RESEARCH ARTICLE

DNA barcoding of selected UAE medicinal plant species: a comparative assessment of herbarium and fresh samples

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Abstract It is commonly difficult to extract and amplify DNA from herbarium samples as they are old and preserved using different compounds. In addition, such samples are subjected to the accumulation of intrinsically produced plant substances over long periods (up to hundreds of years). DNA extraction from desert flora may pause added difficulties as many contain high levels of secondary metabolites. Herbarium samples from the Biology Department (UAE University) plant collection and fresh plant samples, collected from around Al-Ain (UAE), were used in this study. The three barcode loci for the coding genes *matK*, *rbcL* and *rpoC1* were amplified. Our results showed that *T. terrestris*, *H. robustum*, *T. pentandrus* and *Z. qatarense* were amplified using all three primers for both fresh and herbarium samples. Both fresh and herbarium samples of *C. comosum*, however, were not amplified at all, using the three primers. Herbarium samples from *A. javanica*, *C. imbricatum*, *T. aucherana* and *Z. simplex* were not amplified with any of the three primers. For fresh samples 90, 90 and 80% of the samples were amplified using *matK*, *rbcL* and *rpoC1*, respectively. In short, fresh samples were significantly better amplified than those from herbarium sources, using the three primers. Both fresh and herbarium samples from one species (*C. comosum*), however, were not successfully amplified. It is also concluded that the *rbcL* regions showed real potentials to distinguish the UAE species under investigation into the appropriate family and genus.

Keywords DNA barcoding · DNA extraction · Herbarium · *matK* · *rbcL* · *rpoC1*

Introduction

Molecular tools are an excellent option to deal with herbarium specimen and DNA barcoding has demonstrated promising outcomes. DNA barcoding was reported to be a useful technique within many disciplines, such as conservation biology and forensics, to identify species of a sample based on nucleotide similarity to samples of known taxonomy (Pettengill and Neel 2010). But such applications presented difficulties due to problems in obtaining nucleic acids of acceptable purity for manipulation with different DNA modifying enzymes (Cubero et al. 1999). However, it is difficult to extract amplified DNA from the first herbarium sample tested (Savolainen et al. 1995). There is need for the use of dried samples, as fresh rare species samples may be inaccessible for genetic or barcoding investigations (Do and Drábková 2016). Herbarium and other noteworthy collections of plants, have been perceived as a critical hereditary asset helpful for addressing taxonomic inquiries (Lister et al. 2008). Simply because dried plant herbarium specimens are conceivably a significant source of DNA (Staats et al. 2011). Moreover, it is needed to use herbarium specimens in molecular phylogeny assessments, as it is difficult to obtain living material of some rare species (Savolainen et al. 1995). Unfortunately, DNA amplifications from herbarium samples have inhibited efforts for their use in genetic studies (Do and Drábková 2016).

Herbarium DNA presents specific difficulties to molecular examiners. Difficulties can be either specimen-related (specimen preparation techniques, preservation method and

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specimen age) (Staats et al. 2011) or taxon-related (differences of leaf compositions and the wide cluster of secondary compounds present in plants) factors (Jobes et al. 1995). DNA extraction from desert flora may pose added difficulties as many contain high levels of polysaccharides and secondary metabolites; which form insoluble edifices with nucleic acids during extraction (Guillemaut and Maréchal-Drouard 1992). Plants' metabolites and polysaccharides in desert plants repress enzyme activity and therefore lead to added complexities in DNA extractions (Porebski et al. 1997). The polysaccharides are outwardly apparent by their thick, stick-like surface making the DNA unmanageable when pipetting and difficult to amplify by the PCR technique (de la Cruz et al. 1997). The DNA quality of herbarium specimens was significantly worse than that from fresh leaves, because of collection conditions and sample preservation. Ribeiro and Lovato (2007) examined 54 herbarium specimens out of which only eight recovered with successful PCR amplification. With the reported contamination of DNA extract and/or amplification mixture, eight samples out of 17 were successfully amplified (Savolainen et al. 1995). The traditional taxonomic practices are to adapt to the developing requirement for exact species identification, especially using barcoding. The recent advancements of DNA barcoding have been well received for floral species identification (Newmaster et al. 2009).

Strategies for recognizing species by utilizing short orthologous DNA sequences, known as DNA barcodes, are becoming a major protocol for species identification (Kress et al. 2005). Simply because DNA barcoding is a reliable technique, does not require taxonomic expertise and is free from subjective errors and the substantial identification of obscure specimens is the fundamental objective of barcoding (Hebert and Gregory 2005). This is the case for preventing the exchange of plant material from invasive species such as *Prosopis juliflora* (El-Keblawy and Ksiksi 2005) or rare species such as *Acridocarpus orientalis* (Ksiksi et al. 2012) in the UAE, for instance.

The plastid genome is uniparentally acquired, non-recombining, and a structurally stable genome and they constitute the most encouraging competitor genes for plant DNA barcoding (Lucas et al. 2012). Not less than two genes are important to a reliable plant DNA barcode such as *rbcL* and *matK*. *rbcL* could be recognized to genus and family level, while the variable part of the code, *matK* is for species confirmation (Group et al. 2009). But *rbcL* alone was reported to be practical in assessing species differences (Savolainen et al. 1995). Fortunately, most encouraging combinations are *rbcL/matK* (Group et al. 2009). *matK* was used to develop a barcode for plants under pressures from collectors (Yesson et al. 2011). Because of its chloroplast origins and its conservative

mode of evolution, *matK* was useful in various studies (Palmer et al. 1988).

Results reported by Bafeel et al. (2011) indicated that primers of *rbcL* and *matK* did amplify many of the plant species but may fail in other cases. However, they recommended the development of a methodology to design novel universal primers, in order to cover barcoding for wider range of plant species. To build up a DNA barcoding framework for seagrasses, a two-locus approach with particular primers for the amplification of *rbcL* and *matK* was implemented (Group et al. 2009). While *psbA-trnH* and *trnT-trnL* barcodes delivered satisfactory DNA barcoding, but was dependent on the taxonomy used (Pettengill and Neel 2010).

So it is important to compare fresh and herbarium specimens and their potentials to provide reliable DNA material for barcoding purposes. It is therefore critical to develop DNA extraction protocols and appropriate genes to preserve and sustain important herbarium collections, such as the one at the Biology Department (UAE University). It has been identified as one of the biggest collections of floral species in the UAE.

Materials and methods

Plant sample collection

Herbarium samples from the Biology Department (UAE University) and fresh plant samples collected from around Jebel Hafit Mountain in Al-Ain—United Arab Emirates (25°–36″N and 55°–36″E) were used in this study. Species identification was checked against the UAE University herbarium collection. The list of plants species used in the present investigation is in Table 1. Fresh leaves were individually collected, labeled and transported in sterile plastic bags and frozen until processed for DNA extraction.

DNA isolation

Leaf samples were immersed in liquid nitrogen and crushed using bead blaster homogenizer D2400 (Benchmark Scientific Inc., USA) to obtain a fine powder. DNA extraction of the samples were performed using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturers protocol. Purity and quantity of the extracted genomic DNA was conducted with a NanoDrop spectrophotometer. DNA was examined using 1.0% an agarose gel stained with ethidium bromide.

PCR amplification

The three barcode loci for the coding genes *matK*, *rbcL* and *rpoC1*—were amplified using primers recorded in Table 2.

Table 1 List of plant species used in this study and the NCBI GenBank accession numbers of the deposited sequences

Sample ID	Plant species	Family	GenBank		
			rbcL	matK	rpoC1
F1	<i>Tribulus terrestris</i> _	Zygophyllaceae	KX887305	KY000485	KY000491
F2	<i>Haplophyllum robustum</i>	Amaranthaceae	–	KY000486	–
F3	<i>Aerva javanica</i>	Amaranthaceae	KX957747	KY000487	–
F4	<i>Tribulus pentandrus</i>	Zygophyllaceae	KX957748	KY000488	–
F5	<i>Caroxylon imbricatum</i> _	Amaranthaceae	KX957749	–	KY000492
F6	<i>Tamarix aucherana</i>	Tamaricaceae	KX957750	KY000489	KY000494
F7	<i>Zygophyllum qatarense</i>	Zygophyllaceae	KX957751	–	KY000493
F8	<i>Zygophyllum simplex</i>	Zygophyllaceae	KX957752	–	–
F9	<i>Calligonum comosum</i>	Polygonaceae	–	–	–
F10	<i>Dipterygium glaucum</i>	Capparaceae	KX957753	KY000490	–

Table 2 List of primers used for PCR amplification

Barcode	Primer sequence	References
matK	472F (5-CCCRTYCATCTGGAAATCTTGGTTC-3)	Yu et al. (2011)
	1248R (5-GCTRTRATAATGAGAAAGATTCTGC-3)	Yu et al. (2011)
rbcL	1F (5-ATGTCACCACAAACAGAAAC-3)	Fay et al. (1997)
	724R (5-TCGCATGTACCTGCAGTAGC-3)	Fay et al. (1997)
rpoC1	2F (5-GGCAAAGAGGGAAGATTTCG-3)	Sass et al. (2007)
	4R (5-CCATAAGCATATCTTGAGTTGG-3)	Sass et al. (2007)

PCR was carried out in a final reaction volume of 25 μ L made up of 4.5 μ L of genomic DNA (20 ng) used as template, 1.5 μ L each of the forward and reverse primers (15 pM), 12.5 μ L of Taq PCR Master Mix Kit (Qiagen, Germany) with a concentration of 200 μ M for each deoxynucleotide and 1.5 mM $MgCl_2$ and 5 μ L of sterile distilled water. PCR amplification was performed using a T100 Thermal Cycler (BioRad, USA). The thermal profile used was: 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR amplification was verified on a 1.5% agarose gel.

Data analyses

The rbcL, matK and rpoC1 were sequenced according to the method originally described by Sanger et al. (1977). The sequences from each DNA region were aligned by CLUSTALW and genetic distance was computed using the MEGA 5.0 Kimura two-parameter (K2P) model (Tamura and Nei 1993). The nucleotide sequence data of the partial matK, rbcL and rpoC1 were deposited in the Genbank nucleotide sequence databases with the accession numbers reported in Table 1. The phylogenetic trees were constructed using neighbour-joining method (NJ) in MEGA 5.0 software program. Bootstrap testing of 1000 replicates was performed to estimate the confidence level of the topology of the consensus tree.

Results and discussion

DNA purity results are summarized in Table 3. All fresh samples, except those from *C. comosum*, showed high purity ratios (between 1.7 and 2) when compared to herbarium samples.

Table 4 summarizes the PCR amplification for herbarium (HS) vs fresh (FS) UAE plant species samples using matK, rbcL and rpoC1. The results showed that *T. terrestris*, *C. robustum*, *T. pentandrus* and *Z. qatarense* were amplified using all three primers for both fresh and herbarium samples. Both fresh and herbarium samples of *C. comosum*, however, were not amplified at all, using the three primers. Herbarium samples from *A. javanica*, *C. imbricatum*, *T. aucherana* and *Z. simplex* were not amplified with any of the three primers. Isolating high quality DNA from Calligonum species can be difficult as it contains large amounts of polyphenolic compounds and polysaccharides, among others (Abdellaoui et al. 2011). The primers rbcL and matK were highly conservative and failed in Calligonum species discrimination (Li et al. 2014). Genomic DNA extraction was possible, however, for fresh samples for ten accessions of Calligonum polygonoides (Sablok et al. 2009).

Our results also revealed that 90, 90 and 80% of the fresh samples were amplified using matK, rbcL and rpoC1 (Table 5). In comparison, the results for herbarium samples were 40, 50 and 50%; respectively. Reports concluded that

Table 3 DNA purity ratio for herbarium and fresh UAE plant species samples

Plant sepecies	Herbarium samples	Fresh samples
F1- <i>Tribulus terresteris</i>	1.56	1.77
F2- <i>Haplophyllum ructaceae</i>	1.04	2
F3- <i>Aerva javanica</i>	1.08	1.8
F4- <i>Tribulus pentandrus</i>	1.69	1.83
F5- <i>Caroxylon imbricatum</i>	1.32	1.74
F6- <i>Tamarix aucherana</i>	1.27	1.9
F7- <i>Zygophyllum qatarense</i>	1.64	1.87
F8- <i>Zygophyllum simplex</i>	1.43	1.79
F9- <i>Calligonum comosum</i>	1.29	1.45
F10- <i>Dipterygium glaucum</i>	1.77	2.16

molecular studies on herbarium samples have been hindered by difficulties in getting amplifiable DNA (Särkinen et al. 2012). Only two herbarium samples led to DNA amplification (Savolainen et al. 1995). But in another study, tissue samples from 29 species yielded quantifiable amounts of DNA (Rogers and Bendich 1985).

A desirable DNA barcode must simultaneously possess enough sequence variations for species identification and adequate conserved flanking sites for the designation of the universal primers (Kress et al. 2005). The three candidate barcodes, namely, *rbcL*, *matK*, and *rpoC1* used in the present study were successfully amplified with the universal primer pairs. Hence, all these candidate barcodes from the chloroplast genome satisfied one of the two criteria for standard barcodes. When *rbcL*, *matK* and *trnLF* were combined, DNA extraction from *Calligonum* species was slightly better (Li et al. 2014). No universal plant barcode has been identified for plant barcodes (Singh et al. 2012). High quality sequences for *Calligonum* were obtained for *rbcL*, *matK* and *trnLF* with the universal primers (Li et al. 2014).

Table 5 PCR amplification percent (fresh vs herbarium samples) using *matK*, *rbcL* and *rpoC1*

Sample origin	<i>matK</i> (%)	<i>rbcL</i> (%)	<i>rpoC1</i> (%)
Fresh samples	90	90	80
Herbarium samples	40	50	50

In this study 10 flowering plants from the arid regions of the United Arab Emirates were chosen and the three barcode regions *matK*, *rbcL* and *rpoC1* were sequenced and analysed. DNA was isolated and PCR amplification was performed for each sample. PCR primers used in this study were sufficiently good enough to amplify the three chosen regions. The final consensus barcode sequences for the tested plant samples were deposited in the NCBI GenBank database and the accession numbers are provided in Table 1. Phylogenetic tree generated based on *matK* using neighbour-joining algorithms puts sequences of the same family in different clades (Fig. 1). A similar observation could also be made from *rpoC1* sequences. Phylogenetic tree constructed using *rpoC1* and *matK* barcodes indicates the two loci are unable to discriminate among the plant species assessed in the present study.

rbcL had a higher discriminatory power than *matK* and *rpoC1* for the studied plant species (Fig. 1). *Tribulus terrestris*, *Tribulus planoospira*, *Zygophyllum qatarense* and *Zygophyllum simplex* were combined together in first cluster. All of these genera belong to the same family (*Zygophyllaceae*). While *Dipterygium glaucum*, *Tamarix aucherana*, *Aerva javanica*, and *Caroxylon imbricatum* were combined in the second cluster. It is important to note that *D. glaucum* was recovered as a single genus in a separate sub-cluster (Fig. 1), while *A. javanica* and *C. imbricatum* belong to the *Amaranthaceae* family. In *matK*

Table 4 PCR amplification for herbarium (HS) vs fresh (FS) UAE plant species samples using *matK*, *rbcL* and *rpoC1*

Plant Species	<i>matK</i> (FS)	<i>rbcL</i> (FS)	<i>rpoC1</i> (FS)	<i>matK</i> (HS)	<i>rbcL</i> (HS)	<i>rpoC1</i> (HS)
F1- <i>Tribulus terresteris</i>	+	+	–	+	+	+
F2- <i>Haplophyllum ructaceae</i>	+	+	+	+	+	+
F3- <i>Aerva javanica</i>	+	+	+	–	–	–
F4- <i>Tribulus pentandrus</i>	+	+	+	+	+	+
F5- <i>Caroxylon imbricatum</i>	+	+	+	–	–	–
F6- <i>Tamarix aucherana</i>	+	+	+	–	–	–
F7- <i>Zygophyllum qatarense</i>	+	+	+	+	+	+
F8- <i>Zygophyllum simplex</i>	+	+	+	–	–	–
F9- <i>Calligonum comosum</i>	–	–	–	–	–	–
F10- <i>Dipterygium glaucum</i>	+	+	+	–	+	+

Fig. 1 Phylogenetic tree generated using neighbor joining method from matK sequence

Fig. 2 Phylogenetic tree generated using neighbor joining method from rpoC1 sequence

tree constructed using NJ method, *T. aucherana*, *A. javanica*, *T. terrestris*, *T. planoospira* and *D. glaucum* were combined together in a single cluster with high bootstrap confidence levels (99–100%). While *Z. robustum* was placed in a separate cluster (Fig. 1). The chloroplast maturase K gene (matK) is one of the most variable coding genes of angiosperms, and has been suggested for use as a barcode for land plants (Group et al. 2009). However, matK exhibits low rates of both amplification and sequencing due to the low universality of the currently available primers and mononucleotide repeats (Yu et al. 2011).

In rpoC1 trees constructed by using NJ are shown in Fig. 2. *C. imbricatum*, *T. aucherana* and *T. terrestris* are

placed in first cluster but *C. imbricatum* and *C. imbricatum* were separated into a sub-cluster with bootstrap confidence value of 86%. Whereas the second cluster included *Z. qatarense*. In this study, the plastid sequences rbcL, matK and rpoC1 allowed us to analyze the genetic relationships among the selected UAE medicinal plants. The rbcL gene, which encodes the largest subunit of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco evolves slowly, giving accurate results for higher taxonomic levels such as family (Cuénoud et al. 2002). However, it is not considered very useful for phylogenetic studies at the species level, since low-frequency mutations were observed for this gene (Chase et al. 2005). In the present study, the NJ tree of rbcL DNA sequences correctly placed

Fig. 3 Phylogenetic tree generated using neighbor joining method from *rbcL* sequence

species with the correct family with high statistical support (bootstraps of 97–100%), Due to higher sequence divergences, the *rbcL* regions can distinguish plant species and simply place these species in the correct family and genus (Fig. 3). Multilocus DNA barcodes have been proposed to be much effective and accurate in the identification of plant species (Group et al. 2009). The *rbcL* has been proven to be an effective and accurate DNA barcode for the species identification of medicinal plants (Guo et al. 2011). While, *matK* and *rpoC1* would not be sufficient to solely identify the studied UAE plant species.

Summary and future work

In the present investigation, DNA purity, using *matK*, *rbcL* and *rpoC1*, for all fresh samples, except those from *C. comosum*, showed purity ratios between 1.7 and 2. The fact that fresh samples were significantly better amplified than those from herbarium sources, using the three primers, is also supported. Both fresh and herbarium samples from one species (*C. comosum*) were not successfully amplified. While only herbarium samples from three other species (ie. *A. javanica*, *C. imbricatum*, *T. aucherana* and *Z. simplex*) were not amplified with any of the three primers. It is further recommended that neither *matK* nor *rpoC1* would be sufficient to uniquely identify the selected plant species. The *rbcL* regions, however, showed real potentials to distinguish the studied plant species into the appropriate family and genus.

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