRIN1 and PRIN2 for physiological traits. Under field conditions at post establishment year, the NSL and NL populations were superior for most of the morphological traits. The PCA analysis of ten measured traits among the 21 switchgrass populations was able to separate lowland and upland populations. High eigenvectors were reported for tiller height, phytomer number and leaf number. Genotype NL 94 C2-3 had the highest eigenvalue from PRIN1. The SL populations had higher photosynthesis under field conditions from May to August. Both under greenhouse and field conditions, PCA analysis was evident for high biomass and superior morphological traits of genotype NL 93 C2-3.

Multilocation trial study showed genotypic variations and genotype by environment interactions for dry biomass. Tiller density variations were not significantly different between the populations. Across the locations Alamo biomass was greater during the establishment year. Post establishment year, higher biomass yields were reported for NSL and NL populations. Two NSL genotypes, NSL 2009-1 and NSL 2009-2 populations produced significantly higher biomass than check cultivar Alamo. High biomass and stability were also reported for NSL 2009-2 and NL 94 C2-3 populations. Results from correlations showed biomass under low yielding environments was explained by tiller density due to limited stem elongations by severe environmental

interactions. Whereas under high yielding environmental conditions, plant height and tiller density were the major traits for selection to enhance biomass. However, multiyear evaluations would be needed to assess the stability of different genotypes for the biomass potential.

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VITA

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Thesis: GENOTYPIC VARIATIONS AND GENOTYPE X ENVIRONMENT (G X E) INTERACTIONS AMONG NEW SWITCHGRASS (*PANICUM VIRGATUM* L.) POPULATIONS IN OKLAHOMA

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Scope and Method of Study: The present study was carried out to evaluate the biomass potential, morphological and physiological variations among advanced switchgrass (*Panicum virgatum* L.) breeding populations. Two experiments were conducted; the objective of the first experiment was to assess the morphological and physiological variations under greenhouse and field condition. The second objective was to study genotype x environment (G x E) interactions in multi-location trial at four locations within Oklahoma.

Findings and Conclusions:

Under greenhouse conditions, genotype NL 94 C2-4 produced higher biomass than check cultivars. Northern lowland (NL) and Northern Southern Lowland (NSL) populations were superior for most of the morphological traits. The SL genotype, SL 93 C2-2 had the highest photosynthesis. Among the morphological traits, plant height, days to panicle initiation and leaf lengths were best traits to describe the superior performance of the genotypes. For physiological traits, photosynthesis and pigment concentrations were traits that best explained high physiological performance of the genotype. Morphological distinction between lowland and upland was very clear, but the separation was not clear for physiological traits. The G x E interactions was evident across the locations and years for dry biomass. Among the populations studied, NSL and NL populations produced high biomass. Genotypes, NSL 2009-2 and NL 94 C2-3 had high biomass and greater stability than the lowland check cultivar, Alamo. However, multiyear evaluations would be needed to assess the biomass stability of the new breeding populations.

ADVISER'S APPROVAL: Dr. Vijaya Gopal Kakani

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