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RESEARCH ARTICLE

Genetic diversity and structure of natural and agronomic switchgrass (*Panicum virgatum* L.) populations

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Abstract *Panicum virgatum* L. (switchgrass) is an obligate outcrossing C_4 perennial prairie grass currently being pursued for the production of lignocellulosic ethanol. Commercial production of switchgrass for bioenergy has increased substantially in the United States. Understanding the degree of native genetic diversity within and among switchgrass populations will facilitate effective germplasm improvement, conservation, and management programs. In this study, the genetic diversity and differentiation among natural and agronomic switchgrass populations were analyzed at the molecular level by using random amplified polymorphic (RAPD) DNA markers. The mean genetic diversity among populations ranged from 0.051 ± 0.136 to 0.243 ± 0.214 and the mean genetic similarity among all the switchgrass populations was 0.775. The clustering pattern of switchgrass populations grouped the individuals based on their sites of origin, with agronomic cultivars predominantly separated into distinct clusters. The grouping of individuals within and across the populations was corroborated by principal component analysis. These results are consistent with previous reports for switchgrass accessions. RAPD DNA markers were suitable for quickly estimating the genetic diversity of native and agronomic switchgrass populations, and suggest that introgression of agronomic genes into natural switchgrass populations and subsequent changes in genetic structure may be detectable.

Keywords Bioenergy feedstock · Genetic variability · Molecular markers · *Panicum virgatum* · Ploidy · Randomly amplified polymorphic DNA

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Introduction

Panicum virgatum L. (switchgrass) is a warm season C_4 perennial grass native to prairies and other open habitats of eastern and central North America. Traditionally, it has been used for hay, grazing, restoration of range lands and prairies, and soil conservation (Vogel 2004). It has the potential to produce cellulose for biofuels, such as ethanol and butanol, on lands incapable of supporting traditional food crops (Narasimhamoorty et al. 2008), with ethanol yields approaching 3,500 l/ha on high-yielding farms (Schmer et al. 2008). It is a highly heterozygous, self-incompatible, out-crossing polyploid species (Talbert et al. 1983). Though the base chromosome number of switchgrass is $n = 9$, different ploidy levels from diploid ($2n = 2x = 18$) to dodecaploid ($2n = 12x = 108$) have been reported (Church 1940; Burton 1942; Nielsen 1944). Two cytotypes of

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switchgrass, L and U, associated with lowland and upland ecotypes have been recognized; lowland ecotypes are predominantly tetraploid ($2n = 4x = 36$), while the upland ecotypes are hexaploid ($2n = 6x = 54$) or octaploid ($2n = 8x = 72$) (Barnett and Carver 1967; Hopkins et al. 1996; Hultquist et al. 1996; Costich et al. 2010; Zalapa et al. 2011). Earlier work on its improvement focused mainly on forage yield and quality traits (Vogel and Jung 2001); however, after its selection as a candidate bioenergy crop, research efforts have been shifted to improve the biomass production and quality for biofuel conversion efficiency (Parrish and Fike 2005).

Over the past decade, interest in commercial production of switchgrass as a potential bioenergy crop has increased substantially in the United States. Acreage dedicated to biofuel crops, such as switchgrass, will arguably continue to increase as the U.S. attempts to reach government-mandated benchmarks for ethanol production and incorporation in liquid fuels (McDonald et al. 2009). As a biofuel crop, switchgrass exhibits certain traits associated with invasiveness [e.g., rapid early-season growth, effective belowground partitioning of nutrients in the dormant season, high water-use efficiency (Raghu et al. 2006)], even though there is no documented evidence of its weediness or invasiveness. Still, there may be regulatory and ecological concerns about risks of transgene or agronomic gene introgression into native populations (Kausch et al. 2010; Kwit and Stewart 2012). Furthermore, since the geographic origins of agronomic switchgrass cultivars may not coincide with current or future planting locations, such introgression may be possible and discernable, especially where ploidy levels of agronomic and natural populations are equivalent (Martinez-Reyna and Vogel 2002). Assessing such risks for switchgrass may be most appropriate in the mid-southern states of the US, where high projected yields of cultivars (Wullschleger et al. 2010) may result in expansive agronomic plantings in regions with existing native switchgrass populations.

Site- or regionally-adapted germplasm can provide ecological and economic benefits (Czarnecki et al. 2008). To facilitate switchgrass sustainability efforts, and to assist with future genetic improvement efforts, more complete characterization of genetic diversity of native ecotypes may be required (Havens 1998; Burton and Burton 2002; Czarnecki et al. 2008; Zalapa et al. 2011). In the case of switchgrass, addressing these concerns requires further information on the genetic structure of native populations, in particular those that have not yet been characterized in the Midsouth USA area where "non-native" (to the area) cultivars may soon be planted on a more widespread scale. This would additionally entail use of molecular markers that can distinguish between native and agronomic individuals.

Studies on genetic diversity of switchgrass have been focused on differentiating among upland and lowland ecotypes, on polymorphisms for a nuclear gene coding for a plastid protein, and on patterns of relatedness among switchgrass accessions obtained primarily from the USDA National Plant Germplasm System (Gunter et al. 1996; Hultquist et al. 1996; Huang et al. 2003; Casler 2005; Missaoui et al. 2006; Narasimhamoorty et al. 2008; Todd et al. 2011; Zalapa et al. 2011). In the few studies that have emphasized existing native switchgrass populations (see Zhang et al. 2011), gaps exist in Midsouth USA states. Since the early adoption and deployment of switchgrass for bioenergy production has been based on a limited number of cultivars, a greater knowledge of the genetic structure of switchgrass genetic pools will be essential for the preservation of genetic diversity (Casler 2010; Zalapa et al. 2011). In the current study, random amplified polymorphic markers (RAPDs) were selected as they are amenable for quick surveys of native or experimental populations. The objectives of this study were to characterize the genetic diversity and structure of natural and cultivated populations of switchgrass in a region where widespread planting of agronomic cultivars for bioenergy is arguably most imminent.

Materials and methods

Plants

In early September 2011, green (non-senescent) leaf samples were collected from switchgrass individuals (all >5 m apart to ensure collection from different genets) in natural and agronomic populations in central and eastern Tennessee, USA (Table 1; Fig. 1). Five natural populations of switchgrass were sampled from central Tennessee: Brockdell Road ($n = 12$), Morrison Meadow ($n = 12$), Morrison Meadow "East" (MME) ($n = 5$), May Prairie ($n = 12$), and Rowe Gap

Table 1 Population study sites and genetic parameters for switchgrass populations in Tennessee, USA

Population study site	Sample size (N[†])	Polymorphic loci[‡] (%)	Mean genetic diversity[§,*] (±SD[¶])
May Prairie[#] (Coffee County)	12	47.93	0.200 ± 0.219[hgd]
Morrison Meadow[#] (Warren County)	12	26.45	0.109 ± 0.191[ci]
Brockdell Road[#] (Van Buren County)	12	45.45	0.171 ± 0.205[di]
Rowe Gap[#] (Franklin County)	6	14.88	0.051 ± 0.136[e]
Morrison Meadow "East"[#] (Warren County)	5	23.14	0.098 ± 0.184[fi]
Alcoa 'Alamo'[††] (Blount County)	12	59.50	0.243 ± 0.214[g]
Vonore 'Alamo'[††] (Monore County)	12	58.68	0.240 ± 0.218[h]
Vonore 'Kanlow'[††] (Monore County)	12	38.02	0.139 ± 0.200[i]

[†] *N* Number of individuals genetically screened

[‡] Nei's genetic diversity

[§] Percentage of polymorphic loci with a 95 % threshold

[¶] *SD* Standard deviation

[#] Natural switchgrass population

[††] Switchgrass cultivar

* Mean genetic diversity with different letters are significant at $p < 0.005$ (*t* test)

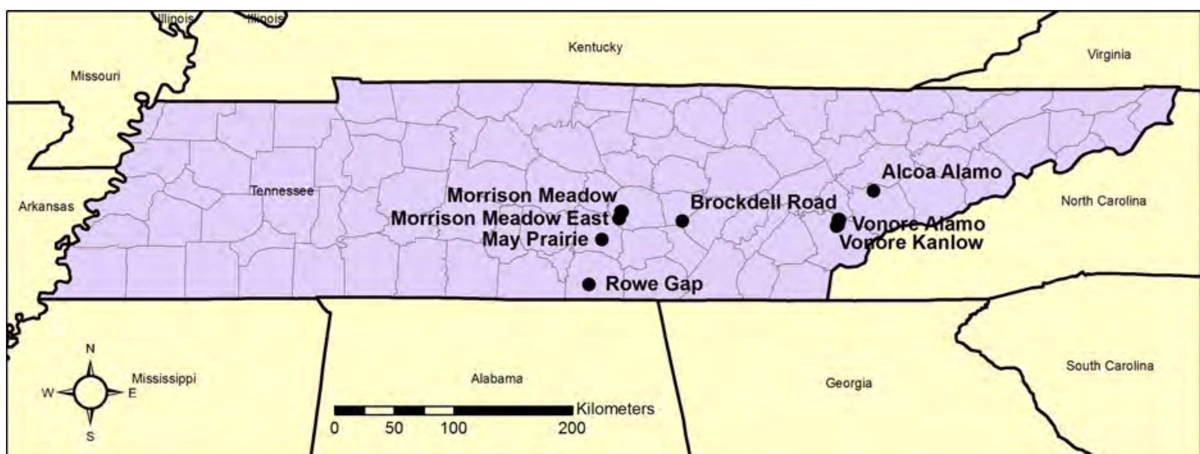

Fig. 1 Map showing switchgrass population study sites

(n = 6). Three agronomic populations were sampled from east Tennessee. They included samples from two 'Alamo' fields in Alcoa (n = 12) and Vonore (n = 12), and one field of an improved variety of 'Kanlow' from Vonore (n = 12). Seed sources of the agronomic 'Alamo' and 'Kanlow' cultivars came from Bamert Seed Company and Ceres Inc., respectively. The MME and Rowe Gap populations were very small, which led to the collection of samples from almost all available individuals. A portion of freshly collected leaf tissue was utilized for flow cytometry, and remaining leaf tissue was stored at −80 °C until DNA extraction.

DNA extraction

Approximately 200 mg of leaf tissue were taken in a 2.0 ml micro-centrifuge tube and ground to fine powder in liquid nitrogen. DNA was extracted from ground tissue with a DNeasy Plant Mini kit (Qiagen Inc., Valencia, CA, USA). Protocols followed those recommended by the kit, with the exception that

extracted DNA was treated with 5 µl of RNaseA (10 mg/ml; Fisher Scientific, Pittsburgh, PA, USA). DNA concentration was determined by using a Nanodrop spectrophotometer ND2000 (Thermo Scientific, Waltham, MA, USA).

Flow cytometry

Leaves, collected in early September 2011, from individual plants of each population were prepared in Otto I buffer for analysis (Otto 1990). Solution suspensions of nuclei were prepared in a Petri dish by finely chopping fresh leaf samples (250 mg) for 4–5 min with a sharp razor blade in 1 ml of Otto I buffer. The Petri dish was placed on ice and 1 ml of Otto I buffer was added again. The contents were gently mixed by pipetting. After ice incubation for 5–10 min, the solution was filtered through 5 ml polystyrene round bottom tubes with cell-strainer caps (BD falcon, Fisher Scientific, Pittsburgh, PA, USA) and then centrifuged for 5 min at $200\times g$. The supernatant was discarded, and the cells were gently resuspended in 500 µl Otto I buffer and stained with 3 µl propidium iodide (50 µg/ml) and RNaseA (50 µg/ml; Fisher Scientific, Pittsburgh, PA, USA). The stained nuclei samples were incubated for 20 min at room temperature and analyzed using a LSR II flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with an argon laser (488 nm). Ploidy levels of the various population samples were estimated by comparing the relative DNA content of cultivars to a single 'Alamo' sample, a known tetraploid, which was used as a control.

RAPD PCR amplification

RAPD fragments were amplified by PCR in a programmable thermal cycler (Eppendorf Mastercycler, Hamburg, Germany). A few individuals of each switchgrass population were initially screened by testing 25 RAPD primers (synthesized by Integrated DNA Technologies; www.idtdna.com). The eight

Table 2 Primer sequences and genetic diversity parameters for RAPD primers

Primers	Sequence (5′ → 3′)	No. of PCR amplified products	Mean genetic diversity† (±SD)	Polymorphism‡ (%)
OPA-10	GTG ATC GCA G	18	0.345 ± 0.153	100
OPAC-10	AGC AGC GAG G	20	0.343 ± 0.139	95.0
OPB-10	CTG CTG GGA C	16	0.362 ± 0.112	100
OPC-10	TGT CTG GGT G	11	0.371 ± 0.081	100
OPG-10	AGG GCC GTC T	13	0.399 ± 0.115	97.0
OPJ-10	AAG CCC GAG G	16	0.333 ± 0.163	100
OPM-10	TCT GGC GCA C	14	0.285 ± 0.148	100
OPN-10	ACA ACT GGG G	13	0.301 ± 0.176	84.62

† Nei's genetic diversity

‡ Percentage of polymorphic loci with a 95 % threshold

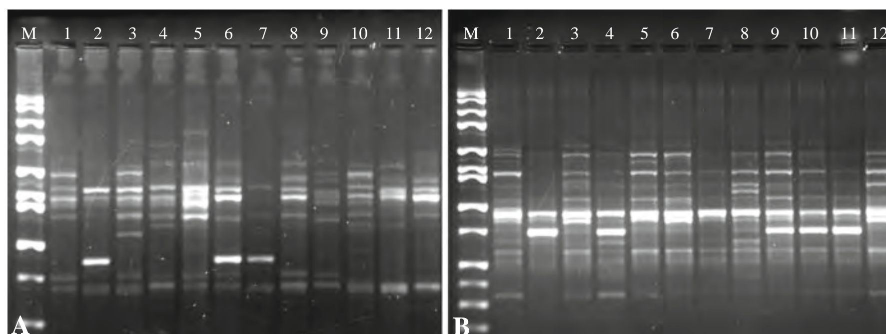

Fig. 2 RAPD-PCR agarose gel electrophoresis generated by the **A** OPM-10 and **B** OPB-10 primers for the 'Alamo' populations from Alcoa and Vonore. M = Marker 1kb ladder

primers (Table 2; Fig. 2) that produced clear-cut DNA fragments and consistent polymorphism were selected to analyze all individuals. The reproducibility of PCR banding patterns was tested on 20 samples randomly selected from various populations. The PCR amplification was conducted in a 20 µl reaction mixture containing 10× reaction buffer containing 500 mM KCl, 15 mM $MgCl_2$ and 100 mM Tris–HCl (pH 9.0), 200 µM dNTPs, 0.8 µM primer, 1 U *Taq* polymerase (Fisher Scientific, Pittsburgh, PA, USA) and 25–50 ng of template DNA. Reactions were run at 93 °C for 3 min (initial denaturation) followed by forty cycles of 93 °C for 1 min (denaturation), 37 °C for 1 min (annealing) and 72 °C for 2 min (extension). The final extension was carried out at 72 °C for 10 min. Amplified PCR products were resolved on 1.5 % agarose gels with 1× TAE buffer (pH 8.0) and were detected under UV light after ethidium bromide staining. RAPD experiments were repeated thrice, and the consistency of the banding patterns was confirmed.

Genetic data scoring and analysis

Binary coding was used for scoring RAPD gels. Presence of a PCR amplified product in a RAPD gel was scored as '1' and its absence as '0'. Various population genetic diversity parameters were generated by using POPGENE software (Yeh and Boyle 1997). To assess the degree of polymorphisms, the proportion of polymorphic amplification products for each population of switchgrass was estimated. An amplified PCR product was considered polymorphic only if the frequency of the most frequent RAPD product was <95 %. Nei's gene diversity index (Nei 1972), which is equivalent to the diversity of amplified gene products within an infinite population, was computed as $h = 1 - \Sigma Pi^2$, where Pi is the frequency of the occurrence of the ith amplified product over individuals within a population. The data were subjected to Student's *t* test. Based on the squared Euclidean distance between individuals, cluster analysis was performed following unweighted pair group method with arithmetic mean (UPGMA algorithm, Sneath and Sokal 1972) as well as Molecular Evolutionary Genetics Analysis (MEGA, version 5.05; Tamura et al. 2011) using neighbor-joining method (1,000 replications). A dendrogram was then constructed. A principal component analysis (PCA) was also performed by using STATISTICA version 4.5 (Statsoft 1993) to display the genetic relationships among individuals across each study site. The genetic similarity indices between all possible pairs of individuals within each population were also computed (Sneath and Sokal 1972). POPGENE (Yeh and Boyle 1997) was used to obtain an estimate of the total genetic diversity from all the populations (H_T) and the mean diversity within each population (H_S). From the H_T and H_S values, the proportion of total genetic diversity residing among populations (G_{ST}) was calculated as $G_{ST} = (H_T - H_S)/H_T$ (Nei 1973).

Results and discussion

Eight RAPD primers amplified a total of 121 loci with the mean number of loci across the primers being 15.1 across all switchgrass populations. The number of fragments amplified ranged from 11 (OPC-10) to 20 (OPAC-10) (Table 2). Previous assessments of genetic diversity in switchgrass accessions based on RAPD primers have reported means of 18.2 loci based on 5 RAPD primers (Gunter et al. 1996) and 17.8 loci from 7 RAPD primers (Casler et al. 2007). The mean genetic diversity across each primer for all switchgrass populations was calculated. The highest mean genetic diversity (0.399) was observed in primer OPG-10; in contrast, primer OPM-10 showed the least mean genetic diversity (0.285; Table 2). With a 95 % threshold, the percentage of polymorphic loci at the primer level ranged from 84.62 (for OPN-10) to 100 (for OPA-10, OPB-10, OPC-10, OPJ-10, and OPM-10; Table 2). Huang et al. (2011) reported similar percentages of polymorphic loci in switchgrass populations, ranging from 88.24 to 100 in sequence-related amplified polymorphic (SRAP) markers and 85.71–100 in expressed sequence tags-simple sequence repeats (EST-SSRs).

The mean genetic diversity and percent polymorphic loci of the eight switchgrass populations (five natural populations, two 'Alamo' populations and one 'Kanlow' population) are presented in Table 1. The least mean genetic diversity was observed in the Rowe Gap natural population (0.051 ± 0.136) while the highest mean genetic diversity was observed in 'Alamo' collected from Alcoa (0.243 ± 0.214). Among the natural switchgrass populations, the May Prairie population was significantly more diverse (0.200 ± 0.219) than all other natural populations.

In bluestem (*Andropogon gerardii* Vitman) and Indian grass (*Sorghastrum nutans* L. Nash), the average marker diversity was approximately equal for bred cultivars and prairie-remnant populations (Gustafson et al. 2004). Our switchgrass populations with few individuals had significantly less mean genetic diversity (0.051 ± 0.136, Rowe Gap; 0.098 ± 0.184, MME) than did larger populations (e.g., 0.243 ± 0.214, Alcoa 'Alamo'; 0.200 ± 0.219, May Prairie; Table 1). Researchers have shown that the demographic status of a species could be used as an appropriate indicator of the status of the genetic diversity of the populations (Lande 1988; Quattro and Vrijenhoek 1989; Ravikanth et al. 2008). There appears to be unanimous agreement that the larger the population size, the better the status of the genetic resources (Gilpin and Soule 1986; Prober and Brown 1994; Nageswara-Rao et al. 2007; Ravikanth et al. 2008). Understanding the effective size of the population and its determinants may have ecological as well as evolutionary implications as switchgrass is governed by its outcrossing nature and clonal reproduction. With increasing clonal reproduction the effective population size reflects the fact that polymorphism is protected within individuals due to fixed heterozygosity. Thus, genetic diversity decreases with increasing rates of clonal reproduction (Balloux et al. 2003; Bengtsson 2003). Irrespective of pollination syndrome, dispersal mode or breeding system, small populations on average tend to suffer more from mating constraints than do large populations (Young and Boyle 2000; Uma Shaanker et al. 2004; Nageswara-Rao et al. 2007).

The frequency of polymorphic loci in the present study was also least for the Rowe Gap natural population (14.88), while the highest percentage of polymorphic loci was observed in the May Prairie population (47.93). Among the two 'Alamo' populations, the one from Alcoa had the highest percent polymorphic loci (59.50; Table 1). The total genetic diversity (H_T) of the eight switchgrass populations was 0.330 and for all natural populations was 0.275 (Table 3). Genetic diversity values of 0.231 and 0.225 based on SRAP and EST-SSR molecular markers, respectively, have been reported in cultivated switchgrass accessions (Huang et al. 2011). Genetic diversity values of 0.295 were observed in lowland switchgrass ecotypes by using amplified fragment length polymorphism (AFLP) markers (Todd et al. 2011). Total genetic diversity in switchgrass was similar to that noted for other allogamous perennial grass species. Total genetic diversity of 0.28 was reported in ryegrass (*Lolium perenne* L.) using ISSR markers (Hu et al. 2011), and 0.24 was reported in carpetgrass [*Axonopus fissifolius* (Raddi) Kuhlm.] samples using AFLP markers (Wang et al. 2010). Genetic diversity ranging from 0.22 to 0.27 based on RAPDs has been reported in bluestem populations (Selbo and Snow 2005).

The proportion of total diversity residing among all populations (G_{ST}) was 0.525 and was 0.542 for the natural populations (Table 3). The mean value of genetic differentiation among other allogamous, perennial grass populations was observed to be 0.54 in *Hemarthria compressa* (L. f.) R. Br. (Huang et al. 2012), 0.575 in *Spartina patens* (Aiton) Muhl. (Wu 2012), 0.49 in European *L. perenne* L. (Diekmann et al. 2012) and 0.314 in *Sasamorpha borealis* (Hackel) Nakai (Lee and Chung 1999). The G_{ST} value among all populations obtained in our study thus indicates a moderate level of genetic differentiation in switchgrass populations, higher than expected for an allogamous, perennial plant (Hamrick and Godt 1989). The population diversity and structure of a species is affected by a number of evolutionary factors,

Table 3 Mean genetic diversity and differentiation within and among switchgrass populations

	H_S[†]	H_T[‡]	G_{ST}[§]
Among natural populations	0.126 ± 0.010	0.275 ± 0.033	0.542
Among agronomic populations	0.207 ± 0.028	0.313 ± 0.038	0.336
Among all populations	0.156 ± 0.011	0.330 ± 0.019	0.525

[†] Mean genetic diversity within populations

[‡] Total genetic diversity from all concerning populations

[§] Total genetic diversity residing among populations (or population differentiation)

The H_S, H_T, G_{ST} were calculated using POPGENE (Yeh and Boyle 1997)

including reproductive system, pollen or seed dispersal, geographic range, successional status, as well as natural selection (Loveless and Hamrick 1984). Methods of seed dispersal that lead to greater gene flow have a more homogenizing effect on the genetic variation among populations, while those that lead to very short distance seed dispersal cause an increase in the genetic structuring of populations (Young et al. 2000). Clonal reproduction is another plant trait that may affect population genetic structuring (Ellstrand and Roose 1987; Hamrick and Godt 1989).

Based on the presence or absence of amplified RAPD-PCR products, mean genetic similarities among all pairs of individuals within and across the study sites were computed and compared. The least mean genetic similarity was observed between 'Kanlow' and the MME natural populations (0.635) while the highest was observed, as expected, between 'Alamo' populations collected at Vonore and Alcoa (0.936; Table 4). Similar genetic similarity coefficients ranging from 0.73 to 0.95 were observed while assessing genetic diversity in 56 accessions of tetraploid switchgrass (Todd et al. 2011). In our study, the total mean genetic similarity among all switchgrass populations was 0.775, which is comparable to the mean genetic similarity of 0.813 observed in cultivated switchgrass accessions (Huang et al. 2011). In buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], native to the short-grass prairie region of North America, the observed mean genetic similarity among the populations was as high as 0.89 (Zhang et al. 2007).

We subjected plants within the five natural, two agronomic and one improved populations to flow cytometry to assess ploidy levels. The results for our switchgrass samples confirmed our expectation of tetraploidy in all instances (Fig. 3). Missaoui et al. (2006) reported no clear separation of switchgrass accessions based on the ploidy level and suggested that they may have descended from the same maternal origin. Although there have been many studies on the agronomy of switchgrass cultivars, less information is available regarding the frequencies of different ploidy levels in wild populations (Zalapa et al. 2011;

Table 4 Genetic similarity measures of switchgrass populations

	Alcoa 'Alamo'	Vonore 'Alamo'	Vonore 'Kanlow'	Brockdell Road	Morrison Meadow	Morrison Meadow "East"	May Prairie	Rowe Gap
Alcoa 'Alamo'	****	0.9365	0.7750	0.8266	0.7623	0.7718	0.8145	0.7725
Vonore 'Alamo'		****	0.7291	0.8361	0.7659	0.7870	0.8528	0.7748
Vonore 'Kanlow'			****	0.7115	0.6869	0.6350	0.6720	0.6484
Brockdell Road				****	0.7739	0.8263	0.8377	0.7850
Morrison Meadow					****	0.7490	0.7671	0.7379
Morrison Meadow "East"						****	0.8114	0.8219
May Prairie							****	0.8370
Rowe Gap								****

Genetic similarity measures were calculated using STATISTICA (Statsoft 1993; Sneath and Sokal 1972)

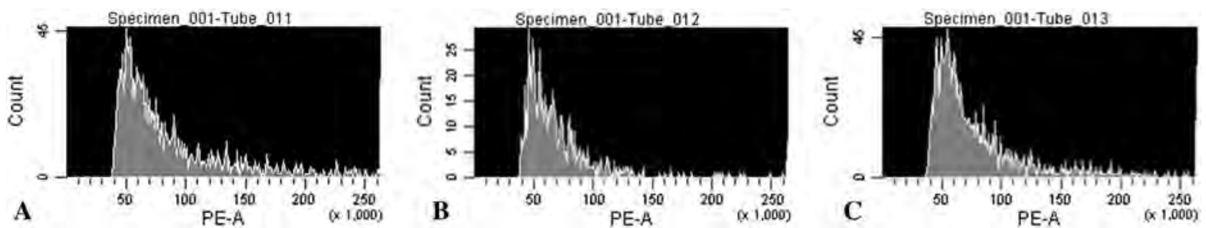

Fig. 3 Flowcytometric profiles of switchgrass populations (**A** Morrison Meadow; **B** 'Kanlow'; **C** Alcoa 'Alamo')

Stottlemyer 2012). This information is required to determine if it is possible for agronomic switchgrass populations to share genes with wild populations. Since ploidy levels in switchgrass can influence compatibility (Martinez-Reyna and Vogel 2002; Stottlemyer 2012; Kwit and Stewart 2012) and hence introgression, gene flow could occur between the natural and the agronomic populations, allowing agronomic genes to escape cultivation and introgress into the natural populations. However, a number of prerequisites such as overlapping flowering phenologies, close proximity of agronomic fields and demographic swamping from crop plants are to be fulfilled for introgression to occur (Stottlemyer 2012; Kwit and Stewart 2012).

In addition to assessing genetic diversity, we also investigated the genetic structure of these switchgrass populations. Based on RAPD-PCR products, Nei's genetic distances were computed and neighbor-joining as well as UPGMA dendrograms were generated. At all the study sites, the membership of a cluster was found to be non-randomly occupied by individuals from all the eight populations indicating a fine degree of genetic structure among the various switchgrass populations (Figs. 4, 5). In prior work, such consistent clustering has often involved having samples from populations of different ecotypes. Based on RAPD genetic coefficients, clustering of switchgrass genotypes into up- and low-land ecotypes have been reported (Gunter et al. 1996). A small amount of population differentiation associated with hardiness zones and ecotypes has also been reported in other switchgrass populations (Casler et al. 2007). The clustering pattern of switchgrass populations obtained in our study was also evident from the principal component analysis (PCA); individuals tended to

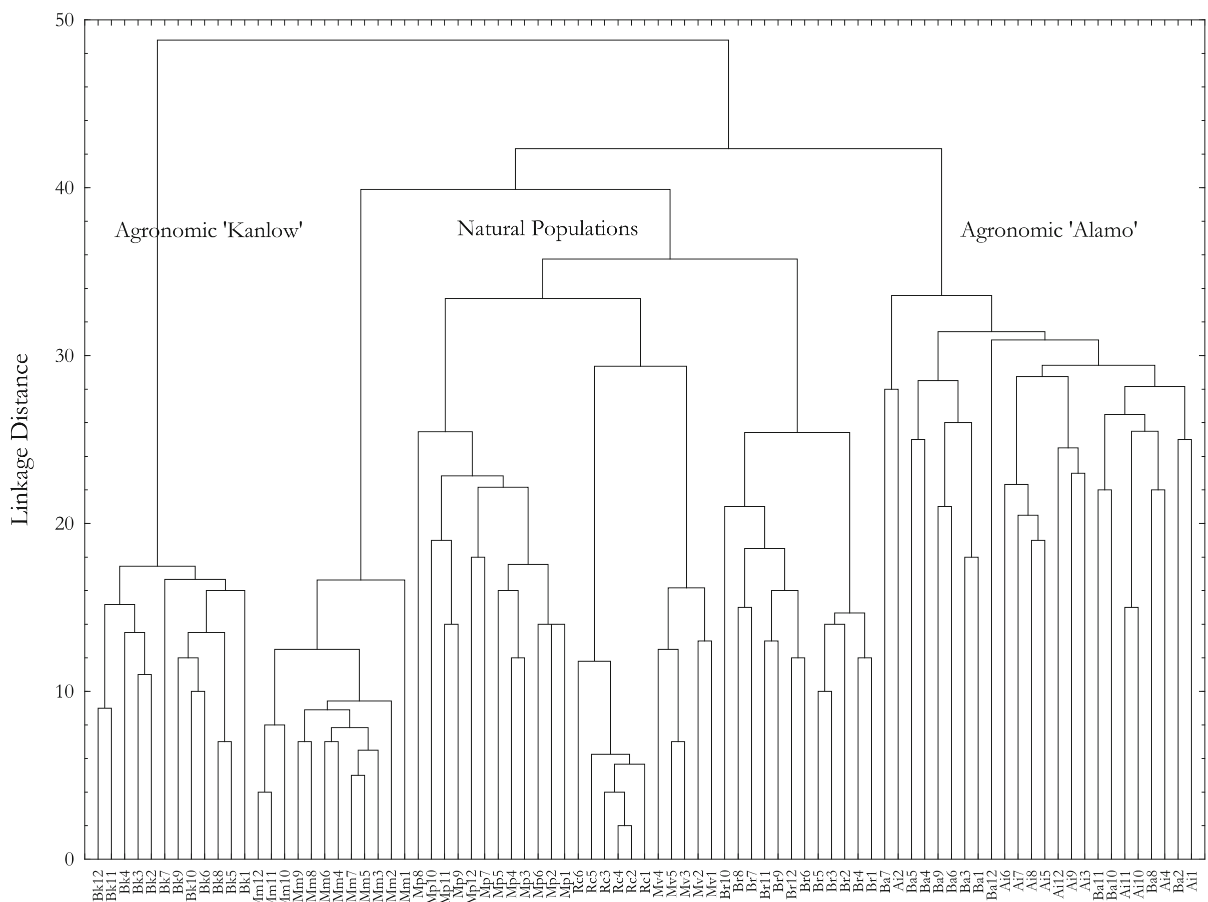

Fig. 4 Dendrogram of switchgrass populations. *Bk* Vonore 'Kanlow', *Mm* Morrison Meadow, *Mp* May Prairie, *Rc* Rowe Gap, *Mv* Morrison Meadow "East", *Br* Brockdell Road, *Ba* Vonore 'Alamo', *Ai* Alcoa 'Alamo'

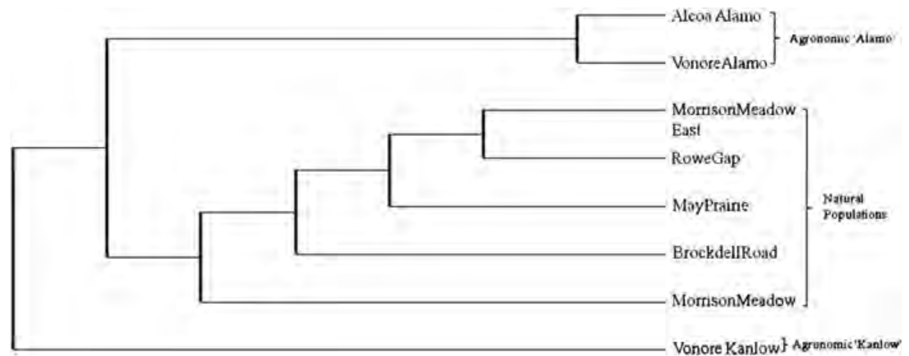

Fig. 5 Neighbor-joining tree of switchgrass populations

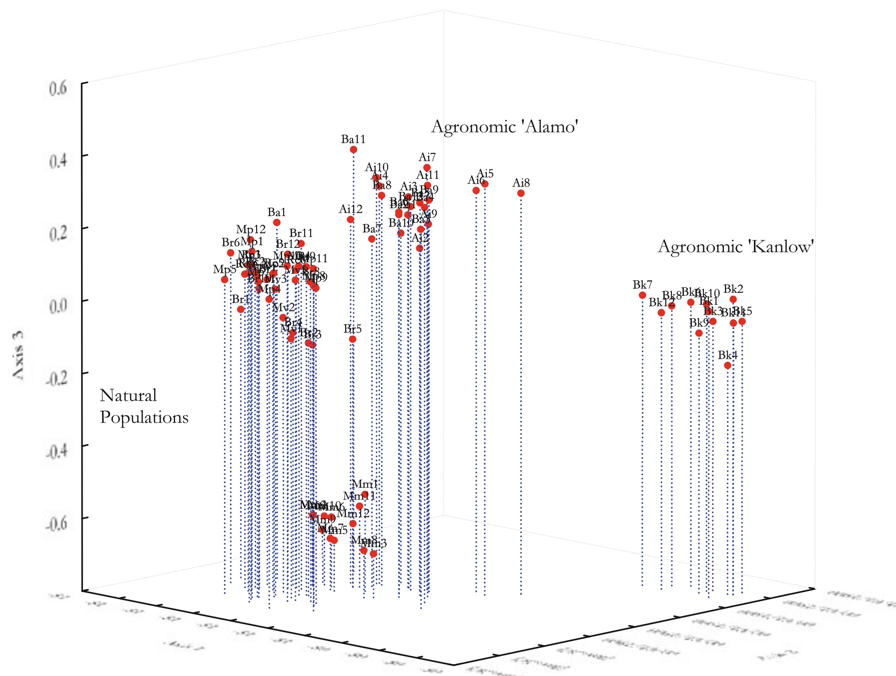

Fig. 6 Principal component analysis of switchgrass populations. *Bk* Vonore 'Kanlow', *Mm* Morrison Meadow, *Mp* May Prairie, *Rc* Rowe Gap, *Mv* Morrison Meadow "East", *Br* Brockdell Road, *Ba* Vonore 'Alamo', *Ai* Alcoa 'Alamo'

group based on their sites of origin (Fig. 6). Thus, individual plants of 'Alamo' grouped into a nearly cohesive cluster as did the natural switchgrass individuals. In each of the study sites, the 'Alamo' individuals grouped predominantly into a cluster distinct from 'Kanlow'. The values for the first three axes of the PCA for our switchgrass populations were 29.60, 8.74, 7.85 % and explained 46.19 % of the total variance. Using EST-SSRs, based on their geographic occurrence, upland and lowland switchgrass populations could be clustered in separate clades (Cortese et al. 2010). Zalapa et al. (2011) reported that the SSR markers could distinguish ecotypes correctly, but chloroplast markers alone were not always able to distinguish the ecotypes.

In our study, the clear separation of individual switchgrass populations based on their geographic occurrence indicates that the populations came from heterogeneous fragments and there were moderate levels of genetic differentiation among populations within a geographical region. There is a need to address whether the natural populations in each study site represent a regional ecotype (Figs. 5, 6) or any other ecological factors contributed to this differentiation/adaptation. Development of a zoning model (Houseal and Smith 2000; Czarnecki et al. 2008) may be

necessary to help achieve an acceptable balance between maintaining sustainability of agronomic switchgrass populations and maintaining genetic diversity/identity among natural populations.

The study presented here offers important information on the genetic diversity and structure of natural and agronomic switchgrass populations and concurs with previous studies in genebank accessions, based on morphological, ploidy level and different molecular markers used (Cortese et al. 2010; Huang et al. 2011; Todd et al. 2011; Zalapa et al. 2011). Our study highlighted that: (1) these switchgrass populations contain a high level of genetic diversity, (2) the agronomic and natural populations we studied had same ploidy levels, (3) the populations are differentiated genetically, and (4) the diversity of natural populations is comparable to the agronomic populations. The effectiveness of RAPDs in quickly identifying closely related switchgrass populations has been presented in this study. Our study was also able to differentiate DNA polymorphisms among natural and agronomic switchgrass populations with relatively few markers. Our results and conclusions presented should be followed by more thorough studies, as we were able to sample only a limited number of natural populations, and we were not able to compare the agronomic populations to their original (and perhaps no longer existing) natural remnant populations.

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