THE IDENTIFICATION OF INVASIVE SPECIES BASED ON DNA BARCODES

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Introduction
A wide variety of invasive plants in China caused serious problems such as loss of biodiversity, extinction of aboriginal species and ecological imbalance. Monitoring and recognition of these invasive species is critical to ensure biodiversity and ecological safety.

DNA barcoding is a hot point in biodiversity research because of its meaning to the discovery of new species and protection of endangered species. It is a fast and exact identification technique using short, standard DNA fragment.

In this study, we tested the feasibility of using DNA barcoding technology, as a complement to the traditional morphological-based taxonomy classification for rapid and accurate identification of invasive species.

Materials and Measurement
1. Materials: In dataset 1, we have tested four key claims of molecular taxonomy, which include three chloroplast regions (pabA-trnH, rbcL, matK) and one nuclear ribosomal DNA region (ITS2), using 48 samples for 9 invasive species belonging to 3 families (Asteraceae, Amaranthaceae, Phytolaccaceae).

Then, the species discriminating power of promising barcode was also explored in a large pool of dataset 2 (Table 1).

2. Sequence alignment and analysis: The sequences were aligned using Clustal W and genetic distances using K2P model were computed using MEGA 4.0. The distribution of intra-versus inter-specific variability was compared using DNA barcoding gaps. BLAST method was used for species identification. Phylogenetic trees (NJ tree) were constructed based on DNA sequences. The ITS2 secondary structure was predicted using the ITS2 database and website found by Koetschan et al.

Results
1. Assessment of the four candidate barcodes
(i) ITS2 is the most discriminatory DNA barcode in these invasive species, followed by pabA-trnH, while rbcL showed the lowest level of inter-specific variation.
(ii) For intra-specific divergence, ITS2, rbcL, and matK all showed low variations among con-specific individuals, and pabA-trnH, in particular, showed the lowest variations.

(iii) ITS2 exhibited distinct barcoding gaps.

(iv) NJ tree constructed based on ITS2 and rbcL sequences showed that the samples of related species were clustered together to form an independent branch supported by high Bootstrap value.

(v) For species discrimination, DNA barcodes all worked well at higher taxonomy levels (100%). Overall, our findings indicated that DNA barcoding is an efficient and powerful taxonomic tool in identification of invasive species. Particularly, ITS2 showed great promise in the monitoring and controlling of invasive species.

2. Measurement of invasive species discriminating power for ITS2 in a large pool of samples
2.1 Testing the efficacy of authentication
For species discrimination, ITS2 work well with high levels of authentication.

Table 2 Identification efficiency of the ITS2 locus for the invasive species and genus in dataset 2

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of samples</th>
<th>No. of species</th>
<th>Success identification (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amananthas</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Datura</td>
<td>20</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>Lolium</td>
<td>6</td>
<td>3</td>
<td>66.7</td>
</tr>
<tr>
<td>Sancus</td>
<td>5</td>
<td>2</td>
<td>54.5</td>
</tr>
<tr>
<td>Others genus</td>
<td>264</td>
<td>119</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>305</td>
<td>136</td>
<td>95.4</td>
</tr>
</tbody>
</table>

Discussion
1. DNA barcoding is an efficient and powerful taxonomic tool in identification of invasive species.

2. ITS2 has showed great promise in the monitoring and controlling of invasive species. ITS2 is one of the most notably choice for inclusion as a universal plant barcoding region (Chen et al., 2010; Yao et al. 2010). In our study, ITS2 was selected as a possible region of invasive species because of its sequence variability and relatively higher species discrimination success.

3. DNA barcoding is a powerful tool for taxonomy and biogeography with utility for identifying species and solving taxonomic problems.

Invasive plant species have also provided solid and practical evidence and basis for applying DNA barcoding on other plant species identification.

Conclusions
1. ITS2 may be a useful molecular identification tool for invasive species, with great application value. Overall, ITS2 is informatively valuable and provide a rapid and promising means for identification.

2. DNA barcoding is an efficient and powerful taxonomic tool in invasive species.

Reference