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Genetic diversity of switchgrass and its relative species in *Panicum* genus using molecular markers

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ABSTRACT

Switchgrass (*Panicum virgatum*), a warm season C4 grass, is a promising crop for bioenergy-dedicated biomass production. Understanding of genetic diversity within *Panicum* genus will facilitate switchgrass breeding. Genetic relationships of 22 *Panicum* species from six continents including ninety-one USDA germplasm accessions were investigated by Sequence-Related Amplified Polymorphism (SRAP) and Expressed Sequence Tags-Simple Sequence Repeat (EST-SSR) markers. Eight hundred and twenty-six markers from 28 pairs of SRAP and 25 pairs of EST-SSR Primers were used to differentiate between accessions of a bulk of 25 genotypes. The results showed that there was high genetic diversity found in *Panicum* species. Most genetic variation was present among the different species and cluster analysis indicated that all the *Panicum* accessions could be distinguished by SRAP or EST-SSR. Dendrogram results reflected the phylogenetic relationships between *Panicum* species and *Panicum amarum* was found to be the closest species to switchgrass. Comparison between molecular markers revealed that SRAP methods were considered more efficient than EST-SSR for screening *Panicum* accessions.

1. Introduction

Switchgrass (Panicum virgatum L.) is a native perennial C4 grass adapted to the prairies of North America. Because of its potential for high biomass yield with minimal input, and its ability to grow on marginal land, switchgrass is considered as a prime candidate for large-scale biomass production for large-scale bioenergy-dedicated biomass production (Bouton, 2007). Identification and selection of useful germplasm for switchgrass breeding programs has been ongoing. Studies on genetic variation via molecular markers reported that upland and lowland ecotypes fell into their distinctive ecotype classes regardless of ploidy level (Missaoui et al., 2006) (Huang et al., 2003; Missaoui et al., 2006). Extensive genetic variations between and within upland and lowland switchgrass types were also observed (Missaoui et al., 2006). However, the genetic relationship between switchgrass and other *Panicum* species and the genetic diversity within *Panicum* genus have not been well studied using molecular markers.

Panicum is a cosmopolitan genus with approximately 500 species widely distributed from wet shores to dry woodlands, grasslands and cultivated fields providing a vast genetic pool for switchgrass breeding (Sandra et al., 2003). Crossing between the two species usually only occurs between plants with close genetic relationship. Therefore, *Panicum* species, which have close genetic relationships with and are crossable to switchgrass, are particularly valuable for switchgrass breeding.

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Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and population. Sequence-Related Amplified Polymorphism (SRAP) is recognized as a new and useful molecular marker system because of its reproducibility, low cost, and without the need of prior knowledge of target sequences (Li and Quiros, 2001). Microsatellites or Simple Sequence Repeats (SSR) was one of most used molecular marker, owing to its co-dominant results, polymorphic characteristics, reproducibility, low cost and simplicity. However, the development of SSR markers from genomic libraries is expensive and inefficient (Squirrell et al., 2003). Thanks to the availability of large EST (expressed sequence tags) datasets, it has become possible to systematically search for SSRs in EST datasets using bioinformatics tools (Kantety et al., 2002; Varshney et al., 2002; Gao et al., 2003; Omirshat et al., 2009). EST-SSR is now an efficient and low cost option for many plant species. Additionally, since these SSR are derived from an EST corresponding to the transcribed component of a gene unit, they have been shown to possess a high potential for inter-specific transferability (Cordeiro et al., 2001; Gupta et al., 2003; Thiel et al., 2003). Thus, Up to now, SRAP and EST-SSR have been successfully used for evaluation of genetic diversity (Sa et al., 2008; Huang et al., 2010) and genetic map construction (Lin et al., 2003; Graham et al., 2004).

In this study, SRAP and EST-SSR are employed to examine the genetic diversity between switchgrass and selected *Panicum* species. These two techniques were further compared for their utilities of discriminating *Panicum* species.

2. Materials and methods

2.1. Plant materials and DNA extraction

All plant materials including 22 *Panicum* species from six continents were obtained from USDA-GRIN (Table 1). Leaf tissues were collected from young seedlings and frozen in liquid nitrogen for DNA isolation. Approximately 200 mg of leaf tissue from 25 genotypes of each accession were extracted using the DNeasy Plant Mini kit (Qiagen Inc, Valencia, CA). The quality and concentration of the DNA were confirmed by electophoresis on 0.8% agarose gels by comparing the samples to the standardized lambda DNA size markers.

2.2. SRAP-PCR amplification

Twenty-eight pairs of SRAP primers were used in the present study (Table 2 and Table 3), which were selected from 144 combinations of 12 forward and 12 reverse primers. These primer sequences were obtained from previous related studies (Li and Quiros, 2001; Ferriol et al., 2003; Lin et al., 2003). The protocol for SRAP analysis was based on Li and Quiros (2001). Each 20 μL PCR reaction mixture consisted of 40 ng genomic DNA, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.5 μM primer, 1 × PCR buffer, and 1 unit of Taq polymerase. Samples were subjected to the following thermal profile: the first five cycles were run at 94 °C, 1 min; 35 °C, 1 min; and 72 °C, 1 min, for denaturing, annealing and extension, respectively. Then the annealing temperature was raised to 50 °C for another 35 cycles, followed by another extension step of 10 min at 72 °C, and then followed by a 4 °C holding temperature. PCR amplification products were analyzed on 2% agarose gel. The gel was stained in 0.75 μg/ml ethidium bromide (EB) solution and photographed under illumination with UV light using Quantity One.

2.3. EST-SSR amplification

A total of 90 conserved grass EST-SSRs (CNL) developed by Kantety et al. (2002), and 176 switchgrass EST-SSRs developed by Tobis et al. (2005), were screened. From these, 25 primers (Table 4) were selected for the EST-SSR analysis based on reproducibility and clarity of bands. PCR amplification reactions were carried out in 20 μl volume, containing 1 × PCR buffer (10 mM Tris-HC, pH 8.0; 50 mM KCl and 0.02% gelatin), 0.15 mM of each dNTP, 0.4 μM of each primer, 1.5 mM of $MgCl_2$, 1 unit of Taq DNA polymerase and 40 ng of template DNA. PCR amplification was performed as follows: initial 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 45 s at 52–58 °C, 90 s at 72 °C, and a final 20 min extension at 72 °C. PCR products were separated on 6% denatured polyacrylamide gels. The gel was pre-run in 1.0 × TBE buffer (with 0.75 μg/ml ethidium bromide) at 300 V constant voltage before the samples were loaded about 2 h.

2.4. Data analysis

Only bands that could be unambiguously scored across all the sampled populations were used in this study. EST-SSR and SRAP amplified fragments, with the same mobility according to the molecular weight (bp), were scored manually for band presence (1) or absence (0). The resulting presence/absence data matrix was analyzed using POPGENE v. 1.31 (Yeh et al., 1999), assuming Hardy–Weinberg equilibrium, to estimate three genetic diversity parameters: the percentage of polymorphic bands (PPB) and Shannon's Information Index of Diversity (I). The data matrix was then used to calculate the Genetic Similarity (GS) index as $GS = 2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of bands common to genotypes i and j, while N_i and N_j are the total numbers of bands observed for genotypes i and j, respectively (Nei and Li, 1979). Genetic relationships among *Panicum* accessions were estimated using the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) cluster analysis of the *GS* matrix (Rohlf, 1997). In order to test for correlations between genetically similar samples based on SRAP and EST-SSR,