Biochemical Systematics and Ecology 69 (2016) 91–100

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Studies on genetic diversity and phylogenetic relationships of limpograss (*Hemarthria altissima*) and related species based on combined chloroplast DNA intergenic spacer data

Jian Zhang [a, **, 1], Xiu Huang [b, 1], De-jun Huang [a], Yu Zhang [c], Lin-kai Huang [b, *], Lu Lu [d], Han-dong Yan [b]

[a] Chongqing Academy of Animal Sciences, Herbivorous Livestock Research Institute, Rongchang, Chongqing, 404100, China
[b] Department of Grassland Science, Animal Science and Technology College, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
[c] Institute of Agrifood Research and Technology (IRTA), Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Barcelona, 08193, Spain
[d] Department of Biochemistry and Molecular Biology, Biosciences Faculty, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, 08193, Spain

ARTICLE INFO

Article history:
Received 25 April 2016
Received in revised form 27 July 2016
Accepted 30 July 2016

Keywords:
Chloroplast DNA
Hemarthria altissima (Poir.) Stapf et C. E. Hubb.
Hemarthria compressa (L. f.) R. Br.
Genetic diversity
Genetic structure
trnL-F
trnC-ycf6
psbC-trnS

ABSTRACT

Hemarthria R. Br. is a genus which includes important forage grasses. However, there is currently a lack of data analysis on the chloroplast DNA (cpDNA) of *Hemarthria* species. This study is to use three cpDNA intergenic spacers (*trnL-F*, *trnC-ycf6* and *psbC-trnS*) to obtain phylogenetic information in 36 *Hemarthria* samples including four *Hemarthria* species: *Hemarthria altissima* (Poir.) Stapf et C. E. Hubb., *Hemarthria compressa* (L. f.) R. Br., *Hemarthria uncinata* R. Br., and *Hemarthria japonica* (Hack.) Roshev. Data analysis revealed that non-significant genetic diversity existed in our samples, which was implied by nucleotide sequences information and the results of haplotypic and nucleotide diversity. The results of phylogenetic trees based on maximum likelihood (ML) and Bayesian inference (BI) revealed that *H. altissima* and *H. compressa* samples were not entirely distinct, suggesting that the two species share an intimate genetic relationship. A haplotype median-joining (MJ) network revealed broadly similar results to those derived from the ML and BI trees and implied that haplotype H3 may represent an ancient haplotype. Analysis of the population statistic F_{ST} revealed little genetic differentiation among the seven populations of *H. altissima* in Africa.

1. Introduction

The genus *Hemarthria* R. Br. in the Poaceae family consists of about 20 species that are geographically widely distributed. Among these species, the two that are the most important agriculturally and widely studied are *Hemarthria altissima* (Poir.)

* Corresponding author.
** Corresponding author.
E-mail addresses: zjturf@163.com (J. Zhang), huanglinkai@sicau.edu.cn (L.-k. Huang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bse.2016.07.024
0305-1978/

Stapf et C. E. Hubb. and *Hemarthria compressa* (L. f.) R. Br. *H. altissima*, also known as limpograss, is a warm-season perennial grass that is native to Africa (Yang et al., 2004; Newman et al., 2011). After being introduced to Florida, limpograss has been widely used by beef cattle producers as a summer forage plant in the southeastern part of the United States due to its high forage yield, high quality, and high tolerance of poorly drained soils (Newman et al., 2011). *H. compressa* (whipgrass), a relative of limpograss, is another particularly important species of *Hemarthria* and is mainly found in China. These two species have been extensively used and commercially grown in subtropical and tropical areas, and made important contributions to agro-animal husbandry ecosystem development and maintenance (Yang et al., 2004).

Despite their economic and ecological importance, there is a serious lack of genomic information available for *H. altissima* and *H. compressa*. Before the 21th century, studies on *H. altissima* and *H. compressa* were mainly focused on naturally occurring germplasm morphology (Schank et al., 1973), cytology (Quesenberry et al., 1982), stress resistance (Hudson, 1986), and breeding (Yang et al., 2004). In recent years, molecular studies, including the estimation of genetic diversity, construction of DNA fingerprints, and association of molecular markers with agronomically important traits, have been conducted on *H. altissima* and *H. compressa* using a variety of molecular markers. However, all of these markers have so far been based on nuclear DNA (Huang et al., 2008, 2014, 2014a; uang et al., 2014; Huang et al., 2014a, 2014b; Chen et al., 2011). To date, there have been no molecular research studies based on analysis of chloroplast DNA (cpDNA) in *Hemarthria*.

Outside of the cell nucleus, the cpDNA of higher plants represents an additional source of genetic information. The chloroplast genomes of most angiosperms exhibit a simple, circular structure with a low substitution rates and high conservation, and they are inherited in a non-Mendelian, uniparental fashion (Clegg et al., 1994). Currently, cpDNA sequence data is widely used to study the origin, evolution, and genetic diversity of natural populations of the majority of important plant species (Juszczak et al., 2012). Although cpDNA is conserved in plants, non-coding and coding regions differ greatly in the evolution rate (Yan et al., 2015). The non-coding regions are more hypervariable and are richer in variations such as substitutions, translocations, inversions, insertions, and deletions (Katayama et al., 2012). These represent ideal segments for interspecific phylogenetic studies at low taxonomic levels, such as hybrid cultivar identification, reconstruction of genetic relationships of plants species, and intraspecific phylogeographic studies (Bakker et al., 1999; Kimura et al., 2003).

Previous molecular research on *Hemarthria* based on DNAmarkers revealed distinctive genetic differences and extensive genetic diversity in wild plant resources. These studies also suggested a close genetic relationship between *H. altissima* and *H. compressa*. However, in-depth analysis of the relationship between these two significant *Hemarthria* species is lacking. Therefore, in this study, we sought to take advantage of the virtues of cpDNA non-coding regions by assessing three cpDNA intergenic spacers (*trnL-F*, *trnC-ycf6*, and *psbC-trnS*) in 36 *Hemarthria* germplasm resources (including 26 *H. altissima*, 8 *H. compressa*, 1 *Hemarthria uncinata* R. Br., and 1 *Hemarthria japonica* (Hack.) Roshev.). Our aims were mainly to (1) estimate the genetic diversity of the 36 *Hemarthria* samples based on cpDNA sequence variation, (2) explore phylogenetic relationships between *H. altissima* and *H. compressa*, and (3) reveal the genetic structure of African *H. altissima*. Our results serve to enrich the existing knowledge on the genetic diversity of *H. altissima* and *H. compressa*, providing useful information for the development of conservation strategies for *Hemarthria* and for future phylogenetic and phylogeographic studies of *Hemarthria* species.

2. Material and methods

2.1. Plant materials

In this study, 26 *H. altissima* samples, one *H. compressa*, and one *H. uncinata* included, were collected from the USDA Germplasm Resources Information Network (GRIN) program in October 2012 (Huang et al., 2014). Another seven *H. compressa* samples and one H. japonica sample were obtained from the Sichuan Agricultural University (Yaan), Sichuan, China. Detailed information on these 36 *Hemarthria* samples is summarized in Table 1. Unfortunately, records of two samples were ambiguous. One sample was described as deriving from 'South Africa', which could refer to either the Limpopo or KwaZulu-Nata region. Another source was recorded as simply 'Japan', which may refer to the city of Yokohama. All plant samples were maintained as rhizomes. Fresh and young leaf tissues from each sampled individual clone were harvested and dried at room temperature in collection bags with color silica gel.

2.2. DNA extraction

Dry and young leaf tissues were first ground by a Tissue Lyser (Grinder, Beijing, China) and were later used to be extracted genomic DNA using a Plant Genomic DNA Kit (Tiangen, Beijing, China) in accordance with the manufacturer's directions. DNA quality was analyzed by 0.8% (w/v) agarose gel electrophoresis, and DNA concentration was quantified by NanoDrop 2000 spectrophotometer (Thermo, USA). The DNA stock was diluted to a working concentration of 20 ng/μL and stored at −20 °C.

2.3. PCR amplification and sequence determination

A set of universal cpDNA primers were used to amplify the target regions of *trnL-F* (5′-CGAAATCGGTAGACGCTACG-3′, 5′-ATTTGAACTGGTGACACGAG-3′), *trnC-ycf6* (5′-CCAGTTCAAATCTGGGTGTC-3′,5′-CATTAAAGCAGCCCAAGC-3′) (Demesure et al., 1995) and *psbC-trnS* (5′-GGTCGTGACCAAGAAACCAC-3′, 5′-GGTTCGAATCCCTCTCTCTC-3′) (Murakami et al., 2006). PCR was