A Preliminarily Phylogeny Study of the Eriobotrya Based on the nrDNA Adh Sequences

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Abstract

Phylogenetic relationships of the genus Eriobotrya Lindl. were examined based on the nrDNA Adh sequences. A phylogenetic tree of 14 loquat accessions (species, varieties and forma) was generated by using Photinieae serrulata L. as an outgroup and Rhaphiolepis indica (L.) Lindl. as an ingroup, which represent the two closest genera of Eriobotrya. The results showed that these loquat accessions were divided into two main clades in the consensus tree. Clade I included E. seguinii Card and group A formed by E. henryi Nakai, E. bengalensis Hook.f., and forma angustifolia Vidal. Clade II is composed of the other taxa which included three groups. E. cavaleriei Rehd and E. fragrans Champ formed group B; group C consisted of E. prinoides Rehd. & Wils. var. dadunensis H.Z.Zhang, and E. japonica Lindl.; and group D included E. deflexa Nakai and E. deflexa Nakai var. deflexa Nakai var. Kwan. Since E. deflexa Nakai, E. deflexa Nakai var. deflexa Nakai var. Kwan. and E. kwangsiensis Chun are closer in the phylogenetic tree; while E. prinoides Rehd. & Wils. var. dadunensis H.Z.Zhang, E. japonica Lindl. E. prinoides Rehd & Wils. and E. elliptica Lindl. were close with each other, they may be located at a similar place of the phylogenetic stage. However, E. malipoensis Kuan need further studies on its phylogenetic relationship for it was separated from the others. Results further support the theory that E. cavaleriei Rehd could be a variety of E. fragrans Champ.

Keywords: Adh gene, Eriobotrya, loquat, phylogenesis

Introduction

Eriobotrya Lindl. belongs to the family Rosaceae, sub-family Maloideae (Lindley, 1822). The genus Eriobotrya Lindl. comprises 21 species including varieties and forms, with Yunnan Province in China the centre of diversity (Yang, 2005; Yang et al., 2005). Further research on the origin and evolution of Eriobotrya is important to establish a solid foundation to achieve improvement of cultivated species such as loquat (Eriobotrya japonica Lindl.) and to further exploit species of Eriobotrya plants for industrial and pharmaceutical purposes.

In previous studies, the interspecific relationship, origin and evolutionary development of Eriobotrya were studied by morphology and molecular markers (Cheng et al., 2003; Vilanova et al., 2001). Li et al. investigated the molecular phylogeny of Eriobotrya by ITS sequence and suggested that the taxonomic treatment of E. cavaleriei Rehd as a variety of E. fragrans Champ (Li et al., 2009). However, results from different studies were not always consistent.

Previous studies have demonstrated that fast evolving introns of low-copy nuclear genes could provide more essential phylogenetic information than chloroplast or nuclear genes to better define interspecific relationships. In addition, Adh gene structure is a useful tool for improving the robustness of phylogenetic reconstruction at all taxonomic levels (Small and Wendel, 2000). Moreover, Adh is also an acetaldehyde-metabolic related gene and exists in both animals and plant. Thus, the Adh gene was selected to study the relationship and evolution of Eriobotrya.

Materials and methods

Sixteen accessions were selected as experimental materials, including 14 Eriobotrya and two relative genera. All of them were preserved ex situ for the future research in the Loquat Germplasm Repository of Horticultural College, South China Agricultural University, P. R. China. The details of these materials, including their original locations are presented in Tab. 1.

Studies of Campbell et al. (1995; 2007) showed that Eriobotrya was close to other Maloideae species including Rhaphiolepis indica (L.) Lindl., Osteomeles anthyllidifolia Lindl., Sorbus scopulina Hedl., Malus prunifolia (Willd.) Barkh, Pyrus pyrifolia (Burm.) Nakai. In this study Rhaphiolepis indica was designated as an ingroup while Photinieae serrulata L. was designated as an outgroup.
Genomic DNAs were isolated from leaf tissue by a modified method (Liu et al., 2005) according to Doyle and Doyle (1987). PCR amplification by MJ Research thermocyclers, were carried out with AdhF2 and AdhR2 primers (Lin et al., 2004) in a 50 μL reaction volume including 2.5 unit Taq polymerase (Promega), 10×PCR buffer (Promega), 200 μM each dNTP, 2.0 mM MgCl2, 10 pmol each primer, and 2 μL template DNA (ca. 10-100 ng).

PCR programs were performed with the following temperature profile: a pre-denaturation step of 4 min at 94°C followed by 5 cycles of denaturation 1 min at 94°C, an annealing at 56°C for 45 sec, and an extension at 72°C for 1.5 min, and then followed by 30 cycles of a denaturation at 94°C for 20 sec, an annealing at 55°C for 20 sec, and an extension at 72°C for 1.5 min, with a final extension for 10 min at 72°C.

The 1300bp specific Adh sequences were obtained by using AdhF2 and AdhR2 primers and these PCR products were purified and cloned into the Easy Vector of PGEM<sup>®</sup>-TSystem II (Promega) according to the manufacturer’s instructions, and then transferred into E. coli TOP10. The recombinant plasmids were obtained after screened by the blue-white method and identified by restriction endonuclease analysis and PCR.

Individual Adh-containing plasmids were isolated and sequenced on an ABI Prism automated DNA sequencer at Shanghai Invitrogen biotechnology Co., Ltd.. Then these sequences were compared with the corresponding DNA sequences of other species reported in GenBank by BLAST software, and finally, the nucleotide homology was found.

The DNA sequences obtained were edited, aligned with ClustalX software (Thompson et al., 1997) and adjusted manually where necessary. Phylogenetic analyses by PAUP4.0b10 (Swofford, 2003), and the construction of the maximum-parsimony (MP) were performed with the heuristic search.

In phylogenetic analysis, ambiguous sites were excluded from the matrix. Gaps were treated as missing information while the inferred indels of clear alignment were recorded as unordered separated characters. All clear characters and character-state transformations were given an equal weight.

A heuristic search was performed for each data set, with RANDOM stepwise data addition (1000 replicates with a start seed of 1) and TBR branch-swapping algorithm options. To assess the relative support for each clade, bootstrap values were calculated from 1000 replicate analyses with the heuristic search strategy and simple addition sequence of the taxa. The amount of phylogenetic information in the MP analysis was constructed with the consistency index (CI) and retention index (RI). Maximum parsimony trees were constructed using PAUP4.0b10 program (Swofford, 2003). Cladistic analysis of the phylogenetic relationship was conducted by using Wagner parsimony and applying heuristic search with tree bisection reconnection (TBS) branch-swapping and simple stepwise taxon application of 1000 replications.

**Results**

Specific primer polymerase chain reaction on Adh gene of Eriobotrya Lindl.

Fourteen Eriobotrya plants and two relatives were amplified with the universal primers, AdhF2 and AdhR2, and the 1300bp specific amplified strands were clearly visualized. Fig. 1 shows the result of PCR amplification.

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**Tab. 1. Some information of the materials**

<table>
<thead>
<tr>
<th>Code</th>
<th>Taxon</th>
<th>Original location</th>
<th>Voucher</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. elliptica Lindl.</td>
<td>Dawei, Guangdong</td>
<td>(S.Q.Lin) Ec1</td>
</tr>
<tr>
<td>2</td>
<td>E. prinoides Rehd &amp; Wils.</td>
<td>Xinyi, Yunnan</td>
<td>(S.Q.Lin) Ed1</td>
</tr>
<tr>
<td>3</td>
<td>E. deflexa Nakai</td>
<td>Hengchun, Yunnan</td>
<td>(S.Q.Lin) Em1</td>
</tr>
<tr>
<td>4</td>
<td>E. deflexa Nakai var. buisanensis Nakai</td>
<td>Malipo, Yunnan</td>
<td>(S.Q.Lin) Eh1</td>
</tr>
<tr>
<td>5</td>
<td>E. henryi</td>
<td>Chengjiang, Yunnan</td>
<td>(S.Q.Lin) Eh1</td>
</tr>
<tr>
<td>6</td>
<td>E. deflexa Nakai var. buisanensis Nakai</td>
<td>Guilin, Guangxi</td>
<td>(S.Q.Lin) Ps1</td>
</tr>
<tr>
<td>7</td>
<td>E. japonica Lindl.</td>
<td>Nanling, Guangdong</td>
<td>(S.Q.Lin) Ej1</td>
</tr>
<tr>
<td>8</td>
<td>E. katangensis Chun</td>
<td>Dayao, Gangxi</td>
<td>(S.Q.Lin) Ek3</td>
</tr>
<tr>
<td>9</td>
<td>E. bengalensis Hook.f.</td>
<td>Hanyuan, Sichuan</td>
<td>(S.Q.Lin) Ed1</td>
</tr>
<tr>
<td>10</td>
<td>forma angustifolia Vidal</td>
<td>Gaoli-Gongshan, Yunnan</td>
<td>(S.Q.Lin) Eb2</td>
</tr>
<tr>
<td>11</td>
<td>E. bengalensis Hook.f.</td>
<td>Kunming, Yunnan</td>
<td>(S.Q.Lin) Ea1</td>
</tr>
<tr>
<td>12</td>
<td>E. fragrans Champ</td>
<td>Nanling, Guangdong</td>
<td>(S.Q.Lin) Ef1</td>
</tr>
<tr>
<td>13</td>
<td>E. cavaleti Rehd</td>
<td>Nanling, Guangdong</td>
<td>(S.Q.Lin) Ec1</td>
</tr>
<tr>
<td>14</td>
<td>E. sequinii Card</td>
<td>Lingle, Guangxi</td>
<td>(S.Q.Lin) Ef1</td>
</tr>
<tr>
<td>15</td>
<td>E. malipoensis</td>
<td>Xinyi, Guangdong</td>
<td>(S.Q.Lin) Ri1</td>
</tr>
</tbody>
</table>

*outgroup; **ingroup

Fig. 2. Majority-rule consensus trees based on Adh sequences (Tree length=1001, Consistency index=0.9371), Retention index=0.9709). Numbers in the branches are bootstrap percentages above 50% and by 1000 replications; * indicate outgroup; ** indicate ingroup.
Phylogenetic analysis of Eriobotrya plants base on Adh gene

Sequencing analysis showed that after arrangement, the matrix is 1332 bp long. The 469 bp were the conserved motifs and 727 bp were the informative polymorphic sites. This result means that the Adh gene sequence have high genetic diversity in Eriobotrya.

The phylogenetic tree based on the sequence of 14 Eriobotrya plants with Rhaphiolepis indica as the ingroup and Photinieae serrulata as outgroup was constructed by the MP method. The phylogenetic tree had 1001 steps, with a consistency index (CI) 0.9371 and a retention index (RI) 0.9709. Fig. 2 shows the majority-rule consensus trees. The result shows the phylogenetic tree could be divided into two clades. E. henryi, E.bengalensis and E.bengalensis forma angustifolia clustered into Group A (Bootstrap value = 59%), and then join together with E. seguini for Clade I (Bootstrap Value = 99%). All the rest clustered into the Clade II. There are four groups in the Clade II, including Group B with E. cavaleriei and E. fragrans (Bootstrap value = 85%), Group C with E. prinoides, var. dadunensis and E. japonica (Bootstrap value = 54%), Group D with E. deflexa and E. deflexa var.buisanensis (Bootstrap value = 57%). E. henryi has the closest relation with the outgroup Photinieae serrulata in Clade I. In Clade II, E.elliptica, E. prinoides, and E. angiosiensis formed monophyletic group, respectively, while E. malipoensis was at the base of Clade II (Bootstrap Value =51%). Therefore, the interspecies relationships and the phylogeny positions of Eriobotrya require further investigation.

Discussion

Adh gene sequences can be used for systematic analysis, suitable for studies on phylogeny relationships and interfamiliy or intergeneric classification. In China, the Adh gene sequence was used to study the phylogenetic and genetic relationships among some plants such as Paonia Section Moutan DC. (Lin et al., 2004), Oryza (Ge et al., 1999), Gossypium (Small et al., 1998). The results show that when using universal primers, a single Adh gene band was obtained for Eriobotrya species, which provide favorable conditions to evaluate phylogeny.

The phylogenetic MP tree of 14 Eriobotrya plants with Photinieae serrulata as an outgroup and Rhaphiolepis indica as an ingroup, was constructed by Adh gene sequence analysis. We draw the following conclusions: the phylogenetic tree could be divided into two clades. E. henryi, E. bengalensis, and E. bengalensis forma angustifolia clustered into Group A, and then join together with E. seguini into Clade I while the rest clustered into Clade II. There are four groups in Clade II, including Group B with E. cavaleriei and E. fragrans, Group C group with E. prinoides, var. dadunensis and E. japonica., Group D with E. deflexa and E. deflexa var. buisanensis, which is largely consistent with the result analyzed by ITS sequence(Li et al., 2009).

The relationships among E. kwangsiensis, E. deflexa, and E. deflexa var. buisanensis were so close that showed they may have the same ancestor, which agrees with the previous study of Yang (2005). Since E. deflexa, E. deflexa var. buisanensis and E. kwangsiensis were closer in the phylogenetic tree; while E. prinoides var. dadunensis, E. japonica, E. prinoides and E. elliptica were close with each other, they may lain at the similar place of the phylogenetic stage, respectively. However, E. malipoensis need further studies on its phylogenetic relationship for it was separated from others.

In the phylogenetic tree, E. cavaleriei and E. fragrans were clustered together and formed Group B group with high bootstrap value (85%) which indicated a relatively close relationship. The results agree with the viewpoint that E. cavaleriei could be treated as a variety under E. fragrans Champ. However, the bootstrap value of some groups are not very high (<60%), which means that to clarify the exact relationships among Eriobotrya plants, a more intensive study with more evidence should be carried out.

Small and Wendel (2000) believed that the low resolution of subsection Erioxylum, gene tree AdhA in Gossypium, was caused by both gene flow and unrecognized paralogy and linkage sorting of the AdhA gene. Sang et al. (2002) also thought that lineage sorting, unrecognized paralogy and gene transformation may cause the low resolution of Adh gene tree in Paonia. From the above analysis, we conclude that although the Adh gene sequence holds a high genetic diversity in Eriobotrya, some species still cannot separated. It can be suggested that the molecular evolution research of Eriobotrya genus is still at an initial phase. A deeper study is required to to determine if there are gene exchanges and unrecognized paralogies.

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