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PHYLOGENETIC ANALYSES OF SOME EGYPTIAN GENERA OF *Lamiaceae* FAMILY USING *rbcL* SEQUENCES

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ABSTRACT

Six local Egyptian commercial cultivars of family *Lamiaceae*, two cultivars of genus *Ocimum* L. (Basil), two cultivars of genus *Menthe* L. (Mint), and two cultivars of genus *Thymus* L. (Thyme) were analyzed for ribulose 1,5-bisphosphate carboxylase Large (*rbcL*) gene at the level of DNA sequences. All samples successfully amplified the ± 630 bp fragment. Additionally, the results of alignment analysis using BLASTN tools divulged that the sequence of DNA *rbc*L for the two local basil cultivars (Basil1 & Basil2) has similarities with (*Ocimum basilicum, Ocimum tenuiflorum, Ocimum kilimandscharicum and Ocimum gratissimum*) 100, 99.69, 99.37 and 99.06 respectively. In addition, two local mint cultivars (Mint 1 and Mint 2) has similarities with *Mentha spicata*, *Mentha_pulegium, Mentha canadensis* and *Mentha menthaefolia*, 99.85, 99.84, 99.69 and 99.53 respectively. For thyme local cultivars (Thyme1 and Thyme2), Thym1 cultivar sample genotype is genetic closely with species, (*Thymus alsinoides* and *Thymus sibthorpii*) with 99.69 and 99.84 respectively and they located nearest from the cluster (Thymus genus) members in phylogenetic trees while, Thym2 is located after the cluster with Artemisia genus belonging to family *Asteraceae*. The reason of this result may be occurring due to that a Thym2 genotype comes from local marketing, which some of them are selling it as a thymus genus however, it is belongs to Artemisia genus and has similarities with with seven species of *Artemisia* genus (*Artemisia absinthium, Artemisia gmelinii, Artemisia selengensis, Artemisia scoparia, Artemisia maritima, Artemisia capillaris and Artemisia fukudo*). Using of *rbcL* DNA barcode proves to be effective in identifying the plants from the family level up to the genus level. This study demonstrates the efficiency of using *rbcL* barcoding primer to classify family *Lamiaceae* phylogenetically. It is also concluded that the *rbcL* gene showed genuine potentials to distinguish the plant Egypt species under investigation into the proper family and genus.

Keywords: DNA barcode; *rbc*L; Family *lamiaceae;* BLASTN; Phylogenetic

INTRODUCTION

From ancient times, medicinal plants have been used to treat diseases and even today, in many tribal and primitive societies, these plants are used on a large scale. Metabolic by-products of plants such as alkali, glycosides, steroids or other active compounds are also used to treat various diseases such as cancer, malaria, diabetes and dysentery. (**Biradar, 2015**). Therefore, the precise knowledge and molecular identification of medicinal plants are essential for developing new healthcare products from plants.

Lamiaceae family, which includes many cultivars of plant species is characterized by its economic importance, which is used for the most part as cooking herbs and the other part is used to extract a lot of drug-active compounds (**Venkateshappa** and **Sreenath, 2013**), which have been used for producing pesticides, cosmetic and food (**Khaled-Khodja et al 2014**). Today, the essential oils of a few *Lamiaceae* plants have become an inexorably significant crude material for the food, pharmaceutical and cosmetic industry (**Edris, 2007**).

Using DNA barcoding for molecular identification of medicinal plants could be very tricky and challenging at the same time in term of generating barcodes data and in analyzing these data to stands on the discrimination power (**Cowan** and **Fay, 2012**).

Practically, DNA barcoding technique depends on a short and unique DNA sequence for one locus or a few loci utilized together as an altogether unit. The generating data from a unique species used for fingerprinting and copyright protection for this species and marketplace regulation in general. (**Kress** and **Erickson, 2007**, **Elansary et al 2017**)

Mainly, to distinguish a difficult taxa, DNA barcoding markers will be the best option by generating a phylogenetic tree (**ElAtroush et al 2015**). moreover, the plant barcode, such as *rbcL,* should be multi-locus, preferably comprising a conserved coding region or vice versa, more rapidly evolving region that is probably non-coding (**Kress et al 2009**).

The *rbcL* (Ribulose -1,5 – bisphosphate carboxylase/ oxygenase large subunit) gene that is coding for large subunit of the enzyme RuBisCo, consider one of the most barcoding genes used in the phylogeny of plants.

By the consent of consortium for the barcode of life (CBOL) in 2009 they consider *matK* (the chloroplast gene) and *rbcL* as the main barcodes of plant species, in addition to intergenic sequence *trnH-psbA* and nuclear gene *ITS* as the addition barcodes (**CBOL, 2009**). Meanwhile, *rbcL* is well-known by its comparability, universality and easy amplification (**Hollingsworth et al 2016**). *rbcL* genes used successfully to classification of angiosperm, and even among the different groups of the seed plants (**Chase et al 2007**). take into consideration that the variation in *rbcL* sequence commonly exists at only the above-species level, and rarely found at the species level (**Newmaster et al 2006**), resulting in poor discrimination power at species level (**Gonzalez et al 2009**). At a study of **Newmaster et al (2006)** they aligned approximately 10,300 *rbcL* sequences collected from GenBank, and found that *rbcL* gene cannot be able to distinguish between all plant species but can clearly distinguished plants belong to the same genus **Newmaster et al** (**2006**).

Another study of **Sundari et al (2019)**, they used the *rbcL* for identification of red jabon and gofasa plants collected from Indonesia stated that the effectiveness of using *rbcL* in identify plants have been limited into the family level up to the genus **Sundari et al (2019)**.

So, our study will try to found some genetic variations able to build a clear phylogenetic relationship between some plants belong to *Lamiaceae* family utilizing *rbcL* genes barcoding.

MATERIALS AND METHODS

MATERIALS

Fresh leaves of six local Egyptian commercial cultivars belong to *Lamiaceae* family were collected from private and public nurseries in Egypt (**Table 1**).

Table 1. The six medicinal plants information's used in this study collected from a private and public nursery in Egypt

No	Sample name	Genus	Collection site (city, Governorate)
1	Basil1	*Ocimum* L. (basil)	Kafr Shukr, Al-Qalyubiyya
2	Basil2		Ossim, Giza
3	Mint1	*Menthe* L. (mint)	Shibin El Qanater, Al-Qalyubiyya
4	Mint2		Sinnuris, Faiyum
5	Thym1	*Thymus* L. (thyme)	Qalyub, Al-Qalyubiyya
6	Thym2		El Hawamdeya, Giza

METHODS

DNA barcoding

Genomic DNA purification

Six fresh plant leaf samples from local cultivars belonging to three genera of *Lamiaceae* family were collected from different governorates of Egypt. DNA extraction was carried out using Plant High Molecular DNA extraction KIT (SIGMA, USA). The quality of DNA was assist using agarose gel electrophoresis, visualized by pre-added RedSafe® (5ul /100ml) under UV light. The quantities and purities of DNA were assisting using UV spectrophotometer on 260 nm and 280 nm (BIO RAD- SmartSpec Plus spectrophotometer).

PCR and sequencing

The primer sequences used to amplify *rbcL* fragment was:

rbcL- Forward (5'-ATGTCACCACAAACAGAAAC-3')

rbcL- Reverse (5'TCGCATGTACCTGCAGTAGC-3'`)

The PCR amplification was carried out in a Peltier Thermal Cycler (Techne Laboratories, USA). The thermal cycling conditions consisted of 1 cycle at 95ºC for 5 min, followed by 30 cycles of denaturation at 92ºC for 15-sec, annealing temperatures for 30 sec. and extension at 72ºC for 30 sec and last extension step at 72ºC for 5 min.

The PCR reaction mixture consisted of 1 µL (~50 ng) DNA, 12.5 µL master mix (Transgen Biotech Company), and 1.0 µL (2.5 µmol/L) each primers in a final volume of 25 µL. Standard PCR profile with 50ºC annealing temperature was used to *rbcL*.

Taxa assignment

Basic local alignment tools (BLAST) were applied to all produced sequences using online NCBI databases (http://www.ncbi.nlm. nih.gov/ Blast). The hits with maximal percent identity scores > 95% was considered successful when all involved a single genus.

Molecular identification and phylogenetic analysis

The maximum likelihood (ML) analysis method was applied to align *rbcL* sequences using MEGA10 (**Tamura et al 2018**) with the following parameters:

1. Tree inference options were set to Nearest Neighbor Interchange.
2. Gaps/missing data were treated as partial deletions with site coverage cut off = 95%.
3. A bootstrap analysis with 100 replicates was carried out in order to study the clade support values.

RESULTS AND DISCUSSION

DNA purification

The concentration of genomic DNA isolated from the leaves of six plants ranged from 11.6 to 27.1 ng/ml with purities from 1.12 to 1.86 (**Table 2**).

Table 2. Results of quantitative measurements of DNA isolation

No	Sample name	Genus	Concentration (ng/ml)	A260/280
1	Basil1	*Ocimum* L.	11.6	1.20
2	Basil2	(basil)	13.4	1.32
3	Mint1	*Menthe* L.	17.7	1.12
4	Mint2	(mint)	19.3	1.73
5	Thym1	*Thymus* L.	27.1	1.29
6	Thym2	(thyme)	21.6	1.86

The amplification of *rbcL* gene

The obtained results of *rbc*L gene amplification from *Lamiaceae* plant samples examined by agarose gel electrophoresis are shown in **Fig. (1)**.

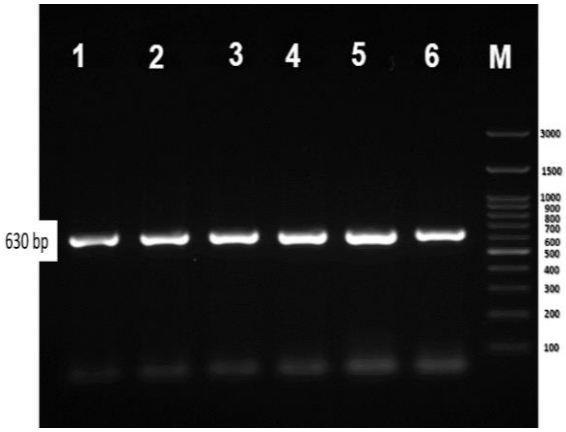

Fig. 1. Agarose gel electrophoresis (1.5%) showing the PCR amplification of the amplified rbcL gene from the six samples (Lane 1: Basil1; Lane 2: Basil2; Lane 3: Menth1; Lane 4: Menth2; Lane 5: Thym1 and Lane 6: Thym2). M= OneMARK 100, GeneDireX.

The amplification of the *rbcL* gene showed DNA bands with a size of ± 630 bp for all the amplified samples.

Phylogenetic Analysis

In order to identify phylogenetic analysis, six samples of this study were compared with data from the Gene Bank of BLAST search results at NCBI. From this phylogenetic analysis the genetic relation within and between two cultivars of genus *Menthe* L. (Mint), two cultivars of genus *Ocimum* L. (Basil) and two cultivars of genus *Thymus* L. (Thyme) were detected. Data from kinship analysis (phylogenetic) are shown in **Figs. (2, 3 and 4)**.

Identification of genus ability of each DNA barcodes was assessed using the BLAST method. In this study was directed as follows: firstly, every DNA sequences of the six collected samples of family *Lamiaceae* was downloaded from NCBI database was established utilizing the downloaded sequences (**Burgess et al 2011**). Secondly, each sequence measured was BLAST against the sequence in Gene bank database, and the percentage of identical sites was calculated and was taken as the genus discrimination rate of the measured sequence. If the

percentage of identical sites of a sequence calculated between intraspecific individuals were higher than interspecific individuals were, then the sequence was taken as the purpose one of the studied species. Finally, the identification success rate of DNA barcoding was calculated as the result of sequencing success rate and genus discrimination rate (**Kress et al 2009**).

The chloroplast gene (*rbcL*) was used for phylogenetic comparative analysis of six local Egyptian commercial cultivars belong to *Lamiaceae* family. The results showed in **Tables** (**3, 4, 5, and 6**) **Figs.** (**2, 3, and 4**).

The Results of phylogenetic tree for the two local Egyptian basil cultivars (Basil1 and Basil2) showed two main clusters, the first one included with five different basil species (*Ocimum basilicum*, *Ocimum americanum*, *Ocimum kilimandscharicum*, *Ocimum tenuiflorum* and *Ocimum gratissimum*), while the second cluster included two species (*Salvia bulleyana* and *Salvia przewalskii*) **(Table 3** and **Fig. 2).**

The two basil Egyptian cultivars (Basil1 and Basil2) were found to be linked together and have closer relationship (100%) with *Ocimum basilicum* species.

The *rbcl* gene of two local Egyptian basil cultivars (Basil1 and Basil2) were highly similar to species *Ocimum basilicum*, *Ocimum tenuiflorum*, *Ocimum kilimandscharicum* and *Salvia bulleyana* with 100, 99.69, 99.37 and 99.06, respectively.

The Blast results for *rbcL* gene of tested samples (Mint1 and Mint2) are shown in **Table** (**4**) and **Fig.** (**3**). The Results of phylogenetic tree for the two mint cultivars (Mint1 and Mint2), indicated two main clusters, the first one included two local Egyptian mint (Mint1 and Mint2) with six different mint species (*Mentha rotundifolia*, *Salvia officinalis*, *Mentha spicata*, *Mentha_canadensis, Mentha longifolia* and *Mentha suaveolens*), while the second cluster included two species (*Mentha_menthaefolia* and *Mentha_pulegium*).

The *rbcL* gene of the two mint cultivars (Menth1 and Menth2) were closely genetic related to genus (*Mentha spicata* and *Mentha longifolia) and (Mentha pulegium and Mentha suaveolens)* with 99.85 and 99.84, respectively.

The Blast results for rbcL gene of tested samples (Thym1 and Thym2) are shown in **Tables** (**5 and 6**) and **Fig.** (**4**).

The results for *rbcL gene of tested samples (Thym1 and Thym2)* are shown in **Tables** (**5 and 6**) and **Fig.** (**4**). The phylogram could be separated into two distinct clusters. Cluster one contained Thym2 with seven species of *Artemisia* genus (*Artemisia absinthium, Artemisia gmelinii, Artemisia selengensis, Artemisia scoparia, Artemisia maritima, Artemisia capillaris and Artemisia fukudo*). Whereas Thym1 with three species of *Thymus* genus (*Thymus alsinoides, Thymus vulgaris and Thymus sibthorpii*) and three species of *Salvia* genus (*Salvia japonica, Salvia nemorosa and Salvia rosmarinus*) were grouped in second cluster. The phylogenetic tree of tested samples from the *rbcL* sequences showed that Thym1 genotype was genetic closely related with species, *Thymus alsinoides* and *Thymus sibthorpii* in cluster one forming clearly distinctive clades (monophyletic groups) whereas in second cluster, only *Artemisia* genus form clearly distinctive clades while *Thym2* genotype not positioned within which was this clade (**Figure 4).** Thym1 plant samples are located nearest from the cluster (*Thymus* genus) members in phylogenetic trees while Thym2 was located next to the cluster with *Artemisia* genus. The reason of this result may be occurs due to that a Thym2 genotype comes from local marketing, which some of them are selling it as an thymus genus however, it is belongs to *Artemisia* genus.

The results of phylogenetic analysis using Neighbor Join (NJ) method note that the rbcL gene can used to clarify taxon positions in the identification of a species. A specimen from a different area may be together on the same cluster (**Che et al 2012**). Our results showed that the identification of Basil, Mint and Thyme plants were effective. Basil1 and Basil2 genotypes were identical (100%) to *Ocimum basilicum* species. For Mint1 and Mint2 genotypes, it has a 99.85% similarity with *Mentha_spicata* and *Mentha_longifolia* NCBI database. Actually, the *rbcL* gene can amplified with a high achievement rate with one or two universal primers. It was additionally stated that when compared to other barcode gene candidates, the *rbcL* gene has a high achievement rate of bidirectional sequencing (Consortium for the Barcode of Life sequencing with forward and reverse primers) (**CBOL, 2009**).

The study of *rbcL* gene sequencing was efficient to differentiate between some genera and species of family *Lamiaceae* due to sufficient data in GenBank (**Molins et al 2011**).

A many researchers have recommended that *rbcL* gene should be incorporated as a standard for comparison to different markers due the advantage that this gene is handily amplified and sequenced in many plants and it is viewed as a benchmark locus in phylogenetic examinations by providing a reliable

placement of a species into plant genus and/or family (**Hassel et al 2013**).

In conclusion, this study gives a fundamental appraisal information that will be valuable for more extensive utilization of DNA barcoding in medicinal plants. It was discovered that *rbcL* was valuable for the barcoding of some medicinal plant species in family *Lamiaceae*, where it has a good resolution toward species identification. However, further protocol development to improve clean DNA extraction, PCR amplification programs, including the development of new primers, and local confirmed databases could assume significant important roles in efficient utilization of plant barcoding.

Table 3. The BLAST results for Basil1 and Basil2 of tested samples. Database search match for similarities and phylogenetic relationship using *rbcL* gene sequences

Species from NCBI	Accession	Query cover	% identity	E value
Ocimum basilicum	KY623639	99%	100%	0.0
Ocimum tenuiflorum	NC043873	99%	99.69%	0.0
Ocimum americanum	MF468188	99%	99.69%	0.0
Ocimum kilimandscharicum	MF468191	99%	99.37%	0.0
Salvia bulleyana	NC_041092	99%	99.06%	0.0
Salvia przewalskii	NC_041091	99%	99.06%	0.0
Ocimum gratissimum	MF468194	99%	99.06%	0.0

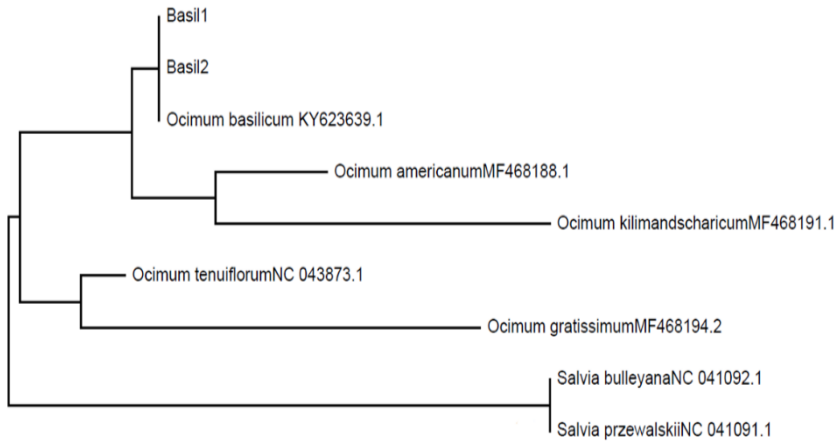

Fig. 2. Phylogenetic relationship of gene *rbcL* sequence for basil (Basil1 and Basil2) samples based maximum likelihood tree

Table 4. The Blast results for Menth1 and Menth2 of tested samples. Database search match for similarities and phylogenetic relationship using *rbcL* gene sequences

Species from NCBI	Accession	Query cover	% identity	E value
Mentha_spicata	NC_037247	100%	99.85%	0.0
Mentha_longifolia	KU956042	100%	99.85%	0.0
Mentha_canadensis	NC_044082	100%	99.69%	0.0
Mentha_rotundifolia	Z37417	99%	99.69%	0.0
Mentha_menthaefolia	Z37420	99%	99.53%	0.0
Salvia_officinalis	NC_038165	100%	99.38%	0.0
Mentha_pulegium	KY656718	98%	99.84%	0.0
Mentha suaveolens	KP172040.1	97%	99.84%	0.0

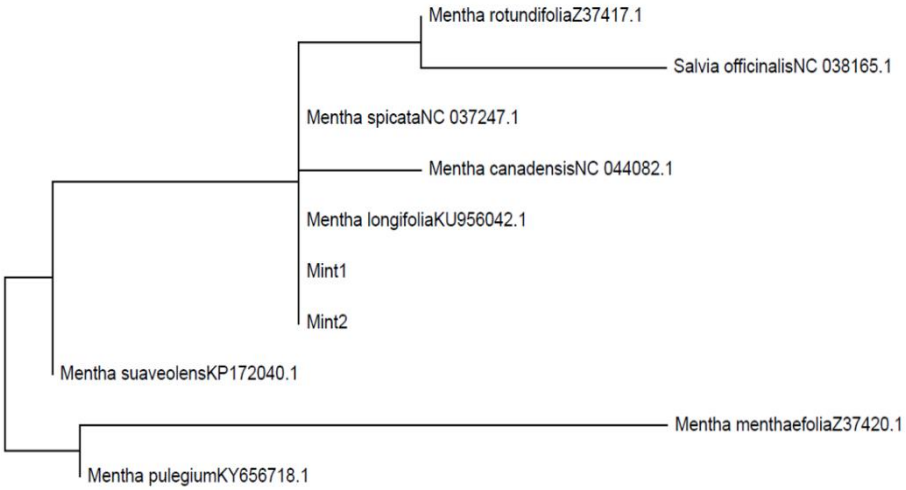

Fig. 3. Phylogenetic relationship of gene rbcL sequence for mint (Mint1 and Mint2) samples based maximum likelihood tree.

Table 5. The Blast results for Thym1 of tested samples. Database search match for similarities and phylogenetic relationship using *rbcL* gene sequences

Species from NCBI	Accession	Query cover	% identity	E value
Thymus_alsinoides	Z37470	99%	99.69%	0.0
Thymus_vulgaris	Z37472	99%	99.53%	0.0
Thymus_sibthorpii	KR063653	97%	99.84%	0.0
Salvia_japonica	KY646163	99%	98.76%	0.0
Salvia nemorosa	--------	99%	98.76%	0.0
Salvia rosmarinus	--------	99%	98.76%	0.0

Table 6. The Blast results for Thym2 of tested samples. Database search match for similarities and phylogenetic relationship using *rbcL* gene sequences

Species from NCBI	Accession	Query cover	% identity	E value
Artemisia_scoparia	NC_045286	100%	98.91%	0.0
Artemisia_maritima	NC_045093	100%	98.91%	0.0
Artemisia_fukudo	NC_044156	100%	98.91%	0.0
Artemisia_capillaris	KY073391	100%	98.91%	0.0
Artemisia_absinthium	MK188885	100%	99.76%	0.0
Artemisia_selengensis	NC_039647	100%	99. 76%	0.0
Artemisia_gmelinii	KY073390	100%	99.76%	0.0

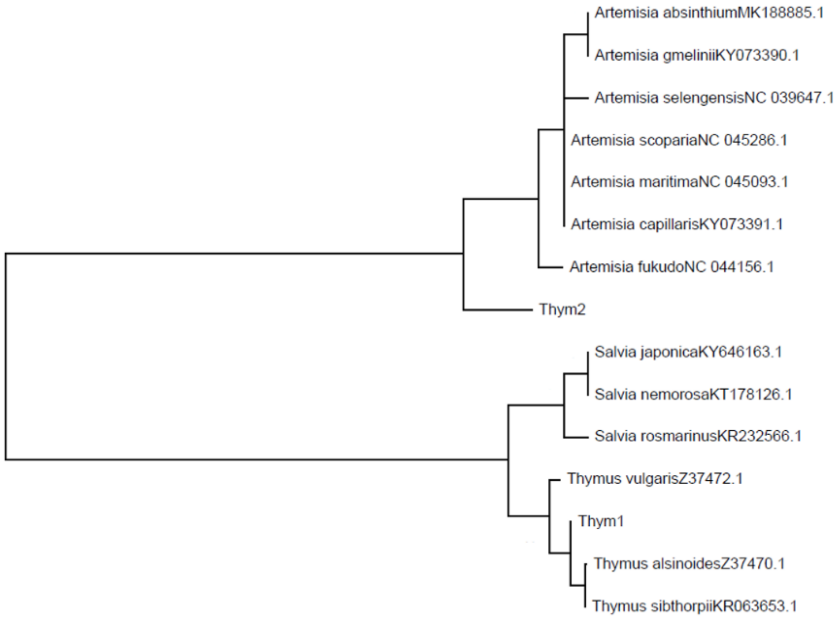

Fig. 4. Phylogenetic relationship of gene *rbcL* sequence for Thyme (Thym1 and Thym2) samples based maximum likelihood tree

REFERENCE

Biradar D.P. 2015. Medicinal plants and phytomedicines. **Ann. Phytomed., 4(1), 1-5.**

Burgess K.S., Fazekas A.J., Kesanakurti P.R., Graham S.W., Husband B.C., Newmaster S.G., Percy D.M., Hajibabaei M. and Barrett S.C.H. 2011. Discriminating plant species in a local temperate flora using the rbcL+ matK DNA barcode. **Methods in Ecology and Evolution, 2(4), 333-340.**

CBOL .2009. **Plant Wording Group.** A. DNA barcode for land plants. **PNAS. 106, 12794-12797.**

Chase M.W., Cowan R.S., Hollingsworth P.M., Van Den Berg C., Madriñán S., Petersen G., Seberg O., Jørgsensen T., Cameron K.M., Carine M., Pedersen N., Hedderson T.A.J., Conrad F., Salazar G.A., Richardson J.E., Hollingsworth M.L., Barraclough T.G., Kelly L. and Wilkinson M. 2007. A proposal for a standardized protocol to barcode all land plants. **Taxon, 56(2), 295-299.**

Che J., Chen H.M., Yang J.X., Jin J.Q., Jiang K., Yuan Z.Y., Murphy R.W. and Zhang Y.P. 2012. Uni-versal COI primers for DNA barcoding amphibians. **Mol. Ecol. Res. 12, 247-258.**

Cowan R.S. and Fay M.F. 2012. Challenges in the DNA barcoding of plant material. **Methods Mol. Biol. 862, 23-33.**

Edris A.E. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. **Phyto Therapy Research, 21, 308-323.**

Elansary H.O., Ashfaq M., Ali H.M. and Yessoufou K. 2017. The first initiative of DNA barcoding of ornamental plants from Egypt and potential applications in horticulture industry. **PLoS One, DOI:10.1371/J.pone.0172170 February 15, 1-15.**

El-Atroush H., Magdi M. and Werner O. 2015. DNA Barcoding of two endangered medicinal Plants from Abou Galoom protectorate. **Life Sci. J., 12(9), 101-109.**

Gonzalez M.A., Baraloto C., Engel J., Mori S.A., Pétronelli P., Riéra B., Roger A., Thebaud C. and Chave J. 2009. Identification of Amazonian trees with DNA barcodes. **PLoS one, 4(10), 1-7.**

Hassel K., Segreto R. and Ekrem T. 2013. Restricted variation in plant barcoding markers limits identificat ion in closely related bryophyte species. **Mol. Ecol. Resour., 13(6), 1047-1057.**

Hollingsworth P.M., Li D.Z., van der Bank M., and Twyford A.D. 2016. Telling plant species apart with DNA: from barcodes to genomes. **Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1702), 20150338.**

Khaled-Khodja N., Boulekbache-Makhlouf L. and Madan K. 2014. Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae. **Ind. Crop. Prod. 61, 41-48.**

Kress W.J., Erickson D.L., Jones F.A., Swenson N.G., Perez R., Sanjur O. and Bermingham E. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. **Proc. Natl Acad. Sci. USA, 106(44), 18621-18626.**

Kress W.J. and Erickson D.L. 2007. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. ***PLoS one*, *2*(6).**

Molins A., Bacchetta G., Rosato M., Rosselló J.A. and Mayol M. 2011. Molecular phylogeography of Thymus herba-barona (*Lamiaceae*): insight into the evolutionary history of the flora of the western Mediterranean islands. **Taxon, 60(5), 1295-1305.**

Newmaster S.G., Fazekas A.J. and Ragupathy S. 2006. DNA barcoding in land Plants:evaluation of *rbcL* in a multigene tiered approach**. Can. J. Bot., 84, 335–341.**

Sundari K.H., Jayali A.M. and Sukamto N.H. 2019. The application of barcode DNA rbcL gene for identification of medicinal plants: red jabon and gofasa. In *Journal of Physics: Conference Series* (Vol. 1146, No. 1, p. 012030). IOP Publishing.

Tamura K., Tao Q. and Kumar S. 2018. Theoretical Foundation of the RelTime Method for Estimating Divergence Times from Variable Evolutionary Rates. **Molecular Biology and Evolution, 35(7), 1770-1782.**

Venkateshappa S.M. and Sreenath K.P. 2013. Potential medicinal plants of Lamiaceae. **American International J. of Res. in Formal, Applied and Natural Sci., 1(3), 82-87.**

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دراسة القرابة الوراثية لبعض الأجناس المصرية التابعة للعائلة الشفوية بإستخدام تتابعات جين الـ rbcL

[16]

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الموجـــــز

تم دراسة القرابة الوراثية لستة أصناف تجارية مصرية من العائلة الشفوية (*Lamiaceae*)، صنفين من جنس الريحان (*Ocimum L.*)، وصنفين من جنس النعناع (*Menthe L.*)، وصنفين من جنس الزعتر (*Thymus L.*) بإستخدام التتابعات النيوكليتيدية لجين ريبوليز 1,5–بيوفوسفات كربوكسيلاز (rbcL). نجحت جميع العينات في تضخيم حزمة بلغ حجمها ± 630 زوج من القواعد. كما أظهرت نتائج التماثل باستخدام أدوات BLASTNأن تتابعات الحمض النووي للجين (*rbcL*) والخاصة بصنفي الريحان المحلى تحت الدراسة لهما قرابة وراثية مع أجناس الريحان التالية (*Ocimum basilicum*, *Ocimum tenuiflorum*, *Ocimum kilimandscharicum* and *Ocimum gratissimum*) بنسب تماثل (100, 99.69, 99.37 and 99.06) على التوالي. كما أشارت النتائج إلى أن صنفى النعناع المحلية تحت الدراسة لها قرابة وراثية مع أجناس النعناع التالية (*Mentha spicata*, *Mentha_pulegium*, *Mentha canadensis* and *Mentha menthaefolia*) بنسـب تماثـــل (99.84, 99.85, 99.53 and 99.69) على التوالي. وبالنسبة للأصناف المحلية من الزعتر تحت الدراسة أظهر نمط التسلسلى الجيني للصنف المحلى (Thym1) تشابها وراثياً بشكل كبير مع الجنسين (*Thymus alsinoides* and *Thymus sibthorpii*) بنسب تراوحت بين (99.69 و 99.84) على التوالي، بينما أظهر الصنف المحلى (Thym2) تشابهاً مع عدة أجناس من الشيح (Artemisia) التابعة للعائلة النجميه (Asteraceae). وقد يكون سبب هذه النتيجة هو أن هذا الصنف المحلى (Thym2) يتم تسويقه محلياً على أنه زعتر تابع للعائلة الشفوية، ولكن هذه الدراسة أشارت إلى أنه ينتمي إلى جنس الشيح (Artemisia) حيث أظهر نمطه التتابعى تشابه مع سبعة أنواع من جنس الشيح. نجحت الدراسة في إظهار كفاءة استخدام التتسلسل النيوكليتدى لجين الباركود rbcL لتحديد درجة القرابة الوراثية بين الستة أصناف المصرية من عائلة Lamiaceae وتحديد إنتمائتها للأجناس النباتية.

الكلمات المفتاحية: باركود الحمض الريبوزي، rbcL، العائلة الشفويه، BLASTN، القرابة الوراثية

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