Genetic Diversity of Switchgrass Populations Grown in New Jersey

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INTRODUCTION

- Although a significant amount of genetic diversity exists within switchgrass (*Panicum virgatum*), little research has been conducted on the level of genetic diversity and local adaptation among different populations/ecotypes of switchgrass currently recommended for habitat restoration in the Northeast region of the US.
- Upland ecotypes (Fig. 1) are commonly octaploids (2*n*=8x=72) and are shorter, finer stemmed and more adapted to drier habitats (Lewandowski et al., 2003).
- Lowland ecotypes (Fig. 1) are typically tetraploid (2*n*=4x=36), and are coarse-stemmed, tall growing and more robust than the upland ecotypes (Lewandowski et al., 2003).

OBJECTIVES

- The objectives of this study were to determine molecular and morphological differences within and between 14 different switchgrass populations.

MATERIALS AND METHODS

Plant Material

- Switchgrass seed from 16 populations (Table 1) were obtained from various sources.
- Carthage, Timber, Contract, Shelter, and High Tide germplasm sources were obtained from the Natural Resources Conservation Service – USDA Plant Materials Center in Cape May NJ. Contract and High Tide represented Northeast ecotypes, while Carthage and Timber represent Eastern ecotypes.
- Standard cultivars developed in the Midwest and other germplasm sources from other countries included Caddo, Shawnee, 196 (PI 337553), Pav12, Turkey (PI 204907), Sunburst, Kanlow, Pathfinder, Blackwell, Cave-in-Rock, and Alamo obtained from the Plant Introduction (PI) collection curated by the Germplasm Resources Information Network (GRIN).
- Kanlow, Timber, and Alamo represented lowland ecotypes. All other populations expressed characteristics of upland ecotypes (Table 1).
- Seed of each population was germinated in Pro-Mix HP (K.C. Shafer, York, PA) in 12 x 15 inch flats.
- Individual plants were grown under greenhouse conditions for approximately 8 weeks and planted to a spaced-plant nursery in the spring of 2005 at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ (Fig. 1 and 2) for a total of 432 plants.

Morphological Markers

- Morphological measurements were taken on 12 individuals from each of the 14 switchgrass populations listed in Table 1 in 2005 and 2006.
- Measurements included plant height, panicle height, and flag leaf height, length and width, and were taken approximately 1-2 weeks after anthesis.

Table 1. Switchgrass populations utilized in morphological and molecular marker analysis.

Populations	
1. Caddo†	9. High Tide
2. Shawnee	10.Pathfinder
3. 196	11.Contract
4. Pav 12‡	12.Blackwell‡
5. Turkey	13.Timber
6. Sunburst	14.Carthage
7. Kanlow	15.Cave-in-Rock*
8. Shelter	16.Alamo*

† Numbers in table correspond to population numbers in *Structure* bar plots

‡ These populations did not yield enough DNA for molecular marker data analysis with *Structure*, *AMOVA* or *Cluster Analysis*

*These populations were only used for molecular marker data analysis

Molecular Markers

- Leaf tissue was collected from 12 individuals from each population listed in Table 1 for molecular marker analysis. DNA was isolated from leaf tissue using the Sigma® GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich Co., St. Louis, MO).
- Publicly available switchgrass specific microsatellite (SSR) markers were utilized for the molecular marker analysis (Tobias et al, 2006).
- Thirty-two SSR primer pairs were tested. SSR markers were genotyped on all individuals using an ABI 3130 genetic analyzer. Fourteen primer pairs amplified polymorphic bands in our populations and these were used for molecular marker analysis.
- A total of 103 SSR alleles were identified.

Analysis

- Morphological and marker data was analyzed using the program *Structure* (Pritchard et al., 2000) which identifies clusters of related individuals from multilocus genotypes. The full data set was analyzed for all models from K=1 through 14.
- Analysis of molecular variance (AMOVA) was performed using the program *GENALEX* (Peakall and Smouse, 2006).
- Cluster analysis was performed in SAS OnlineDoc® 9.1.3 (SAS Institute Inc., 2004), derived from a pairwise distance matrix generated in *GENALEX* (Peakall and Smouse, 2006), to generate a distance-based tree (Fig. 3).

RESULTS AND DISCUSSION

2005 and 2006 Morphological Data

- *Structure* analysis of 2005 and 2006 morphological data separated the populations into distinct groups. Kanlow(7) and Timber(13) grouped together based on morphological measurements (Fig. 4). These two populations also looked phenotypically similar and represented the lowland ecotypes.
- Morphological analysis in 2005 and 2006 provided some delineation between upland and lowland ecotypes, but did not distinguish between Northeast and Midwest populations.

Molecular Marker Data

- *Structure* analysis of molecular marker data from 14 primer pairs also divided the populations into distinct groups (Fig. 5).
- Molecular marker analysis did group Kanlow and Timber (lowland ecotypes) together, but Contract (upland ecotype) was also included in that grouping. This indicated that the analysis did differentiate between upland and lowland but it was not complete.
- The *Structure* molecular marker analysis did not clearly delineate Midwest and Northeast populations.
- Molecular and morphological marker analysis in *Structure* did not produce exactly the same results although they did consistently identify lowland types.

Figure 4. *Structure* bar plot at K=3 for 2006 morphological data. Populations are listed in Table 1.

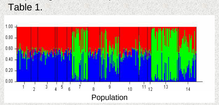


Figure 5. *Structure* bar plot at K=3 for molecular marker data. Populations are listed in Table 1.

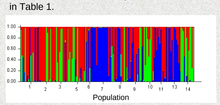


Figure 1. Upland (left) and lowland (right) ecotypes of switchgrass.

Figure 2. *Panicum virgatum* 'Carthage'.

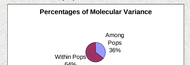
Analysis of Molecular Variance (AMOVA)

- AMOVA (Fig. 6) showed that 64% of the molecular variance was found within the 14 populations (Table 1), while 34% of the molecular variance was found among the populations.

Cluster Analysis

- Cluster analysis of molecular marker data grouped the populations into 3 distinct clusters (Fig. 3).
- Lowland populations, including Alamo, Timber, and Kanlow formed one cluster.
- Northeastern upland populations Contract and High Tide formed another grouping.
- All other populations expressing upland characteristics composed the third cluster.

Figure 6. AMOVA of molecular marker data from 14 populations

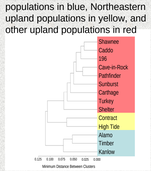


Summary AMOVA Table

Source	df	SS	MS	Est. Var. %	
Among Pops	13	786.730	60.518	4.452	36%
Within Pops	151	1216.627	8.057	8.057	64%
Total	164	2003.358		12.509	100%

Stat	Value	P(rand >= data)
PhiPT	0.356	0.001

Figure 3. Cluster analysis of molecular data with lowland populations in blue, Northeastern upland populations in yellow, and other upland populations in red



CONCLUSIONS

- More variation exists within populations than between populations of switchgrass.
- Morphological and molecular analysis distinguished lowland types from upland types but did not consistently distinguish between upland types from different geographic locations.
- Continued work with molecular markers is needed to further differentiate between switchgrass populations.

REFERENCES

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