Development of genetic markers for *Ochradenus arabicus* (Resedaceae), an endemic medicinal plant of Saudi Arabia

S. Khan, F. Al-Qurainy, M. Nadeem and M. Tarroum

Department of Botany and Microbiology, College of Science,
King Saud University, Riyadh, Saudi Arabia

Corresponding author: S. Khan
E-mail: salimkhan17@yahoo.co.in

Genet. Mol. Res. 11 (2): 1300-1308 (2012)
Received January 11, 2012
Accepted March 27, 2012
Published May 14, 2012
DOI <http://dx.doi.org/10.4238/2012.May.14.4>

ABSTRACT. Some species of the genus *Ochradenus* are difficult to identify based on morphological markers. Similar limitations are found for biochemical markers. We developed genetic markers based on DNA sequences for *Ochradenus arabicus*, which is an endemic plant to Saudi Arabia, locally utilized as a medicinal shrub. The internal transcribed spacer sequence of nuclear ribosomal DNA and chloroplast (*rpoB* and *rpoCI*) markers were more informative than other chloroplast DNA markers. Based on these markers, we were able to discriminate this species from another species of the same genus (*O. baccatus*) that is widely distributed in Saudi Arabia, despite a high degree of morphological similarity. These genetic markers facilitate its identification, even when acquired in a dried state from local markets.

Key words: ITS; *Ochradenus arabicus*; *rbcL*; DNA barcode

INTRODUCTION

Ochradenus arabicus is a medicinal plant (family: Resedaceae) and found mostly in desert regions. This species has been reported from Saudi Arabia, Oman, United Arab Emirates and Yemen, where it grows in limestone rocky ground and sandy arid places from 0 to 2000 m above sea level (Miller, 1984). Another species of *Ochradenus*, *O. baccatus*, is distributed in Ethiopia, Egypt, Libya, Middle East to South Iran and Pakistan, Somaliland, and Socotra. It is a shrub with a frequently struggling habit. It has numerous flavonoids in its aerial parts, such as quercetin 3-O-beta-glucosyl (1→2)-alpha-rhamnoside-7-O-alpha-rhamnoside and quercetin 3-O-p-coumaryl (1→6)-beta-glucosyl (1→6)-beta-glucoside-7-O-alpha rhamnoside (Shabana et al., 1990; Barakat et al., 1991). It bears deciduous leaves (morphological adaptations for xeric conditions) and polygamous flowers. Simplification has been seen in the floral structure of this genus as reduction in size or loss of petals, dioecy of flower to polygamous and hermaphroditism to dioecy.

O. arabicus is an endemic plant of Saudi Arabia and found in patches in a few places. As it is an endemic species, DNA-based markers are necessary for its accurate identification and further use for research purposes either in genetic engineering or in the pharmaceutical industry. The traditional identification of *Ochradenus* species is based on morphological features such as position of sepals, petals, stamens, and perigynous and hypogynous flowers (Müller Argoviensis, 1857, 1868), and even until now, these morphological keys are still the main basis of its taxonomy. However, the identification of *Ochradenus* is sometimes confusing, and thus, there is a need for the development of more unique and reproducible genetic markers for accurate identification and authentication. Based on morphological markers, the species of *Ochradenus* can be identified in its fresh state, but sometimes these markers overlap in the natural habitat, and also, dried materials available in the local markets are difficult to identify. Also, there is long-standing problem of mixing genuine plant samples with adulterants in herbal markets. *O. aucheri* Boiss has oblong-ovoid to ellipsoid fruit, 12-mm fruit pedicels and 17-18 stamens (Miller, 1984). In *O. baccatus*, flowers are small (2 mm), unspecialized, and borne on spicate inflorescences. Ripe fruit is baccate, reddish or white in dried state, and seeds have a papillose testa. While in *O. arabicus*, ripe fruit is papery, yellow or straw-colored, seeds have a smooth, glossy testa, and the plant has a shrubby, spinescent habit.

The morphological as well as biochemical markers used in the identification and authentication of plant species have many limitations due to their lower reproducibility. *O. arabicus* and *O. baccatus* have more similarity in morphological markers. Moreover, the identification of seeds of both species needs microscopic examination, as they are difficult to differentiate with the naked eye. Sequencing- and non-sequencing-based markers have been used in many medicinal and non-medicinal plant species for the detection of adulterants in the local herbal markets (Khan et al., 2011; Al-Qurainy et al., 2011a). Currently, internal transcribed spacer sequences of nrDNA and chloroplast DNA markers are used for the accurate identification and authentication of plant species (Chase et al., 2005; CBOL Plant Working Group, 2009; Devey et al., 2009; Fazekas et al., 2009; Chen et al., 2010; Al-Qurainy et al., 2011b). Thus, variations in the DNA sequences are very helpful in the development of unique markers, which could be used as a DNA barcode for that species. DNA barcode is a short sequence of DNA of a gene, which is unique for that species. The term DNA barcode was first coined by Hebert et al., in 2003, and has gained worldwide attention in the scientific community (Chen et

al., 2010). Chloroplast DNA sequences, which show variations, can be used for identification as well as authentication of plant species and their adulterants. The non-coding region *psbA-trnH* spacer sequence has proven to be a potential plant barcode by CBOL (www.barcoding.si.edu/plant_working_group.html) for the identification of various plant species. This chloroplast gene has been used successfully for the identification of various cultivars of date palm in Saudi Arabia (Al-Qurainy et al., 2011a).

Since *O. arabicus* is an endemic medicinal plant of Saudi Arabia and few research papers on its molecular characterization have been published, the development of genetic markers is necessary to preserve its identity for future research activities. There are many biotechnological approaches available, which can be used to enhance its medicinal quality by increasing its content of phytoconstituents (Khan et al., 2012). Therefore, in the present study, sequence-based markers were developed for *O. arabicus* and further used for discrimination from a closely related species (*O. baccatus*), widely available in the Kingdom of Saudi Arabia, which share similar morphological characteristics in the dried state.

MATERIAL AND METHODS

Plant material

O. arabicus and *O. baccatus* were collected from the natural habitat of the Central region of Saudi Arabia. The identification was performed by taxonomy based on morphology. The endemic *O. arabicus*, which was collected from wild, is given in Figure 1.

Figure 1. *Ochradenus arabicus* collected from wild.

Chemicals for DNA isolation

EDTA, CTAB (cetyltrimethylammonium bromide), NaCl, Tris-HCl, PVP (polyvinyl pyrrolidone), β -mercaptoethanol, and isopropanol were used for DNA isolation. All chemicals were purchased from Sigma (USA). The chemicals for PCR amplification were purchased from GE Healthcare (Spain).

DNA isolation and purification

Leaf material was used for the extraction of genomic DNA. The DNA was isolated and purified by the modified CTAB method (Khan et al., 2007).

Amplification of nrDNA-ITS and chloroplast DNA spacer sequences

The internal transcribed spacer (ITS) sequence of nuclear ribosomal DNA (nrDNA) and chloroplast spacer sequences were amplified in PCR using universal primers (Table 1). The universal primers were synthesized by the GeneDirex Company. The PCR bead was used for amplification. The reaction mixture consisted of 20 μ L distilled water, 2 μ L 20 pM primer and 2 μ L 30 ng genomic DNA. After mixing the above mixture, the tubes were centrifuged for 5 s to remove air bubbles. The reaction was set up in triplicate for reproducibility of the results. The amplification was performed in a Techne thermal cycler (UK), and reactions were set up according to the program in Table 1. The amplified products were electrophoresed on a 1.2% agarose gel and size was checked with molecular weight markers.

Table 1. Universal primer sequences and PCR conditions for amplification.

Gene	Primer	Primer sequences (5'-3')	Reaction conditions
<i>nrITS</i>	Forward	GTCCACTGAACCTTATCAATTAG	94°C for 5 min
	Reverse	TCCTCCGCTTATTGATATGC	94°C for 1 min, 49°C for 1 min, 40 cycles, 72°C for 5 min
<i>rbcL</i>	Forward	ATGTCACCACAAACAGAGACTAAAGC	94°C for 1 min
	Reverse	CTTCTGCTACAAATAAGAATCGATCTC	94°C for 45 s, 51°C for 45 s, 30 cycles, 72°C for 5 min
<i>rpoC1</i>	Forward	GTGGATACACTTCTTGATAATGG	94°C for 1 min
	Reverse	CCATAAGCATATCTTGAGTTGG	94°C for 30 s, 53°C for 40 s, 40 cycles, 72°C for 5 min
<i>rpoB</i>	Forward	ATGCAACGTCAAGCAGTTCC	94°C for 1 min
	Reverse	GATCCCAGCATCACAATTCC	94°C for 30 s, 53°C for 40 s, 40 cycles, 72°C for 5 min
<i>rps16</i>	Forward	GTGGTAGAAAGCAACGTGCGACTT	94°C for 1 min
	Reverse	TGCGGATCGAACATCAATTGCAAC	94°C for 45 s, 51°C for 45 s, 30 cycles, 72°C for 5 min

Sequencing of PCR products

The amplified PCR products were sequenced using universal primers at Macrogen, South Korea. The PCR products were purified by removing unwanted small fragments and other PCR chemicals. Each sample was sequenced in the sense and antisense directions for reproducibility of the results and analyzed with the ABI Sequence Navigator software (Perkin-Elmer/Applied Biosystems). All sequences were deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/>).

RESULTS AND DISCUSSION

The authenticity of any medicinal plant is important for drug development in the pharmaceutical industry. The adulteration of such plant species in local markets may lead to a decline in the efficacy of herbal drugs. Therefore, prior to development of any herbal drugs, the authenticity of raw material is necessary to maintain the efficacy of the developed drugs. Therefore, the identification and authentication of any plant species should be from the wild to the industrial herbal markets, so that their efficacy could be maintained for better disease treatment. In the wild

conditions, plant identification is based merely on the morphological markers, which are keys until now for taxonomy (Wang and He, 2006). For herbal drug development, dried materials are needed, where morphological markers are merged with each other, which make the material more complex for identification. Therefore, biochemicals, phytochemicals and DNA-based markers can be used for accurate identification, which are reproducible in the laboratory. DNA sequence-based identification is a more accurate method and has been used in many studies (Heinrich, 2008; Li et al., 2010; Liu et al., 2011).

The sequences generated in the present study, namely *ITS*, *rbcL*, *rpoB*, and *rpoCl*, were deposited in the GenBank. The ITS sequence of *O. arabicus* (Saudi Arabian endemic plant) showed 99% similarity to that of *O. baccatus* (accession No. DQ987078) when using BLAST on the NCBI site, whereas this species showed 98% similarity to the same species, *O. arabicus* (accession No. DQ987076) (Figure 2). However, it also showed less similarity to the other species of the same genus, namely *O. socotranus* (DQ987077; 98%), *O. ochradeni* (DQ987082; 97%), and *O. aucheri* (DQ987081; 96%). Thus, this species showed more similarity to *O. baccatus* in sequence as well as in morphological markers as compared to other species.

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
DQ987078.1	Ochradenus baccatus voucher M. Thulin 4351 (UPS) internal transcribe	1134	1134	100%	0.0	99%
DQ987079.1	Ochradenus baccatus voucher M. Thulin 10630 (UPS) internal transcrib	1127	1127	100%	0.0	99%
DQ987077.1	Ochradenus socotranus voucher A.N. Gifri 9984 (UPS) internal transcrib	1122	1122	100%	0.0	98%
DQ987076.1	Ochradenus arabicus voucher A.N. Gifri 9984 (UPS) internal transcribed	1114	1114	100%	0.0	98%
DQ987082.1	Ochradenus ochradeni voucher K.H. Rechinger 51646 (M) internal trans	1061	1061	100%	0.0	97%
DQ987081.1	Ochradenus aucheri voucher K.H. Rechinger 54876 (M) internal transcri	1011	1011	97%	0.0	96%
DQ987085.1	Randonia africana voucher S.L. Jury 14595 (RNG) internal transcribed s	990	990	100%	0.0	95%
FH77065.1	Reseda tymphaea isolate R.tym internal transcribed spacer 1, partial se	987	987	100%	0.0	95%
DQ987143.1	Reseda armena voucher M. Nydegger 47327 (M) internal transcribed sp	983	983	100%	0.0	95%
DQ987093.1	Reseda lutea subsp. lutea voucher S. Martin-Bravo 15SMB04 (UPOS) in	977	977	100%	0.0	94%
DQ987095.1	Reseda lutea subsp. lutea voucher P. Montserrat s.n. (M) internal trans	976	976	100%	0.0	94%
DQ987094.1	Reseda lutea subsp. lutea voucher S. Martin-Bravo 339SMB05 (UPOS) i	976	976	100%	0.0	94%
DQ987092.1	Reseda lutea subsp. lutea voucher S. Martin-Bravo 40SMB04 (UPOS) in	976	976	100%	0.0	94%
DQ987144.1	Reseda phyteuma voucher S. Martin-Bravo 23SMB04 (UPOS) internal tr	972	972	100%	0.0	94%
DQ987145.1	Reseda phyteuma voucher S. Martin-Bravo 26SMB04 (UPOS) internal tr	972	972	100%	0.0	94%
DQ987091.1	Reseda lutea subsp. neglecta voucher F.J. Fernandez-Casas 13695 (RN	970	970	100%	0.0	94%
DQ987090.1	Reseda lutea subsp. neglecta voucher S. Martin-Bravo 9SMB04 (UPOS)	970	970	100%	0.0	94%
DQ987088.1	Reseda crystallina voucher Snogerup 16461 (LD) internal transcribed sp	970	970	100%	0.0	94%
DQ987142.1	Reseda inodora voucher Cernoch 24622 (BRNM) internal transcribed sp	965	965	99%	0.0	94%
DQ987103.1	Reseda stricta voucher S. Martin-Bravo 18SMB04 (UPOS) internal trans	965	965	100%	0.0	94%
DQ987089.1	Reseda crystallina voucher E. Dorn 35 (M) internal transcribed spacer 1	965	965	100%	0.0	94%
DQ987086.1	Reseda crystallina voucher L. Borgen 2942 (O) internal transcribed spa	965	965	100%	0.0	94%
FH77066.1	Reseda collina isolate R.col3 internal transcribed spacer 1, partial sequ	959	959	100%	0.0	94%
DQ987135.1	Reseda collina voucher S. Martin-Bravo 12SMB04 (UPOS) internal trans	959	959	100%	0.0	94%
DQ987105.1	Reseda stricta voucher E. Bayer 128 (M) internal transcribed spacer 1,	959	959	100%	0.0	94%
DQ987104.1	Reseda stricta voucher S. Martin-Bravo 38SMB04 (UPOS) internal trans	959	959	100%	0.0	94%
DQ987087.1	Reseda crystallina voucher P. Sunding 1607 (O) internal transcribed sp	959	959	100%	0.0	94%
DQ987101.1	Reseda lanceolata subsp. lanceolata voucher P. Vargas 184PV04 (UPOS	957	957	100%	0.0	94%
FH77068.1	Reseda collina isolate R.col5 internal transcribed spacer 1, partial sequ	955	955	100%	0.0	94%
DQ987151.1	Reseda media voucher S. Martin-Bravo 156SMB05 (UPOS) internal trans	955	955	100%	0.0	94%
DQ987149.1	Reseda media voucher P. Jimenez-Mejias 106PJM05 (UPOS) internal tra	955	955	100%	0.0	94%
DQ987139.1	Reseda alopecurus voucher A. Liston s.n. 1984 (HUJ) internal transcrib	955	955	100%	0.0	94%
DQ987136.1	Reseda collina voucher S. Martin-Bravo 17SMB04 (UPOS) internal trans	955	955	100%	0.0	94%
DQ987100.1	Reseda lanceolata subsp. constricta voucher S. Martin-Bravo 29SMB04	955	955	100%	0.0	94%
DQ987099.1	Reseda lanceolata subsp. constricta voucher S. Martin-Bravo 19SMB04	955	955	100%	0.0	94%

Figure 2. Screenshot showing the ITS sequence similarity of *Ochradenus arabicus* from other species in BLAST search.

All sequences had few variations in the percent of guanine plus cytosine content (%GC) and size except ITS sequences, comparing sequences of *O. arabicus* and *O. baccatus* (Table 2). The generated sequence of ITS for *O. arabicus* was compared with widely distributed *O. baccatus* and significant results were found in terms of sequence divergence (Figure 3). There were 22-bp substitutions in the ITS sequence of nrDNA, which could be used as a potential marker for identification of these species. All nucleotide substitutions at site (i.e., 133, 210, 212, 239, 251, 301, 304, 307, 311, 340, 395, 439, 523, 542, 561, 564, 571, 589, 598,

604, 622, and 629) were single base substitutions. We could not find any deletion in the sequences of the ITS region compared in the two species. However, if single base pair substitution is present in the compared sequences, then it would be also a good marker for that species for identification. Therefore, nrDNA-ITS loci are reproducible for DNA barcoding, among other loci. Similarly, the ITS2 region can be used as a potential DNA barcode for identification of medicinal plants and their closely related species, which share similarity in morphological markers (Chen et al., 2010; Yao et al., 2010). The secondary structure of the ITS2 region provides useful information for plant species identification (Yao et al., 2010). In our study, the *rbcL* gene has low sequence variation, which was observed at only two sites (Figure 4). One site has a base insertion at fifth base, and the second site has a base deletion at base 624, when both species sequences were compared. Although, the *rbcL* loci has been used as a potential DNA barcode for plant identification (Chase, 2005; Newmaster et al., 2008), this gene did not reveal sufficient variations to discriminate *O. arabicus* and *O. baccatus*.

Table 2. Sequence characteristics of *Ochradenus* species for the present study.

Species	Gene	Accession No.	Total length	%GC	Tm
<i>Ochradenus arabicus</i>	nrITS	JQ899053	634	59	88
<i>Ochradenus baccatus</i>	nrITS	JQ899054	634	58	87
<i>Ochradenus arabicus</i>	<i>rbcL</i>	JQ899052	632	43	81
<i>Ochradenus baccatus</i>	<i>rbcL</i>	JQ899051	631	43	81
<i>Ochradenus arabicus</i>	<i>rpoB</i>	JQ899050	526	40	80
<i>Ochradenus baccatus</i>	<i>rpoB</i>	JQ899049	526	41	80
<i>Ochradenus arabicus</i>	<i>rpoC1</i>	JQ899048	584	43	81
<i>Ochradenus baccatus</i>	<i>rpoC1</i>	JQ899047	590	42	81

%GC = percent of guanine plus cytosine content; Tm = melting temperature.

Figure 3. Sequence alignment of ITS sequences from *Ochradenus arabicus* and *O. baccatus*.

Figure 4. Sequence alignment of *rbcL* sequences from *Ochradenus arabicus* and *O. baccatus*.

The *rpoB* gene has insertions and deletions at sites 28, 29, 501, 502, 505, 513, 521, 522 and 15, 16, 31, 520, respectively (Figure 5). The number of inserted bases was more as compared to deleted bases. Our results are in line with the results of Al-Qurainy et al. (2011a), where *rpoB* showed more sequence variations in date palm cultivars, which were discriminated from each other. The *rpoC1* gene has base deletions and insertions at sites 4, 5, 10, 26, 27, 28 and 8, 13, 17, 29, 77, 78, 79, 80, 82, 83, 86, respectively (Figure 6). Similarly, this gene has been used successfully for the authentication of *Ruta graveolens* from its adulterant *Euphorbia dracunculoids* (Al-Qurainy et al., 2011b).

Figure 5. Sequence alignment of *rpoB* sequences from *Ochradenus arabicus* and *O. baccatus*.

Figure 6. Sequence alignment of *rpoC1* sequences from *Ochradenus arabicus* and *O. baccatus*.

These plastid regions have performed well in DNA barcoding in terms of being amplifiable with a limited range of PCR conditions and primer sets (Liu et al., 2011). Although *rpoC1* and *rpoB* are not rapidly evolving, they show more discrimination between species in many groups of organisms, and the greatest discrimination is observed for plant identification at the species level, which represents one option for DNA barcoding (Liu et al., 2011). There was no sequence variation found in the *rps16* gene between these two species, which was not studied further. However, this gene has differentiated *Meconopsis impedita* and *M. racemosa* clearly, although they show high similarity in their morphological markers (Li and Dao, 2011).

However, the ITS sequences of nrDNA and chloroplast spacer sequences *rpoB* and *rpoC1* have large sequence variations, which could be used as a DNA barcode for this endemic species of Saudi Arabia. For confirmation of the results, combined studies of genes are necessary for identification and authentication of any plant species.

CONCLUSION

In the present study, nrDNA-ITS and chloroplast spacer sequences (*rpoB* and *rpoC1*) were found highly informative as compared to other markers. The two species were differentiated using these markers. DNA sequence-based markers are more reproducible than the other DNA-based polymorphic markers. Thus, genetic markers developed for these plant species could be used as a DNA barcode for the identification and authentication of plant samples available in the fresh as well as in the dried state.

ACKNOWLEDGMENTS

Research supported by the NSTIP Strategic Technologies Program (Project #10-BIO1289-02), Kingdom of Saudi Arabia.

REFERENCES

- Al-Qurainy F, Khan S, Al-Hemaid FM, Ali MA, et al. (2011a). Assessing molecular signature for some potential date (*Phoenix dactylifera* L.) cultivars from Saudi Arabia, based on chloroplast DNA sequences *rpoB* and *psbA-trnH*. *Int. J. Mol. Sci.* 12: 6871-6880.
- Al-Qurainy F, Khan S, Tarroum M, Al-Hemaid FM, et al. (2011b). Molecular authentication of the medicinal herb *Ruta graveolens* (Rutaceae) and an adulterant using nuclear and chloroplast DNA markers. *Genet. Mol. Res.* 10: 2806-2816.
- Barakat HH, el-Mousallamy AM, Souleman AM and Awadalla S (1991). Flavonoids of *Ochradenus baccatus*. *Phytochemistry* 30: 3777-3779.
- CBOL Plant Working Group (2009). A DNA barcode for land plants. *Proc. Natl. Acad. Sci. U. S. A.* 106: 12794-12797.
- Chase MW, Salamin N, Wilkinson M, Dunwell JM, et al. (2005). Land plants and DNA barcodes: short-term and long-term goals. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 360: 1889-1895.
- Chen S, Yao H, Han J, Liu C, et al. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One* 5: e8613.
- Devey DS, Chase MW and Clarkson JJ (2009). A stuttering start to plant DNA barcoding: microsatellites present a previously overlooked problem in non-coding plastid regions. *Taxon* 58: 7-15.
- Fazekas AJ, Kesanakurti PR, Burgess KS, Percy DM, et al. (2009). Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? *Mol. Ecol. Resour.* 9: 130-139.
- Hebert PDN, Cywinska A, Ball SL and deWaard JR (2003). Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B Biol. Sci.* 270: 313-321.
- Heinrich M (2008). Ethnopharmacy and natural product research - multidisciplinary opportunities for research in the metabolomic age. *Phytochem. Lett.* 1: 1-5.
- Khan S, Qureshi MI, Kamaluddin, Alam T, et al. (2007). Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. *Afr. J. Biotech.* 6: 175-178.
- Khan S, Mirza KJ and Abidin MZ (2011). DNA fingerprinting for the authentication of *Ruta graveolens*. *Afr. J. Biotech.* 10: 8709-8715.
- Khan S, Al-Qurainy F and Nadeem M (2012). Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia. *Saudi J. Biol. Sci.* 19: 1-11.
- Li R and Dao Z (2011). Molecular authentication of the traditional Tibetan medicinal plant, *Meconopsis impedita*. *Afr. J. Biotech.* 10: 7493-7496.
- Li Y, Ruan J, Chen S, Song J, et al. (2010). Authentication of *Taxillus chinensis* using DNA barcoding technique. *J. Med. Plants Res.* 4: 2706-2709.
- Liu C, Gu Z, Yang W, Yang L, et al. (2011). Advances of biological taxonomy and species identification in medicinal plant species by DNA barcodes. *J. Am. Sci.* 7: 147-151.
- Miller AG (1984). A revision of *Ochradenus*. *Notes R. Bot. Gard. Edinb.* 41: 491-594.
- Müller Argoviensis J (1857). Monographie de la famille des Résédacées. Zürcher and Furrer, Zurich.
- Müller Argoviensis J (1868). Resedaceae. In: *Prodromus Systematis Naturalis Regni Vegetabilis* (de Candolle AP, ed.). Vol. 16. Victor Masson, 548-589.
- Newmaster SG, Fazekas AJ and Steeves RAD (2008). Testing candidate plant barcode regions in the Myristicaceae. *Mol. Ecol. Resour.* 8: 480-490.
- Shabana MM, Mirhom YW, Genenah AA, Aboutabl EA, et al. (1990). Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. *Arch. Exp. Veterinarmed.* 44: 389-394.
- Wang L and He Z (2006). *Chin. Wild Plant Resour.* 25: 1-4.
- Yao H, Song J, Liu C, Luo K, et al. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS One* 5: e13102.