Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 2 (2008) 35–40
FSI GENETICS
www.elsevier.com/locate/fsig

Bidens identification using the noncoding regions of chloroplast genome and nuclear ribosomal DNA

Li-Chin Tsai [a,b], Jenn-Che Wang [a], Hsing-Mei Hsieh [b], Kuo-Lan Liu [c], Adrian Linacre [d], James Chun-I Lee [e,*]

[a] Department of Life Science, National Taiwan Normal University, 88 Ting-Chow Road, Sec 4, Taipei 116, Taiwan, ROC
[b] Department of Forensic Science, Central Police University, 56 Shu-Jen Road, Kwei-San, Taoyuan 33334, Taiwan, ROC
[c] Forensic Biology Office, Criminal Investigation Bureau, No. 5, Lane 553, Chung Hsiao Eastern Road Section 4, Taipei, Taiwan, ROC
[d] Centre for Forensic Science, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XW, United Kingdom
[e] Department of Forensic Medicine, College of Medicine, National Taiwan University, No. 1 Jen Ai Road Section 1, Taipei 100, Taiwan, ROC

Received 6 March 2007; received in revised form 26 July 2007; accepted 27 July 2007

Abstract

Bidens pilosa L. is a plant producing barbed fruits which, due to its method of seed dispersal, is commonly found during forensic investigations. In Taiwan there are three varieties of the species, *radiata*, *minor* and *pilosa*. Fragments of these three varieties are difficult to differentiate by traditional morphological characteristics and until now little is known of their genetic composition. To discover genetic polymorphisms among these varieties, five loci within the nuclear and chloroplast genomes were screened. A total of 161 specimens were used in this study comprising different geographical populations. Seven samples of *Bidens biternata* were included as an out-group control. DNA fragments of all samples at the trnL intron and trnL-trnF IGS loci of the chloroplast genome, internal transcribed spacer (ITS1 and ITS2) and the 5.8S of nuclear ribosomal DNA (nrDNA) were amplified and sequenced. There were 3, 4, 20, 12 and 9 sequence types at these five loci, respectively. The sequence types for any locus of trnL intron, ITS1, ITS2 and 5.8S were found to be useful markers to identify *Bidens biternata* and *B. pilosa*. The resulting 84 haplotypes at the 5 loci could differentiate the var. *radiata* from the varieties of *B. pilosa* with only the exception of 1 type. The genetic polymorphisms can be used when comparing botanical remains to identify the variety of *B. pilosa* present at a crime scene.

Keywords: *Bidens pilosa*; nrDNA; 5.8S; ITS1; ITS2; trnL intron; trnL-trnF IGS; Forensic botany

1. Introduction

Botanical specimens found at a crime scene may provide highly valuable information when determining a primary geographic location. This is especially the case for plants that rely on animal dispersal. *Bidens* species produce barbed fruits and are a wide-spread herb in Taiwan. Parts of the plant are used widely in traditional medicine [1,2] and are of value due to their phytochemical properties [3,4] and for medical purposes [5,6]. The Flora of Taiwan (second edition) sub-divides *Bidens pilosa* L. into three varieties, var. *pilosa*, var. *minor* and var. *radiata*. The variety *radiata* is alien, probably native to the United States, and first colonized Taiwan in the last two decades [7]. It is difficult to differentiate these three varieties by traditional morphological characteristics when they are fragmented and lack physical features. A genetic study of the three varieties would provide useful information in determining species and variety. The genetic variation of *B. pilosa* using inter-simple sequence repeat (ISSR) loci indicated that unrestricted gene flow occurs within varieties making it difficult to differentiate genetic variation among different varieties and populations [8]. A DNA fingerprint produced by ISSR maybe useful when studying genetic variation of species, but lacks reproducibility, cannot be used to establish a database, and prevents inter-laboratories comparisons. Such tests that detect genetic polymorphisms throughout the genome rather than specific loci cannot be used when the samples are degraded, old, or contaminated with fungal or bacterial growth. Genetic loci are not affected by such problems that are used more commonly in phylogenetic studies. Such loci include non-coding DNA regions such as the intron of the trnL (UAA), the intergenic spacer (IGS) of the trnL-trnF (GAA) in the chloroplast genome

* Corresponding author. Fax: +886 2 23218438.
E-mail address: jimlee@ntu.edu.tw (J.-I. Lee).

1872-4973/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.fsigen.2007.07.005

and the internal transcribed spacer (ITS1 and ITS2) of nuclear ribosomal DNA (nrDNA) [9–13]. These loci have been found to be capable of detecting inter and intra species variation in a range of botanical species [14]. We report an extensive study of *Bidens* species using these four loci in addition to the 5.8S locus.

2. Materials and methods

2.1. Sample collection

A total of 168 leaf samples of three varieties of *B. pilosa* and *B. biternata* were collected from different populations in Taiwan and listed in Table 1. The morphological identification was followed by the guideline in the Flora of Taiwan [7].

2.2. DNA extraction

Approximately 10 mg of leaf samples were pulverized under liquid nitrogen in a mortar. The powder was transferred to a 1.5 ml microcentrifuge tube and DNA was extracted by a commercial kit (Plant Genomic DNA Miniprep System, Viogene, Taiwan). The isolated DNA was quantified by UV spectrophotometer and separated by electrophoresis on a 0.7% agarose gel, and stored at −20 °C.

2.3. PCR amplification

The universal primers [9,10,15] for algae, land plants and fungal targeting exon regions were used to amplify the intron of the trnL, the trnL-trnF IGS, the internal transcribed spacer (ITS1 and ITS2) and 5.8S of nrDNA. The PCR amplifications were performed in separate tubes but under the same conditions. These were in a 50 μl volume containing 0.3 μM each of primers, reaction buffer (10 mM Tris–HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1% (w/v) gelatin), 200 μM dNTP, 2.5 unit of VioTaq DNA polymerase (Viogene) and 10 ng of genomic DNA. The amplifications were conducted in a 2400 Perkin-Elmer thermal cycler (Applied Biosystems) under the following conditions: denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 2 min for 35 cycles, and followed by a 7 min extension at 72 °C.

2.4. DNA sequencing

PCR products were checked on a 2% agarose gel, purified with the PCR-M™ Clean Up System (Viogene), and sequenced using the forward and reverse primers in a PCR amplification and the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit. The cycle sequencing products were analyzed with POP-7™ (Applied Biosystems) and detected by ABI 3730 DNA Analyzer.

2.5. Sequence analysis

Sequences were aligned using the PileUp program of the GCG computer package. The gap creation penalty and gap extension penalty were 5 and 1, respectively, for these five loci. The program of GCG is available from http://bioinfo.nhri.org.tw (National Health Research Institute, Taiwan).

3. Results

3.1. Sequence analysis of these five loci among B. biternata and the varieties of B. pilosa

All samples of *B. pilosa* and of *B. biternata* were amplified and sequenced using the universal primers. A complete DNA sequence was identified for these five loci. Table 1 shows the distribution of sequence types in each population used in this study. The loci of nrDNA (ITS1, 5.8S and ITS2) produced more sequence types than the chloroplast loci (trnL intron and trnL-trnF). A similar intra-genus phenomenon was reported in *Gentiana*, *Ilex* and *Festuca ovina* [16–18]. Table 2 shows the sequence characteristics of these loci for *B. biternata* and the three varieties of *B. pilosa*. No example of sequence heterogeneity was detected at the trnL intron and trnL-trnF IGS loci in the chloroplast genome among all 168 samples.

Table 1
Distributions of samples and sequence types determined using five genetic loci: the trnL intron, trnL-trnF IGS, ITS1, ITS2 and 5.8S

Taxon	Population	Sample size	trnL intron	trnL-trnF IGS	ITS1	5.8S	ITS2
B. pilosa var. *radiata*	Taoyuan Fusing	15	AB	ABD	CFGIJKLOQS	ABE	ACEIJKM
	Changhua Tianwei	20	A	AB	GHINQRT	A	ABCIM
	Pintung Chunrih	17	A	B	GHNOR	AF	CIKM
	Hualein Fongbin	20	AB	ABD	GHIJPQT	AE	ACDIKM
B. pilosa var. *minor*	Taoyuan Dasi	13	AB	AC	CDELP	BCDEG	DEK
	Hsinchu Lidong mountain	17	AB	AD	CEFKLMP	BCDEG	DEJKL
	Nantou Sinyi	6	A	A	DE	BC	DE
	Taitung Beinan	20	A	A	DELMP	BCDEG	DEIJKL
B. pilosa var. *pilosa*	Taoyuan Dasi	20	AB	ACD	CDEFKLP	BDEG	DEIJL
	Yunlin Kouhu	5	A	A	E	BCE	DK
	Taitung Beinan	8	A	A	EP	BDEG	EL
B. biternata	Pintung Lilong mountain	7	C	A	AB	HI	FH
Total		168					