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Original article

Species-specific molecular signature of *Commiphora* species of Saudi Arabia inferred from internal transcribed spacer sequences of nuclear ribosomal DNA

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ABSTRACT

The deciduous habit and tendency to produce flowers prior to developing leaves, and a predominantly dioecious system of breeding in the genus *Commiphora* leads to difficulties in its taxonomic identification at species level. The characteristics of easy amplification by universal primer, shorter length and higher discrimination power at the species level makes the internal transcribed spacer (ITS) sequence of nuclear ribosomal DNA (nrDNA) to a smart gene for generating species-specific phylogenetic inferences in most of the plants groups. The present study deals the ITS sequence of nrDNA based molecular genotyping of seven species of the genus *Commiphora* of Saudi Arabia. The molecular phylogenetic analysis of ITS sequences of nrDNA of *Commiphora* species distributed in Saudi Arabia reveals the the occurrence of *C. madagascariens* in Saudi Arabia.

1. Introduction

Commiphora Jacq., (family Burseraceae) is a genus of about 185 species distributed in tropical and subtropical regions (Byng, 2014). Several species of the genus *Commiphora* produce fragrant resins used for incense, perfume and also used medicinally in the diseases of liver, gastrointestinal disorder, urinary tract, rheumatism, scurvy and jaundice (Abdul-Ghani and Amin, 1997; Al-Howiriny et al., 2004, 2005; Hanuša et al., 2005; Shen et al., 2008; Iluz et al., 2010), cancer (Hartwell, 1982), respiratory, kidney, muscular, and in kidney complaints (Tejero et al., 2008); and thus gum/resins produced by the different species of *Commiphora* have high commercial value (Chaudhary, 2001). In Saudi Arabia, the genus *Commiphora* is represented by seven species i.e. *C. erythraea, C. gileadensis, C. habessinica, C. kataf, C. myrrha, C. opobalsamum* and *C. quadricincta* (Chaudhary, 2001). The systematic understanding of the genus *Commiphora* at species level has been hindered because of the life history which includes deciduous habit, a predominantly dioecious breeding system, and a tendency to produce flowers prior to developing leaves (Weeks and Simpson, 2007). Infrageneric groups of *Commiphora* have been proposed but limited to northeastern and tropical East Africa species (Wild, 1959; Gillett, 1991; Vollesen, 1986). The characteristics of easy amplification by universal primer, shorter length and higher discrimination power at the species level makes the internal transcribed spacer (ITS) sequence of nuclear ribosomal DNA (nrDNA) to a smart gene for generating species-specific phylogenetic inferences in most of the plants groups (Chen et al., 2010; Poczai and Hyvönen, 2010; Yao et al., 2010; Ali et al., 2014). Hence, the present study attempts to establish ITS sequence of nrDNA based species-specific molecular signature for the genus *Commiphora* distributed in Saudi Arabia.

2. Materials and methods

2.1. Sampling of the plant materials and sequencing of ITS gene

The leaf materials of a total number of seven species of the genus *Commiphora* distributed in Saudi Arabia (Table 1) were collected from herbarium specimens housed at the Herbarium, Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia (KSUH). The taxonomic identification of specimens were confirmed through the consultation of Flora of Saudi

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Table 1
Taxon sampling information and GenBank accession number for the sequences used in the molecular phylogenetic analysis.

S. No.	Taxon	Locality	Voucher	GenBank Accession No.
1.	*Commiphora erythrea* (Ehrenb.) Engl.	Abha, Saudi Arabia	Al-Farhan and Masin 1755 (KSUH)	MH522401
2.	*Commiphora gileadensis* (L.) C.Chr. [Syn = *Commiphora opobalsamum* (L.) Engl.]	Farasan, Saudi Arabia	R.A. Baseni 3159 (KSUH)	MH522402
3.	*Commiphora habessinica* (O.Berg) Engl.	Jabal Shada, Saudi Arabia	A.R. Al-Zaudi 21191 (KSUH)	MH522403
4.	*Commiphora kataf* (Forssk.) Engl.	Jabal Shada,Saudi Arabia	A.R. Al-Zaudi 21211 (KSUH)	MH522404
5.	*Commiphora madagascariens* Jacq. {[synonym *Commiphora roxburghii* Alston], [Illegitimate], http://www.theplantlist.org/tpl1.1/record/kew-2733654}	Jabal Fayfa, Saudi Arabia	M.I. Hassan 4092 (KSUH)	MH522405
6.	*Commiphora myrrha* (Nees) Engl.	Abha, Saudi Arabia	Al-Farhan and Masin 1786 (KSUH)	MH522406
7.	*Commiphora quadricincta* Schweinf.	Jabal, Saudi Arabia	*sn* 19278 (KSUH)	MH522407

Arabia (Chaudhary, 2001). The extraction of total genomic DNA were performed using Qiagen kit and then it was subjected to polymerase chain reaction for the amplification of ITS gene and the amplified product were further used for DNA sequencing following previously described method (Ali et al., 2010).

2.2. Molecular phylogenetic analysis

The similarity of the ITS sequences of nrDNA generated in the present study were matched using BLAST (Basic Local Alignment Search Too) search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) by NCBI server (Altschul et al., 1990). The ITS sequences of nrDNA of 14 species of *Commiphora* (Table 2) were retrieved from GenBank (www.ncbi.nlm.nih.gov). The ITS sequences of nrDNA of *Bursera attenuate* (GenBank accession number AF445871) was used as outgroup in the phylogenetic analyses. The sequence included in the molecular phylogenetic analyses were aligned using CLUSTAL X v.1.81 (Thompson et al., 1997). The ITS sequences of nrDNA generated in the present study were submitted to GenBank (Table 1). The aligned sequences were saved in nexus format. The nexus file of aligned sequences were imported in to MEGA 5 software (Tamura et al., 2011), and phylogenetic analyses were performed using Maximum Parsimony (MP) method (Tamura et al., 2011).

3. Results and discussion

3.1. Combined length of the ITS gene, GC content and BLAST

The combined length and GC content of ITS region (ITS1-5.8S-ITS2) in studied taxa ranges from 608 to 656 nucleotide base pairs (bp) and 65–67% respectively (length 620 bp and GC content 67% in *C. erythrea*, length 619 bp and GC content 67% in *C. gileadensis*, length 649 bp and GC content 66% in *C. habessinica*, length 608 bp and GC content 65% in *C. kataf*, length 646 bp and GC content 66% in *C. madagascariens*, length 651 bp and GC content 66% in *C. myrrha*, and length 656 bp and GC content 67% in *C. quadricincta*). The BLAST search of ITS sequence shows high similarity with GenBank sequences of *C. myrrha, C. kataf*, *C. neglecta*, *C. gowlello*, *C. habessinica* and *C. kua* [99% identity of sequence of *C. gileadensis* with *C. myrrha* (KC311151), 99% identity of sequence of *C. kataf* with GenBank sequence of *C. kataf* (JN882709), 100% identity of sequence of *C. myrrha* with GenBank sequence of *C. myrrha* (JN882706) and 96% identity with *C. kua* (JN882696), 97% identity of sequence of *C. erythrea* with *C. neglecta* (JF919029), 97% identity of sequence of *C. habessinica* with *C. gowlello* (JN882674), 96% identity of sequence of *C. madagascariens* with *C. gowlello* (JN882674), and 96% identity of sequence of *C. quadricincta* with *C. habessinica* (JN882673) and 96% identity with *C. kua* (JN882696)].

Table 2
The ITS sequences of nDNA retrieved from GenBank, and used in the molecular phylogenetic analysis of *Commiphora*.

S. No.	Species	**GenBank Accession No.**
1	*Commiphora africana*(A.Rich.) Endl.	AF445873
2	*Commiphora edulis* (Klotzsch) Engl.	JF919026
3	*Commiphora eminii* Engl.	JN882700
4	*Commiphora falcata* Capuron	KF906076
5	*Commiphora grandifolia* Engl.	JN882671
6	*Commiphora kataf* (Forssk.) Engl.	JN882709
7	*Commiphora kua* (R.Br. ex Royle) Vollesen	JN882696
8	*Commiphora leptophloeos* (Mart.) J.B.Gillett	AF445875
9	*Commiphora monstruosa* (H.Perrier) Capuron	AF080004
10	*Commiphora myrrha* (Nees) Engl.	KC311151
11	*Commiphora neglecta* Verd.	JF919029
12	*Commiphora orbicularis* Engl.	JN882697
13	*Commiphora schimperi* (O.Bergman) Engl.	JN882702
14	*Commiphora wightii* (Arn.) Bhandari	EU419975

3.2. Molecular phylogenetic relationships

The molecular phylogenetic analysis of the ITS gene (data matrix- 513 positions, parsimony informative sites- 106) using MP method (Eck and Dayhoff, 1966; Felsenstein, 1985; Nei and Kumar, 2000; Tamura et al., 2011) resulted in 16 maximally parsimonious trees (length- 287, consistency index- 0.637, retention index- 0.741, and composite index- 0.540) (Fig. 1).

The maximum parsimony tree recovered (Fig. 1) from the phylogenetic analysis clearly revealed the occurrence of the *C. kataf* and *C. myrrha* in Saudi Arabia; *C. kataf* and *C. myrrha* showed proximity [bootstrap support (BS 99%)] with the sequence of *C. kataf* and *C. myrrha* included in the analysis from GenBank (GenBank accession number JN882709 and KC311151) respectively. *C. kataf* clade with *C. gileadensis-C. neglecta* (BS 36%) and *C. erythrea-C. eminii* (BS 54%). Morphologically *C. myrrha* (fruit beaked, pseudoaril with broad triangular lobes) differs from *C. gileadensis* (fruit 4-valved, with prominent line) and *C. erythrea* (stout gnarled tree, fruit globose-ovoid, tomentose).

3.3. New distributional record of Commiphora madagascariens

C. quadricincta clade with *C. africana, C. schimperi, C. myrrha, C, wightii, C. kua, C. madagascariens* and *C. habessinica* (BS 95%). *C. madagascariens* (fruit beaked rounded oblong when viewed from above, with 2 small apical pits) is native to Tanzania, formerly cultivated in Mauritius, and surviving near Bhagalpur (India) on the Ganges as a relic of cultivation (http://powo.science.kew.org/-taxon/urn:lsid:ipni.org:names:127719-1)] showed close proximity with *C. habessinica* (fruit not beaked, pseudoaril with four parallel-sided arms) (BS 95%). At the molecular level, there were four specific nucleotide differences at the alignment position i.e. 99 (C → Gap), 207 (Gap → G), 482 (C → Gap and 483 (T → Gap) were noted in between *C. habessinica* and *C. madagascariens*. Thus the present study herein reports the occurrence of *C. madagascariens* for the first time from Saudi Arabia.

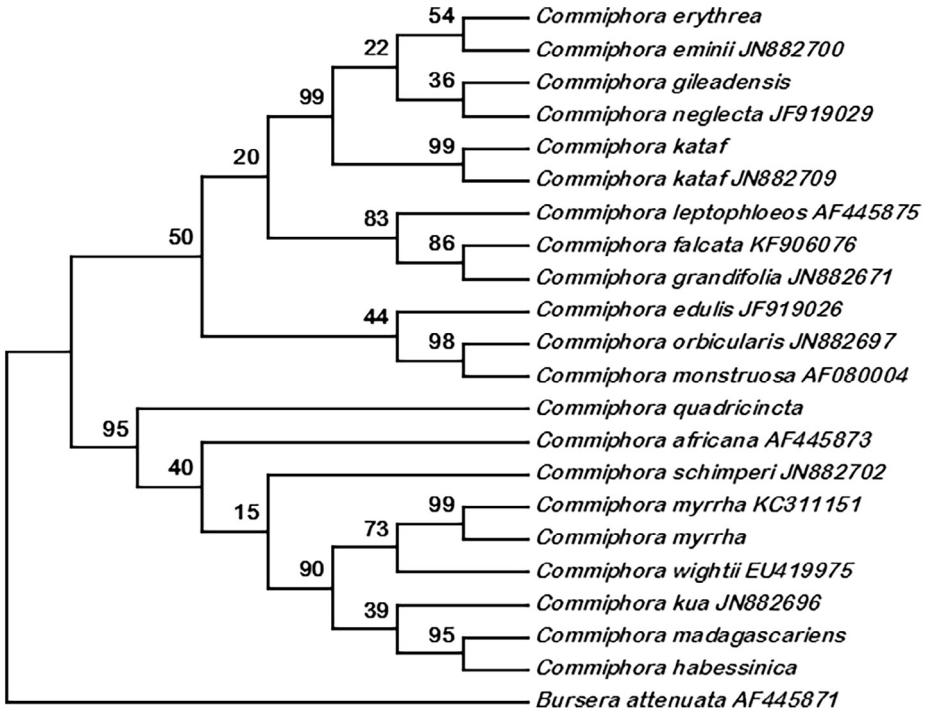

Fig. 1. The maximum parsimony tree (length- 287, consistency index- 0.637, retention index- 0.741, composite index- 0.540) recovered from the molecular phylogenetic analysis of ITS sequence of nuclear ribosomal DNA of 21 species of *Commiphora;* the number on the node indicated bootstrap support in 1000 bootstrap replicates.

3.4. Species-specific molecular signature

The taxonomic identification of plants based on morphological characteristics depends on sufficient experience and expertise in plant taxonomy, and it is well known that the morphological characteristics can easily be varied by the geographical environment (Marcon et al., 2005; Rai et al., 2012); however, the DNA sequences is hardly influenced by environment, and remain same even in the developmental stages (Liu et al., 2011); and therefore, the DNA based species-specific molecular genotyping (DNA barcoding) is an effective tools to classical morphological methods (Hebert et al., 2003) which is being used for the species identification successfully across all the groups of live forms. Further, the tools and techniques of DNA barcoding has now been proven useful in assessment of biodiversity, forensics and in conservation genetics (Ali et al., 2014). The ITS sequences of nrDNA generated in the present study have been submitted to GenBank (Table 1), which may be used as species-specific molecular signature of *Commiphora* species of Saudi Arabia.

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References

Abdul-Ghani, A.S., Amin, R., 1997. Effect of aqueous extract of *Commiphora opobalsamum* on blood pressure and heart rate in rats. J. Ethnopharmacol. 57, 219–222.

Al-Howiriny, T.A., Al-Sohaibani, M.O., Al-Said, M.S., Al-Yahya, M.A., El-tahir, K.H., Rafatullah, S., 2004. Hepatoprotective properties of *Commiphora opobalsamum* (Balessan), a traditional medicinal plant of Saudi Arabia. Drugs exptl. Clin. Res. 5&6, 213–220.

Al-Howiriny, T., Al-Sohaibani, M., Al-Said, M., Al-Yahya, M., El-Tahir, K., Rafatullah, S., 2005. Effect of *Commiphora opobalsamum* (L.) Engl. (Balessan) on experimental gastric ulcers and secretion in rats. J. Ethnopharmacol. 98, 287–294.

Ali, M.A., Van, D.L., Kim, S.K., 2010. Molecular systematic study of *Cardamine glechomifolia* Levl. (Brassicaceae) using internal transcribed spacer sequence of nuclear ribosomal DNA (ITS) and chloroplast *trn*L and *trn*L-F sequences. *Saudi Journal of Biological.* Science 17 (4), 275–290.

Ali, M.A., Gábor, G., Norbert, H., Balázs, K., Al-Hemaid, F.M.A., Pandey, A.K., Lee, J., 2014. The changing epitome of species identification- DNA barcoding. Saudi J. Biol. Sci. 21 (3), 204–231.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Byng, J.W., 2014. The Flowering Plants Handbook: A practical guide to families and genera of the world. Plant Gateway Ltd., Hertford, UK.

Chaudhary, S.A., 2001. Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and Water, Kingdom of Saudi Arabia.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., Leon, C., 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One 5, (1) e8613.

Eck, R.V., Dayhoff, M.O., 1966. Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Silver Springs, Maryland.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783–791.

Gillett, J.B., 1991. Burseraceae. In: Polhill, R.M. (Ed.), Flora of Tropical East Africa. A. A. Balkema, Rotterdam, pp. 1–95.

Hanuša, L., Ezankab, T., Dembitskya, V.M., Moussaieffa, A., 2005. Myrrh – *Commiphora* Chemistry. Biomed. Papers 149, 3–28.

Hartwell, J.L., 1982. Plants Used Against Cancer, A Survey vol. II, 89–93.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. Biol. Sci. 270, 313–321.

Iluz, D., Hoffman1, M., Gilboa-Garber1, N., Amar, Zohar, 2010. Medicinal properties of *Commiphora gileadensis*. African J. Pharm. Pharmacol. 4 (8), 516–520.

Liu, C., Liang, D., Gao, T., Pang, X., Song, J., Yao, H., Han, J., Liu, Z., Guan, X., Jiang, K., Li, H., Chen, S., 2011. PTIGS-IdIt, a system for species identification by DNA sequences of the *psb*A-*trn*H intergenic spacer region. BMC Bioinformatics 12, S4.

Marcon, A.B., Barros, I.C., Guerra, M., 2005. Variation in chromosome numbers, CMA bands and 45S rDNA sites in species of *Selaginella* (Pteridophyta). Ann. Bot. 95, 271–276.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Poczai, P., Hyvönen, J., 2010. Nuclear ribosomal spacer regions in plant phylogenetics: problems and prospects. Mol. Biol. Rep. 37 (4), 1897–1912.

Rai, P.S., Bellampalli, R., Dobriyal, R.M., Agarwal, A., Satyamoorthy, K., Narayana, D. A., 2012. DNA barcoding of authentic and substitute samples of herb of the family Asparagaceae and Asclepiadaceae based on the ITS2 region. J. Ayurveda Integr. Med. 3, 136–140.

Shen, T., Wan, Wen-Zhu, Wang, Xiao-Ning, Sun, Ling-Mei, Yuan, Hui-Qing, Wang, Xiao-Ling, Ji, Mei, Lou, Hong-Xiang, 2008. Sesquiterpenoids from the Resinous

Exudates of *Commiphora opobalsamum* (Burseraceae). Helvetica Chimica Acta 91, 881–887.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28 (10), 2731–2739.

Tejero, M.R.G., Casares-Porcel, M., Sanchez-Rojas, C.P., Ramiro-Gutíerrez, J.M., Molero-Mesa, J., Pieroni, A., Giusti, M.E., Censorii, E., de Pasquale, C., Della, A., Paraskeva-Hadijchambi, D., Hadjichambis, A., Houmani, Z., El-Demerdash, M., El-Zayat, M., Hmamouchi, M., ElJohrig, S., 2008. Medicinal plants in the Mediterranean area: Synthesis of the results of the project Rubia. J. Ethnopharmacol. 116, 341–357.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Vollesen, K., 1986. Commiphora, some thoughts on the classification of an ‘‘impossible” genus. In: Hedberg, I. (Ed.), Research on the Ethiopian Flora: Proceedings of the Wrst Ethiopian Flora Symposium held in Uppsala May 22–26, 1984. Almqvist & Wikdell International, Uppsala, pp. 204–212.

Weeks, A., Simpson, B.B., 2007. Molecular phylogenetic analysis of *Commiphora* (Burseraceae) yields insight on the evolution and historical biogeography of an ‘‘impossible” genus. Mol. Phylogen. Evol. 42, 62–79.

Wild, H., 1959. A revised classification of the genus *Commiphora* Jacq. Bol. Soc. Broteriana 33, 67–95.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P., Chen, S., 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One 5, (10) e13102.