Two Particularly Evolutionary Loci of matK of cpDNA of Genera of Magnoliaceae

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Abstract: In order to quickly identify Magnoliaceae plants and scientifically correct the misidentification of the samples of plastid complete genomes in the NCBI (National Center for Biotechnology Information, USA) database, total 260 samples of Magnoliaceae, 196 of Yulania Spach, 40 of Magnolia L., 19 of Michelia L., and 5 of Liriodendron L. were collected and the partial sequences of matK gene were amplified and sequenced respectively. The results indicated that there are two particular loci in the partial sequences, matK (...AAGGAATGATTGTATAA...CCAAAAATMGACAAGGTG...) (N = A, G; M = A, G, T) of Magnoliaceae, which can be used to identify the family because they are not possessed by other families. They are also PEL (particularly evolutionary loci) of genera of Magnoliaceae, which can be used to quickly identify the genera of the family, for all samples of the genus of Yulania Spach are with the loci of matK (...AAGGAATGATTGTATAA...CCAAAAATAGACAAGGTG...), all samples of Magnolia L. with matK (...AAGGAATGATTGTATAA...CCAAAAATGGACAAGGTG...), all samples of Michelia L. with matK (...AAGGAATGATTGTATAA...CCAAAAATGGACAAGGTG...), and all samples of Liriodendron L. with matK (...AAGGAATGATTGTATAA...CCAAAAATGGACAAGGTG...). So, in four genera of Magnoliaceae, Yulania Spach, Michelia L. and Liriodendron L. have the respective PEL, and Magnolia L. can also be easily identified for having the oppositely evolutionary loci of other 3 genera. Based on the two PEL, the misidentified samples of plastid complete genome in NCBI were listed, which included 11 samples of Yulania Spach and 3 samples of Michelia L.. Being simple and reliable, PEL is a scientific method to identify the evolutionary taxa, which can effectively overcome the limitations of being partial and subjective in Taxonomy and Phylogeny.

Keywords: PEL (Particularly Evolutionary Loci), matK, Genera, Magnoliaceae, Evolutionomy

1. Introduction

Magnoliaceae Juss. [1-14] have many primitive characters, such as the branchlets with annular stipular scars, the flowers with spathaceous perules and bracts, and androecium and gynoeicum spirally arranged on the elongate receptacle, which are the most primitive taxa of Fructophyta [15] and take an extremely important role in the study of evolutionomy of fruit plants. Some of them are rare taxa, with narrow geographical distribution, and extremely high scientific research value, such as Yulania puberula D. L. Fu. Many tree species such as Michelia L. and Magnolia L. are important tree species of the evergreen broad-leaved forests from the central subtropical to the southern subtropical zone. They are also important broad-leaved timber species, which are of good quality and are used for fine furniture. Some trees with large, beautiful and aromatic flowers, are important species for garden viewing, such as Yulania denudata (Desr.)
D. L. Fu, Y. liliiflora (Desr.) D. L. Fu, Y. campbellii (Hook. f. et Thom.) D. L. Fu and Magnolia grandiflora L.. Some species are important aromatic and medicinal species, such as Magnolia officinalis Rehd. & Wils and Yulania biondii (Pamp.) D. L. Fu, have significant economic benefits. Some tree species, such as Yulania biondii (Pamp.) D. L. Fu have strong vitality, strong adaptability and developed root system, which are important tree species for barren hill greening and preservation of water and soil.

Scientific identification of taxa of Magnoliaceae is the basis for the research and utilization of the plant resources. Due to the complexity of morphological evolution, the traditional morphological taxonomy often leads to the misidentification of the taxa of Magnoliaceae even by the taxonomic experts of the family. The genomes of plants have a large number of evolutionary codes, which are scientific means for identifying the evolutionary taxa of plants. However, in the process of chloroplast genomic research of Yulania Spach, it was found that the chloroplast genomes of Magnoliaceae in the NCBI (National Center for Biotechnology Information, USA) database have also the phenomenon of misidentification of evolutionary taxa as the traditional taxonomy, which maybe is inseparable with the possible existence of the partiality and subjectivity of traditional taxonomy and phylogenetic theory. How to scientifically avoid this unscientific phenomenon has always been the problem that the authors have begun to solve. It has been found that, based on the evolutionary continuity principle [15] and evolutionary particularity principle, the PEL (particularly evolutionary loci) of evolutionary taxa can be used to quickly identify the plants of Magnoliaceae and scientifically to distinguish different evolutionary taxa of Magnoliaceae.

2. Materials & Methods

2.1. Plant Materials

The leaves of 4 genera of Magnoliaceae, total 260 samples, 196 of Yulania Spach, 40 of Magnolia L., 19 of Michelia L., and 5 of Liriodendron L., were collected from Henan, Shanxi, Sichuan, Yunnan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang, Guangdong province, and Xizang Autonomous Region of China. The samples include some representative species of the family, such as Liriodendron chinense L., T. zelkova; Magnolia decidua (Q. Y. Zheng) V. S. Kumar, M. delavayi Franchet, M. fordiana (Oliver) Hu, M. globosa Hook. f. & Thomson, M. grandiflora L., M. henryi Dunn, M. hodgsoni (Hook. f. & Thom.) H. Keng, M. insignis Wallich, M. kwangsiensis Figlar & Nootboon, M. officinalis Rehd. & Wils., M. omeiensis (W. C. Cheng) Dandy, M. rostrata W. W. Smith, M. sieboldii K. Koch; Michelia alba DC., M. baillonii Finet & Gagnep, M. balansae Dandy, M. chapensis Dandy, M. figo (Lour.) Spreng, M. odorata (Chun) Noot. & B. L. Chen, Yulania acuminata (L.) D. L. Fu, Y. biondii (Pamp.) D. L. Fu, Y. campbellii (Hook. f. & Thomson) D. L. Fu, Y. cylindrica (Wils.) D. L. Fu, Y. dawsoniana (Rehd. & Wils.) D. L. Fu, Y. kobus (DC.) Spach, Y. liliiflora (Desr.) D. L. Fu, Y. pendula D. L. Fu et al., Y. puberula D. L. Fu, Y. salicifolia (Sieb. & Zucc.) D. L. Fu, Y. sargentiana (Rehd. & Wils.) D. L. Fu, Y. zizhenii D. L. Fu et F. W. Li, Y. sinostellata (P. L. Chu & Z. H. Chen) D. L. Fu, Y. sprengerii (Pamp.) D. L. Fu, Y. stellata (Sieb. & Zucc.) D. L. Fu, Y. urceolata D. L. Fu, B. H. Xiong et X. Chen, Y. viridula D. L. Fu, T. B. Zhao et G. H. Tian, Y. zenii (Cheng) D. L. Fu, etc. (see Table 1).

Table 1. Experimental materials of Magnoliaceae in the study.

| Genus      | Samples | Species                                                                 | Collected place         |
|------------|---------|-------------------------------------------------------------------------|-------------------------|
| Liriodendron | 5       | Liriodendron chinense, L. tulpitifera                                   | Henan, Shanxi            |
| Magnolia   | 40      | Magnolia acissae, M. albospericera, M. chinii, M. coco, M. decidua, M. delavay, M. fordiana, M. globosa, M. grandiflora, M. henryi, M. hodgsoni, M. insignis, M. kwangsiensis, M. lucida, M. megaphylla, M. moto, M. nitida, M. officinalis, M. omeiensis, M. otangensis, M. pachyphylla, M. rostrata, M. sieboldii, M. tripetala, M. yunnanensis, M. yuanyagensis, etc. | Henan, Shaxi, Sichuan, Yunnan, Hubei, Jiangsu, Guangdong, Xizang |
| Michelia   | 19      | Michelia alba, M. baillonii, M. balansae, M. chapensis, M. crassipes, M. elegans, M. figo, M. gioi, M. macclurei, M. martini, M. maulana, M. mediocriis, M. odorata, M. platypetala, M. wilsonii, etc. | Henan, Sichuan, Yunnan, Guangdong, Jiangsu |
| Yulania    | 196     | Yulania acuminata, Y. amoena, Y. anhueiensis, Y. axilliflora, Y. baotaina, Y. biondii, Y. campbellii, Y. cuneatifolia, Y. cylindrica, Y. dawsoniana, Y. denudata, Y. dimorpha, Y. diva, Y. elliptigemnata, Y. elliptilimba, Y. funishanensis, Y. honaensis, Y. huaingisensis, Y. jigionshanhensis, Y. kobus, Y. liliiflora, Y. pendula, Y. pilocarpa, Y. puberula, Y. pyrifolium, Y. salicifolia, Y. sargentiana, Y. shireishanensis, Y. shizhenii, Y. sinostellata, Y. sprengerii, Y. stellata, Y. urceolata, Y. viridula, Y. wufengensis, Y. wugangensis, Y. xingyuanensis, Y. zenii, Y. zhangyangylan, etc. | Henan, Sichuan, Sichuan, Yunnan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang, Guangdong, Xizang |

* The italic names of Yulania Spach are initially determined to be synonyms.

2.2. PCR Primer Design

A pairs of primers, matK-Y01-F and matK-Y01-R, were designed using Primer Premier 6, the sequences of primers and the length of amplification and sequencing of cpDNA, see Table 2.

Table 2. The designed primers for amplification and sequencing of partial cpDNA of Magnoliaceae.

| Primer name | Primer sequences                           | Length of amplification and sequencing /bp |
|-------------|-------------------------------------------|-------------------------------------------|
| matK_Y01    | F: 5'-GAGCCAAAGTGTCTAGCACAACG-3'          | 832                                       |
|             | R: 5'-CACTGCTGGATACAAAGATGCC-3'           |                                            |
2.3. PCR Amplification

Total genomic DNA was isolated from silica-dried leaves of 260 samples of 4 genera, 196 of Yulania Spach, 40 of Magnolia L., 19 of Michelia L. and 5 of Liriodendron L., using a modified CTAB method [16]. The primers of PCR amplification are matK_Y01 (see Table 2). PCR amplifications were performed in 15 µL volume containing 1µL genomic DNA, 7.5 µL 2x Es Taq MasterMix, 0.2 µL forward primer and 0.2 µL reverse primer, 6.1 µL ddH2O, and with the following cycles: 5 min initial denaturation at 94°C; 10 cycles of 30 s at 94°C, 45 s at 61°C and 2 min at 72°C; 27 cycles of 30 s at 94°C, 45 s at 56°C and 2 min at 72°C; and 5 min final extension at 72°C. PCR reactions were carried out in T-gradient (Biometra). The amplified products were extracted and purified with the Gel Extraction Kit (OMEGA).

2.5. DNA sequence Analysis

The partial sequences of absolutely coincident sequencing using the forward primer and reverse primer were analysis. The particularly evolutionary loci could be easily found out by the forward primer and reverse primer were an alysis.
AAATCATACAGCACTACTACAAGATGTTCTATT
TTCCATAGAAATGTGTTGTCAGCATGAAAAGGTTCCAG
AGGATGGTATCGTAATGAGAAGATTGTTACGGAGAAACACTAATACGGATTCCATATCAT

Compared to the samples of the same genus and other genera, it can be found that all samples of the genus of Yulania Spach with the loci of matK (...AAGGAA TGA TTGTA TAA...CCAAAAA TGGACAAGGGT...), all samples of the genus of Michelia L. with the loci of matK (...AAGGAA TGA TTGTA TAA...CCAAAAA TGGACAAGGGT...), and the PEL of the genus is Magnoliaceae in the NCBI database were compared. The compared two particularly evolutionary loci of the family of Magnoliaceae, because the other families do not possess the loci. So two loci both can be used to quickly distinguish the plants of Magnoliaceae.

| Species                        | DNA number in NCBI | Species                        | DNA number in NCBI |
|--------------------------------|--------------------|--------------------------------|--------------------|
| Liriodendron chinense          | NC030504.1         | Magnolia pyramidata            | NC023236.1         |
| Liriodendron tulipifera        | DQ899947.1         | Magnolia sinica                | NC023241.1         |
| Magnolia aromatica             | NC037000.1         | Magnolia tripetala             | NC024027.1         |
| Magnolia conifera              | NC037001.1         | Magnolia yunnanensis           | NC024545.1         |
| Magnolia dandyi                | NC037004.1         | Michelia cathcartii            | NC022324.1         |
| Magnolia dealbata              | NC023235.1         | Michelia laevifolia            | NC035956.1         |
| Magnolia duclouxii             | NC037002.1         | Michelia odora                 | NC023239.1         |
| Magnolia fordiana var. calcarea| MF990562.1         | Yulania acuminata              | JX280391.1         |
| Magnolia glaucifolia           | NC037003.1         | Yulania biondii                | KY085894.1         |
| Magnolia grandiflora           | JN867584.1         | Yulania demudata               | JN227740.1         |
| Magnolia grandiflora           | JN867587.1         | Yulania demudata               | JN867577.1         |
| Magnolia insignis              | MF990566.1         | Yulania demudata               | JX280394.1         |
| Magnolia kwangsiensis          | HM775382.1         | Yulania diva?                  | NC023242.1         |
| Magnolia officinalis           | JN867579.1         | Yulania kobus                  | NC023237.1         |
| Magnolia officinalis           | JN867581.1         | Yulania liliiflora             | NC037005.1         |
| Magnolia officinalis           | JN867582.1         | Yulania liliiflora             | NC023238.1         |
| Magnolia officinalis           | JN867583.1         | Yulania liliiflora             | JX280397.1         |
| Magnolia officinalis var. biloba| JN867580.1         | Yulania liliiflora             | NC023240.1         |

4. Misidentification of Chloroplast Complete Genomes of Magnoliaceae

Mainly based on the two PEL of partial sequences of matK, 39 chloroplast complete genomes of different samples of Magnoliaceae in the NCBI database were compared. The misidentified samples of chloroplast complete genomes were listed in italic and the correct names were given (see Table 3), which included 11 samples of Yulania Spach and 3 samples of Michelia L. Those are NC023234.1.Michelia cathcartii, NC035956.1.Michelia laevifolia, KY921716.1.Michelia sp., JX280391.1.Yulania acuminata, KY085894.1.Yulania biondii, JN227740.1.Yulania demudata, JN867577.1.Yulania demudata, JX280394.1.Yulania demudata, NC023242.1.Yulania diva? NC023237.1.Yulania kobus, NC037005.1.Yulania liliiflora, NC023238.1.Yulania liliiflora, JX280397.1.Yulania liliiflora, JX280394.1.Yulania liliiflora, NC023240.1.Yulania salicifolia.

5. Conclusion

Based on the evolutionary continuity principle and evolutionary particularity principle, with large numbers of repeated samples of Yulania Spach and Liriodendron L. and some representative samples of Magnolia L. and Michelia L., two particularly evolutionary loci of matK of cpDNA of genera of Magnoliaceae are found, and total 14 misidentified samples of chloroplast complete genomes of Magnoliaceae in NCBI were scientifically corrected. Being simple and reliable, PEL is a scientific method to identify the evolutionary taxa, which can effectively overcome the limitations of being partial and subjective in Taxonomy and Phylogeny.

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