Journal of Applied Research on Medicinal and Aromatic Plants 3 (2016) 94–100

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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

The nuclear internal transcribed spacer (ITS2) as a practical plant DNA barcode for herbal medicines

Claire-Iphanise Michel[a], Rachel S. Meyer[b], Yanille Taveras[a], Jeanmaire Molina[a,b,*]

[a] Department of Biology, Long Island University-Brooklyn, 1 University Plaza, Brooklyn, NY 11201, United States
[b] Center for Genomics and Systems Biology, New York University, 12 Waverly Place, New York, NY 10003, United States

ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form 21 January 2016
Accepted 1 February 2016
Available online 4 March 2016

Keywords:
ITS2
Herbal products
DNA barcoding
Medicinal plants
rbcL
matK

ABSTRACT

With the development of DNA barcoding methods and databases, misidentification may be easily caught. However, this technique has presented challenges with regard to the tissue type and marker locus variation. In this study, DNA barcoding was used to investigate the taxonomic accuracy of herbal medicines sold in the New York City (NYC) area using the plastid markers *rbcL*, *matK* and the nuclear ribosomal region ITS2. Forty-seven (47) samples (single-ingredient herbal medicinal products or HMPs) were purchased from eight herbal stores, and represented different structures: 22 leaf samples, 11 roots, 9 barks, and 5 samples that were ground beyond recognition. MEGABLAST sequence comparison was performed to verify the taxonomic identity of the samples. Maximum likelihood trees were constructed for sequence queries with equivocal multiple high-scoring MEGABLAST hits. Of the barcodes, ITS2 had the most success being amplified and sequenced (barcoded) in 38 of the 47 HMPs (81%), mostly leaf and root samples as barks were difficult to barcode. This is in contrast to 26/47 (55%) and 15/47 (32%) successful barcoding for *rbcL* and *matK*, respectively. ITS2 alone was accurate enough to match 88% of ITS2 sequences to the expected species, while only 59% of sequenced *rbcL* and 45% of *matK* was able to match with the expected species. All four HMPs obtained from one store did not match the declared species on the label based on ITS2, and this result was echoed by the *rbcL* and *matK* barcodes. However, all other herbal stores appeared to be selling accurately labeled HMPs. Our study showed that DNA barcoding is a worthwhile effort to test HMP authenticity, which may help build reputation of quality brands and ensure consumer safety. ITS2 is a practical DNA barcode that regulatory agencies may propose to NYC herbal medicine manufacturers and merchants to ensure quality.

ï¾© 2016 Elsevier GmbH. All rights reserved.

1. Introduction

According to the US National Center for Complementary and Alternative Medicine (NCCAM, http://nccam.nih.gov), more than 38 million adults in the United States use herbal products for medicinal purposes. However, little is known about their effectiveness, proper use, and safety. Currently, the US Food and Drug Administration (FDA) includes herbal medicinal products (HMPs) as dietary supplements, which are regulated differently from prescription medicines. Manufacturers of herbal supplements do not need approval from the FDA in order to sell their products. Labels must include all source ingredients including the common name of the botanical species, fillers, excipients, and binders used (CFR, 2015). If they make a claim about their HMP, then they need to include a disclaimer: "This statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure, or prevent any disease (www.fda.gov)". However, FDA can take action against the manufacturer and remove the herbal product when there are serious customer complaints.

Herbs are traditionally identified by morphological characteristics, but morphology can sometimes be misleading, as in the case of phenotypically variable species or cryptic species (Vijayan and Tsou, 2010). Since the FDA does not strictly regulate HMPs, there is a risk that many HMPs sold in the market are taxonomically misidentified, mislabeled, or contaminated. For example, Baker et al. (2012) reported, that of the HMPs advertised as black cohosh (*Actaea racemosa,* Ranunculaceae) sold in New York City, only 75% were identified properly. The rest were replaced by different species, which may not only reduce the therapeutic effects, but can potentially be harmful.

* Corresponding author at: Department of Biology, Long Island University-Brooklyn, 1 University Plaza, Brooklyn, NY 11201, United States.
E-mail addresses: jeanmaire.molina@liu.edu, jeanmaire.molina@gmail.com (J. Molina).

http://dx.doi.org/10.1016/j.jarmap.2016.02.002
2214-7861/ï¾© 2016 Elsevier GmbH. All rights reserved.

Misidentification of HMPs can result in serious consequences. One example was reported by Lin et al. (2010) to show that *Digitalis purpurea* (foxglove) could be mistaken for *Symphytum officinale* (comfrey) because their leaves are morphologically similar prior to flowering. Comfrey is used to treat fractures, tendon injury, among others (Lin et al., 2010). Foxglove contains cardiac glycosides and has been used to treat congestive heart failure. Nine people had to be treated for cardiotoxicity after ingesting the "comfrey" tea, with some even requiring temporary pacemakers to regulate their heart rhythm (Lin et al., 2010). Deliberate adulteration of HMPs with additives not listed on the label has also been rampant. Newmaster et al. (2013) have shown that 30/44 of the North American HMPs from 12 companies contained substitutions, contaminants or fillers (but see also Gafner et al., 2013). Another study has also shown that many commercial teas in the market are adulterated with plant ingredients not listed in their food labels (Stoeckle et al., 2011). More recently, big retailers in NYC, like GNC, Walgreens, and Target were criticized by the attorney general for selling HMPs not containing the declared plant ingredient (New York City Press Office, 2015). These studies highlight the importance of accurate taxonomic identification of herbal products. Resolutions have included planned incorporation of DNA technology in product assessment, acknowledging its potential to build consumer confidence (Long, 2015).

Retailers of dietary supplements usually have no role in determination of accurate labeling, and mislabeling could have occurred at any of the many steps between harvest and sales. To help combat mislabeling, FDA has begun to adopt the technology of DNA barcoding in the seafood industry (Handy et al., 2011). DNA barcoding is a molecular technique used for taxonomic identification. It makes use of short (<1000 bp) regions of the genome, universally present in target taxa, and evolve fast enough to differ between closely related species. When a barcode sequence is retrieved from an unidentified sample, a sequence alignment algorithm is used to compare the unidentified sample to a reference database (Rydberg, 2010).

The standard genomic regions that have been used in plant barcoding include the large subunit of the rubisco enzyme gene (*rbcL*), and the plastid gene that codes for the maturase protein (*matK*). *rbcL* is easily retrievable across divergent lineages of land plants (Fazekas et al., 2008), but does not sufficiently vary among species. It is used with *matK* due to *matK's* higher sequence variability (Consortium for the Barcode of Life Plant Working Group, 2009). Together, *rbcL* and *matK* were able to discriminate >98% of angiosperm tree species from a forest plot in the Barro Colorado Island of Panama (Kress et al., 2009). However, the *rbcL–matK* barcode is far from perfect due to the fact that *matK* has a low amplification success rate (Kress and Erickson, 2007). Thus, another plant barcode, the second spacer of the internal transcribed region (ITS2) of the nuclear ribosomal DNA has been used. In a study performed by Chen et al. (2010), ITS2 discriminated more than 6600 plant samples belonging to 4800 species from 753 distinct genera. The ITS2 barcode had a 92.7% success rate of identification at the species level. Although *rbcL–matK* tandem is widely used in plant barcoding studies, ITS2 is also an alternative marker especially when *matK* cannot be easily amplified. This conclusion was also supported by many studies (Chen et al., 2010; Yao et al., 2010; Han et al., 2012; Newmaster et al., 2013).

Here, we investigated the taxonomic accuracy of herbal medicines sold in eight local non-chain stores within the NYC area using DNA barcoding of three standard markers (*rbcL*, ITS2, and *matK*). NYC markets represent a cross-section of the supplements market that is unparalleled in diversity with respect to user groups. Sampled stores cater to immigrant communities that may use these herbs in medicinal or culinary preparations and cater to chefs and restaurateurs. As far as we know, this is the first study that has investigated HMPs sold by small businesses in NYC. We also compared the ease of barcoding using the different markers on different plant parts, including amplification success and ability to resolve species in comparative sequence analyses.

Fig. 1. Examples of the HMPs obtained for this study (A–F). (A) bark of African mahogany (B) capsules containing ground material of blackseed (C) comfrey leaf fragments (D) loose powdered material of Saba senegalensis (E) loose powdered material for Euphorbia paganorum (F) pieces of burdock root.

2. Materials and methods

Herbal medicinal products (HMPs) representing 47 plant samples, each containing a single plant species on the label, were purchased from eight herbal stores in NYC and the adjacent Hudson County of NJ. Sampled herbal stores specialize in herbs used by various immigrant communities: African, Arabic, Caribbean/Latin, Chinese, and Indian. Unlike HMPs sold in big retail stores or pharmacies that are packaged under manufacturing guidelines and mandated to include the FDA disclaimer, the HMPs we sampled come in plastic or paper packets, pre-weighed or weighed according to customer needs. HMPs sampled included fragmented plant parts, 22 of which were leaf samples, 11 roots, 9 barks, and 5 samples that were ground (powdered plant material) beyond recognition (see Fig. 1). Prices ranged from $2 to $10 per HMP. None were labeled as dried extracts. To prevent any untoward legal action by storeowners, we have withheld the names of these stores. Each sample was pulverized in the Qiagen Tissue Lyser LT at 50 oscillations per second at 2-min intervals. Hard samples were ground using a typical coffee grinder. DNA extraction was performed using the Qiagen Plant DNEasy extraction kit (Cat# 69104, Qiagen, Hilden, Germany) following the manufacturer's protocol. For recalcitrant DNA from roots and bark samples, Qiagen extraction was modified by incubating the samples overnight at 65 °C in the AP1 (lysis) buffer that included 3.1% polyvinylpyrrolidone (PVP40, Sigma-Aldrich, St. Louis, MO), which has been shown to increase PCR amplification in woody samples rich in PCR-inhibiting polyphenolics (Rachmayanti et al., 2006).

The genomic region *rbcL* was amplified using the primers SI_for and SI_rev, while *matK* was amplified using primers Kim_3F and Kim_1R (Kress et al., 2009). The ITS2 region was amplified using primers from Yao et al. (2010). Amplification reactions for *rbcL*, *matK*, and ITS2 were performed following methods in Molina et al. (2013) on an Eppendorf Mastercycler using PCR programs described in Kress et al. (2009) for *rbcL* and *matK* and Gu et al. (2013) for ITS2. PCR products were run on 1% agarose gels and the DNA was stained with Foto/Vision (cat#E1-1843, Fotodyne Inc., Hartland, WI). PCR products were purified using ExoSAP-it (Affymetrix, Santa Clara, CA) and then sequenced by Genewiz Inc. (www.genewiz.com). Assembly of forward and reverse sequences (using "highest quality" setting), and manual trimming were performed in Geneious v. R7. Sequences were then compared to the

Download English Version:

https://daneshyari.com/en/article/3099123

Download Persian Version:

https://daneshyari.com/article/3099123

Daneshyari.com