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 distributed in Saudi Arabia. The molecular phylogenetic analysis of ITS sequences of nrDNA of Commiphora species distributed in Saudi Arabia reveals the occurrence of C. madagascariensis 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. katuf, 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
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) 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 (Thompson et al., 1997). The ITS sequences of nrDNA generated in the present study herein reports the occurrence of Commiphora 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. gowlelo, 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. gowlelo (JN882674), 96% identity of sequence of Commiphora with C. gowlelo (JN882674), and 96% identity of sequence of C. quadricincta with C. habessinica (JN882673) and 96% identity with C. kua (JN882696).

### 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, pseudorial 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

The ITS sequences of nrDNA generated from GenBank, and used in the molecular phylogenetic analysis of Commiphora 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:isbn:ipni.org:names:127719-1) showed close proximity with C. habessinica (fruit not beaked, pseudorial 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.

Table 1

| S. No. | Taxon                          | Locality        | Voucher                  | GenBank Accession No. |
|-------|-------------------------------|-----------------|--------------------------|-----------------------|
| 1     | Commiphora erythrea (Ehrenb.) Engl. | Abha, Saudi Arabia | Al-Farhan and Masin 1755 (KSUH) | MHS2401               |
| 2     | Commiphora gileadensis (L.) C.Chr. [Syn + Commiphora opobalsamum (L.) Engl.] | Farasan, Saudi Arabia | R.A. Baseni 3159 (KSUH) | MHS2402               |
| 3     | Commiphora habessinica (O.Berg) Engl. | Jabal Shada, Saudi Arabia | A.R. Al-Zaedi 21191 (KSUH) | MHS2403               |
| 4     | Commiphora kataf (Forsk.). Engl. | Jabal Shada, Saudi Arabia | A.R. Al-Zaedi 21211 (KSUH) | MHS2404               |
| 5     | Commiphora madagascariens Jacq. ([synonym Commiphora roxburghii Alston]. [Illegitimate], http://www.theplantlist.org/tpl1.1/record/kew-2733654) | Jabal Fayfa, Saudi Arabia | M.H. Hassan 4992 (KSUH) | MHS2405               |
| 6     | Commiphora myrrha (Nees) Engl. | Abha, Saudi Arabia | Farasan and Masin 1786 (KSUH) | MHS2406               |
| 7     | Commiphora quadricincta Schweinf. | Jabal, Saudi Arabia | Al-Farhan and Masin 1786 (KSUH) | MHS2407               |

Table 2

| 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 (Forsk.). Engl. | JN882709             |
| 7     | Commiphora kua (R.Br. ex Royle) Vollesen | JN882696         |
| 8     | Commiphora leptophloeoas (Matr.) J.B.Gillet | AF445875         |
| 9     | Commiphora monstruosa (H.Perrier) Capuron | AF080004         |
| 10    | Commiphora myrrha (Nees) Engl.   | KC311151             |
| 11    | Commiphora neglecta Verd.       | JF919029             |
| 12    | Commiphora orbiculare (Engl. | JN882697             |
| 13    | Commiphora schimperi (O.Bergman) Engl. | JN882702        |
| 14    | Commiphora wightii (Arn.) Bhandari | EU419975             |
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 are 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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