Phylogeny Validation for Some Egyptian Brassicaceae Endemic Species

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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 steno-endemic, 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 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™ 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) 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 model for nucleotide substitution. 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 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 (Calypogea fissa and Haplomitrium blumen), 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°04’89"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°54’54.05"N    | 29°32’44.05"E   |
| Diplotaxis harra                       | Dh   | Brassiceae     | SR                   | 30°43’9.16"N     | 31°27’59.37"E   |
| Eruca vesicaria subsp. Sativa          | Es   | Brassiceae     | NC                   | 30°50’31.68"N    | 29°23’38.75"E   |
| Eruca hispanica                        | Eh   | Brassiceae     | NC                   | 30°50’31.68"N    | 29°23’38.75"E   |
| Farsetia aegyptia Turra               | Fa   | Alysseae       | SR                   | 30°43’10"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°63’1.84"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   | Sisymbriea     | SR                   | 30°35’2.57"N     | 31°25’29.01"E   |
| Zilla spinose                          | Zs   | Brassiceae     | SR                   | 30°13’1.09"N     | 31°19’9.02"E    |

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

at NCBI (http://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       | Bt_MT747637 | Bt_MT747638 | Cm_MT747638 | Ld_MT747639 | Db_MT747640 | Eh_MT747641 | Es_MT747642 | Fa_MT747643 | La_MT747644 | Li_MT747645 | Mllc_MT747646 | Mllc_MT747647 | Mllc_MT747648 | Mllc_MT747649 | Mllc_MT747650 | Mllc_MT747651 | Mllc_MT747652 |
|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Bt_MT747637 | 0.000       |             |             |             |             |             |             |             |             |             |                 |                 |                 |                 |                 |                 |
| Cm_MT747638 | 0.004       | 0.004       |             |             |             |             |             |             |             |             |                 |                 |                 |                 |                 |                 |
| Ld_MT747639 | 0.020       | 0.020       | 0.016       |             |             |             |             |             |             |             |                 |                 |                 |                 |                 |                 |
| Db_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       |             |             |                 |                 |                 |                 |                 |                 |
| Li_MT747645 | 0.014       | 0.014       | 0.012       | 0.010       | 0.016       | 0.014       | 0.002       | 0.002       |             |             |                 |                 |                 |                 |                 |                 |
| Mllc_MT747646 | 0.016     | 0.016       | 0.014       | 0.020       | 0.016       | 0.018       | 0.014       | 0.012       | 0.012       | 0.013       |                 |                 |                 |                 |                 |                 |
| Mllc_MT747647 | 0.014     | 0.014       | 0.012       | 0.018       | 0.014       | 0.016       | 0.013       | 0.011       | 0.011       | 0.012       | 0.000           |                 |                 |                 |                 |                 |
| Mllc_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       | 0.005       | 0.019       | 0.014       | 0.016       | 0.014       | 0.009           | 0.011           |                 |                 |                 |                 |
| Si_MT747651 | 0.018       | 0.018       | 0.016       | 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 | Bl_MT747654 | Cm_MT747655 | Ld_MT747656 | Dh_MT747657 | Eh_MT747658 | Es_MT747659 | Fa_MT747660 | La_MT747661 | LL_MT747662 | Mlssr_MT747663 | Msnc_MT747664 | Rr_MT747665 | Rs_MT747666 | Sa_MT747667 | Si_MT747668 | Zs_MT747669 |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|-------------|-------------|-------------|-------------|-------------|
| 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       |             |                 |                |             |             |             |             |             |
| Mlssr_MT747663 | 0.056 | 0.069 | 0.066 | 0.053 | 0.067 | 0.072 | 0.060 | 0.268 | 0.989 | 0.055 |
| Msnc_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 |
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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                | GTAAATCAAGTCCACCRCG                         |
| matK   | CGTACAGTACTTTTGTGTTFACGAG                 | ACCCAATCATCGAAATGTTGTTTC                   |

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 ditymium (Ld), Farsetia aegyptia Turra (Fa), Matthiola longipetala (MISR 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.

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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 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.

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تحقيق علاقات القرابة لبعض الأنواع المستوطنة للعائلة الصليبية (الخردلية) المصرية

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الموجز

تعتبر ظاهرة الاحتباس الحراري الناشئة عن زيادة النشاط البشري من التهديدات الحاسمة لقضاء العائلات النباتية. ويتوقع 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 سيتدريجياً إلى فقدان الرياح والображات النباتية لثروتنا النباتية. تعتبر العائلة الصليبية (الخردلية) من العائلات النباتية ذات الأهمية الاقتصادية في القطاع الزراعي والطبي والصيدلاني نظرًا لاحترامها على العديد من المراعيات والمثبتات، حيث تلعب دورًا حيويًا كمضادات للميكروبات والديدان من مسببات الأمراض الزراعية مثل إصابات الحشرات والبكتيريا. بالإضافة إلى احتوائها علي كمية كبيرة من مضادات الأكسدة التي تلعب دورًا هاماً في علاج بعض الأمراض الروماتيزمية وتستخدم كمضادات للألم. يقتصر التعريف لنباتات العائلة الصليبية على التوصيف المورفولوجي أساسيًا، لذا أصبح من الضروري الحفاظ على الأصول الباقية وتوحيدها. توحيد النباتات من الوراثة وتحديث علاقات القرابة والتطورية للنباتات، يتطلب دراسة هذا البحث على عمل تحديد شجرة القرابة والعلاقات الطورية (phylogenetic analysis) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام الترميز الجزيئي (DNA) (الصاربية) باستخدام طرق التحليل (matK) و (rbcl-a) للانتقادات الخاصة بالأنواع (maximum likelihood) النباتية التي تتميز بكفاءة عالية التخصص، مع مقارنتها بالانتقادات المرجعية لأنواع نفس العائلة على قواعد البيانات المتخصصة. حيث تم الحصول علي شجرة القرابة الفعلية للأنواع النباتية المصرية، ومقارنتها بعدد 244 نبات من العائلة الصليبية. تم استمرار على تحديد التتابعات الجينية المتخصصة تعتبر هذه الدراسة الخطوة الأولى نحو تعرفها، وفه مسماة بعلميات المتخصصة في قاعدة البيانات التي يجب أن تنطلق دراسات كيميائية حيوية تهتم المجالات المختلفة.