Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt
29(1), 387 - 399, 2021
Website: http://ajs.journals.ekb.eg
DOI: 10.21608/ajs.2021.49712.1298

Phylogeny Validation for Some Egyptian Brassicaceae Endemic Species

[26]

Shaimaa A Abdelhady[1*], Abdoallah A Sharaf[1], Aly ZE Abdelsalam[1], Khaled A Soliman[1], Asmaa M Abushady[1,2]

1- Genetics Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68 Hadyek Shoubra 11241, Cairo, Egypt

2- Biotechnology School, Nile University, Sheikh Zayed, Giza, Egypt

*Corresponding author: shahmed_2010@agr.asu.ed.eg

Received 12 January, 2021 Accepted 9 March, 2021

Abstract

A crucial menace for the biodiversity in arid and semi-arid territories is the global warming arising from anthropogenic activity. Egypt is expected to undergo an acute rainfall decrease and temperature boost in the next few decades, leading to many plant species' geographical allocation. Endemic plants of pleiotropic economic importance are strongly affected by climate change prospects, which will gradually result in losing our plant wealth genetic resources. Due to few studies on the Egyptian Brassicaceae family which has an economically and medicinally importance due to the presence of many active compounds that are included, in the pharmaceutical and cosmetic components (e.g., glucosinolates) besides having a large amount of antioxidant which inhibit the growth of microbes and also treat rheumatic diseases. The identification of this family still kind of fishy as researchers mostly relies on the morphological characters. To globally sustain this Egyptian plant family wealth's genetic pattern, it is substantial to characterize them based on their authenticated genetic background. Here we present a phylogenetic analysis of 16 species of the Egyptian Brassicaceae family using two plastid coding genes; Ribulose-1,5- bisphosphate carboxylase oxygenase (*rbcL-a*) and maturase K (*matK*). The maximum likelihood of the two markers for our samples was concordant with the Brassicaceae's references-sequences, which exist on plastid are considered highly conserved biomarkers. In conclusion, we have generated a robust phylogeny tree based on the molecular level that validates the Egyptian plant species and reliably differentiates them on morphological identification. This study is considered the first phase of Egyptian Brassicaceae family species authentication followed by biochemical studies serving the pharmacological and medicinal fields.

Keywords: Endemic plants; *RbcL-a*; *MatK*; DNA Barcoding; Phylogenetic analysis

1 Introduction

Among 190 endemic and near-endemic taxa, 76 are believed to be endemic, 21 stenoendemic, and 93 near-endemic in Egypt (Boulos 2009), which the latter reduced to 61 (Hosni et al 2013). Recently, it has been shown that Fabaceae, Lamiaceae, Caryophyllaceae, Asparagaceae, Asteraceae, and Brassicaceae are the dominant endemic families in Egypt (Abdelaal et al 2018). Indeed, Brassicaceae occupies great attention due to many aspects such as their antioxidant potential and bioactive phytochemical capacity (Danlami et al 2016; Nurzyńska-Wierdak, 2015). For instance, Lob-

ularia. spp. contains components like Isothiocyanates (ITCs) and glucosinolates that play an essential role as antimicrobial activity against several pathogens like insects and nematodes (Fahey et al 2001; Al-Gendy et al 2016). Cakile maritima has plenty of antioxidants that inhibit microbes' growth and are used to treat rheumatic diseases (Omar et al 2016). Global climate changes negatively impact endemic plants, especially in Egypt, due to the large deserts (Serag et al 2018). Such a negative impact could lead to the loss of these substantial plants; therefore, it was indispensable to authenticate and preserve this species diversity in Egypt (Amer et al 2015). Endemic Brassicaceae has been characterized morphologically and/or biochemically, while robust molecular phylogeny studies are missing (Abdel Khalik et al 2002; Marzouk et al 2010; Mohamed, 2009). Of note, few reports are available to characterize the Egyptian endemic plants on the molecular level. DNA barcoding is one of the most important techniques used to differentiate species by utilizing a small DNA sequence from a standard and conserved localized region in the genome (Kress et al 2005). Two plastid coding genes; Ribulose-1,5-bisphosphate carboxylase oxygenase (*rbcL-a*) and maturase K (*matK*), were utilized as a core-barcode as recommended by Consortium of Barcode of Life (CBOL) in 2009 (CBOL Plant Working Group, 2009). Many investigators reported that *rbcL-a* and *matK* sequences are efficient in identifying species (Asahina et al 2010; Starr et al 2009).

This study aimed to characterize some of the Egyptian endemic genetic resources on the molecular level to sustain the Egyptian plant genetic wealth. Further, to address whether morphological identification is sufficient for characterization or confirmed with the molecular characterization. Therefore, 16 Brassicaceae endemic species were collected from two different geographical locations and barcoding them using two chloroplast markers. Latter, we generated a robust concatenated phylogenetic tree was generated concerning the Brassicaceae family not only for our collected taxa but also for all reference nucleotide sequences available on the database based on the two selected markers *(rbcL-a* and *matK*).

2 Materials and Methods

2.1 Plant material

The aerial parts of seventeen endemic plants pigeonholed from one family (Brassicaceae) spanning 16 different species were collected from two different localities in Egypt (Suez Road and North-West Coast, respectively (**Table 1**) in spring during the flowering stage of 2018. Collected plants were identified morphologically by consulting published taxonomic keys and related literature (Täckholm 1974; Boulos 2009). Plant materials were air-dried separately in the shade, packed in tightly zip-lock plastic bags containing silica gel, and stored in a -80 freezer for the downstream molecular studies.

2.2 DNA extraction and barcoding

For each plant, 100 mg were separately extracted using the EZ-10 Spin column Plant Genomic DNA Mini-preps Kit following the manufacturer's instructions (BIO BASIC INC, CANADA). DNA was stored at -20 C for the downstream analysis. DNA was amplified using the corresponding universal DNA barcoding primers for the two aforementioned chloroplast markers, the encoded *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase) and *matK* (maturase K) as shown in **(Table 4)**, (Maloukh et al 2017). DNA Polymerase Chain Reaction (PCR) was performed using a 20 µl total volume reaction mixture of 10 µl 2x PCR Master Mix i-Taq^{TM} Solution (intron, Korea), 6 µl distilled water, 1µl forward and reverse primers, and 2 µl of DNA. PCR amplification was carried out in Techne, 3primX thermal cycler. The PCR profile consisted of an initial denaturation step of 5 min at 94 ˚C followed by 35 cycles with annealing temperatures of 50 ˚C for 30 s in *rbcL-a* and 45 ˚C in 30 s for *matK*. PCR products were separated on 1.6% agarose gel electrophoresis. The respective bands (550 and 690 bps for *rbcL* and *matK* respectively)

were cleaned by the QIAquick PCR Purification Kit (Qiagene, USA) to remove unincorporated primers and dNTPs. The purified PCR product was sequenced in both directions (forward and reverse) directions using a 3500xL Genetic Analyzer with Sanger's sequencing method by Color lab (Egypt) (Sanger and Coulson 1975).

2.3 Phylogenetic analyses

The forward and reverse sequences obtained were aligned, consensus sequences were validated using NCBI nucleotide-BLAST (blastn) (https://blast.ncbi.nlm.nih.gov). All the sequences generated in this study were deposited in the NCBI Gene bank with accession numbers (Supplementary Table S1A). All available nucleotide reference sequences (244 sequences) of each chloroplast (*rbcL* and *matK*) genes were retrieved from NCBI GeneBank (www.ncbi.nlm.nih.gov: 08:2020), which represents sequences of specimens from 117 Brassicaceae genera (Supplementary Table S1B). Also, embryophyta's sequences from 4 species were retrieved as an out-group (Supplementary Table S1B). The retrieved sequences were combined with the obtained sequences (34 sequences) in a single sequences dataset. Sequences were aligned using MAFFT software (Katoh and Standley, 2013) with the E-INS-i multiple alignment method and BLOSUM80 scoring matrix. Spurious and poorly aligned regions were removed from the alignment by trimAl (http://trimal.cgenomics.org/) using the "gappyout" parameter (Capella-Gutiérrez et al 2009). Alignments were tested using ProtTest v3 (Darriba et al 2011) to choose the appropriate nucleotide substitution model. Two separate phylogenetic trees were computed based on the *rbcL* and *matK* genes nucleotide sequences. Maximum likelihood (ML) using RAxML-NG (Kozlov et al 2019) and IQ-TREE 2 (Minh et al 2020). ML analyses were performed using 1000 bootstrap replicates and models TIM+F+I+G4 and TVM+F+G4 for *rbcL* and *matK*, respectively. To better understand the relationships between the obtained DNA sequences and different Brassicaceae genera, both sequences alignments of *rbcL* and *matK* nucleotide were concatenated into one alignment. The concatenated alignments were tested using ProtTest v3 to choose the appropriate model for nucleotide substitution. One phylogenetic tree was computed based on both *rbcL* and *matK* markers sequences. ML analyses were performed in IQ-TREE 2 and RaxML-NG (TVM+F+I+G4 model, 1000 bootstrap replicates). The supporting values from both methods were merged into one rooted tree with hornwort (*Leiosporoceros dussii*), moss (*Ulota bruchii*), liverworts (*Calypogeia fissa* and *Haplomitrium blumei*), and sequences, which were considered as the out-group.

3 Results and Discussion

3.1 Morphological characterization

The collected samples belonging to the family Brassicaceae were gathered after a rain shower (March- April 2018), allowing the seeds to germinate and grow. Based on the morphological features (Täckholm, 1974; Abdel Khalik et al 2002; Boulos, 2009), the collected endemic plants from the two mentioned regions (Suez Road and North-West Coast) represent five tribes; each tribe involves certain species **(Table 1)**. *Brassiceae* tribe covers ten different species **(Table 1)**, while *Lobularia spp*. and *Farsetia aegyptia* is implicated under the Alysseae tribe. Besides, *Lepidieae*, *Matthioleae*, and *Sisymbrieae* tribes are represented in the study **(Table 1)**. We have to mention that *Brassica tournefortii* and *Brassica nigra* were collected from Suez Cairo and North coast respectively. The total number of studied species is 16 plants, while the total number of selected plants in this study is 17 plants as *Matthiola longipetala* were collected twice from the two locations **(Table 1).**

3.2 Nucleotide diversity

All studied species in the Brassicaceae family were successfully amplified. The obtained sequences were searched using BLASTn

Table 1. List of 16 studied Brassicaceae family taxa, with their code, tribe information, and collection sites in Egypt

Scientific Name	Code	Tribe	Collection Location*	GPS Points	
Brassica nigra. Koch	Bn	Brassiceae	NC	30°54'54.05"N	29°32'44.05"E
Brassica tournefortii. Gouan	Bt	Brassiceae	SR	30° 0'4.89"N	32°29'7.69"E
Cakile maritima.Scop	Cm	Brassiceae	NC	30°54'54.05"N	29°32'44.05"E
Sinapis alba	Sa	Brassiceae	NC	30°50'31.68"N	29°23'38.75"E
Lepidium didymus	Ld	lepidieae	NC	30°50'31.68"N	29°23'38.75"E
Diplotaxis harra	Dh	Brassiceae	SR	30° 4'39.16"N	31°27'59.37"E
Eruca vesicaria subsp. Sativa	Es	Brassiceae	NC	30°50'31.68"N	29°23'38.75"E
Erucaria hispanica	Eh	Brassiceae	NC	30°50'31.68"N	29°23'38.75"E
Farsetia aegyptia Turra	Fa	Alysseae	SR	30° 4'3.10"N	31°26'56.64"E
Lobularia arabica	La	Alysseae	NC	30°54'54.05"N	29°32'44.05"E
Lobularia libyca	Ll	Alysseae	NC	30°54'54.05"N	29°32'44.05"E
Matthiola longipetala (SR)	MlSR	Matthioleae	SR	30° 6'31.84"N	31°30'17.82"E
Matthiola longipetala (NC)	MlNC	Matthioleae	NC	30°50'31.68"N	29°23'38.75"E
Raphanus raphanistrum	Rr	Brassiceae	NC	30°54'54.05"N	29°32'44.05"E
Raphanus sativus	Rs	Brassiceae	NC	30°54'54.05"N	29°32'44.05"E
Sisymbrium irio	Si	Sisymbrieae	SR	30° 3'52.57"N	31°25'29.01"E
Zilla spinose	Zs	Brassiceae	SR	30° 1'31.09"N	31°19'9.02"E

*NC, North-West Cost; SR, Suez Road

at NCBIhttp://www.ncbi.nlm.nih.gov/BLAST/) and compared to the full length of *rbcL* and *matK* genes. MEGA software was used to calculate the pairwise and mean nucleotide-sequence divergences with Kimura's two-parameter model and 1000 bootstrap replicates for both genes (Kimura 1980, Kumar et al 2018). Data in **Tables (2 & 3)** show the nucleotide sequence divergences (K2P distances) between the partial sequences from each species calculated based on the Multiple Sequence Alignment (MSA) (**Fig 1**). Regarding *rbcL*, the mean divergence in the studied species was 0.01% with a range of 0% to 0.025%, and the most nucleotide divergence ranged from 0% to 0.020% (**Table 2**). Also, we found that the variation in a partial segment of the chloroplast *rbcL* gene can be used to identify Brassicaceae species in line with previous DNA studies of the family (Sun et al 2015).

Among all pairwise combinations in 16 species, the mean *matK* divergence was 0.18%, with a range of 0% to 1.159%, and the most nucleotide divergence ranged from 0.012% to 0.926% (**Table 3**). When we disregarded the divergence of samples of the same genera (Raphanus and Brassica) or the same species (*Matthiola longipetala*), the *rbcL* data show a narrow range of divergence ranging from 0% between the *Sinapis alba* (*Brassiceae* tribe) and both *Brassiceae* tribe's members *Brassica nigra* and *Brassica tournefortii* to 0.025% between the *Brassiceae tribe's* member (*Raphanus sativus*) and the *lepidieae* tribe member (*Lepidium didymus*) (**Table 2**). Moreover, the *matK* data show a wide range of divergence ranging from 0.012% between the *Eruca vesicaria* subsp. *Sativa* (*Brassiceae* tribe) and another *Brassiceae tribe's* member (*Diplotaxis harra*) to 1.159% between both Alysseae tribe's members *Lobularia arabica* and *Farsetia aegyptia* (**Table 3**). Further, the rest of the species shows a moderate percentage between each other (**Table 2 & 3**). Few species represent all the tribes in this barcode, and further samplings from across their geographic ranges are required. These expanded data may increase the already relatively high

Table 2. Estimates of evolutionary divergence between rbcL sequences.

rbcL	Bn_MT747636	Bt_MT747637	Cm_MT747638	Ld_MT747639	Dh_MT747640	Eh_MT747641	Es_MT747642	Fa_MT747643	La_MT747644	Ll_MT747645	Ml_{SR}_MT747646	Ml_{NC}_MT747647	Rr_MT747648	Rs_MT747649	Sa_MT747650	Si_MT747651	Zs_MT747652
Bt_MT747637	0.000																
Cm_MT747638	0.004	0.004															
Ld_MT747639	0.020	0.020	0.016														
Dh_MT747640	0.007	0.007	0.005	0.022													
Eh_MT747641	0.007	0.007	0.004	0.020	0.005												
Es_MT747642	0.005	0.005	0.004	0.020	0.002	0.007											
Fa_MT747643	0.019	0.019	0.017	0.011	0.019	0.021	0.017										
La_MT747644	0.014	0.014	0.012	0.011	0.014	0.016	0.012	0.004									
Ll_MT747645	0.014	0.014	0.012	0.010	0.016	0.016	0.014	0.002	0.002								
Ml_{SR}_MT747646	0.016	0.016	0.014	0.020	0.016	0.018	0.014	0.012	0.012	0.013							
Ml_{NC}_MT747647	0.014	0.014	0.012	0.018	0.014	0.016	0.013	0.011	0.011	0.012	0.000						
Rr_MT747648	0.009	0.009	0.007	0.023	0.005	0.011	0.004	0.021	0.016	0.018	0.018	0.016					
Rs_MT747649	0.011	0.011	0.009	0.025	0.007	0.013	0.005	0.022	0.018	0.020	0.018	0.018	0.002				
Sa_MT747650	0.000	0.000	0.004	0.020	0.007	0.007	0.005	0.019	0.014	0.014	0.016	0.014	0.009	0.011			
Si_MT747651	0.018	0.018	0.016	0.018	0.018	0.020	0.016	0.015	0.011	0.014	0.020	0.018	0.020	0.022	0.018		
Zs_MT747652	0.007	0.007	0.005	0.016	0.007	0.009	0.005	0.017	0.013	0.016	0.014	0.013	0.005	0.007	0.007	0.016	

The number of base substitutions per site between sequences are shown. Analyses were conducted using the Kimura 2-parameter model and evolutionary analyses were conducted in MEGA X.

Table 3. Estimates of evolutionary divergence between matK Sequences. The number of base substitutions per site between sequences are shown. Analyses were conducted using the Kimura 2-parameter model and evolutionary analyses were conducted in MEGA X.

matK	Bn_ MT747653	Bt_ MT747654	Cm_ MT747655	Ld_ MT747656	Dh_ MT747657	Eh_ MT747658	Es_MT747659	Fa_MT747660	La_ T747661	Ll_ MT747662	MI_{SR}_MT747663	MI_{NC}_ MT747664	Rr_ MT747665	Rs_ MT747666	Sa_MT747667	Si_ MT747668
Bt_MT747654	0.023															
Cm_ MT747655	0.029	0.031														
Ld_MT747656	0.057	0.066	0.055													
Dh_MT747657	0.026	0.034	0.032	0.062												
Eh_MT747658	0.037	0.031	0.015	0.065	0.032											
Es_MT747659	0.020	0.022	0.022	0.055	0.012	0.031										
Fa_MT747660	0.249	0.260	0.267	0.253	0.257	0.266	0.262									
La_MT747661	0.948	0.918	0.910	0.944	0.926	0.945	0.935	1.159								
Ll_MT747662	0.054	0.056	0.048	0.047	0.059	0.065	0.051	0.247	0.940							
MI_{SR}_MT747663	0.056	0.069	0.066	0.053	0.067	0.072	0.060	0.268	0.989	0.055						
MI_{NC}_MT747664	0.059	0.065	0.068	0.053	0.068	0.088	0.057	0.275	1.079	0.048	0.014					
Rr_MT747665	0.027	0.038	0.035	0.064	0.023	0.046	0.018	0.264	0.949	0.061	0.069	0.068				
Rs_MT747666	0.024	0.035	0.033	0.067	0.023	0.042	0.016	0.266	0.971	0.063	0.067	0.068	0.000			
Sa_MT747667	0.019	0.035	0.033	0.062	0.032	0.045	0.025	0.259	0.939	0.062	0.066	0.067	0.035	0.033		
Si_MT747668	0.036	0.038	0.038	0.046	0.039	0.054	0.033	0.257	0.918	0.045	0.055	0.052	0.042	0.041	0.045	
Zs_MT747669	0.036	0.046	0.040	0.056	0.036	0.054	0.033	0.258	0.909	0.058	0.066	0.063	0.041	0.041	0.041	0.035

Table 4. Forward and reverse primers sequence for rbcL-a and matK genes.

Gene	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
rbcL-a	ATGTCACCACCAACAGAGACTAAA GC	GTAAAATCAAGTCCACCRCG
matK	CGTACAGTACTTTTGTGTTTACGAG	ACCCAGTCCATCTGGAAATCTTGGTTC

incidence of low interspecific divergence in *matK* and *rbcL* sequence among the Brassicaceae family. Further work is necessary on Brassicaceae species to produce a validated taxonomic system to aid in interpreting sequence variation.

3.3 Sequence divergences within Brassicaceae species

To check for ubiquity and prejudicial potential in plant species, one of the most studied DNA regions is the plastid genome (Kress et al 2005; Hollingsworth et al 2009). Combining of the two markers rbcL and matK was suggested for plant DNA barcoding in 2009 (CBOL Plant Working Group 2009) and to identify Brassicaceae species at molecular level (Sun et al 2015). Our results indicate that the mean *rbcL* divergence within Brassicaceae is 0.01% with a range of 0.002% to 0.025%, while mean *matK* divergence was 0.18%, with a range of 0.012% to 1.159%. This degree of divergence is more significant than that found in economic Brassicaceae species, 0.0028 (*rbcL*) and 0.0183 (*matK*) (Sun et al 2015). Likewise, our identified nucleotide-divergence within Brassicaceae is less than that found in other Egyptian ornamental genera (Elansary et al 2017). In this study, out of 189 *matK* sequences, 22 species revealed a higher (>2%), and 52 revealed a lower (<2%) interspecific divergence. At the same time, the analysis of 217 *rbcL* sequences revealed 23 species with higher (>2%) and 91 with lower (<2%) intraspecific distances (Elansary et al 2017).

3.4 Phylogenetic analysis

In Egypt, the Brassicaceae family is divided into nine tribes (Abdel, 2005). One of the drawbacks of relying on morphological identification is missing some characters, such as homoplasy, which dominates the family (Al-Shehbaz, 2012). Unfortunately, sometimes the morphological identification could be misleading; therefore, molecular studies (e.g., DNA Barcoding) are required to identify the investigated species correctly. One of the driving forces of this study that previous work shed light on the impact of climate change on some Egyptian flora (Serag et al 2018; Shaltout et al 2018).

In this study, the phylogenetic analysis is carried out by combining the obtained sequences from the endemic Brassicaceae family, and all available nucleotide-reference sequences for the two markers *rbcL* and *matK* of the Brassicaceae family database (arranged alphabetically in the supplementary file S1B). The concatenated tree based on the two markers (**Fig 2**) showed that 12 out of 16 species were completely matching with the morphological identification. Mainly, *Lepidium didymium* (Ld), *Farsetia aegyptia* Turra (Fa), *Matthiola longipetala* (MlSR and MlNC from North Coast and Suez Cairo, respectively), *Sisymbrium irio* (Si), *Zilla spinose* (Zs), *Raphanus raphanistrum* (Rr), *Raphanus sativus* (Rs), *Eruca sativa* (Es), *Diplotaxis harra* (Dh), *Sinapis alba* (Sa), *Brassica nigra* (Bn) and *Brassica tournefortii* (Bt) were forming sister-groups with the respective reference sequences (**Fig 2**). Furthermore, high bootstrap

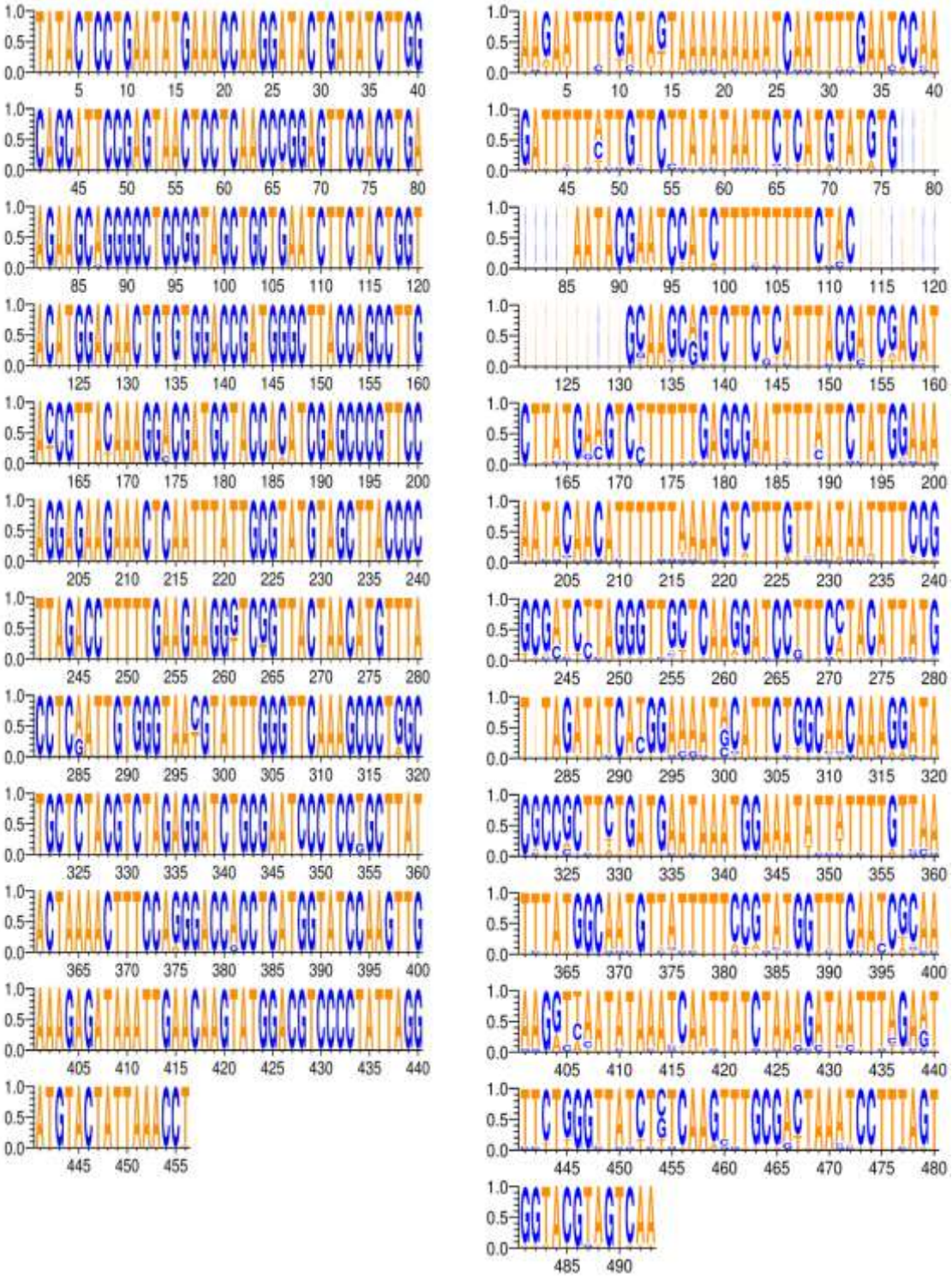

Fig 1. The consensus sequence of the obtained chloroplast gene markers. The *rbcL* (a) and *matK* (b) consensus sequence of the investigated 16 Brassicaceae species

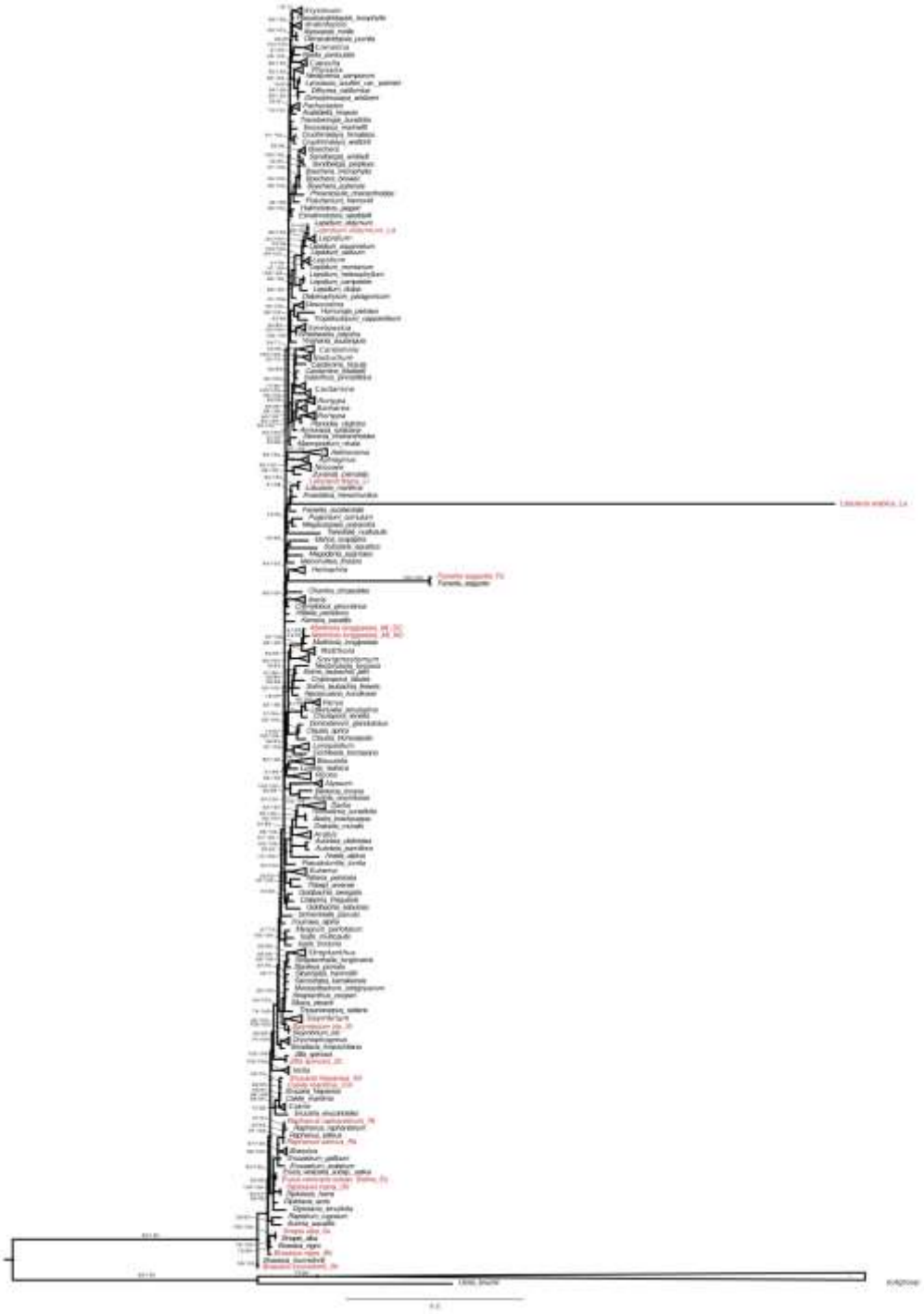

Fig. 2. Maximum likelihood of concatenated phylogenetic tree based on *rbcL* and *matK* nucleotide sequences. The evolutionary relationships between the Egyptian Brassicaceae endemic species and the available references-sequences in the NCBI GenBank database (08/2020). The obtained sequences from the current study were colored in red, and the maximum likelihood branch bootstrap support values are given in %.

supports were obtained for the two markers of 12 species are following the morphological identification. However, there remains a remarkable uncertainty level for four species, such as *Lobularia, Erucaria,* and *Cakile*. Although they have been identified based on the pods' morphology, the phylogenetic tree negates such identification, which could be attributed to sequencing artifacts or morphological misidentification (German et al 2011; Couvreur et al 2010). Therefore, we should mention that relying on pods morphology is not compelling evidence of the plant species identification; such identification should be supported with molecular studies to validate the taxa.

In conclusion, the primary target beyond this study is to authenticate the Egyptian endemic plant wealth to go further with the downstream studies related to the active components used in the pharmacological and medical sectors. This study constitutes the first report exploring the molecular phylogeny between the Egyptian Brassicaceae tribes based on DNA sequencing, not only on the morphological identification. To obtain a robust phylogenetic tree and get a highly accurate relationship between the target species, we relied on the standard barcode loci (*rbcL-a* and *matK*) not only for the collected species but along with 117 Brassicaceae genera in the database. This study provides robust assessment data by making use of DNA barcoding applications to validate Egyptian endemic plants. However, future work should be done using more than two genetic markers supplemented with biochemical studies that should together enrich the plant barcoding data.

4 Conclusions

In Egypt, the geographical distribution of endemic plants (Brassicaceae) is still undescribed. Besides, under the current climate conditions, and its future variation concerning the climate change issue. It was substantial to take part and start the first step regarding validating the Egyptian endemic plants presented in Brassicaceae. This study confers preliminary assessment data that will be useful in DNA barcoding investigations for endemic plants. We found that DNA barcoding is essential for accurate plant species identification with the current concatenated tree, when relying only on morphology characters might be misleading. However, further protocol development to increase the number of plants, various gene markers, including different regions, and local authenticated databases would enrich plant barcoding efficiency.

5 Acknowledgments

The authors would like to express deep thank to Prof. Abd El-Halim Abd El-Mogali Mohamed for his invaluable help for sample authentication using morphological characters. The authors would also like to pay their gratitude to our mentor and professor, the late Prof. Dr. Aly Z. Abdelsalam, who initiated this work before his leave ‘may God bless his soul’.

References

Abdel khalik, K; Van Der Maesen, LJG; Koopma, NWJM; Van Denberg, RG (2002) Numerical taxonomic study of some tribes of Brassicaceae from Egypt. *Plant Syst Evol* 233, 207-221.

Abdel Khalik, K (2005) Morphological studies on trichomes of Brassicaceae in Egypt and taxonomic significance. *Acta Botanica Croatica* 64, 57-73.

Abdelaal, M; Fois, M; Fenu, G; Bacchetta, G (2018) Critical checklist of the endemic vascular plants of Egypt. *Phytotaxa* 360, 19-34.

Al-Gendy, AA; Nematallah, KA; Zaghloul, SS; Ayoub, NA (2016) Glucosinolates profile, volatile constituents, antimicrobial, and cytotoxic activities of Lobularia libyca. *Pharm Biol* 54, 3257-3263.

Al Shehbaz, IA (2012) A generic and tribal synopsis of the Brassicaceae (Cruciferae). *Taxon* 61, 931-954.

Amer, W; Soliman, A; Hassan, W (2015) Floristic composition of Nile islands in Middle Egypt, with special reference to the species migration route. *Journal of American Science* 11, 14 -23.

Asahina, H; Shinozaki, J; Masuda, K; Morimitsu, Y; Satake, M (2010) Identification of medicinal Dendrobium species by phylogenetic analyses using matK and *rbcL* sequences. *J Nat Med* 64,133-138.

Boulos, L (2009) Flora of Egypt Checklist, Revised Annotated Edition. Al- Hadara publishing, Cairo. p 410.

Capella-Gutiérrez, S; Silla-Martínez, JM; Gabaldón, T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25, 1972-1973.

CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proc Natl Acad Sci* USA. 106, 12794-12797.

Couvreur, TL; Franzke, A; Al-Shehbaz, IA; Bakker, FT; Koch, MA; Mummenhoff, K (2010) Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). *Mol Biol Evol* 27, 55-71.

Danlami, U; Orishadipe Abayomi, T; Lawal, DR (2016) Phytochemical, nutritional and antimicrobial evaluations of the aqueous extract of Brassica nigra (Brassicaceae) Seeds. *Am J Appl Chem* 4, 161-163.

Darriba, D; Taboada, GL; Doallo, R; Posada, D (2011) Prot Test 3: fast selection of best-fit models of protein evolution. Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics). *Bioinformatics.* 27, 1164-1165.

Elansary, HO; Ashfaq, M; Ali, HM; Yessoufou, K (2017) The first initiative of DNA barcoding of ornamental plants from Egypt and potential applications in horticulture industry. *PLoS ONE* 12 e0172170

Fahey, JW; Zalcmann, AT; Talalay, P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56, 5-51.

German, DA; Grant, JR; Lysak, MA; Al Shehbaz, IA (2011) Molecular phylogeny and systematics of the tribe Chorisporeae (Brassicaceae). *Plant Systematics and Evolution* 29, 65-86.

Hollingsworth, ML; Andra Clark, A; Forrest, LL; Richardson, J; Pennington, RT; Long, DG; Cowan, R; Chase, MW; Gaudeul, M; Hollingsworth, PM (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species level sampling in three divergent groups of land plants. *Mol Ecol Resour* 9, 439-57.

Hosni, H; Hosny, A; Shamso, E; Hamdy, R (2013) Endemic and Near-endemic Taxa in the Flora of Egypt. *Egyptian Journal of Botany* 53, 357-383.

Katoh, K; Standley, DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30, 772-780.

Kimura, M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111-120.

Kozlov, AM; Darriba, D; Flouri, T; Morel, B; Stamatakis, A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35, 4453-4455.

Kress, WJ; Wurdack, KJ; Zimmer, EA; Weigt, LA; Janzen, DH (2005) Use of DNA barcodes to identify flowering plants. *Proc Natl Acad Sci* USA. 102, 8369-8374.

Kumar, S; Stecher, G; Li, M; Knyaz, C; Tamura, K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547-1549.

Maloukh, L; Kumarappan, A; Jarrar, M; Salehi, J; El-Wakil, H; Rajya Lakshmi, TV (2017) Discriminatory power of rbcL barcode locus for authentication of some of United Arab Emirates (UAE) native plants. 3 *Biotech.* 7, 144**.**

Marzouk, MM; Al-Nowaihi, ASM; Kawashty, SA; Saleh, NAM (2010) Chemosystematic studies on certain species of the family Brassicaceae (Cruciferae) in Egypt. *Biochem. Syst.* Ecol. 238, 680-685.

Minh, BQ; Schmidt, HA; Chernomor, O; Schrempf, D; Woodhams, MD; von Haeseler, A; Lanfear, R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era.37, 1530-1534.

Mohamed, AH (2009) Molecular Systematic of Some Brassicaceae Taxa in Egypt Based on Electrophoretic Isoenzymes Pattern and RAPD Markers**.** *Australian Journal of Basic and Applied Sciences* 3, 1499-1511.

Nurzyńska-Wierdak, R (2015) Biological value of Eruca Sativa mill. leaves under the different plant nutrition by nitrogen and potassium**.** *Acta Sci Pol Hortorum Cultus* 14, 41-53.

Omer, E; Elshamy, A; El Gendy, A; Cai, X; Sirdaarta, J; White, A; Cock, I (2016) *Cakile maritima* Scop. extracts inhibit the growth of ome bacterial triggers of autoimmune diseases: *GC-MS analysis of an inhibitory extract. Pharmacognosy Journal* 8, 361-374.

Sanger, F; Coulson, AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* 94, 441-448.

Serag, M; Moustafa, A; Qiqa, S (2018) Impact of Climate Change on Surviving of

Phlomis aurea as an Endemic Species Growing in Southern Sinai, Egypt. Catrina: *The International Journal of Environmental Sciences* 17, 33-39.

Shaltout, K; Ahmed, D; Diab, M; El-Khalafy, M (2018) Re-assessment of the near- endemic taxa in the Egyptian flora. *Taeckholmia* 38, 61-83.

Starr, JR; Naczi, RFC; Chouinard, BN (2009) Plant DNA barcodes and species resolution in sedges (Carex, Cyperaceae). *Molecular Ecology Resources* 9, 151-163.

Sun, XQ; Qu, YQ; Yao, H; Zhang, YM; Yan, QQ; Hang, YY (2015) Applying DNA barcodes for identification of economically important species in Brassicaceae. *Genet Mol Res* 14, 15050-15061.

Täckholm, V (1974) Students' Flora of Egypt. 2nd Edition, *Cairo University Publishing*, Beirut, p 888

مجلة اتحاد الجامعات العربية للعلوم الزراعية، جامعة عين شمس، القاهرة، مصر
مجلد(29)، عدد(1)، 387 - 399، 2021
Website: http://ajs.journals.ekb.eg
DOI: 10.21608/ajs.2021.49712.1298

تحقق علاقات القرابة لبعض الأنواع المستوطنة للعائلة الصليبية (الخردلية) المصرية [26]

شيماء أحمد على عبدالهادي[1*] – عبدالله أبو النصر شرف[1] – على زين العابدين عبدالسلام[1] – خالد عبدالعزيز سليمان[1] – أسماء محمد أبو شادي[2،1]

1- قسم الوراثة – كلية الزراعة – جامعة عين شمس – ص.ب 68 – حدائق شبرا 11241 – القاهرة – مصر

2- كلية البيونكنولوجى – جامعة النيل – الشيخ زايد – الجيزة – مصر

*Corresponding author: shahmed_2010@agr.asu.ed.eg

Received 12 January, 2021 Accepted 9 March, 2021

الموجـــــز

تعد ظاهرة الاحتباس الحراري الناشئ عن زيادة النشاط البشري من التهديدات الحاسمة للقضاء على التنوع البيولوجي في المناطق القاحلة وشبه القاحلة. ومن المتوقع أن تشهد مصر انخفاضًا حادًا في هطول الأمطار وزيادة معدلات درجات الحرارة في العقود القليلة القادمة، مما يؤدي إلى التحديد الجغرافي للعديد من الأنواع النباتية، حيث تتأثر النباتات المستوطنة بشدة في ظل هذه الظروف، والتي ستؤدي تدريجياً إلى فقدان الموارد الوراثية لثروتنا النباتية. تعتبر العائلة الصليبية (الخردلية) (Brassicaceae) من العائلات النباتية ذات الأهمية الاقتصادية في القطاع الزراعي والطبي والصيدلي نظراً لاحتوائها علي العديد من المركبات النشطة علي سبيل المثال مركبات الجلوكوزينولات والتي تلعب دورًا حيويا كمضادات للميكروبات والعديد من مسببات الأمراض الزراعية مثل إصابات الحشائش والحشرات والنيماتودا، بالإضافة إلى احتوائها على كمية كبيرة من مضادات الأكسدة التي تلعب دوراً هاماً في علاج بعض الأمراض الروماتيزمية وتستخدم كمثبطات للأورام. يقتصرتعريف نباتات معظم العائلات النباتية المصرية علي التوصيف المورفولوجي أساسا، لذا أصبح من الضروري الحفاظ علي الأصول النباتية وتوصيفها توصيفا جزيئيا كاملا وتوثيقها ببنك الجينات والأصول الوراثية وتحديد علاقات القرابة والعلاقات التطورية لنباتات العائلة الواحدة. تقوم دراسة هذا البحث علي عمل تحليل شجرة القرابة والعلاقات التطورية (phylogenetic analysis) لعدد 16 نوعًا من العائلة الصليبية (الخردلية) باستخدام الترميز الجزيئي (DNA Barcode) لاثنان من جينات البلاستيدة الخضراء وهما (rbcL–a) و (matK) باستخدام طريقة التحليل (maximum likelihood) للتتابعات الخاصة بالأنواع النباتية تحت الدراسة والتي تتميز بكونها عالية التخصص، مع مقارنتها بالتتابعات المرجعية لأنواع نفس العائلة علي قواعد البيانات المتخصصة. حيث تم الحصول علي شجرة القرابة المفصلة للأنواع النباتية المصرية ومقارنتها بعدد 244 نبات من العائلة الصليبية معتمداً علي تحليل التتابعات الجينية المتخصصة تعتبر هذه الدراسة هي الخطوة الأولى للتعريف الجزيئي وحفظ الأنواع النباتية تحت هذه العائلة وتسجيلها باكواد متخصصة علي قاعدة البيانات والتي يجب أن تليها دراسات كيميائية حيوية تخدم المجالات المختلفة.