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Genetic diversity of *Hemarthria altissima* and its related species by EST-SSR and SCoT markers

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ABSTRACT

There is a need for an appropriate evaluation of *Hemarthria* germplasm resources using genetic analysis. Understanding their genetic background will promote effective development and utilization of its germplasm resources in plant breeding. We examined the genetic diversity and relationships among 46 *Hemarthria* germplasm resources from four continents. Expressed Sequence Tags-Simple Sequence Repeat (EST-SSR) and Start Codon Targeted (SCoT) markers were used to investigate species *Hemarthria altissima* (36), *Hemarthria compressa* (8) and *Hemarthria uncinata* (2). We selected 19 EST-SSR primers and generated 550 polymorphic (94.7%). Twenty one SCoT primers were selected and amplified to produce 597 bands with 89.4% of polymorphic bands. The Mantel test between EST-SSR and SCoT matrices revealed significant correlations ($r = 0.854$) and the data from both markers were combined for cluster analysis. The 46 materials were clustered into two main groups by UPGMA clustering with a similarity coefficient ranging from 0.573 to 0.940 and the dendrogram was basically concordant with geographical origins and species. Among the three *Hemarthria* species, *H. altissima* was genetically closest to *H. compressa* while it was not close to *H. uncinata*. When the utility of the two markers were compared, we found EST-SSR to be more efficient than SCoT in determining the genetic diversity study of *Hemarthria* species.

1. Introduction

Hemarthria R. Br. is a genus of perennial grasses of the family Gramineae, which include about 20 species naturalized on the wide geographic regions. Of these, *Hemarthria compressa* and *Hemarthria altissima* are particularly important in the tropical and subtropical areas due to their high quality biomass and tolerance to poorly drained soils (Huang et al., 2008, 2012).

Appropriate evaluation of *Hemarthria* germplasm resources is necessary to understand their genetic background. In China, the agronomic characters and molecular genetic diversity of *H. compressa* have been studied extensively (Xu et al., 2003; Chen et al., 2011). Currently, *H. compressa* cultivars, 'Guang Yi', 'Chong Gao' and 'Ya An', have been officially registered in the

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Agricultural Department of China. In contrast to *H. compressa*, *H. altissima* is native to tropical Africa and was later introduced to Florida where their entries were evaluated. Until now, four *H. altissima* cultivars ('Redalta', 'Greenalta', 'Bigalta' and 'Floralta') were released. These entries were introduced to Venezuela, Ecuador, New Zealand and other countries, but seldom to China. Due to lack of its germplasm resources, there is limited research completed on the morphological and molecular aspects of *H. altissima* (Wilms et al., 1970; Woods et al., 1996). Therefore, understanding the genetic diversity of *H. altissima* and its related species is important in effectively utilizing the *Hemarthria* germplasm resources and providing sufficient forages for the developing grassland agriculture in the tropical and subtropical zones in China.

DNA-based molecular markers have been proved to be of great use for a variety of purposes, including genetic diversity assessment, cultivar fingerprint, genetic map construction, and marker assisted breeding (Collard et al., 2005; Okogbenin et al., 2006; Bai et al., 2013; Huang et al., 2014). Expressed Sequence Tags-Simple Sequence Repeat (EST-SSR) marker is a highly conserved and transferable molecular marker technique derived from transcribed regions of DNA (Varshney et al., 2005). Microsatellite-mining from SSR-containing ESTs refers to a new class of locus-specific DNA markers which was developed to mirror the functional genomic components (Varshney et al., 2007). It is inexpensive, time-saving, and has been proved to be an effective approach in tall fescue, asparagus, and switchgrass (Saha et al., 2004; Caruso et al., 2008; Narasimhamoorthy et al., 2008). Start Codon Targeted (SCoT) polymorphism is a DNA marker technique that is similar to random amplified polymorphic DNA (RAPD) markers or inter-simple sequence repeat (ISSR) markers based on the short conserved region flanking the ATG start codon in plant genes. It combines the advantages of ISSR and RAPD markers and provides a simple, time-saving, cost effective method that is easily reproducible and stable. This technique has been widely applied in rice and mango for cultivar identification and genetic diversity analysis (Collard and Mackill, 2009; Luo et al., 2010, 2011).

In present study, EST-SSR and SCoT markers were employed to evaluate the genetic diversity and relationships among *H. altissima*, *H. compressa* and *H. uncinata* genotypes and to compared the usefulness of EST-SSR and SCoT markers for discriminating *Hemarthria* species.

2. Materials and methods

2.1. Plant materials

Summarized in Table 1, we tested 46 *Hemarthria* germplasm resources. Seven *H. compressa* materials were collected from the teaching and research center at Department of Grassland Science, Sichuan Agricultural University (Yaan), Sichuan, China. Thirty nine materials were obtained from the Germplasm Resources Information Network (GRIN) program within the United States Department of Agriculture (USDA) in October 2012. The 46 *Hemarthria* genotypes include 36 *H. altissima*, 2 *H. uncinata* and 8 *H. compressa*. Only one out of 36 *H. altissima* genotype was from South America and the rest were originated from Africa. Two *H. uncinata* were from Oceania and 8 *H. compressa* were from Asia. Fresh healthy young leaves of each material were harvested directly, placed into a collection bag with color silica gel, and stored at −80 °C before the leaves were thoroughly dry. In accordance with their genetic background of individuals, *Hemarthria* materials are strictly propagated through rhizomes; therefore, in our study one *Hemarthria* materials was selected for each individual species.

2.2. Genomic DNA extraction

Each *Hemarthria* germplasm was randomly selected using the same amount of leaf tissue to extract DNA using a genomic DNA extraction kit (Tiangen Biochemical Technology Co., Ltd. Beijing, China), following the manufacturer's protocol. The quality and concentration of the genomic DNA were determined by 1% (wt/vol) agarose gels electrophoresis and spectrophotometer analysis using a NanoDrop 2000 nucleic acid/protein analyzer. The qualified DNA samples were diluted to 20 ng/μL and stored at −20 °C for later use.

2.3. Primers screening and PCR program

We used conserved grass EST-SSRs (CNL) primer sequences developed by Kantety et al. (2002) and SCoT primers were obtained from previous related study by Luo et al. (2010). Both marker primers were synthesized by Shanghai Shenggong Biological Engineering Technology Services Ltd. (Shanghai, China). Eighty six pairs of conserved grass EST-SSR primers and 48 SCoT primers were screened by choosing high quality electrophoresis and DNA materials (H203、H232、H242 and 'Ya An') along with significant morphological traits differences found in the field as template. Primers that generated clear and reproducible banding patterns were selected for the EST-SSR and SCoT analysis.

For the EST-SSR PCR amplification reaction, we used 1.2 μL (20 ng/μL) DNA template, 7.5 μL 2 × Taq PCR MasterMix (Kangwei Century Biotech Co., Ltd. Guangzhou, China), 1.2 μL (10 pmol/μL) of each forward and reverse primers, and ddH_2O was added as needed to create a final volume of 15 μL. The EST-SSR PCR amplification reaction was performed in an eppendorf thermocycler (Biometra; Goettingen, Germany) using 5 min pre-denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 ~ 58 °C for 1 min, extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min and stored at 4 °C.