Plant Biotechnology 22(3), 231–233 (2005)

Gene Note

Sequence variation in the *rbcL-accD* region in the chloroplast genome of *Moraceae*

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Received December 27, 2004; accepted May 23, 2005 (Edited by T. Kohchi)

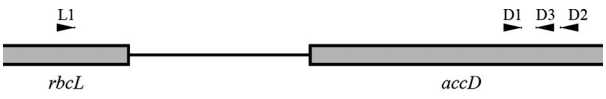
Abstract To investigate whether the *rbcL-accD* region from the chloroplast genome would be suitable for phylogenetic studies of *Moraceae*, we determined 2 kb of the nucleotide sequence for this region in 10 species in the family *Moraceae*. Genera examined included *Morus*, *Artocarpus*, *Ficus*, *Broussonetia* and *Dorstenia*. In the intergenic spacer and *accD* region, 220 variable sites and 16 indels (insertions and deletions) were found. However, *rbcL-accD* was highly conserved among *Morus* species. No difference was found among three Japanese mulberry species, *M. alba* cv. Minamisakari, *M. bombysis* cv. Kenmochi and *M. latifolia* cv. Kokusou 21. Only one polymorphism was found in *M. nigra*, which is native to West Asia. Possible transfer of the chloroplast genome between *Morus* species and potential use of the intergenic spacer and *accD* regions to study phylogenetic relationships within *Moraceae* are discussed.

Key words: *AccD*, intergenic spacer, *Moraceae*, *rbcL*.

The nucleotide sequence for the *rbcL-accD* region of the chloroplast genome is highly variable in the family *Polygonaceae* at both the intrageneric (Yasui and Ohnishi 1998) and intraspecific (Inamura et al. 2000; Yamane et al. 2003) levels. To investigate whether the *rbcL-accD* region from the chloroplast genome would be suitable for phylogenetic studies of *Moraceae*, which includes economically important plants such as mulberry (*Morus* species), fig (*Ficus carica*), kozo (*Broussonetia kazinoki*), breadfruit (*Artocarpus altilis*) and jackfruit (*A. heterophyllus*), we determined the nucleotide sequence of this region, including the 3′ portion of *rbcL*, the intergenic spacer and the 5′ portion of *accD* of 10 species belonging to this family.

M. alba L. cv. Minamisakari, *M. bombysis* Koids. cv. Kenmochi, *M. latifolia* Poir. cv. Kokusou 21 and *M. nigra* L. were maintained at the Center for Bioresource Field Science, Kyoto Institute of Technology. *B. kazinoki* Sieb. and *F. carica L.* were collected in Kouchi and Kyoto Prefectures, respectively. *A. altilis* Fosb., *A. heterophyllus* Lam. and *Dorstenia asaroides* Gardn. were purchased from Exotic Plants (Chiba, Japan). *F. benjamina L.* was purchased from a local flower shop. The sequences of the primers used for PCR and the location of their target binding sites are shown in Figure 1.

The DNA fragment obtained from total genomic DNA by PCR using primer pair L1 and D3 contained 0.25 kb of the 3′ portion of *rbcL*, 1 kb of the 5′ portion of *accD* and the intergenic spacer (Figure 2). Among the plants examined, the *rbcL* region was highly conserved and only 10 variable sites were found. In contrast, the intergenic spacer and *accD* regions were highly variable. We found 110 variable sites in both the intergenic spacer and *accD* region. In addition, 13 and 3 indels (insertions and deletions), respectively, were found in the intergenic spacer and *accD* region.



L1	5′-TCACGTTTGGCATATGCCTGCTCTGACCG-3′
D1	5′-TATTCAAACAGGTACAGGTCAATTAAA-3′
D2	5′-CGGAAGAACACACTAAAATAAGAGGTA-3′
D3	5′-CGTGTAATTTTCTCGCCCACTACAG-3′

Figure 1. Sequences of primers used in PCR, and location of their target binding sites. Based on the *Brassica napus accD* sequence (Z50868), *accD*-specific primers D1 and D2 were prepared. We sequenced the PCR product obtained using these two primers and *M. alba* DNA as template, and designed an *accD*-specific primer, D3. An *rbcL*-specific primer, L1, was prepared based on the *M. alba rbcL* nucleotide sequence (D86319).

Abbreviations: indel, insertion and deletion.

[a] The first two authors contributed equally to the report. The nucleotide sequence data reported in this paper appear in Genbank/EMBL/DDBJ nucleotide sequence databases with the accession numbers, AB194397 to AB194402 and AB207916 to AB207919.

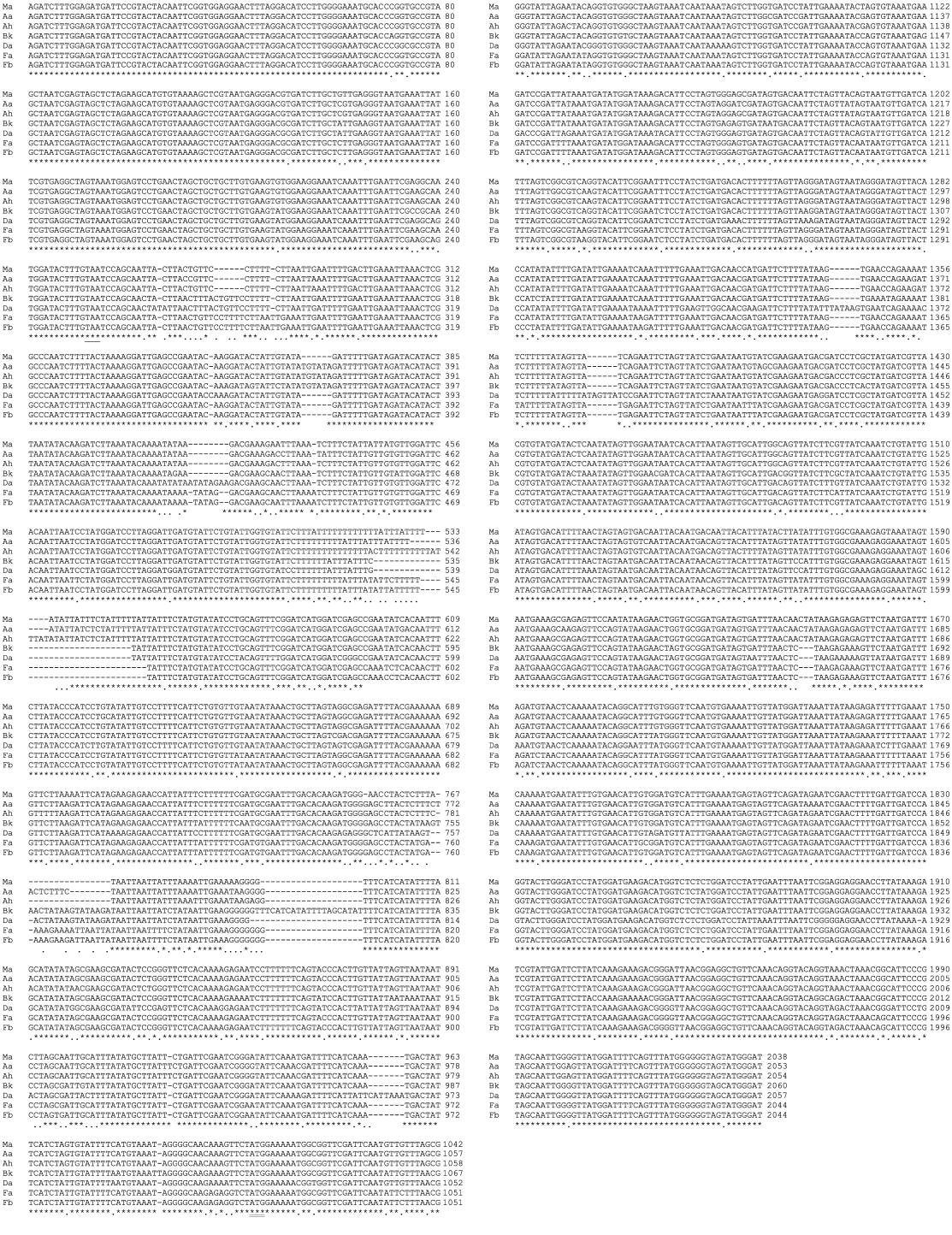


Figure 2. Alignment of chloroplast DNA sequences of the 3′ portion of the *rbcL* gene, intergenic spacer and 5′ portion of the *accD* gene of *M. alba* Minamisakari (Ma), *A. altilis* (Aa), *A. heterophyllus* (Ah), *B. kazinoki* (Bk), *D. asaroides* (Da), *F. carica* (Fc) and *F. benjamina* (Fb). The primer pair L1 and D3 was used to amplify a 2 kb fragment of *rbcL-accD* from the chloroplast genome of each plant. Asterisks (*) and periods (.) mark nucleic acids that are identical and conserved, respectively. The stop codon (TAA) of *rbcL* and initiation codon (ATG) of *accD* are underlined and double-underlined, respectively.

The nucleotide sequences of *M. bombysis* cv. Kenmochi and *M. latifolia* cv. Kokusou 21 were identical to that of *M. alba* cv. Minamisakari (data not shown). Only one polymorphism was found in *M. nigra*, which is native to West Asia: bases 513–524, located within the intergenic spacer, are T_{12} in Minamisakari, Kokusou 21 and Kenmochi, while the corresponding site is T_{11} in *M. nigra* (data not shown). Conservation of the chloroplast genomic sequence among the *Morus* species may be due to transfer of the chloroplast genome. While *M. bombysis* is believed to be native to Japan, *M. alba* and *M. latifolia* are considered to have been introduced from

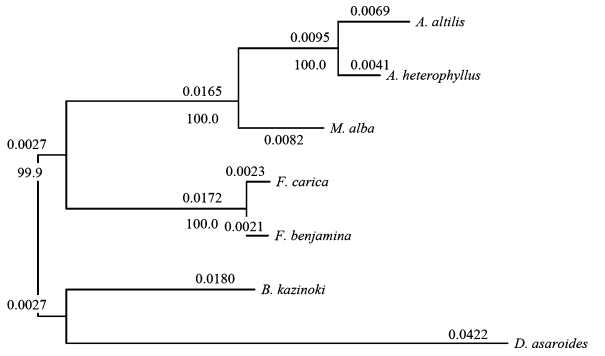


Figure 3. Phylogenetic tree generated based on the nucleotide sequences of the *rbcL-accD* region. The indels were removed from the sequence data set. Numbers above branches are genetic distances. Numbers below branches are confidence levels (%) based on 10,000 bootstrap replicates. The tree was obtained by the neighbor-joining method using Genetyx ver. 6.1.1 software (Genetyx, Tokyo, Japan).

China in the 7th century (Hotta 1951) and domesticated in Japan. In addition to naturally occurring hybridization, mulberry species have been commonly crossed by breeders for the sericulture industry. The high level of nucleotide sequence similarity between *M. nigra* and the other mulberries might be due to the introduction of Chinese mulberry to West Asia for sericultural purposes, followed by domestication and/or crossing with other species there.

A phylogenetic tree was generated based on the nucleotide sequences determined in this study (Figure 3). Based on these results, species of *Ficus* or *Artocarpus* are more closely related to species in the same genus than to samples from other genera, and *Morus* is more closely related to *Artocarpus* than to the other genera. In a published phylogenetic tree based on the nucleotide sequence of the nuclear-encoded 5S-rRNA gene spacer region (Miyahara et al. 1998), species within the genera of *Ficus*, *Morus* and *Broussonetia*, for unknown reasons, were not grouped together, suggesting that such a tree does not reflect the true relationships within the *Moraceae*. Some of the recent phylogenetic studies on *Moraceae* are based on nucleotide sequences from chloroplast DNA, such as *rbcL*, *trnL-F* and *ndhF* (Sytsma et al. 2002; Datwyler and Weiblen 2004). Our results here indicate that the *rbcL-accD* region is another site informative for studies of phylogenetic relationships in *Moraceae*.

The number of nucleotide sequences studied here is limited. Since the primer pair L1 and D3 could be used for all species of *Moraceae* tested in this study, amplification of corresponding DNA fragments in other species of *Moraceae* for detailed analyses should also be possible.

Acknowledgments

We wish to thank Dr. Masamitsu Yamaguchi for granting us use of a DNA sequencer, Dr. Masanobu Itoh for helpful advice in phylogenetic analysis, and Dr. Kazufumi Yazaki for helpful advice in preparing this manuscript.

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