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Original Research Article

DNA Fingerprinting of *Albizia chevalieri* (Harms) Mimosoideae Using Ribulose-1, 5-Biphosphate Carboxylase (Rbcl) Gene Sequence

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ABSTRACT

Albizia chevalieri (Harms) Mimosoideae is a tree or shrub of 5-12 m tall that is use in treatment of diabetes mellitus, cancer and has hypoglycaemic effect. Medicinal plant needs to be differentiated in case of those that are substituted or adulterated with other species or varieties that are morphologically or phytochemically indistinguishable. In this study, ribulose-1, 5-biphosphate carboxylase (rbcL) gene sequence profile of *A. chevalieri* were determined. Our study showed that this specie is phylogenetically related with 98-99% sequence similarities hit with multiple plant species (*Albizia amara, Albizia bracteata, Albizia lucidior* etc) group on the basis of rbcL gene sequences. The studied specimen and related taxa inferred from partial rbcL gene sequence demonstrated a distinct lineage; therefore, could distinguish the specie as *A. chevalieri*. It also showed closest similarity that demonstrated the highest level of sequence similarity with *A. amara*. Therefore, the determined rbcL gene sequence profile from *A. chevalieri* will improve the identification process with morphology based taxonomic methods.

Keywords: *Albizia chevalieri*, Mimosoideae, rbcL, Phylogenetic, Identification.

Introduction

Albizza chevalieri (harms) subfamily Mimosoideae is a tree or shrub of 5-12 m tall and distributed in the dry savannah from Senegal, Niger and Nigeria. The plant is known in Hausa as *Katsari* and Zarma as *Nkolo*. *A. chevalieri* leaf is used in Borno-North eastern Nigeria as purgative, dysentery, diarrhoea, taenicide and also remedy for coughs. A decoction of leaves is used in Northern Nigeria as remedy for dysentery.[1] The leaf extract of *A. chevalieri* is used either as cold-water decoction or dried, ground and sieved leaf mixed with pap, for the management of diabetes mellitus by traditional medical practitioners in some parts of Niger Republic and Sokoto, Nigeria.[2] The bark is use as vermifuge, purgative, for cough and tanning of hides.[3] There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna state and also reported to have anti-oxidant activity, a significant hypoglycemic effect.[4,5]

DNA (Deoxyribonucleic acid) fingerprints are a bar-code like patterns generated by amplification of chromosomal DNA of an individual which can distinguish the uniqueness of one individual from another.[6] DNA fingerprinting is based on the identity of an organism at molecular level i.e., genetic characteristics. It is primarily used in botanicals for protection of biodiversity, identifying markers for traits, identification of gene diversity and variation etc.[7] They are also used in molecular biology and biotechnology experiments where they are used to identify a particular sequence of DNA. As the DNA sequences are very highly specific, they can be identified with the help of the known molecular markers, which can find out a particular sequence of DNA from a group of unknown.[8] It mimics the basic process used to copy DNA in a cell during chromosomal replication. Normally the length of the "sequence read" can vary from about 50 to more than 1,000 bases. Plastid rbcL (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) is the most commonly sequenced gene for identification and phylogenetic studies of plants,[9] in which the success rate of PCR and sequencing is higher compared with the other selected plant characterization genes such as matK (maturase K).[10,11] The DNA fingerprinting is so specific technique and widely used for authentication of plant species of medicinal importance.[12] This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable.[13] DNA based tools for authentication of medicinal plants is an evolving new pharmacognostic measure aimed at quality control and quality assurance in medicinal plant research as well as in clinical usage.[14] Early description about morphological and geographical distribution of this plant is difficult to be differentiated from similar species in the same genera/family on the basis of morphological characters. In this study, we attempted to characterize this regionally plant species by rbcL gene sequence.

Materials and Methods

Plant Material

In this study, Leaf specimen was collected from Kufena village, Zaria Local Government Area of Kaduna state, Nigeria. It was identified and authenticated at Herbarium unit, Department of Biological Sciences of Ahmadu Bello University Zaria and given voucher number (900247). The leaf samples were individually placed in plastic pouches and transported to the laboratory where the specimen was stored at -80^{o}C until processed for DNA extraction.

DNA Extraction of A. chevalieri

DNA extraction was carried out using DNeasy plant mini kit (Qiagen) and an automated DNA extraction instrument (QIAcube, Qiagen) were used for DNA isolation. The protocol was according to manufacturer manual. [15] Fresh leaf specimens of 200 mg were crushed using sterile

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mortar and pestle and transferred into well labelled microcentrifuge tubes. 400 µL of Buffer AP1 and 4 µL of RNase making a stock solution (100 mg/mL) were added. The mixture was incubated for 10 min at 65°C and mixed 2–3 times during incubation by inverting tube. 130 µL of buffer AP2 was added to the lysate, mixed, and incubated for 5 min on ice. The lysate was centrifuged for 5 min at full speed and the supernatant was transferred to QIAshredder spin column sitting in a 2 mL collection tube and centrifuged for 2 min at maximum speed. The flow-through was transferred into labelled microcentrifuge tubes. 1.5 volumes of buffer AP3/E was added to the cleared lysate and mixed by pipetting. 650 µL of the mixture from above was pipetted to DNeasy mini spin column sitting in a 2 mL collection tube and centrifuged for 1 min at 6000 x g. The flow-through was then discarded and the spin columns placed back into the collection tubes. This step was repeated for the remaining solution. The DNeasy column were placed in new 2 mL collection tubes and 500 µL of buffer AW was added to the DNeasy column and centrifuged for 1 min at 6000 x g. The flow-through was discarded and 500 µL of buffer AW was added to the DNeasy column and centrifuged for 2 min at maximum speed to dry the membrane. The DNeasy column was transferred to 1.5 mL microcentrifuge tube and 200 µL of preheated (65°C) buffer AE was directly added onto the DNeasy membrane. It was Incubated for 5 min at room temperature and then centrifuged for 1 min at 6000 x g to elute DNA. DNA quality and concentration were checked by running 2 µL of genomic DNA on NanoDrop®2000 spectrophotometer (Thermo Scientific, Wilmington, USA).[16] Absorbance of the solution with the sample was read. The concentration of DNA in the sample was calculated using the given formula:

Concentration of dsDNA = A260 x 50µg x dilution factor

Purity of the DNA = A260 : A280 ratio = A260 / A280

Isolated plant genomic DNA was preserved at -80^{o}C until use.

Polymerase chain reaction (PCR)
A set of primer, rbcLaF (5′ATGTCACCACAAACAGAGACTA3′;) and rbcLaR (5′ GAAACGGTCTCTCCAACGCAT3′;),[17-18] was used in this study for the amplification of rbcL gene of the chloroplast. A total volume of 25 µl of PCR reaction mixture containing 100 ng of genomic DNA prepared as follows: 12.5 µL of 10× of FideliTaq PCR Master Mix (USB Corporation, Cleveland, OH), 1 µL of 50 mM of $MgCl_2$ (Magnesium Chloride), 2 µL of 2.5 mM dNTPs (Deoxynucleoside triphosphate), 0.1 µL Taq polymerase, 1 µL DMSO (Dimethyl sulfoxide), 1 µL each of forward and reverse primer and 11.3 µL of H_2O. Touch-down PCR was used for amplification as follows: initial denaturation step of 5 mins at 94°C, followed by 9 cycles each consisting of a denaturation step of 20 sec at 94°C, annealing step of 30 sec at 65˚c, and an extension step of 72°C for 45 sec, this is followed by another 30 cycles each consisting of a denaturation step of 20 sec at 94°C, annealing step of 30 sec at 55˚C, and an extension step of 72°C for 45sec. Amplification reactions was performed in a Applied Biosystems GeneAmp® PCR System 9700.[19]

Agarose Gel Electrophoresis
Agarose gel (1%) using 1X TAE (Tris-acetate) buffer containing 0.5 µg/mL ethidium bromide was used for electrophoresis of PCR-products. Gel images were obtained using Proxima C16 Phi+ (Isogen Life Science) UV transilluminator and Opticom (version 3.2.5; OptiGo) imaging system. The amplified PCR products were determined on gel for the presence or absence of the band. The size of PCR products resulting from the primer pair were determined by using an Amersham 1kp ladder (GE Healthcare) and the TotalLab TL100 1D software (version 2008.01).[20]

Sequencing
The amplified product was first purified using manufacturer's protocol (QIAquick PCR Purification Kit).[21] Sequence was determined by adopted variant of Sanger sequence,[22] with a DNA sequencer (Applied Biosystems® 3130 xl Genetic Analyser) and a Big Dye Terminator (version 3.1) cycle sequencing kit (RR-100, Applied Biosystems), according to manufacturer's instructions. Unincorporated dye terminators were purified and precipitated using ethanol EDTA solution. The purified amplified product was diluted in water (2 µL of PCR x 6 µL H_2O) and mix with 2 µL of the Dye ready reaction termination and then run in thermal cycler. The pellets were then re-dissolved in Hi-Di^{TM} formamide buffer then run on for 6 hrs. Obtained rbcL gene sequence was submitted to DDBJ/EMBL/GenBank database and given Accession no. MH178368.

Assignment of Taxa
BLAST (Basic local alignment search tool) searches were applied to the produced sequence using the available online databases. Sequences of rbcL that matched closely with the query sequences retrieved from DDBJ/EMBL/GenBank database. The sequences were aligned using CLUSTAL X (version 1.81), Phylogenetic analyses were conducted in MEGA4, Phylogenetic trees were constructed using maximum Likelihood, and Neighbor Joining methods.[23-26]

Results and Discussion

Isolated total genomic DNA extracted from plant sample has a purity of 1.67 obtain using Nanodrop spectrophotometer wavelengths of 260 nm and 280 nm as shown in Table 1.
Figure 1 depicts the size of amplified rbcL gene of the plant sample on gel electrophoresis of forward and reverse rbcL primers with a size of 620 bp (Base pair) after Polymerase Chain reaction.
Chromatogram of the sequence amplified rbcL gene (Figure 2) showed the order of nucleotide sequence and the quality of the sequence. The length of the analysed rbcL sequences with primer is about 616 bp.
On the basis of rbcl region analysed sequence alignment (BLAST) database-search of the plant A. chevalieri with Query id (lcl|Query_108313) in order to determine the approximate identification and related taxa, search showed 98-99% sequence similarities with multiple plant species (Albizia amara, gb|JX856628.1; Albizia bracteata, gb|KR528654.1; Albizia lucidior, gb|KR528662.1. etc) and 99% sequence similarity with A. amara retrieved from the related sequences from the GenBank database and determined phylogenetic position of the species are shown in Figure 3. Here the correct identification means that the highest BLAST % identity of the query sequence was from the expected species or the species belonging to the expected genera; ambiguous identification means that the highest BLAST % identity for a query sequence was found to match several genera of the expected family; incorrect identification means that the highest BLAST % identity of the query sequence was not from the expected species/expected genera/expected family.[27] All the species that were inferred from partial rbcL gene sequence of the studied specimen and related taxa demonstrated a distinct lineage of the studied specimen.
Assignment of an unknown specimen under valid taxa primarily depends on the availability of the sequence in the database. Molecular genetic techniques for species identification based on single-gene sequence similarity or phylogenies are rapidly gaining wide use[28] e.g. in authentication of medicinal plant, in ecology research, environmental research, gastro-intestinal flora or oral cavity samples research, [29-31] except few criticisms.[32,33] One of the prime motivations for the development of genetic methods is their large-scale application to species identification. BLAST, distance and liberal tree-based methods showed equally success when all species are represented in the reference data set.[27]

Table 1: NanoDrop of isolated genomic DNA from *A. chevalieri* Leaf

Nucleic Acid (ng/µL)	A260 (Abs)	A280 (Abs)	260/280	260/230	Factor
161.1	3.222	1.931	1.67	1.92	50

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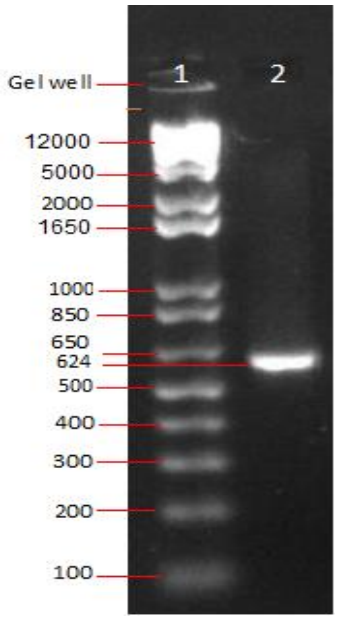

Figure 1: Electrophoresis of PCR products of amplified rbcl gene from *A. chevalieri*. Lane 1: 1kb plus DNA ladder; Lane 2: amplified product with molecular weight (624bp).

Conclusion

In this study, it was found that rbcL could be used for amplification using a set of primers reported by kress and Erickson, 2007. This plant with given accession number MH178368 could therefore, be used to distinguish between closely related genera or species, clearly delineate their medicinal use and appropriate selection of the right species for optimum and desired medicinal efficacy.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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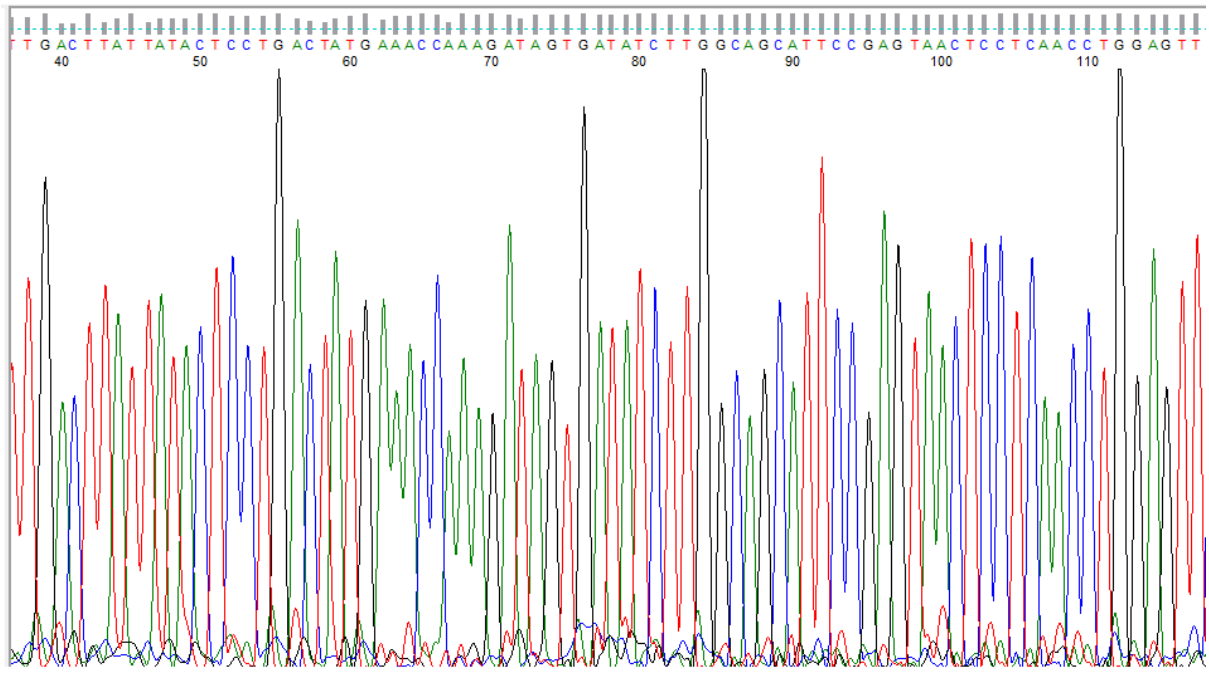

Figure 2: Portion of chromatogram of sequences DNA from *A. chevalieri*

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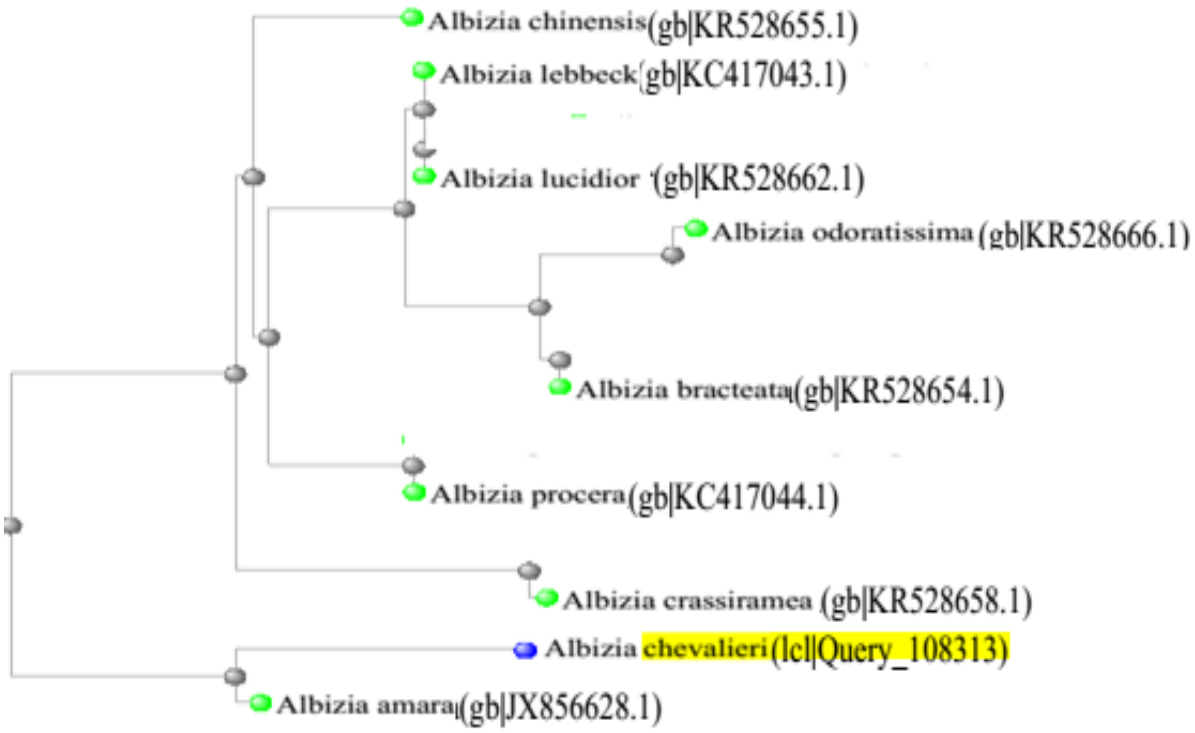

Figure 3: The Dendrogram showing the relationship of *A. chevalieri* with the related genera. Query numbers of the corresponding taxa are written in parentheses with a p-distance of 0.0007.

References

1. Le Houèrou H. *Albizia chevalieri* Harms. [Online] 2009 [Cited on 2016]. Available from http://www.fao.org/ag/AGP/AGPC/doc/GBASE/Data/pf000366.HTM.
2. Saidu Y, Lawal M, Isezuo SA, Shehu RA, Sahabi DM, Bilbis LS. Partial purification and elucidation of mechanism of hypoglycaemic agent of aqueous leaf extract of *Albizzia chevalieri* Harms (Leguminosae). J Pharm Toxi. 2007; 2(6):513-523.
3. Alhassane B. Vegetal Biodiversity: [Online] 2013 [cited 2016]. *Albizia chevalieri*. Available from: http://ecologistesud.e-monsite.com/en/blog/vegetal-biodiversity-albizia-chevalieri.html.
4. Aliyu AB, Musa AM, Ibrahim MA, Ibrahim H, Oyewale AO**.** Preliminary Phytochemical Screening and Antioxidant activity of Leave extract of *Albizia chevalieri* harms (leguminoseae-mimosoideae). Bay J of Pure and Applied Sci**.** 2009; 2(1):149-153.
5. Yusuf S, Lawal SB, Mansur L, Simeon AI, Rabiu AU. Hematotoxicity study of the leaf extract of *Albizia chevalieri* harms (Leguminosae). Biochemia Medica. 2007; 17(2):203-211.
6. DNA fingerprinting. [Online] 2005 [cited 2016]. Available from Http://www.biology-online.org/dictionary/DNA_Fingerprinting.
7. DNA fingerprinting in plants, [Online] 2006 [cited 2016]. Available from Http://biosolutions.6te.net/articles_files/dna_fin.html.
8. Kurane J, Shinde V, Harsulkar A. Application of ISSR marker in pharmacognosy: Current update, Phcog. 2009; 216-228 p.
9. Schuettpelz E, Korall P, Pryer KM. Plastid atpA data provide improved support for deep relationships among ferns. Taxon. 2015; 55:897-906.
10. CBOL Plant Working Group. A DNA barcode for land plants. Proc Natl Acad Sci. 2009; 106:12794-12797.
11. Parveen I, Singh HK, Raghuvanshi S, Pradhan UC, Babbar SB, DNA barcoding of endangered Indian *Paphiopedilum* species. Mol Ecol Resour. 2012; 12:82-90.
12. Himesh S, Priyanka N, Singhai AK. A new era of pharmacognosy –Molecular Pharmacognosy MIT, I J of Pharma Sci. 2015; 1:64–70.
13. Srivastava S. and Mishra N. Genetic Markers - A Cutting-Edge Technology in Herbal Drug Research, J Chem Pharm Res. 2009; 1(1):1-18.
14. Kumar LD, Kathirvel M, Rao GV, Nagaraju J. DNA profiling of disputed chilli samples (*Capsicus annum*) using ISSR-PCR and FISSR-PCR marker assays. For Sci Int. 2001; 116:63–68.
15. QIAGEN Genomic DNA Handbook. QIAGEN. [Online] 2001 [cited 2016]. Available from https://www.qiagen.com/resources/molecular-biology-methods/dna/
16. Lyons E. biotechnology 1 -DNA Concentration and purity. [Online] 2010 [2016]. Available from http://users.stlcc.edu/departments/fvbio/Bio219_Lab_Manual/Lab%202%20Determination%20of%20DNA%20Concentration%20and%20Purity.pdf
17. Fazekas AJ, Burgess KS, Kesanakurti PR. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS One. 2008; 3:2802.
18. Kress J, Erickson DL. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS One. 2007; 6: 1-10.

19. Korbie DJ, Mattick JS. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nat Protoc. 2008; 3:1452-1456.
20. Bafeel SO, Arif IA, Bakir MA, Al Homaidan AA, Al Farhan AH, Khan HA. DNA barcoding of arid wild plants using *rbcL* gene sequences Gen Mol Res. 2012; 11 (3):1934-1941.
21. QIAquick Spin Handbook. [Online] 2008 (2016). QIAquick PCR Purification Kit Protocol Available from http://2012.igem.org/wiki/images/a/a3/QIAquick_PCR-purification.pdf
22. Metzker ML (2005) Emerging technologies in DNA sequencing. Genome Res 15:1767-1776.
23. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc Acids R. 1997; 24:4876-4882.
24. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Bio Evol. 2007; 24:1596-1599.
25. Tamura K and Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Bio Evol. 1993; 10:512-526.
26. Saitou N and Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Bio Evol. 1987; 4:406-425.
27. Arif IA, Bakir MA, Khan HA, Al Farhan AH, Al Homaidan AA, Bahkali AH, Al Sadoon M, Shobrak MA. brief review of molecular techniques to assess plant diversity. Int J Mol Sci. 2010; 11:2079-2096.
28. Ross HA, Murugan S, Li WLS. Testing the reliability of genetic methods of species identification via simulation. Sys Biol. 2008; 57(2):216–230.
29. Li-Cheng G, Zhao M, Sun W, Teng H, Huang B, Zhao X. Differentiation of the Chinese minority medicinal plant genus *Berchemia* spp. by evaluating three candidate barcodes. Springer Plus 2016; 5:658
30. Baker SC, Steel D, Nieukirk S, Klinck H. Environmental DNA (eDNA) From the Wake of the Whales: Droplet Digital PCR for Detection and Species Identification. Front Mar Sci. 2018; 5:133.
31. Allan GJ and Max TL. Molecular Genetic Techniques and Markers for Ecological Research. Nat Edu Kno. 2010; 3(10):2.
32. Will KW and Rubinoff D. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics. 2004; 20:47-55.
33. Meyer CP and Paulay G. DNA barcoding: error rates based on comprehensive sampling. PLoS Biol. 2005; 3:e422.