A Phylogenetic Study of Polygonum sect. Tovara (Polygonaceae) Based on ITS Sequences of Nuclear Ribosomal DNA

Suh, Youngbae*, Sangtae Kim† and Chong-Wook Park‡
Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
†Department of Biology, Seoul National University, Seoul 151-742, Korea

Polygonum sect. Tovara comprises three morphologically very similar species; P. virginianum, P. filiforme, and P. neofiliforme. Sequences of internal transcribed spacers (ITSs) of nuclear ribosomal DNA of these were determined to examine phylogenetic relationships and the levels of differentiation among them. The size of ITS 1 was 241 bp in P. filiforme and P. neofiliforme, and 242 bp in P. virginianum. The size of ITS 2 was 243 bp, and that of the 5.8S rRNA coding region was 163 bp. The ITS sequences clearly separate North American P. virginianum from the eastern Asian species. Nucleotide divergence between them ranges from 3.3% to 3.8% for ITS 1 and from 9.3% to 10.7% for ITS 2. The molecular data also revealed that two eastern Asian species are closely related but should be treated as distinct species.

Keywords: Polygonaceae, Polygonum sect. Tovara, molecular phylogeny, ITS, disjunct distribution

Polygonum sect. Tovara (Adans.) Benth. & Hook. (Polygonaceae) is a highly variable taxon usually defined by large ovate to elliptic leaves, elongate spike-like inflorescences, and persistent bifid styles which become rigid and bent obliquely downward in fruit (Park et al., 1992; Mun and Park, 1995). Plants of sect. Tovara are erect perennial herbs, which usually occupy moist habitats such as margins of swamps and lakes, rich shady forest floors, and soils of streambeds. The section shows an interesting disjunct distribution pattern: Eastern North America (with a few isolated occurrences in Mexico) and eastern Asia (Li, 1952a; Park et al., 1992).

Species of sect. Tovara exhibit very complicated patterns of morphological variation, resulting in taxonomic confusion and difficulty in delimiting species boundaries (Nieuwland, 1912; Elmer, 1915; Nakai, 1922; Ohki, 1926; Steward, 1930; Maekawa, 1932; Li, 1952b; Hara, 1962, 1965; Graham and Wood, 1965). Recently, Park et al. (1992) recognized three species in the section on the basis of numerical analyses of morphological characters: Polygonum virginianum L. in eastern North America and P. filiforme Thunb. and P. neofiliforme Nakai in eastern Asia. In addition, the subsequent study on flavonoid chemistry of these taxa (Mun and Park, 1995) revealed that they are closely allied but distinct species. However, phylogenetic relationships among these species have not been proposed by these authors because of the paucity of discrete qualitative morphological characters and the difficulty in estimating phylogenetic derivation for the various structural types of flavonoids (Park et al., 1992; Mun and Park, 1995).

Recent molecular phylogenetic studies have demonstrated that the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (nrDNA) are very useful for assessing phylogenetic relationships at lower taxonomic levels such as among genera or species, because their rates of divergence are relatively high in comparison to protein or rRNA coding genes such as rbcL and 18S/26S ribosomal DNA (Baldwin, 1992; Suh et al., 1993; Kim and Jansen, 1994; Sang et al., 1994, 1995; Baldwin et al., 1995; Downie and Katz-Downie, 1996). In this study, we analyzed the ITS sequences of nrDNA from the three species recognized in sect. Tovara to look into phylogenetic relationships among them and to compare the results with those from previous
studies of morphology (Park et al., 1992) and flavonoid chemistry (Mun and Park, 1995).

MATERIALS AND METHODS

Plant materials and DNA extraction

Three species recognized in sect. Tovara (Park et al., 1992; Mun and Park, 1995) and one outgroup species were sequenced (Table 1). Polygonum hydro-
piper L. of sect. Persicaria (Mill.) DC. was chosen as an outgroup species, because sect. Tovara is considered to be most closely related to sect. Persicaria (Haraldson, 1978; Ronse Decraene and Akeroyd, 1988; Mun and Park, 1995). Leaves used as sources of DNA were collected in the field, and transported to the laboratory on ice. Leaves were powdered in li-
quid nitrogen and kept in -70°C until DNA extraction. Total DNA was extracted by the 2× CTAB method of Doyle and Doyle (1989), and further pu-
rified with Gene Clean Kit II (Bio101, CA).

PCR and sequencing

PCR was carried out in 100 µl final volume of 10 mM Tris buffer (pH 8.3) containing 0.5 ng template DNA, 2.5 units of Taq polymerase (Perkin-Elmer Cetus), 50 mM KCl, 1.5 mM MgCl2, 0.001% gela-
tin, 200 µM of each dNTP, and 0.5 µM of each primer. PCR primers were 'ITS1' and 'ITS4' design-
ed by White et al. (1990). PCR thermal cycle pro-
file was 3 minutes at 95°C for pre-denaturation, fol-
lowed by 30 cycles, each consisting of 1 minute at
95°C for denaturation, 1 minute at 55°C for annealing, and 45 seconds at 72°C for extension. Primer ex-
tension time was increased by 3 seconds with each
cycle, followed by the final extension for 7 minutes
at 72°C. Double stranded PCR product was directly sequenced by using the Sequenase PCR Product Sequencing Kit (USB, OH). Excess dNTPs, primers and extraneous single-stranded DNA’s produced by PCR were removed by Shrimp alkaline phosphatase and Exonuclease I. In addition to PCR primers, 'ITS 2' and 'ITS3' of White et al. (1990) were used as in-
ternal primers for sequencing in both directions. Elec-
trohoresis was performed with denaturing formam-
dide gels, and glycerol tolerant buffer containing tau-
rine was used instead of TBE buffer (protocols for
Sequenase PCR Product Sequencing Kit, 2nd ed.,
USB, OH).

Sequence alignment and phylogenetic analyses

The sequence boundaries of ITS 1, the 5.8S cod-
ing region, and ITS 2 were determined by com-
parison with published sequences from various plant
species (Yokota et al., 1989; Baldwin, 1992; Suh et
al., 1993; Kim and Jansen, 1994). Sequences were
aligned using the Clustal V program (Higgins et al.,
1992), and then finally adjusted by eye. Gaps were
not included in the phylogenetic analysis. Phylogeny
was reconstructed with Fitch parsimony as implim-
ented in PAUP (ver. 3.1.1; Swofford, 1993). Branch
and bound searches, with equal weighting of charac-
ter state changes, were conducted. Sequence diver-
gence values between species were calculated using
Kimura’s two-parameter method (Kimura, 1980). Ki-
mura’s distances were obtained using DNADIST pro-
gram of PHYLIP (ver. 3.5; Felsenstein, 1992), with
default settings (transition vs. transversion=2:1).

RESULTS

The complete sequences of ITS 1, the 5.8S cod-
ing region, and ITS 2 were determined for all three
species of sect. Tovara and outgroup species, P. hy-
dropiper of sect. Persicaria (Fig. 1). The size of
ITS 1 was 241 bp long in P. filiforme, P. neofili-
forme, and P. hydropiper, and 242 bp long in P. vir-
ginianum. The size of ITS 2 was 241 bp long in P.
hydropiper, and 243 bp long in all other species.
The 5.8S coding region was 163 bp in length for all
species examined (Table 2). Sequence alignment re-
quired insertion of a one-base gap in ITS 1 of P. vir-
ginianum, and four one-base indels between the in-
group species and the outgroup in ITS 2 (Fig. 1).

GC content ranged from 61.8% to 66.5% in ITS 1,
and from 65.4% to 79.8% in ITS 2 (Table 2). In the

<p>| Table 1. Species used for ITS sequence analysis of Polygonum sect. Tovara and outgroup species. All vouchers are at SNU |
|-----------------|------------------|----------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Collection number</th>
<th>Locality and Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section Tovara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polygonum filiforme Mun 640</td>
<td>Korea, Chunam Prov., Mt. Turyun</td>
<td>Aug. 20, 1993</td>
</tr>
<tr>
<td>P. neofiliforme</td>
<td>Mun 23</td>
<td>Korea, Chunam Prov., Mt. Chiri</td>
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<tr>
<td>Section Persicaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. hydropiper</td>
<td>Mun 701</td>
<td>Korea, Seoul, Mt. Kwanak</td>
</tr>
</tbody>
</table>