Phylogenetic Relationships of *Prunus* (Rosaceae) in Korea and Japan Inferred from Chloroplast DNA Sequences

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**ABSTRACT**

We used sequences from both *rbcL* gene and *trnL-trnF* intergenic spacer of chloroplast DNA (cpDNA) for phylogenetic reconstruction of 39 accessions of *Prunus* distributed in Korea and Japan. No sequence length variation was found in the 39 accessions based on *rbcL* gene sequences. However, the length of the *trnL-trnF* spacer ranged from 358 to 387 base pairs (bp). Of the 1,790 aligned positions of the two cpDNA regions, 406 sites (22.7%) were variable: 299 sites (21.4%, 299/1,398) in *rbcL* and 107 sites (27.3%, 107/392) in the *trnL-trnF* intergenic spacer. The longest length mutation of the *trnL-trnF* spacer was a 21-bp deletion in *P. padus* and two taxa of *P. buergeriana*. Phylogenetic trees based on the sequence data were constructed by using maximum parsimony (MP) analysis. The bootstrap values for individual clades based on 1,000 replicates ranged from 80 to 100%. The strict consensus tree had five large, independent groups. The trees contained monophyletic groups consistent with the subgenera, *Cerasus*, *Padus*, *Amygdalus*, *Prunophora*, and *Laurocerasus*, with a common ancestral divergence point with a bootstrap value of 100%. In addition, subgenera *Amygdalus* and *Prunophora* had a well-supported sister group relationship with 100% bootstrap support, as did the clade of subgenera *Cerasus*, *Laurocerasus* and *Padus*. The two natives of *P. yedoensis* from Jeju, Korea were clearly distinguished from the cultivars as suggested in previous reports. Accordingly, cpDNA sequence analysis is a useful approach for reconstructing phylogenetic relationships among members of the genus *Prunus* distributed in Korea and Japan.

**Key words:** chloroplast DNA, maximum parsimony, *Prunus*, *rbcL*, *trnL-trnF*.

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INTRODUCTION

Prunus L., the plum or cherry genus, belongs to the subfamily Amygdaloideae (=Prunoideae) of the Rosaceae. It is generally distributed in the temperate zone of the Northern hemisphere, although some species are found in tropical and subtropical regions (Rehder, 1940; Bailey and Bailey, 1976). Prunus is economically important, because many species are sources of fruit (e.g., plum, peaches, apricots, cherries, and almonds), oil, timber, and ornaments (Mabberley, 1997).

The genus Prunus contains five subgenera: Prunus [=Prunophora (Neck.) Focke], Amygdalus (L.) Benth. & Hook., Cerasus (Adans.) Focke, Padus (Moench) Focke, and Laurocerasus (Ser.) Rehd. (Rehder, 1940). The controversy over the classification of Prunus largely results from the lack of a phylogenetic framework. Previous workers emphasized a few characters, especially fruit morphology, inflorescence type, and leaf duration, in formulating the classification (McVaugh, 1951).

The species of Prunus distributed in Korea and Japan exhibit very complex patterns of morphological variation, resulting in taxonomic confusion and difficulty in delimiting boundaries between species (Bentham and Hooker, 1867; Kitamura and Murata, 1979). Especially, it has long been disputed whether P. yedoensis Matsumura originated from Mt. Halla in Jeju (Korea), and whether the taxon from Jeju is the same as cultivated taxa from Japan (Koidzumi, 1932; Takenaka, 1958).

In 1976, Watkins postulated that central Asia was the center of origin for Prunus. Cherries (subgenus Cerasus) are considered to be the most distantly related species group. Almonds and peaches, members of the subgenus Amygdalus appear closely related (they hybridize easily), but are relatively distant from plums and apricots in the subgenus Prunophora. In addition, Watkins postulated that subgenera Amygdalus and Prunophora were genetically monophyletic, with the caveat, based on limited evidence, that almonds and Damsom plums were genetically isolated from the rest of the group. He did not believe that direct genetic transfer between the Amygdalus-Prunophora group and the subgenus Cerasus was likely, and Prunophora was closely associated with the section Microcerasus. Although he suggested that the ancestors of Prunus were located in Cerasus, Watkins (1976) concluded that plum species in the Prunophora subgenus were the central species for Prunus evolution at the present, due to their ability to hybridize with species from the other subgenera.

Recently, various molecular techniques for phylogenetic inference have been introduced to plant systematics, including restriction site analysis, comparative sequencing, analysis of structural rearrangement, and polymerase chain reaction (PCR)-based techniques such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) (Jung et al., 1997; Garcia et al., 2000). Of these, sequencing techniques have been widely adopted for phylogenetic studies at various levels, using DNA regions with different base substitution rates. Chloroplast DNA (cpDNA) is an extremely valuable molecule for studying phylogenetic relationships between closely related species (Chase et al., 1993; Sang et al., 1997; Azuma et al., 1999). Particularly, the plastid rbcL gene, which encodes the large subunit of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and a primary enzyme in carbon fixation, has been sequenced from numerous plant taxa. The sequences have been very useful information in plant phylogeny (Chase et al., 1993; Sang et al., 1997). In addition, trnL-trnF intergenic spacer, a noncoding cpDNA region, has been used to define the phylogenetic relationships at lower taxonomic levels (Jung et al., 2003). This zone tends to evolve more rapidly than coding sequences, and accumulates insertion/deletions (indels) at a rate at least equal to that for nucleotide substitutions (Curtis and Clegg, 1984; Clegg and Zurawski, 1991).

This study aimed to reconstruct the phylogenetic relationships among 39 taxa representing all of the subgenera (Amygdalus, Cerasus, Laurocerasus, Padus, and Prunophora) of Prunus distributed in Korea and Japan by using molecular approaches. The sequences of the two cpDNA regions, rbcL gene and trnL-trnF intergenic spacer, were analyzed for these purpose. Our findings were then compared with previous published results.

MATERIALS AND METHODS

Plant materials and DNA extraction

Thirty nine taxa representing all of the subgenera (Amygdalus, Cerasus, Laurocerasus, Padus, and Prunophora) of the classification system of the genus Prunus proposed by Rehder (1940). Exochorda