International Journal of Biotech Trends and Technology
ISSN: 2249 – 0183 /doi:10.14445/22490183/IJBTT-V11I3P603

Volume 11 Issue 3, 18-22, July-Sep 2021

The Phylogenetic relationship study of Maturase K and Ribulose 1,5 bisphosphate carboxylase/oxygenase large subunit – A DNA barcoding marker region of Medicinal plant Beetroot (*Beta vulgaris*) from the region of Gujarat (INDIA)

Shubham S Bumb*[1], Dr. Sanjay Lal*[2], Dr. Sandeep Chovatiya*[3]

Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, CVM University, Anand, Gujarat, India, 388121.

Received Date: 03 June 2021
Revised Date: 11 July 2021
Accepted Date: 21 July 2021

ABSTRACT

Introduction: *Beetroot, scientifically called Beta vulgaris, is one in every of the accepted plants belonging to the Chenopodiaceae family. It is associate with an erect annual herb with stalk rootstocks. It makes a superb dietary supplement being not solely made in minerals, nutrients, and vitamins; however, it conjointly has distinctive phytoconstituents that have many medicative properties. Several elements of this plant area unit are utilized in a meditative system like an anti-oxidant, anti-depressant, anti-microbial, anti-fungal, medicine, diuretic, medicinal drug, and carminative.*

Aim of the study: *To identify the medicinal plant using plant core barcode (matK and rbcL).*

Materials and methods: *A sample of the beetroot was collected from the main market of Anand city, Gujarat (INDIA). DNA samples were extracted, and it was amplified using matK and rbcL primer. The amplified product was sequenced at Eurofins Sequencing Lab, Bangalore, India. The sequence was edited manually using Chromatogram explorer. Species identification established by NCBI BLAST and constructing a phylogenetic tree using CLC main workbench.*

Results: *PCR amplification results gave 100 % success for both the loci and thus confirmed the amplification. BLAST analysis also confirmed the similarity to genus Beta, and phylogenetic tree analysis showed a best close match to identify the plant species.*

Conclusion: *DNA Barcoding is a reliable tool for species identification. Our result shows that the matK and rbcL proved efficient in identifying the plant species.*

KEYWORDS *- DNA Barcoding, Species identification, rbcL, matK, Phylogenetic tree, BLAST, NCBI*

I. Introduction

The innovative practice of identifying biological samples using short DNA sequences from either nuclear or cell organ genomes is termed DNA Barcoding. (Techen *et al.*, 2014) Paul Hebert, who eventually discovered the technique of DNA Barcoding, found the mitochondrial gene CO1 (Cytochrome C oxidase subunit 1) as a universal barcode region for animals. (Hebert *et al.*, 2003). However, this CO1 was not appropriate as a plant barcode region due to its slow evolutionary rate and high rearrangement rate of the mitochondrial genome. For land plants, checking out a core plant barcode region has evidenced to be harder. Many recommendations were given by different researchers like ITS and trnH-psbA (Kress *et al.*, 2005), rbcL by (Chase *et al.*, 2005), matK and trnH-psbA (Newmaster *et al.*, 2008), but unfortunately, there were no universal plant barcodes to depend on.

In recent years, different solo loci and grouping of loci have been anticipated as plant DNA Barcodes. (Techen et al., 2014) In 2009, the association for the Barcode of Life Plant Working Group (CBOL) projected a mixture of matK and rbcL as a core barcode for plant identification across the land plants. (Vere *et al.*, 2015) In explicit, ITS2 was projected as a core DNA Barcode for medicinal plants. (Chen *et al.*, 2010) ITS, trnH-psbA, matK, and rbcL are the highest four barcoding regions mentioned within the kinds of literature for the authentication and identification of medicinal plant materials (Techen *et al.* 2014).

Plants are used for medicative functions not only by humans since prehistoric times but also are accustomed to treat numerous ailments by our closest relatives. (Hart,

2005) DNA- based methods are developed for the identification of medicinal plants. Nuclear and plastid DNA is amplified by the polymerase chain reaction, and also the reaction products are analyzed by gel dielectrolysis, sequencing, or coupling with species-species probes. Though sequences from single plastid or nuclear genes have been helpful for the differentiation of species, phyletic studies usually need to be thought of as DNA sequence knowledge from quite one sequence or genomic region. (Sucher and Carles, 2008)

India is well-known for its richness and variety of plant species and contains long-established data and understanding of medicinal plants for treating varied human ailments, although medicinal plants play associate in nursing awfully very important role in the trendy economy. The Asian nation has the oldest, richest, and most numerous cultural traditions at intervals the utilization of medicinal plants. The correct identification of medicinal plants is also a demand for its safe application. (IEA, 2011)

Beetroot (*Beta vulgaris L.*) is a crop belonging to the Chenopodiaceae family having, bright crimson color. It is renowned for its juice worth and healthful properties; and well- known by many common names like beet, chard, spinach beet, sea beet, garden beet, white beet, and Chakundar (in Hindi). (Kumar, 2015) Although it is obtainable in many varieties from color yellow to red, the most cultivated and widely used is that the deep red-colored beets. It is familiar for its richness of antioxidants within the sort of betalains and different phytochemicals having anti-cancer and therapeutic properties. It contains bio-active constituents, antioxidants as well as betalains, carotenoids, phenolic resin compounds, and the goodness of different nutrients. (Jasmitha, Shenoy and Hegde, 2018)

This study describes a protocol for using the *matK* and *rbcl* barcode to identify medicinal plants. The protocol provides an example of the use of DNA barcoding.

II. MATERIALS AND METHODS

A. Sample Collection and DNA Isolation:

The plant material was collected from the main market of Anand City, Gujarat (INDIA). The sample was washed with D/W for about 1 min, and the total genomic DNA was isolated using the CTAB method with certain modifications in it. The quality of the DNA was estimated by checking the absorbance at the ratio of 260 nm⁄280 nm.

B. PCR Amplification and Sequencing:

The isolated genomic DNA was amplified using universal primers for *rbcL*, *matK*. The primer sequence of matK region, i.e., Forward primer 5'GATCTATTCATTCAATATTTC3' and Reverse primer 5'TCTAGCACACGAAAGTCGAAGT3', and for rbcL region, Forward primer 5' TCTGTTACTAACATGTTTACTTC3' and Reverse primer 5'TCCCTCATTACGAGCTTGTACACA 3'. The reaction conditions of PCR amplification are as follows: 950 C for 5 min, 940C for 1 min, 550 C for 45 sec, 720 C for 1:30 sec of 40 cycles, and 720 C for 7 min. PCR amplification was carried out on 25µl reaction mixtures containing about 100-200ng of isolated genomic DNA template, 10x buffer with MgCl2, 2.5 mm each of dNTPs, 1x Taq polymerase, 40nm primer each in a Corbett Research PCR Thermal Cycler. After amplification, the amplified product was resolved in 1.5% agarose gel, and the desired band was checking under a UV transilluminator, and the gel documentation was done. The amplified PCR product was sent for purification, followed by sequencing to Eurofins Sequencing Lab, Bangalore (INDIA).

III. RESULTS

A. Genomic DNA isolation and PCR Amplification:

High-quality genomic DNA was isolated from *B. Vulgaris* plant species. The absorbance values at 260/280nm gave a ratio of 1.8, indicating a good quality isolated DNA showing concentration ranging from 50ng to 360ng as shown in the figure (Fig1A). The PCR success rate was 100 % for all the loci. Amplification of matK and rbcL generated 928bp and 934 bp sized fragments, as shown in the figure, respectively (Fig1B).

B. Sequence Analysis:

After sequencing sequence analysis was carried out, sequencing was carried from both forward and reverse directions, and BLAST analysis confirmed the sequences showed similarity to the respective loci of genus beetroot, which also indicate the sequence novelty. The generated sequences were deposited in GenBank MW386984 for matK. The contigs were assembled from the forward and reverse sequence reads using DNA baser software and edited manually by the Chromatogram Explorer tool. The generated sequences were aligned using the CLC Main Workbench version 6.7.2. Global Multiple sequence alignment (figure not shown) and phylogenetic analysis for *rbcL* and *matK* markers were carried out for the best fifty selected closely related species obtained after BLAST (NCBI) using NJ Method.

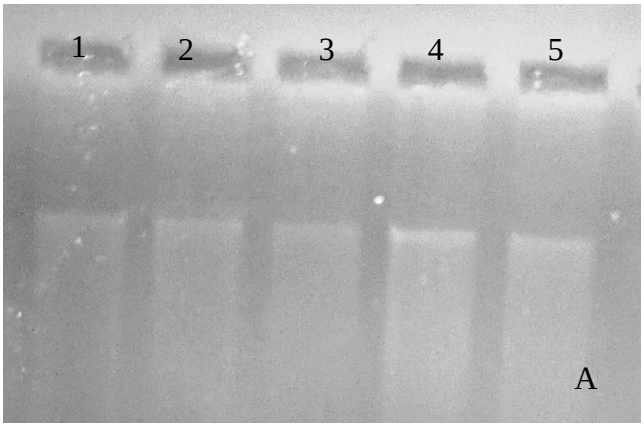
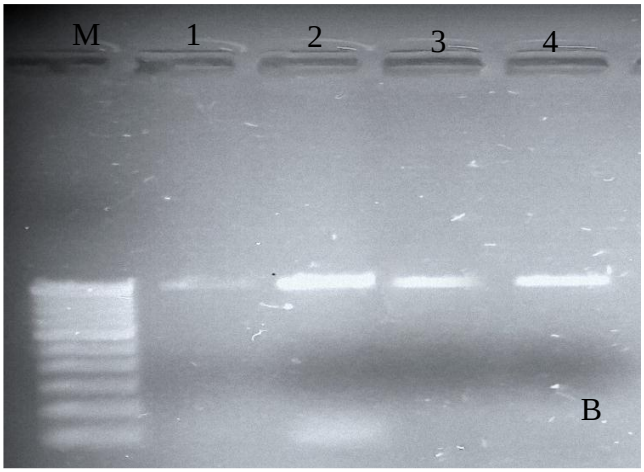


Figure 1: (A) 1% Agarose gel electrophoresis of Isolated Genomic DNA from Beta vulgaris plant, Lane 1 to 5



(B) 1.5 % Agarose gel electrophoresis amplified PCR product where Lane M indicate 100 by DNA ladder and Lane 1 and 2 show amplified rbcl region (934 bp) and Lane 3 and 4 show amplified matK region (928 bp).

C. BLAST and Phylogenetic Analysis:

The sequences obtained from the result of BLAST for *matK (*maturase k*)* marker region confirm the common ancestor and origin of the species, and it indicates a maximum of 97% sequence coverage with 100% identity with *Beta vulgaris subsp. Maritima* (table 1). Along with this, it also indicates matches with *Patellifolia procumbens subsp. procumbens* (97%, 96.22%), *Aphanisma blitoides* (97%, 95.32%), *Oreobliton the iodides subsp. the iodides* (97%, 95.07%), *Hablitzia tamnoides* (97%, 94. 96%) and other species. Whereas the BLAST results for *rbcl (*ribulose-1,5-bisphosphate carboxylase/large oxygenase subunit*)* marker region shows a maximum of 99% sequence coverage with 95.69% identity with *Beta vulgaris.* It also shows matches with other species such as *Blitum bonus-Henricus*, *Aphanisma blitoides* with a 99% sequence coverage and identity of 94.18%, *Suaeda linifolia* (99%, 93.95%), *Blitum californicum* (99%, 93.83%), *Patellifolia patellaris* (99%, 93.83%) and others species.

The similarity-based phylogenetic relationship between the fifty selected sequences (figure 2) for *matK (*maturase k*)* marker region indicates the closest relation with *Beta vulgaris subsp. maritima*. With accession numbers JN895142, JN894325, JN895701. It also shows sister relationship with *Bosea Cypria* (Acc. No. AY042559), *Habiltzia tamnoides* (Acc. No. AY042598), *Beta trigyna* (Acc. No. AY042555), *Chenopodium species* (Acc. No. HQ593233, KX299000, AF204864), *Dysphania ambrosioides* (Acc. No. MF159466, MF159465, MF159497). Also, the phylogenetic relationship between the fifty selected sequences for *rbcl (*ribulose-1,5-bisphosphate carboxylase/large oxygenase subunit*)* marker region (figure 3) shows maximum relationship with *Beta vulgaris* with the Accession number of LT576798, KM360669, and AY270065, and also with sister relationship with *Suaeda linifolia* HM630106, *Dysphania Ambrosides* (Acc. No. MF135402, MF135403, MF135359, MF135358), *Holbergia tweedii* (Acc. No. AY270100), *Atriplex hortensis* (Acc. No. FR775290), *Axyris amaranthoides* (Acc. No. JX84845250), *Blitum nuttallianum* (Acc. No. JX848452), *Agriophyllum squarrosum* (Acc. No. LT576791), *Halimione portulacoides* (Acc. No. KM360659), *Psilotrichum gnaphalobryum* (Acc. No. JQ933458) and *Achyranthes aspera* (Acc. No. MH287277).

TOP HIT plant from GenBank (Accession no)	Query **Coverage %**	**% Identity**	**Alignment Length (bp)**	**Mismatch**	**Gap**	**E_Value**
Beta vulgaris (Maturase K) matK marker region						
JN895142	87	100.00	804	1	0	0.0
JN894325	88	100.00	812	1	0	0.0
JN895701	87	100.00	1892	1	0	0.0
***Beta vulgaris (*ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit*) rbcl* marker region**						
LT576798	99	96	1242	1	9	0.0
KM360669	99	96	1408	1	9	0.0
AY270065	99	96	1343	1	9	0.0

Table 1 : BLAST analysis of *matK* and *rbcL* of *B. Vulgaris*

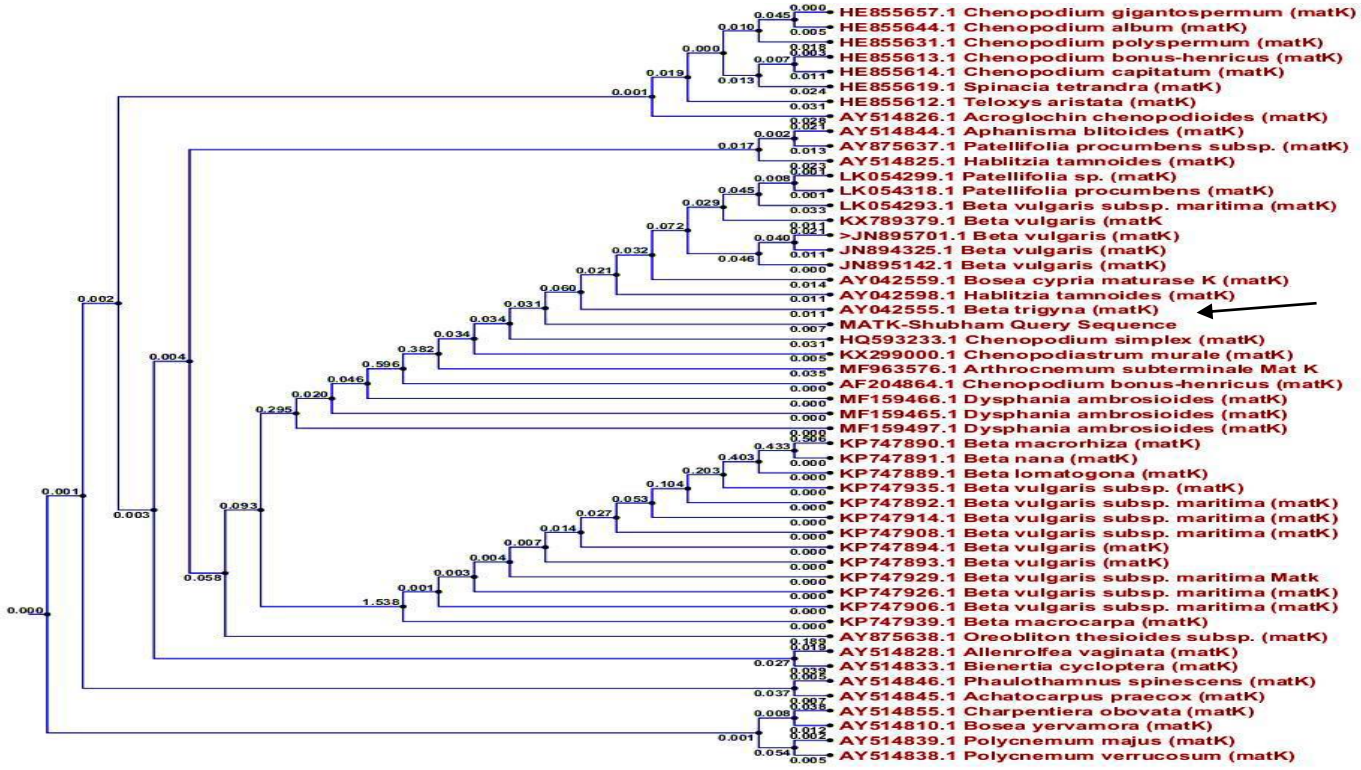


Figure 2 : Phylogenetic tree of matK (maturase k) marker region using CLC main workbench by Neighbour Joining (NJ) method with a bootstrap support value (≥ 50%).

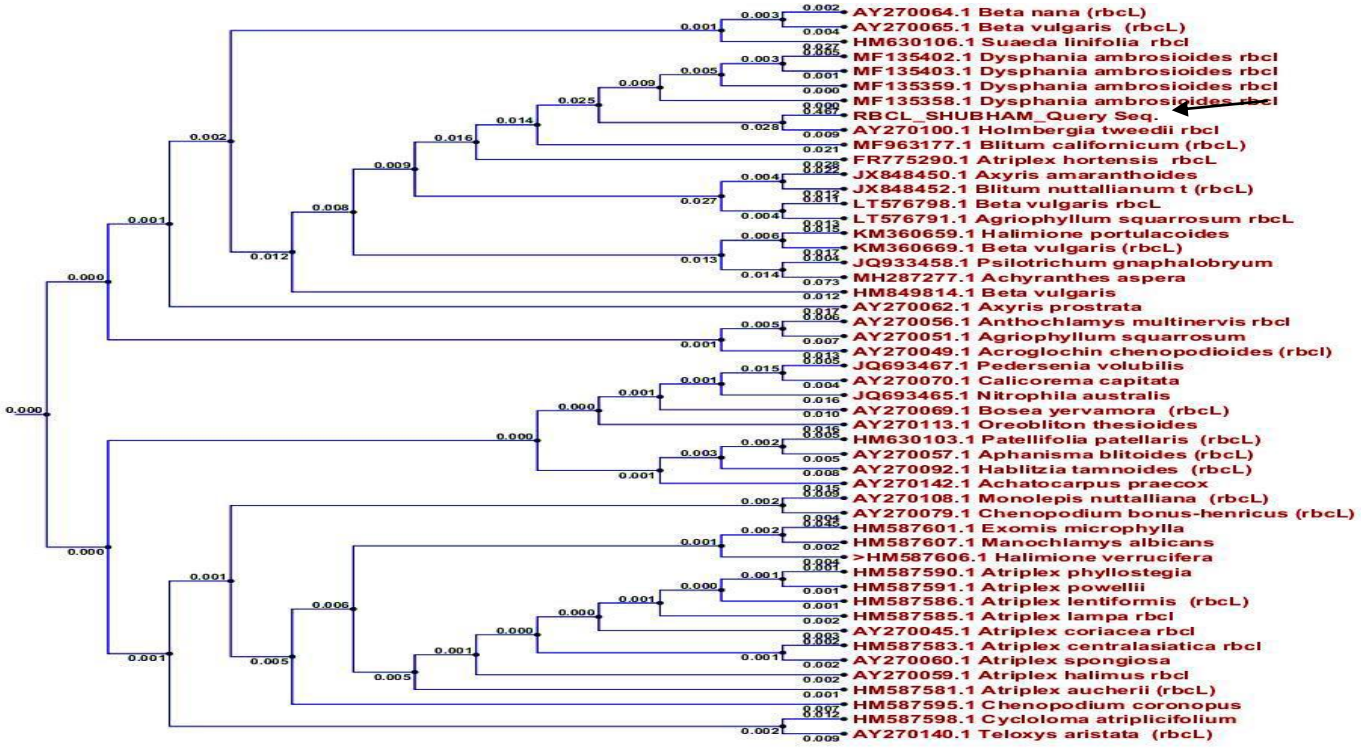


Figure 3 : Phylogenetic tree analysis using for rbcl (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) marker region using ClC main workbench by Neighbor Joining (NJ) method with a bootstrap support value (≥ 50%).

The main aim of the study was to find a suitable marker for the identification of plant species. Here, *Beta vulgaris* was used to check the presence of *matK* and *rbcl* genes in chloroplast genome. It confirmed the presence of these genes as PCR amplification gave the 100% result. There is as such no universal barcode for plants due to their lack of universality, sequence quality, and discriminating power. The use of *rbcl* and *matK* was suggested as core barcodes, as these two-plastid barcodes provided the best result in discriminating capability between the plant species than the single barcode marker. There are estimated more than 7,500,000 species around the world, but only a few of them are acknowledged on the basis of the conventional methods of identifying plant species by scientists, taxonomists. So, to overcome this problem, the technique of DNA Barcoding was introduced as this is a reliable and easiest way to identify the plant species. (IEA, 2011) In a tree primarily based analysis, NJ methodology was used to check the monophyletic relationships between the species as a result of the NJ methodology has verified extremely helpful for estimating relatedness among species. (Vences *et al.*, 2012) NJ tree reconstruction methodology was additionally employed in a DNA Barcoding study for the identification of plant species. (Al-Qurainy *et al.*, 2014) The polyphyletic relationship within the *Beta vulgaris* was examined perpetually in rbcL and matK sequence, primarily based on phylogenetic trees still as within the combined information analysis.

IV. Conclusion

DNA Barcoding is a reliable tool for species identification. The ability of *matK* and *rbcl* barcode in the identification of medicinal plants has been checked in a wide range of taxa. This method proved to be effective in identifying the Chenopodiaceae family and its species.

V. Acknowledgement

The authors are grateful to Charutar Vidya Mandal University, Anand, Gujarat, for this research work. We are also thankful to I/C head Dr. Bhakti Bajpai, ARIBAS, New V. V. Nagar, for providing the facilities for research work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

VI. References

[1] Techen, N. et al., DNA barcoding of medicinal plant material for identification," Current Opinion in Biotechnology, 25 (2014) 103–110. doi: 10.1016/j.copbio.2013.09.010.

[2] Hebert PDN, Ratnasingham S, de Waard JR. Barcoding animal life: cytochrome c oxidase subunit 1 divergence among closely related species. Proc R Soc Lond B Biol Sci 270 Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely. Proc Biol Sci. 7(270) (2003) Suppl 1:S96-9

[3] Kress, W.J. New Technologies for Taxonomy. Nat. Hist.Mini-barcodes, D.N. a., DNA Barcodes. 858 (2012) 339–353.

[4] Chase MW, Salamin N, Wilkinson M, et al., Land plants and DNA barcodes: short term and long-term goals. Phil Trans Lond B 360 (2005) 1889–1895

[5] Newmaster SG, Fazekas AJ, Ragupathy S ., DNA barcoding in the land plants: an evaluation of rbcL in a multigene tiered approach. Can J Bot 84 (2006) 335–341

[6] Vere, N. de et al., Dna barcoding for plants, Methods in Molecular Biology, 1245 (2015) 101–118. doi: 10.1007/978-1-4939-1966-6_8.

[7] Chen, S. et al., Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species, PLoS ONE, 5(1) (2010) 1–8. doi: 10.1371/journal.pone.0008613.

[8] Hart, B. L., The evolution of herbal medicine: Behavioural perspectives, Animal Behaviour, 70(5) (2005) 975–989. doi: 10.1016/j.anbehav.2005.03.005.

[9] Sucher, N. J., and Carles, M. C., Genome-based approaches to the authentication of medicinal plants, Planta Medica, 74(6) (2008) 603–623. doi: 10.1055/s-2008-1074517.

[10] IEA., Chapter 1 股関節 概念 Chapter 1 股関節, An Automated Irrigation System Using Arduino Microcontroller, 1908 (2011) 2–6.

[11] Kumar, Y., Beetroot: A Super Food, Internal Journal of Engineering Studies and Technical Approach, 01(3) (2015) 1–7.

[12] Jasmitha, S., Shenoy, A. and Hegde, K., Review Article A REVIEW ON Beta vulgaris (BEETROOT), International Journal of Pharma And Chemical Research, 4(2) (2018) 136–140.

[13] Vences, M. et al., DNA Barcodes Amphibians and reptiles, DNA Barcodes: Methods and Protocols, 858 (2008) (2018). doi: 10.1007/978-1-61779-591-6.

[14] Al-Qurainy, F. et al., Selection of DNA barcoding loci and phylogenetic study of a medicinal and endemic plant, Plectranthus asirensis J.R.I. wood from Saudi Arabia, Genetics and Molecular Research, 13(3) (2014) 6184–6190. doi: 10.4238/2014.August.7.31.

[15] Kang, Y. et al., DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests, Scientific Reports, 7(1) (2017) 1–9. doi: 10.1038/s41598-017-13057-0.

[16] Kaur, S., DNA Barcoding and its Applications, Biotechnology in Horticulture: Methods and Applications, 3 (2013) 414.

[17] Keele, J. et al., Identification of Unknown Organisms by DNA Barcoding: A Molecular Method for Species Classification, Trends in Genetics, 23(4) (2014) 167–172. doi: Research and Development Office, Final report 2014-01 (0045).

[18] Letchuman, S., Short Introduction of DNA Barcoding, International Journal of Research, 5(4) (2018) 673–686.

[19] McGinnis, S. and Madden, T. L., BLAST: At the core of a powerful and diverse set of sequence analysis tools, Nucleic Acids Research, 32(WEB SERVER ISS.) (2004) 20–25. doi: 10.1093/nar/gkh435.

[20] Horner, D. S. et al. A unified index of sequence quality and contig overlap for DNA barcoding. Pakistan Journal of Botany **7** (2015) 3190–3194.

[21] Anvarkhah, S., Khajeh-Hosseini, M., and Rashed, M.H., Identification of three species of genus Allium using DNA barcoding. 1. international Journal of Agriculture and Crop Sciences 5 (11) (2013) 1195

[22] Porebski S, et al., Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Biol. Reptr., 15 (1997) 8-15.