Molecular Phylogeny of the Genus *Prunus* in Korea and Japan Inferred from Nuclear Ribosomal and Chloroplast DNA Sequences

by

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Molecular Phylogeny of the Genus *Prunus* in Korea and Japan Inferred from Nuclear Ribosomal and Chloroplast DNA Sequences

Yong Hwan Jung

This study examined the molecular phylogeny of the genus *Prunus*, in a phylogenetic study of 45 accessions representing all subgenera (*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunophora*) of *Prunus* distributed in Korea and Japan, and the origin and characteristics of *P. yedoensis*, which is native to Jeju, using nucleotide sequences from the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA) and three chloroplast DNA (cpDNA) regions [*rbc*L gene, *psb*A gene, *trn*L(UAA)-*trn*F(GAA) intergenic spacer]. To clarify the origin of interspecific hybridization in *P. yedoensis*, both nrDNA ITSs and the chloroplast *rbc*L gene from 10 taxa of *P. yedoensis* (6 cultivars and 4 native *P. yedoensis*) were analyzed. Five species of *Prunus* (*P. pendula* for. *ascendens*, *P. serrulata* var. *quelpaertensis*, *P. sargentii*, *P. lannesiana*, and *P. serrulata* var. *spontanea*), which are putative parents of *P. yedoensis,* were also examined. The most parsimonious trees had four large and independent groups: group I (subgenera *Prunophora*,

Amygdalus, and *Cerasus* I), group II (subgenera *Padus* and *Laurocerasus*), group III (subgenus *Cerasus* II), and group IV (subgenus *Cerasus* III). Interestingly, the subgenus *Cerasus* was split among three subgroups. *P. yedoensis* was distinct from the other subgenus *Cerasus* species. The four natives of *P. yedoensis* from Jeju were clearly distinguished from the cultivars. The strict consensus 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, subgenus *Amygdalus* (*P. persica* and *P. persica* for. *rubroplena*) plus section *Microcerasus* of subgenus *Cerasus*, and subgenus *Prunophora* (*P. salicina*, *P. mume*, and *P. armeniaca* var. *ansu*) had a well-supported sister group relationship with 100% bootstrap support, as did the clade of subgenera *Cerasus*, *Laurocerasus* and *Padus*. Particularly, *P. choreiana*, *P. tomentosa*, *P. glandulosa* for. *sinensis*, and P. *japonica* var. *nakaii*, section *Microcerasus* of subgenus *Cerasus*, were more closely related to the subgenus *Amygdalus*. These molecular data strongly suggest that a taxonomic realignment of the infra-genus delimitations and compositions should be considered. In addition, *P. pendula* for. *ascendens* is the maternal parent of *P. yedoensis*, if chloroplast DNA is inherited maternally, and *P. serrulata* var. *quelpaertensis* is the putative pollen parent of *P. yedoensis*. This study found differences between native and cultivar *P. yedoensis* based on the lengths of ITS1 and ITS2, the secondary folding structure of the nrDNA ITS1 sequence, and amino acid substitutions in the cpDNA

coding sequences. These data suggest that native *P. yedoensis* from Jeju is a different taxon from the cultivar. The rate of genetic variation was much higher between *P. yedoensis* trees native to Jeju, compared with that found in cultivars of *P. yedoensis* and other species; this counters the arguments that *P. yedoensis* is not native to Jeju. Particularly, the argument that *P. yedoensis* is native to Mt. Halla (Jeju, Korea) and is a hybrid between *P. pendula* for. *ascendens* and *P. lannesiana* is not persuasive, since *P. lannesiana* does not occur on Jeju. Accordingly, *P. yedoensis* is considered native to Jeju and a species distinct from other *Prunus*.

Key words: genus *Prunus*, *Prunus yedoensis*, ITS, *psb*A, *rbc*L, *trn*L(UAA)-*trn*F(GAA), molecular phylogeny, monophyletic, origin

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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; Cronquist, 1981; Krussman, 1986; Hotta *et al.*, 1989; Ghora and Panigrahi, 1995; Mabberley, 1997). *Prunus* is economically important, because many species are sources of fruit (e.g., plums, peaches, apricots, cherries, and almonds), oil, timber, and ornamentals. Since the genus *Prunus* was established by Linnaeus in 1754, many scientific names have been added, indicating that the classification of the genus has received much attention. Rehder (1954) recognized 77 species, while Sauer (1993) suggested that as many as 200 species exist.

The taxonomy of *Prunus*, especially the generic delimitations, is controversial. Based primarily on fruit morphology, de Tournefort (1700) recognized six distinct genera within *Prunus*: *Amygdalus* L., *Armeniaca* Miller, *Cerasus* Miller, *Laurocerasus* Duhamel, *Persica* Miller, and *Prunus*. The de Tournefort treatment was accepted to some degree, but was amended by later workers. Linnaeus (1753) recognized three genera, *Amygdalus*, *Padus* Miller, and *Prunus,* and later (1754) accepted four genera, *Armeniaca*, *Cerasus*, *Padus* (including *Laurocerasus*), and *Prunus*. Several workers adopted the generic concepts of Tournefort and

Linnaeus, e.g., Miller (1754), Komarov (1971), Yü *et al*. (1986), and Ghora and Panigrahi (1995). Minor changes were often made. For example, *Laurocerasus* was synonymized under *Padus* (Miller, 1754); *Armeniaca* was included in *Prunus* (Ghora and Panigrahi, 1995) or *Persica* (de Jussieu, 1789); and *Laurocerasus* was treated in *Cerasus* (de Candolle, 1825) (Table 1).

Bentham and Hooker (1865) were the first to unite the six genera of de Tournefort (1700) into a single genus *Prunus*, which was subdivided into seven sections: *Amygdalopsis*, *Amygdalus*, *Armeniaca*, *Cerasoides*, *Cerasus*, *Laurocerasus*, and *Prunus*. Koehne (1893) initially divided the genus *Prunus* into seven subgenera, but later (Koehne, 1911) recognized four: *Amygdalus*, *Cerasus*, *Padus*, and *Prunophora* (=*Prunus*). Rehder (1940) reviewed the previous treatments and divided the genus into five subgenera: *Amygdalus* (L.) Benth. & Hook., *Cerasus* (Adans.) Focke, *Laurocerasus* (Ser.) Rehd., *Padus* (Moench) Focke, and *Prunophora* [= *Prunus* (Neck.) Focke]. Many workers followed Rehder's system (Groh and Senn, 1940; Fernald, 1950; Radford *et al*., 1968) (Table 1).

On the other hand, Hutchinson (1964) recognized three genera (*Laurocerasus*, *Padus*, and *Prunus*) within *Prunus*, and synonymized the other previously recognized genera (*Aflatunia* Vassilcz, *Amygdalopsis* Carr., *Amygdalus*, *Armeniaca*, *Cerasus*, *Emplectochadus* Torr., *Microcerasus* M. Roem., and *Prunophora* Neck) to *Prunus* (Table 1).

The controversy over the classification of *Prunus* largely results from the lack of a phylogenetic framework. Previous workers

Table 1. Principal taxonomic treatments of *Prunus*

Author	Rank	Taxa recognized
Tournefort (1700)	Genera	*Amygdalus, Armeniaca, Cerasus, Laurocerasus, Persica*, and *Prunus*
Linnaeus (1754)	Genera	*Armeniaca, Cerasus, Padus*, and *Prunus*
Bentham and Hooker (1865)	Subgenera	*Amygdalus, Amygdalopsis, Armeniaca, Ceraseides, Cerasus, Laurocerasus*, and *Prunus*
Focke (1894)	Subgenera	*Amygdalus, Cerasus, Chamaeamygdalus, Emplectocladus, Microcerasus, Padus*, and *Prunophora*
Koehne (1911)	Subgenera	*Amygdalus, Cerasus, Padus,* and *Prunophora*
Rehder (1940)	Subgenera	*Amygdalus, Cerasus, Laurocerasus, Padus*, and *Prunophora*
Hutchinson (1964)	Genera	*Laurocerasus, Padus*, and *Prunus*
Komarov (1971)	Genera	*Amygdalus, Armeniaca, Cerasus, Laurocerasus, Padus, Persica*, and *Prunus*
Yu *et al*. (1986)	Genera	*Amygdalus, Armeniaca, Cerasus, Laurocerasus, Padus*, and *Prunus*
Ghora and Panigrahi (1995)	Subgenera	*Amygdalus, Cerasus, Laurocerasus, Padus*, and *Prunus*

emphasized a few characters, especially fruit morphology, inflorescence type, and leaf duration, in formulating the classification (McVaugh, 1951). A few studies attempted to construct evolutionary relationships within the genus (Mowrey and Werner, 1990; Uematsu *et al.*, 1991; Badenes and Parfitt, 1995). In addition, several isozyme studies have been conducted (Arulsekar *et al.*, 1986; Hauagge *et al.*, 1987; Byrne and Littleton, 1989; Byrne, 1990; Mowrey and Werner, 1990; Mowrey *et al.*, 1990); however, they have been used primarily to study intraspecific variation, and the taxa sampled in these studies were limited. Crossing experiments among *Prunus* species, especially native and cultivated ones, have proved an important tool for ascertaining relationships within the genus. The analysis by Mowrey and Werner (1990) remains the most comprehensive phylogenetic study of the genus. These workers examined the isozyme variation of 34 species belonging to three (subgenera *Amygdalus*, *Cerasus*, and *Prunus*) of the five subgenera (Rehder, 1940) and recognized three groups, which roughly correspond to the three subgenera, with a few exceptions.

In 1976, Watkins (cited in Badenes and Parfitt, 1995) 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* were the central species for *Prunus* evolution at the present, due to their ability to hybridize with species from the other subgenera.

Preliminary DNA analyses examined several groups of *Prunus* species (Kaneko *et al*., 1986; Nybom *et al*., 1990; Uematsu *et al*., 1991; Badene and Parfitt, 1995, Innan *et al*., 1995; Jung *et al*., 1997). Kaneko *et al*. (1986) used restriction fragment length polymorphism (RFLP) of chloroplast DNA (cpDNA) to classify Japanese flowering cherries. Uematsu *et al*. (1991) used RFLP from isolated cpDNA to classify wild and cultivated peach and apricot species, as well as *P. domestica* and several wild species. However, these studies had several major problems, including the use of questionable band-pattern data, and reliance on cluster analysis for establishing species relationships. The resulting dendrograms associated apricot species most closely with the *P. davidiana* peach relative, then with *P. domestica*, and most distantly with *P. persica*, which is inconsistent with any conventional interpretation. Uematsu *et al*. (1991) concluded that apricot species were at the center of stone fruit diversity, although their dendrogram dose not directly support this conclusion. Badeness and Parfitt (1995) studied only eight species, but they concluded that subgenera

Prunophora and *Amygdalus* are closely related and that subgenus *Cerasus* evolved more extensively and earlier. On the other hand, using random amplified polymorphic DNA (RAPD) analysis, 9 *Prunus* species distributed in Korea were divided into two large groups: the subgenera *Padus* and *Cerasus* (Jung *et al.*, 1997).

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, 1880; Hooker, 1894; Fernald, 1950; 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, 1942).

In 1916, Wilson (cited by Iwasaki, 1986) suggested that *P. yedoensis* is an interspecific hybrid between *P. lannesiana* Wilson and *P. pendula* for. *ascendens*, based on morphological comparison. Takenaka (1963) succeeded in producing artificial hybrids by reciprocal crosses between *P. lannesiana* and *P. pendula* for. *ascendens*, which are very similar to *P. yedoensis*. His results supported Wilson's hypothesis experimentally. In addition, Funazu (1966) reported that his grandfather wrote that *P. yedoensis* was bred by crossing *P. lannesiana* as the maternal parent and *P. pendula* for. *ascendens* as the pollen parent. On the other hand, Park *et al.* (1984) suggested that *P. yedoensis* originated from hybridization between *P. pendula* for. *ascendens* and *P. sargentii* Rehder, based on palynological studies of

pollen from 6 species of cherry trees. However, the pattern of striae, along with other pollen characters, showed that *P. yedoensis* was closely related to *P. serrulata* var. *quelpaertensis*, making the latter one of the putative parents of *P. yedoensis*. In a cpDNA RFLP analysis, however, Kaneko *et al*. (1986) showed that *P. yedoensis* and *P. pendula* for. *ascendens* shared the same restriction pattern, and that *P. lannesiana* had a different pattern. Their result suggested that the maternal parent of *P. yedoensis* was *P. pendula* for. *ascendens*, if cpDNA is inherited maternally. Innan *et al*. (1995) affirmed that the *P. yedoensis* in Japan is likely a hybrid between *P. lannesiana* and *P. pendula* for. *ascendens*.

In the RAPD analysis, the genetic distance between *P. yedoensis* and *P. serrulata* var. *qeulpaertensis* was 0.4082, and was the lowest among all pairs. Interestingly, the native *P. yedoensis* from Jeju was completely separated from the cultivars, as suggested in previous morphological studies. In the RAPD patterns, the genetic distance between the native and cultivated *P. yedoensis* was 0.430, which is very high compared to other pairs (Jung *et al*., 1997). In addition, Kim *et al*. (1998) insisted that native *P. yedoensis* from Jeju was clearly distinguished from cultivated *P. yedoensis* based on the morphology of the flowers, leaves, seeds, and trichomes. In particular, the calyx tube of cultivated *P. yedoensis* is cup-shaped, whereas it is wedge-shaped in native specimens from Jeju.

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 RAPD and amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995; Maughan *et al.*, 1996; Paul *et al.*, 1997; Garcia *et al.*, 2000; Huh and Huh, 2001). Of these, sequencing techniques have been widely adopted for phylogenetic studies at various levels, using DNA regions with different base substitution rates. To assess molecular phylogenetic relationships and evolutionary traits among plants, the nuclear genes encoding the 18S and 25S ribosomal RNA (rRNA) components of cytosolic ribosomes have been studied extensively at the family and higher taxon levels (Hamby and Zimmer, 1992). To study the phylogeny of closely related genera or species, however, more rapidly evolving regions need to be analyzed.

The internal transcribed spacer (ITS) region is part of the transcriptional unit of nuclear ribosomal DNA (nrDNA), but the spacer segments of the transcript are not incorporated into mature ribosomes. Instead, the ITS1 and ITS2 regions of the nrDNA transcript appear to function, at least in plants, in the maturation of nuclear rRNA (Baldwin *et al.*, 1995). The ITS regions are relatively conservative in length and have exciting potential as a source of nuclear DNA characters for phylogenetic reconstruction in plants (Baldwin, 1992, 1993; Suh *et al.*, 1993; Bain and Jansen, 1995; Buckler and Holtsford, 1996). Furthermore, their rates of divergence are relatively high in comparison to protein or rRNA coding genes, such as 5.8S, 18S, and 25S 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). The small size of the ITS regions (less than 800 bp) makes them particularly appropriate for direct sequencing of DNA amplified by the PCR. In addition, the conserved nature of the surrounding coding regions of nrDNA (18S, 5.8S, and 25S) allows the construction of universal primers that can be used for very diverse organisms, from fungi to flowering plants (White *et al.*, 1990). Tandemly repeated nrDNA encodes three ribosomal RNA (rRNA) genes (18S, 5.8S, 25S) and each copy contains a transcribed region that is separated by a long nontranscribed intergenic spacer (IGS). The transcribed region contains three rRNA coding genes along with two internal transcribed spacers (ITSs), which occur in the order 5′-18S-ITS1-5.8S-ITS2-25S-3′ and are transcribed as a single precursor rRNA. The two ITSs (ITS1 and ITS2) are subsequently removed and the three rRNA coding regions eventually mature into three rRNAs (Rogers and Bendich, 1987). All three rRNA coding regions are highly conserved, in both structure and the level of sequence divergence (Lake, 1985; Olsen, 1987; Field *et al.*, 1988; hamby and Zimmer, 1992; Kim and Jansen, 1994). In contrast to the coding regions, numerous studies have demonstrated that the structure and sequence of the ITSs evolves rapidly (Ueki *et al.*, 1992; Yakura *et al.*, 1984; Yakura and Nishikawa, 1992; Chase *et al.*, 1993; Kim and Jansen, 1994).

The chloroplast genomes of the vast majority of vascular plants are circular molecules with 120-180 kilobase pairs (kbp) in size composed of two inverted repeat segments separating two single-copy regions.

The genetic content of chloroplast genomes is highly conserved, and includes genes for proteins involved in photosynthetic processes, RNA polymerase subunits, ribosomal and transfer RNA, and ribosomal proteins. Many of the chloroplast genes are organized into phylogenetically conserved, cotranscribed culsters. CpDNA has also been used extensively to infer plant phylogenies at different taxonomic levels. Direct sequencing of PCR products is now a rapidly expanding area of plant systematics and evolution (Clegg and Zurawski, 1991).

Particularly, the plastid *rbc*L 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 (Palmer *et al.*, 1988; Clegg and Zurawski 1991; Chase *et al.*, 1993; Clegg, 1993). Arguing from expected synonymous substitutions per site under a particular rate assumption, Clegg (1993) suggested that *rbc*L sequences are phylogenetically informative for the time interval 400-1000 million years ago. The *psb*A gene, encoding the DI reaction center protein of photosystem II, is also used to examine phylogenetic relationships among major groups of green plants (Lidholm *et al.*, 1991).

The photosynthetic genes, *rbc*L and *psb*A, have been used for phylogenetic analysis at the family level (Zurawski *et al.*, 1984; Soltis *et al.*, 1990; Wilson *et al.*, 1990; Jansen *et al.*, 1991; Bousquet *et al.*, 1992a; Michaels *et al.*, 1993; Morgan and Soltis, 1993) and also at higher levels (Bousquet *et al.*, 1992b; Gaut *et al.*, 1992; Chase *et al.*, 1993). However, the utility of *rbc*L and *psb*A genes have been limited to

the interordinal level of the intrafamilial level in the Zingiberales (Smith *et al.*, 1993).

Phylogenetic relationships using *rbc*L sequences also have been studied at lower taxonomic levels (inter- and intrageneric) in the Cornaceae (Xiang *et al.*, 1993), Cupressaceae (Gadex and Quinn, 1993), Ericaceae (Kron and Chase, 1993), Geraniaceae (Price and Palmer, 1993), Onagraceae (Conti *et al.*, 1993), and Saxifragaceae (Soltis *et al.*, 1993), indicating that *rbc*L can be used at the generic level. However, the phylogenetic relationships using *rbc*L sequences remain unclear, such as in the Asteraceae (Kim *et al.*, 1992), Cornaceae (Xiang *et al.*, 1993), and Saxifragaceae (Soltis *et al.*, 1993). Furthermore, in the tribe Triticeae of the Poaceae, relationships among the genera *Hordeum*, *Triticum*, and *Aegilops* could not be resloved (Doebley *et al.*, 1990; Gaut *et al.*, 1992). Therefore, the *rbc*L gene is sometimes too conserved to clarify relationships between closely related genera.

The *trn*L(UAA)-*trn*F(GAA) intergenic spacer, a noncoding chloroplast DNA region, have been used to define the phylogenetic relationships at lower taxonomic levels. This zone tends to evolve more rapidly than coding sequences, and accumulates insertions/deletions (indels) at a rate at least equal to that for nucleotide substitutions (Curtis and Clegg, 1984; Wolfe *et al.*, 1987; Zurawski and Clegg, 1987; Clegg and Zurawski, 1991). The sequence analysis can give us very useful information to define relationships below the family level. CpDNA is an extremely valuable molecule for studying phylogenetic relationships between closely related species (Palmer, 1987; Palmer *et al*, 1988;

Clegg *et al.*, 1991). Despite its conservative mode during evolution, numerous cases of intraspecific variation have been reported (reviewed by Soltis *et al.*, 1991). Accordingly, sequences of noncoding regions should contain more phylogenetically informative sites than *rbc*L equences. The universal primers were previously designed to amplify the cpDNA noncoding regions (Taberlet *et al.*, 1991).

Some plant genera have been subjected to extensive phylogenetic study. The phylogenetic relationships for the genus *Prunus* distributed in Korea have been studied using the results from the morphological (Kim *et al.*, 1990; Kim *et al.*, 1998) and cytological (Park *et al.*, 1984) data, and RAPD analysis (Jung *et al.*, 1997). These previous data provide the opportunity to evaluate molecular evolution and phylogenetic markers, and to construct the phylogeny for the *Prunus* species. However, these studies had several major problems such as questionable band pattern and reliance on cluster analysis for establishing species relationships.

This study aimed to (1) reconstruct the phylogenetic relationships among 45 taxa representing all of the subgenera (*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunophora*) of *Prunus* distributed in Korea and Japan by using molecular approaches; (2) estimate the origin and the evolution of *P. yedoensis* and the 5 related species, *P. lannesiana*, *P. pendula* for. *ascendens*, *P. sargentii*, *P. serrulata* var. *quelpaertensis*, and *P. serrulata* var. *spontanea,* which are putative parents of *P. yedoensis*. The sequences of the nrDNA ITSs (ITS1, 5.8S rRNA gene, and ITS2) and three cpDNA regions [*rbc*L gene, *psb*A gene, and *trn*L(UAA)-*trn*F(GAA) intergenic spacer] were analysed for these

purpose. Our findings were then compared with previous published results.

MATERIALS AND METHODS

1. Plant Materials

45 taxa representing all of the subgenera (*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunophora*) of the classification system of the genus *Prunus* proposed by Rehder (1940). *Magnolia pyramidata* (Magnoliaceae) was used as an outgroup taxon. Species' names were referred to Kitamura and Murata (1979) and Lee (1980). Ten grams of fresh leaves were collected at the end of the leaf-growing season, when the polysaccharide content was significantly lower than that of leaves collected earlier. The collected leaves were immediately frozen in liquid nitrogen and stored at -70°C until DNA extraction. The collected taxa and their localities are given in Table 2.

2. Total DNA Extraction and Purification

Total DNA was extracted from approximately 0.5 g of fresh leaves with hexadecyltrimethylammonium bromide (CTAB) extraction buffer, separated with chloroform/isoamyl alcohol, centrifuged at 5,000 rpm for 15 min, precipitated in isopropanol, and washed with ethanol, according to the procedure described by Doyle and Doyle (1987). DNA was further

Table 2. Collected taxa and their sources of *Prunus* and outgroup used in this study. Taxonomic treatment followed Rehder (1940) and species names were referred to Kitamura and Murata (1979) and Lee (1980)

Taxa	Source[a]
Subgenus *Cerasus*	
P. apetala (Sieb. et Zucc.) Fr. et Sav.	FFPRI Tama Science Forest Gargen, Tokyo, Japan
P. campanulata Maxim.	Taiwan[b]
P. choreiana Nakai	Halla Arboretum, Jeju, Korea
P. glandulosa Thunb. for. *sinensis* Koehne	Halla Arboretum, Jeju, Korea
P. incisa Thunb.	Chiba, Japan
P. incisa Thunb. var. *kinkiensis* Koidzumi	Hyogo, Japan
P. japonica Lev. var. *nakaii* Rehder	Halla Arboretum, Jeju, Korea
P. lannesiana Wils. 1	Tokyo, Japan
P. lannesiana Wils. 2	FGIFAJ, Ibaraki, Japan
P. leveilleana Koehne 1	Jeju, Korea
P. leveilleana Koehne 2	FFPRI Tama Science Forest Gargen, Tokyo, Japan
P. leveilleana Koehne var. *pendula* Nakai	Halla Arboretum, Jeju, Korea
P. maximowiczii Ruprecht 1	Jeju, Korea
P. maximowiczii Ruprecht 2	Yamanashi, Japan
P. pendula for. *ascendens* (Makino) Ohwi 1	Jeju, Korea
P. pendula for. *ascendens* (Makino) Ohwi 2	Kagoshima, Korea
P. sargentii Rehder 1	Jeju, Kores
P. sargentii Rehder 2	FFPRI Tama Science Forest Gargen, Tokyo, Japan
P. serrulata var. *pubescens* (Makino) Ohwi	Jeju, Korea
P. serrulata var. *quelpaertensis* (Nakai) Uyeki	Jeju, Korea
P. serrulata var. *spontanea* (Max.) Uyeki 1	Jeju, Korea
P. serrulata var. *spontanea* (Max.) Uyeki 2	FFPRI Tama Science Forest Gargen, Tokyo, Japan
P. serrulata var. *tomentella* Nakai	Jeju, Korea
P. takesimensis Nakai	National Arboretum, Kunggi, Korea
P. tomentosa Thunberg	Halla Arboretum, Jeju, Korea
P. yedoensis Matsumura - Cultivar 1	Jeju, Korea
P. yedoensis Matsumura - Cultivar 2	Chonnam, Korea

Table 2. (Continued)

Taxa	Source[a]
Subgenus *Cerasus*	
P. yedoensis Matsumura - Cultivar 3	Jeju, Korea
P. yedoensis Matsumura - Cultivar 4	TUAT, Tokyo, Japan
P. yedoensis Matsumura - Cultivar 5	Kyoto, Japan
P. yedoensis Matsumura - Cultivar 6	Shizuoka, Japan
P. yedoensis Matsumura - Native 1	Jeju, Korea
P. yedoensis Matsumura - Native 2	Jeju, Korea
P. yedoensis Matsumura - Native 3	Natural Monument No. 159-1, Jeju, Korea
P. yedoensis Matsumura - Native 4	Natural Monument No. 159-2, Jeju, Korea
Subgenus *Amygdalus*	
P. persica (L.) Batsch	Halla Arboretum, Jeju, Korea
P. persica for. *rubroplena* Schneid	Halla Arboretum, Jeju, Korea
Subgenus *Laurocerasus*	
P. spinulosa Sieb. et Zucc.	FFPRI Tama Science Forest Gargen, Tokyo, Japan
Subgenus *Padus*	
P. grayana Maxim.	FFPRI Tama Science Forest Gargen, Tokyo, Japan
P. padus L.	Jeju, Korea
P. buergeriana Miquel 1	Jeju, Korea
P. buergeriana Miquel 2	FFPRI Tama Science Forest Gargen, Tokyo, Japan
Subgenus *Prunophora*	
P. armeniaca var. *ansu* (L.) Marsh	Halla Arboretum, Jeju, Korea
P. mume (Sieb.) Sieb. & Zucc.	Halla Arboretum, Jeju, Korea
P. salicina Lindl.	Halla Arboretum, Jeju, Korea
Outgroup	
Magnolia pyramidata Thunb.	Jobes *et al.*, 1998, Kim *et al.*, 2000

[a]FFPRI, Forestry and Forest Product Research Institute; FGIFAJ, The Flower and Green Institute of the Flower Association of Japan; TUAT, Tokyo University of Agriculture & Technology. [b]Plant sample was received from Ph.D. Katsuki in FFPRI, Japan.

purified in cesium chloride/ethidium bromide gradients (Sambrook *et al.*, 1989). The DNA concentration ranged from 20 to 80 ng/㎕ depending on the sample. The purified DNA was diluted 10 times with distilled water and 1 ㎕ of each diluted DNA (2-8 ng) was used for 50 ㎕ amplification reactions.

3. Used NrDNA and CpDNA Regions

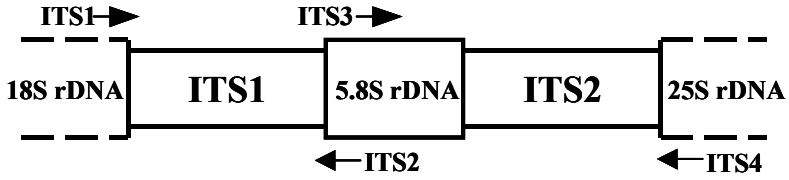
The ITS regions (ITS1 and ITS2) including the 5.8S rRNA coding gene of nrDNA and the three cpDNA regions [*rbc*L gene, *psb*A gene, and *trn*L(UAA)-*trn*F(GAA) intergenic spacer] were used as molecular markers to examine the molecular phylogeny and evolution of the genus *Prunus* distributed in Korea and Japan (Fig. 1). ITS sequences have been reported for various crops and wild plants, including rice (Takaiwa *et al.*, 1985), mustard (Rathgeber and Capesius, 1989), mung bean (Schibel and Hemleben, 1989), tobacco (Venkateswarlu and Nazar, 1991), tomato (Kiss *et al.*, 1988), melon (Kavanagh and Timmis, 1988), oats (Chatterton *et al.*, 1992a), barley (Chatterton *et al.*, 1992b), and Populus deltoides (D'Ovidio, 1992).

In addition, two photosynthetic genes, *rbc*L and *psb*A, were used in this study, because they are the most widely studied genes in angiosperms (Palmer *et al.*, 1988; Chase *et al.*, 1993; Clegg, 1993).

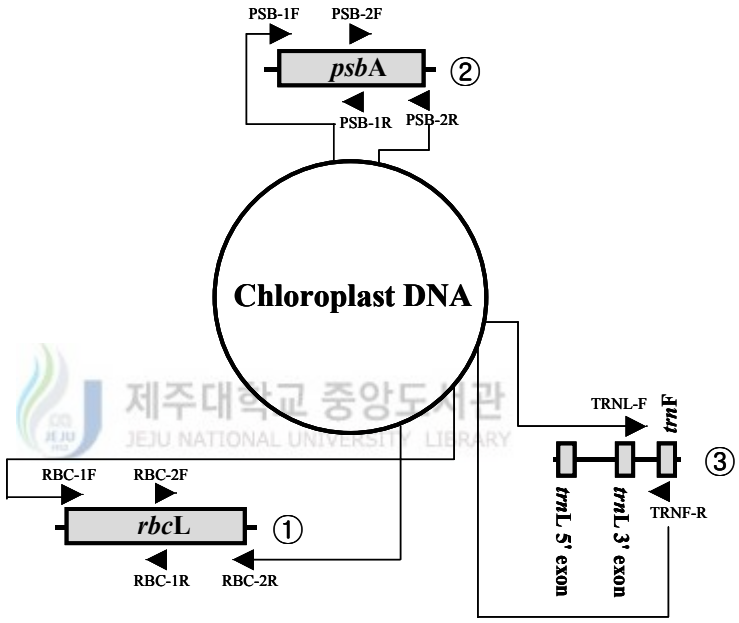
The sequences for the *rbc*L gene from various green plants have been used for molecular phylogenetic studies including Araliaceae

Figure 1. The general structure of the nrDAN ITS region and the three cpDNA regions [*rbc*L gene, *psb*A gene, *trn*L(UAA)-*trn*F(GAA)] used in this study. Arrows indicate the positions and orientations of the primers used. A) nrDNA ITS; B) cpDNA region. ① *rbc*L gene; ② *psb*A gene; ③ *trn*L(UAA)-*trn*F(GAA) intergenic spacer. C) Pattern of PCR bands on agarose gel electrophoresis. M: GeneRuler DNA ladder size marker. Lane 1 to 4: ITS region, *rbc*L gene, *psb*A gene, and *trn*L(UAA)-*trn*F(GAA) intergenic spacer, respectively.

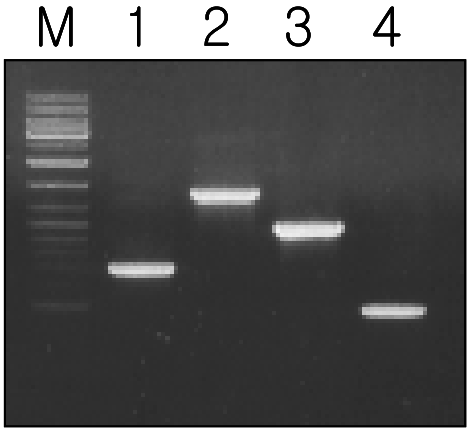
A)



B)



C)



(Plunkett *et al.*, 1997), Asteraceae (Panero and Jansen, 1997), Campanulales (Cosner *et al.*, 1994), Saxifragaceae (Soltis and Soltis, 1997), and Paeoniaceae (Sang *et al.*, 1997).

A cpDNA noncoding region, the *trn*L(UAA)-*trn*F(GAA) intergenic spacer, was also used in this study, because intergenic spacers generally change more rapidly than coding regions (Taberlet *et al.*, 1991; Fragan *et al.*, 1994; Gielly and Taberlet, 1996; Gielly *et al.*, 1996; Kim *et al.*, 1996; Bayer and Starr, 1998; Baker *et al.*, 1999; Richardson *et al.*, 2000).

4. Polymerase Chain Reaction

NrDNA-The nrDNA ITS region, which includes ITS1, ITS2, and 5.8S rRNA, was amplified directly by symmetric polymerase chain reaction (PCR) using the universal primers ITS1, ITS2, ITS3, and ITS4 (White *et al.*, 1990) (Fig. 1). The nucleotide sequences of primers ITS1, ITS2, ITS3, and ITS4 are listed in Table 3.

CpDNA-The primer pairs to amplify *rbc*L and *psb*A genes, and the *trn*L(UAA)-*trn*F(GAA) intergenic spacer are listed in Table 3. The primer pairs used for PCR are as follows; for the *rbc*L gene: RBC-1F and RBC-1R, RBC-2F and RBC-2R; for the *psb*A gene: PSB-1F and PSB-1R, PSB-2F and PSB-2R; and for the *trn*L(UAA)-*trn*F(GAA) intergenic spacer: TRNL-F and TRNF-R. The primers, RBC-1R, RBC-2F, PSB1R, and PSB-2F, were also used as sequencing primers to

Table 3. Oligonucleotide sequences of primers used in this study

Name	Sequences	References
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	White *et al.*, 1990
ITS2	5'-GCTGCGTTCTTCATCGATGC-3'	White *et al.*, 1990
ITS3	5'-GCATCGATGAAGAACGCAGC-3'	White *et al.*, 1990
ITS4	5'-TCCTCCGCTTATTGATATGC-3'	White *et al.*, 1990
RBC-1F	5'-ATGTCACCACAAACAGAGAC-3'	Morgan and Soltis, 1993
RBC-1R*	5'-ATGCCCTTTGATTTCACCTG-3'	This study
RBC-2F*	5'-CAGGTGAAATCAAAGGGCAT-3'	This study
RBC-2R	5'-CTTCACAAGCAGCAGCTAGTTC-3'	Morgan and Soltis, 1993
PSB-1F	5'-CCATGACTGCAATTTTAGAG-3'	Cipriani *et al.*, 1995
PSB-1R*	5'-CAGTAGCAGCTGCAACAGGA-3'	This study
PSB-2F*	5'-TCCTGTTGCAGCTGCTACTG-3'	This study
PSB-2R	5'-ACTTCCATACCAAGGTTAGC-3'	Cipriani *et al.*, 1995
TRNL-F	5'-AAAATCGTGAAGGTTCAAGT-3'	Sang *et al.*, 1997
TRNL-R	5'-GATTTGAACTGGTGACACGA-3'	Sang *et al.*, 1997

*The primers were designed on the basis of nucleotide sequences from *Nicotiana tabaccum* (Shinozaki *et al.*, 1986) and preliminary sequencing data from this experiment.

determine nucleotide sequences in the internal regions of *rbc*L and *psb*A genes (Fig. 1).

The PCR mixture contained 30.5 μl distilled sterile water, 5 μl of 10x reaction buffer (10 mM Tris-HCl, pH 9.0 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1% Triton X-100; Promega, USA), 2 μl of dNTP mixture (final concentration 200 μM, Promega, USA), 2.5 units of *Taq* DNA polymerase (Promega, USA), 3 μl of primer (final concentration 1 μM; DNA International Inc., USA), and 4 μl of genomic DNA. The amplification was performed in a GeneAmp PCR system 9600 thermal cycler (Perkin-Elmer Cetus, USA). After an initial 3-minute denaturation step at 94°C, PCR cycle parameters were as follows: 1 minute at 95°C for denaturation, 1 minute at 55°C for annealing, and 1 minute at 72°C for the extension step. After 30 thermal cycles, there was a final 7-minute step at 72°C. The PCR reaction was kept at 4℃ after amplification. Two μl of amplified DNA were then electrophoresed on 2% agarose gels at 50 V for 1 hour using 0.5x Tris-borate/EDTA (TBE) electrophoresis buffer.

5. Purification of PCR Products

The PCR products were purified by electrophoresis on a low-melting-point agarose gel (Promega, USA) matrix using 1x Tris-acetate / EDTA (TAE) electrophoresis buffer. After ethidium bromide

staining, the agarose block containing DNA was excised from the gel using a blade under low wavelength UV light. The excised block was transferred to a microtube, and melted for 10 minutes at 65℃. The PCR products were purified by precipitation using 3 M sodium acetate (pH 7.6) and ethanol, followed by washes in 70% ethanol. Finally, the DNA pellets were dissolved in distilled H_2O (Soltis and Soltis, 1997). In addition, concentrated DNA was recovered using the GeneClean II system according to the manufacturer's protocol (Bio 101, CA, USA).

6. Cloning and Sequencing

The PCR products were ligated into pT-Adv vector and cloned using the AdvanTAge PCR Cloning Kit (Clontech Laboratories, Palo Alto, CA). The ligation mixture contained 4 ㎕ distilled sterile water, 50 ng of PCR-product, 1 ㎕ of 10x ligation buffer, 50 ng (25 ng/㎕) of pT-Adv Vector, and 1 ㎕ of T4 DNA ligase. Then, the ligation mixture was incubated at 14℃ for 8 hours. The conditions for transformation and cloning followed the manufacturer's manual. The cloned DNA fragment was sequenced using Cy5-labeled vector inner primers, M13-40 primer (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and M13-reverse primer (5′-TTTCACACAGGAAACAGCTATGAC-3′), and a Cy5 AutoCycle Sequencing Kit on an ALFexpress DNA sequencer (Pharmacia Biotech, USA). Samples were electrophoresed at 1500 V for 700 minutes on 6% acrylamide-7 M urea gels using 0.6x TBE buffer as

the gel buffer.

7. Sequence Analysis and Secondary Folding Structure

The sequence boundaries of the nrDNA ITSs (ITS1, 5.8S rRNA coding gene, and ITS2) and the three cpDNA regions [*rbc*L gene, *psb*A gene, and *trn*L(UAA)-*trn*F(GAA) intergenic spacer] were determined by comparison with published sequences for various plant species (Takaiwa *et al.*, 1985; Kavanagh and Timmis, 1988; Kiss *et al.*, 1988; Yokota *et al.*, 1989; Venkateswarlu and Nazar, 1991; Baldwin, 1992; Chase *et al.*, 1993; Kron and Chase, 1993; Soltis *et al.*, 1993; Xiang *et al.*, 1993; Kim and Jansen, 1994; Sang *et al.*, 1995) and the entire tobacco cpDNA sequence (GenBank accession number NC_001879; Shinozaki *et al.*, 1986). The differences in the genetic structure of *P. yedoensis* cultivars and *P. yedoensis* native to Mt. Halla, Jeju, Korea were also demonstrated in comparative analyses of the nrDNA ITS1 region. To analyze the possible interdependence of mutations in the nrDNA ITS1 region, a secondary structure model of the gene was superimposed using the program Mfold ver. 3.1 (Zuker, 2000). Characters involved in putative base pairing were compared between the cultivars and the natives for *P. yedoensis*, and examined for possible phylogenetic information. In addition, the conserved sequence motifs reported previously were examined for sequence data from this study (Liu and Schardi, 1994).

8. Phylogenetic Analyses

Sequence gap opening and extension penalties were used to align the entire sequences using the program CLUSTAL W (Thompson *et al.*, 1994) with the default settings, and then alignment was adjusted manually in order to align several regions of conserved sequences. Both the small size and number of indels made manual adjustments feasible.

Parsimony analysis was performed with PAUP version 3.1.1 (Swofford, 1993) on a Macintosh Quadra 700 for the combined data or all regions [ITSs, *rbc*L, *psb*A and *trn*L(UAA)-*trn*F(GAA) intergenic spacer]. Various statistics were compiled for the combined data such as number of characters examined, percentage of variable sites, maximum divergence (Kimura's $K \times 100$), GC content, number of most parsimonious trees, associated tree lengths, consistency index (CI), and retention index (RI). To find the shortest tree, the HEURISTIC SEARCH algorithm, with MULPARS, COLLAPSE zero-length branches, Tree Bisection Reconnection (TBR) branch swapping, and 1000 random additions to search for multiple islands of trees (Maddison, 1991), was adopted with ACCTRAN optimization and setting Maxtrees=1000. Bootstrap analysis (Felsenstein, 1985) was used to assess the support for each node. Bootstrap analysis was carried out for 1000 replicates using heuristic searches with simple addition and TBR branch-swapping options.

The HEURISTIC search algorithm was used to find the most parsimonious trees in all analyses, except for the bootstrap analysis,

with the SIMPLE addition sequence and TBR swapping. A majority-rule consensus tree was derived from the bootstrap analysis using PAUP set to the HEURISTIC search option and SIMPLE addition sequence (Felsenstein, 1985).

Sequence divergence values among species were calculated using the DNADIST program of PHYLIP 3.572 (Felsenstein, 1993) and the numbers of nucleotide substitutions were estimated using Kimura's two-parameter method (Kimura, 1980). A transition/transversion ratio of 2.0 was used. A bootstrap analysis of these data was performed, using 1000 resampled data sets generated with the SEQBOOT program, before calculating the distance matrices and neighbor-joining trees. The CONSENSE program of PHYLIP was then used to construct a strict consensus tree.

RESULTS

1. Sequence Variation

The nucleotide sequences of three independent clones for the nrDNA ITSs (ITS1, 5.8S rRNA gene, ITS2) and three cpDNA regions [*rbc*L gene, *psb*A gene, and *trn*L(UAA)-*trn*F(GAA) intergenic spacer] were determined for 45 accessions representing all subgenera (*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunophora*) of genus *Prunus* distributed in Korea and Japan; *P. apetala*, *P. campanulata*, *P. choreiana*, *P. glandulosa* for. *sinensis*, *P. incisa*, *P. incisa* var. *kinkiensis*, *P. japonica* var. *nakaii*, two accessions of *P. lannesiana*, two accessions of *P. leveilleana*, *P. leveilleana* var. *pendula*, two accessions of *P. maximowiczii*, two accessions of *P. pendula* for. *ascendens*, two accessions of *P. sargentii*, *P. serrulata* var. *pubescens*, two accessions of *P. serrulata* var. *quelpaertensis*, two accessions *P. serrulata* var. *spontanea*, *P. serrulata* var. *tomentella*, *P. takesimensis*, *P. tomentosa*, 10 accessions (6 cultivars and 4 natives) of *P. yedoensis*, *P. persica*, *P. persica* for. *rubroplena*, two accessions of *P. buergeriana*, *P. grayana*, *P. padus*, *P. armeniaca* var. *ansu*, *P. mume*, *P. salicina*, and *P. spinulosa* (see Appendix). The nucleotide sequence data from this study were submitted to the GenBank of the National Center for Biotechnology Information (NCBI), under the accession numbers given in Table 4.

Table 4. Species and GenBank accession numbers included in the combined analyses of ITSs of the nrDNA and three cpDNA regions among 45 accessions of *Prunus*

Taxa	GenBank Accession No. ITS / *rbc*L / *psb*A / *trn*L-*trn*F	Abbreviation
Subgenus *Cerasus*		
P. apetala	AF411509/AF411500/AF410197/AF429902	P.apet
P. campanulata	AF411511/AF411501/AF410198/AF429903	P.camp
P. choreiana	AF185615/AF411496/AF410193/AF429904	P.chor
P. glandulosa for. *sinensis*	AF185622/AF411490/AF410187/AF429905	P.glan.sine
P. incisa	AF411508/AF411499/AF410196/AF429906	P.inci
P. incisa var. *kinkiensis*	AF411512/AF411502/AF410199/AF429907	P.inci.kink
P. japonica var. *nakaii*	AF145383/AF411482/AF410179/AF429908	P.japo.naka
P. lannesiana 1	AF411507/AF411498/AF410195/AF429909	P.lann1
P. lannesiana 2	AY052509/AY052517/AY052525/AF429910	P.lann2
P. leveilleana 1	AF185619/AF411483/AF410180/AF429911	P.leve1
P. leveilleana 2	AY052503/AY052511/AY052520/AF429912	P.leve2
P. leveilleana var. *pendula*	AY052512/AF411515/AF411510/AF429913	P.leve.pend
P. maximowiczii 1	AF143532/AF411484/AF410181/AF429914	P.maxi1
P. maximowiczii 2	AY052504/AY052513/AY052521/AF429915	P.maxi2
P. pendula for. *ascendens* 1	AF145378/AF314015/AF315342/AF429916	P.pend.asce1
P. pendula for. *ascendens* 2	AY052505/AY052514/AY052522/AF429917	P.pend.asce2
P. sargentii 1	AF143530/AF314014/AF315340/AF429918	P.sarg1
P. sargentii 2	AY052507/AY052515/AY052523/AF429919	P.sarg2
P. serrulata var. *pubescens*	AF145382/AF411486/AF410183/AF429920	P.serr.pube
P. serrulata var. *quelpaertensis*	AF145379/AF315338/AF315341/AF429921	P.serr.quel
P. serrulata var. *spontanea* 1	AF145380/AF411487/AF410184/AF429922	P.serr.spon1
P. serrulata var. *spontanea* 2	AY052508/AY052516/AY052524/AF429923	P.serr.spon2
P. serrulata var. *tomentella*	AF145381/AF411488/AF410185/AF429924	P.serr.tome
P. takesimensis	AF143534/AF411504/AF410201/AF429925	P.take
P. tomentosa	AF185617/AF411495/AF410192/AF429926	P.tome
P. yedoensis- Cultivar 1	AF117895/AF314012/AF314009/AF429927	P.yedo.C1
P. yedoensis- Cultivar 2	AF329014/AF329003/AF329008/AF429928	P.yedo.C2

Table 4. (Continued)

Taxa	GenBank Accession No. ITS/*rbc*L/*psb*A/*trn*L(UAA)-*trn*F(GAA)	Abbreviation
Subgenus *Cerasus*		
P. yedoensis- Cultivar 3	AF329015/AF329004/AF329009/AF429929	P.yedo.C3
P. yedoensis- Cultivar 4	AF329016/AF329005/AF329010/AF429930	P.yedo.C4
P. yedoensis- Cultivar 5	AF411506/AF411497/AF410194/AF429931	P.yedo.C5
P. yedoensis- Cultivar 6	AY052510/AY052518/AY052526/AF429932	P.yedo.C6
P. yedoensis- Native 1	AF145384/AF314011/AF314010/AF429933	P.yedo.N1
P. yedoensis- Native 2	AF329011/AF314013/AF315339/AF429934	P.yedo.N2
P. yedoensis- Native 3	AF329012/AF329001/AF329006/AF429935	P.yedo.N3
P. yedoensis- Native 4	AF329013/AF329002/AF329007/AF429936	P.yedo.N4
Subgenus *Amygdalus*		
P. persica	AF143535/AF411492/AF410189/AF429937	P.pers
P. persica for. *rubroplena*	AF185621/AF411493/AF410190/AF429938	P.pers.rubr
Subgenus *Laurocerasus*		
P. spinulosa	AF411513/AF411503/AF410200/AF429939	P.spin
Subgenus *Padus*		
P. grayana	AF411514/AF411505/AF410202/AF429942	P.gray
P. padus	AF143533/AF411485/AF410182/AF429943	P.padu
P. buergeriana 1	AF143531/AF411481/AF410178/AF429940	P.buer1
P. buergeriana 2	AY052502/AY052506/AY052519/AF429941	P.buer2
Subgenus *Prunophora*		
P. armeniaca var. *ansu*	AF185620/AF411489/AF410186/AF429944	P.arme.ansu
P. mume	AF185616/AF411491/AF410188/AF429945	P.mume
P. salicina	AF185618/AF411494/AF410191/AF429946	P.sali
Outgroup		
Magnolia pyramidata	U90801/AY008893/U63020/AY009080	M.pyra

ITSs of nrDNA–The length of the ITS1–5.8S–ITS2 region for the 45 accessions of the genus *Prunus* used in this study ranged from 625 (*P. glandulosa* for. *sinensis*) to 647 (*P. salicina*) bp, with ITS1 ranging from 222 (*P. glandulosa* for. *sinensis*) to 243 (*P. salicina*) bp, and ITS2 ranging from 230 (*P. yedoensis* cultivar 1) to 245 (2 taxa of *P. leveilleana* and *P. leveilleana* var. *pendula*) bp. In contrast, the length of the 5.8S rRNA coding region (164 bp) did not vary. No intraspecific length variation was found among 14 accessions (*P. lannesiana*, *P. leveilleana*, *P. maximowiczii*, *P. pendular* for. *ascendens*, *P. sargentii*, *P. serrulata* var. *spontanea*, and *P. buergeriana*), whereas the 10 accessions of *P. yedoensis* had length variation of 625 to 634 bp. The ITS2 region from 45 accessions was slightly longer than the ITS1 region, except for the 6 cultivated taxa of *P. yedoensis*, *P. tomentosa*, and *P. salicina* (Table 5).

The GC content of the ITS1 regions varied from 58.1 (*P. choreiana*) to 65.3% (*P. armeniaca* var. *ansu*), and that of the ITS2 region ranged from 62.0 (two taxa of *P. leveilleana* and a *P. leveilleana* var. *pendula*) to 68.3% (*P. armeniaca* var. *ansu*). However, the GC content of the 5.8S rRNA coding gene was identical at 54.9% in all 45 accessions of *Prunus*. In case of subgenus *Cerasus*, the GC content of the ITS regions (ITS1 and ITS2) was slightly lower than in any of the other subgenera (*Amygdalus*, *Padus*, *Prunophora*, and *Laurocerasus*). The GC content of the ITS2 region was higher than that of ITS1 among the 45 accessions of *Prunus*, except for *P. tomentosa* (Table 5).

Table 5. The length and GC contents of ITS1, ITS2, and 5.8S rRNA coding regions from 45-taxon of *Prunus*

Taxa	ITS1		5.8S		ITS2	
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)
Subgenus *Cerasus*						
P. apetala	235	60.0	164	54.9	239	62.3
P. campanulata	231	58.9	164	54.9	238	62.6
P. choreiana	234	58.1	164	54.9	239	63.2
P. glandulosa for. *sinensis*	222	60.4	164	54.9	239	63.2
P. incisa	234	60.3	164	54.9	239	63.6
P. incisa var. *kinkiensis*	235	60.4	164	54.9	239	63.6
P. japonica var. *nakaii*	238	60.3	164	54.9	239	63.6
P. lannesiana 1	235	59.6	164	54.9	239	63.2
P. lannesiana 2	235	59.6	164	54.9	239	63.2
P. leveilleana 1	235	58.3	164	54.9	245	62.0
P. leveilleana 2	235	58.3	164	54.9	245	62.0
P. leveilleana var. *pendula*	235	58.7	164	54.9	245	62.0
P. maximowiczii 1	236	60.3	164	54.9	239	63.6
P. maximowiczii 2	236	60.2	164	54.9	239	63.6
P. pendula for. *ascendens* 1	236	58.9	164	54.9	239	63.2
P. pendula for. *ascendens* 2	236	58.9	164	54.9	239	63.2
P. sargentii 1	235	60.0	164	54.9	239	63.6
P. sargentii 2	235	60.0	164	54.9	239	63.6
P. serrulata var. *pubescens*	237	60.8	164	54.9	239	64.0
P. serrulata var. *quelpaertensis*	235	60.0	164	54.9	238	63.0
P. serrulata var. *spontanea* 1	237	59.1	164	54.9	240	63.8
P. serrulata var. *spontanea* 2	237	59.1	164	54.9	240	63.3
P. serrulata var. *tomentella*	234	59.0	164	54.9	239	63.2
P. takesimensis	237	59.1	164	54.9	238	63.9
P. tomentosa	241	64.7	164	54.9	239	64.0

Table 5. (Continued)

Taxa	ITS1		5.8S		ITS2	
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)
Subgeneus *Cerasus*						
P. yedoensis cultivar 1	236	60.2	164	54.9	230	64.8
P. yedoensis cultivar 2	236	60.2	164	54.9	234	64.5
P. yedoensis cultivar 3	236	59.7	164	54.9	232	64.7
P. yedoensis cultivar 4	236	59.7	164	54.9	231	64.5
P. yedoensis cultivar 5	236	59.3	164	54.9	231	64.5
P. yedoensis cultivar 6	236	59.3	164	54.9	231	64.5
P. yedoensis native 1	229	59.4	164	54.9	233	64.4
P. yedoensis native 2	229	59.4	164	54.9	234	64.1
P. yedoensis native 3	229	59.4	164	54.9	234	64.5
P. yedoensis native 4	229	59.4	164	54.9	234	64.1
Subgenus *Amygdalus*						
P. persica	237	64.6	164	54.9	241	64.7
P. persicar for. *rubroplena*	239	64.0	164	54.9	242	64.9
Subgenus *Padus*						
P. buergeriana 1	234	59.8	164	54.9	239	63.2
P. buergeriana 2	234	58.5	164	54.9	239	63.6
P. grayana	233	59.7	164	54.9	239	64.9
P. padus	236	62.3	164	54.9	239	65.3
Subgenus *Prunophora*						
P. armeniaca var. *ansu*	236	65.3	164	54.9	240	68.3
P. mume	223	61.0	164	54.9	241	65.1
P. salicina	243	60.9	164	54.9	240	65.0
Subgenus Laurocerasus						
P. spinulosa	236	59.3	164	54.9	239	63.6

In this study examined 690 nucleotide sequences of the ITS alignment (ITS1, 5.8S rRNA gene, and ITS2) from 45 accessions (see Appendix I). Out of 690 aligned positions of the entire ITSs, 329 sites (47.7%) were variable among the sequences. More than half (53.2%, 175/329) of these variable sites were in ITS1, compared to 45.3% (149/329) in ITS2, and only 1.5% (5/164) in the 5.8S rRNA gene. Surprisingly, different variable sites were found in the ITS1 and ITS2 of *P. yedoensis* native to Jeju (Korea) and those of the cultivars. Namely, there was a five-bp deletion (correspond to 12-16 position in Appendix I) in the ITS1 region of the four native *P. yedoensis*. On the other hand, a five-bp deletion (correspond to 647-651 position in Appendix I) was found in the ITS2 region of the 6 cultivated *P. yedoensis* compared with the sequence of the other *Prunus* species. In addition, the ITSs had the highest proportion of variable sites (47.7%, 329/690) out of the 4 regions examined in this study (Fig. 2).

For the alignment of the entire regions, 41 indels were needed: 27 in ITS1 and 14 in ITS2. Most indels consisted of one to three nucleotides. Two relatively large indels were required for *P. glandulosa* for. *sinensis* and *P. mume*: 18-bp deletions in ITS1 (correspond to 121-138 position) and ITS2 (correspond to 118-135 position) region, respectively (see Appendix I).

CpDNA regions-No sequence length variation was found in the 45 accessions based on coding genes (*rbc*L and *psb*A) sequences. However, the length of the *trn*L(UAA)-*trn*F(GAA) intergenic spacer

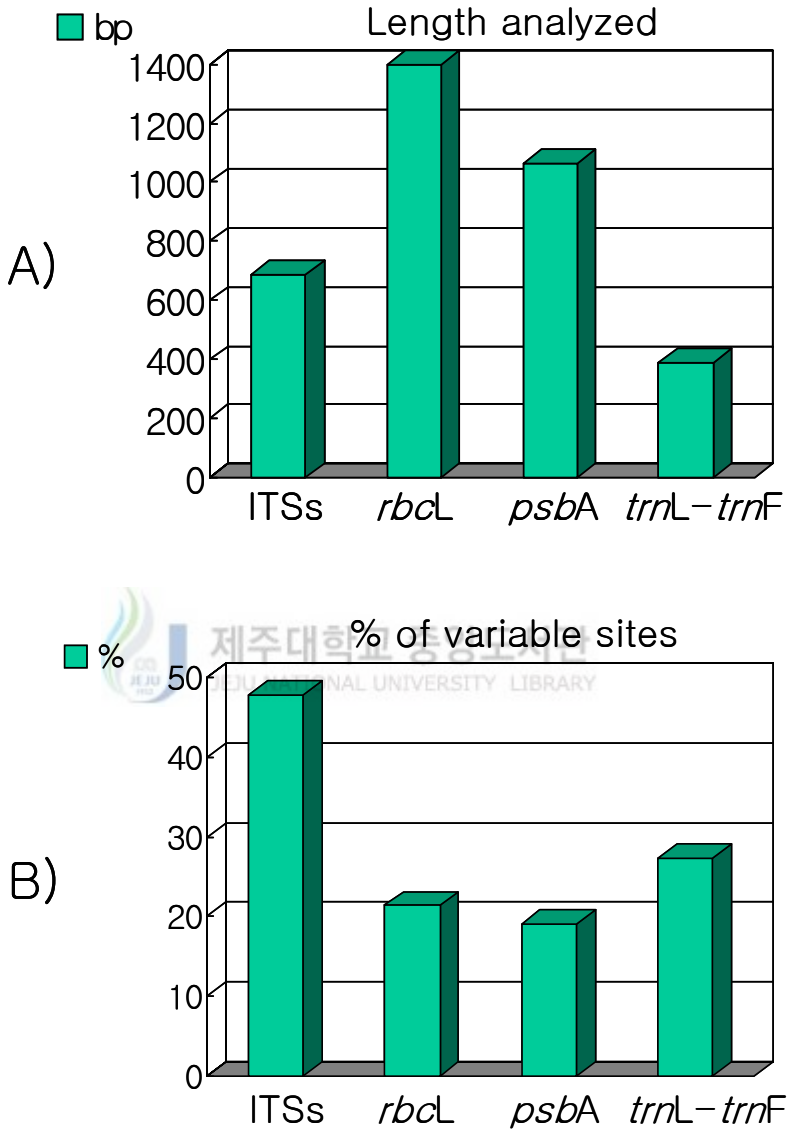


Figure 2. Comparison of the sequence characteristics of the nrDNA and three cpDNA regions in 45 accessions of genus *Prunus*.

ranged from 358 (two taxa of *P. buergeriana* and *P. padus*) to 387 (two taxa of *P. serrulata* var. *spontanea* and *P. spinulosa*) bp. Unlike the ITSs analysis, no intraspecific length variation was found in the 24 accessions (*P. lannesiana*, *P. leveilleana*, *P. maximowiczii*, *P. pendula* for. *ascendens*, *P. sargentii*, *P. serrulata* var. *spontanea, P. buergeriana*, and *P. yedoensis* natives and cultivars) (Table 6).

The nucleotide sequences determined for *rbc*L (1,398), *psb*A (1,062), and *trn*L(UAA)-*trn*F(GAA) intergenic spacer (392) were aligned to analyze variable sites among 45 accessions. Of the 2,852 aligned positions of the three cpDNA regions, 609 sites (21.4%) were variable: 299 sites (21.4%, 299/1398) in *rbc*L, 203 sites (19.1%, 203/1062) in *psb*A, and 107 sites (27.3%, 107/392) in the *trn*L(UAA)-*trn*F(GAA) intergenic spacer (Fig. 2). Although the coding genes (*rbc*L and *psb*A genes) had a lower ratio of variable sites than the non-coding region [*trn*L(UAA)- *trn*F(GAA) intergenic spacer], there were more variable sites in the coding genes. This result suggests that the two coding genes, *rbc*L and *psb*A, can be useful marker for the phylogenetic analysis of genus *Prunus* distributed in Korea and Japan.

No indels were required to align the nucleotide sequences for the *rbc*L and *psb*A genes. However, alignment of the nucleotide sequence of the *trn*L(UAA)-*trn*F(GAA) intergenic spacer required 14 indels. Eight of the 14 length mutations in the *trn*L(UAA)-*trn*F(GAA) intergenic spacer were one-bp indels. In addition, a three-bp deletion was detected in the *trn*L(UAA)-*trn*F(GAA) intergenic spacer of the subgenus *Padus* (two taxa of *P. buergeriana*, *P. grayana*, and *P. padus*). The longest length

Table 6. The length and GC contents of *rbc*L, *psb*A, and *trn*L(UAA)-*trn*F(GAA) regions from 45-taxon of *Prunus*

Taxa	*rbc*L		*psb*A		*trn*L-*trn*F	
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)
Subgenus *Cerasus*						
P. apetala	1398	43.8	1062	43.0	381	30.7
P. campanulata	1398	44.2	1062	42.7	382	32.2
P. choreiana	1398	44.5	1062	42.0	384	30.5
P. glandulosa for. *sinensis*	1398	44.8	1062	42.0	384	30.5
P. incisa	1398	44.3	1062	42.7	382	31.9
P. incisa var. *kinkiensis*	1398	44.6	1062	42.7	381	31.8
P. japonica var. *nakaii*	1398	44.8	1062	42.5	381	31.8
P. lannesiana 1	1398	44.6	1062	42.7	381	31.5
P. lannesiana 2	1398	44.6	1062	42.7	381	31.5
P. leveilleana 1	1398	44.6	1062	42.7	381	33.6
P. leveilleana 2	1398	44.6	1062	42.7	381	33.3
P. leveilleana var. *pendula*	1398	44.6	1062	42.5	381	33.3
P. maximowiczii 1	1398	44.6	1062	42.3	383	31.6
P. maximowiczii 2	1398	44.6	1062	42.4	383	31.6
P. pendula for. *ascendens* 1	1398	44.3	1062	43.0	381	30.7
P. pendula for. *ascendens* 2	1398	44.3	1062	43.0	381	31.0
P. sargentii 1	1398	44.3	1062	42.2	381	31.2
P. sargentii 2	1398	44.3	1062	42.3	381	31.0
P. serrulata var. *pubescens*	1398	44.4	1062	42.6	384	30.5
P. serrulata var. *quelpaertensis*	1398	44.2	1062	42.6	381	31.8
P. serrulata var. *spontanea* 1	1398	44.0	1062	43.0	387	30.5
P. serrulata var. *spontanea* 2	1398	43.9	1062	43.0	387	30.7
P. serrulata var. *tomentella*	1398	44.3	1062	42.3	384	30.5
P. takesimensis	1398	44.3	1062	42.7	386	29.8
P. tomentosa	1398	44.3	1062	41.4	384	30.5

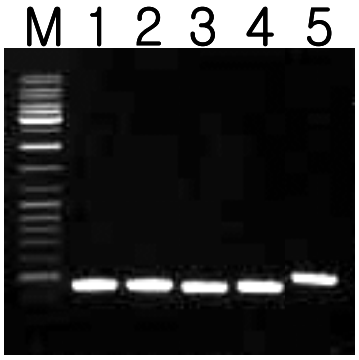
Table 6. (Continued)

Taxa	*rbc*L		*psb*A		*trn*L-trnF	
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)
Subgeneus *Cerasus*						
P. yedoensis cultivar 1	1398	44.1	1062	42.7	381	31.0
P. yedoensis cultivar 2	1398	44.1	1062	42.7	381	31.0
P. yedoensis cultivar 3	1398	44.0	1062	42.7	381	31.2
P. yedoensis cultivar 4	1398	44.0	1062	42.7	381	30.7
P. yedoensis cultivar 5	1398	44.1	1062	42.6	381	31.0
P. yedoensis cultivar 6	1398	44.1	1062	42.7	381	31.0
P. yedoensis native 1	1398	44.2	1062	42.7	381	30.2
P. yedoensis native 2	1398	44.1	1062	42.7	381	30.4
P. yedoensis native 3	1398	44.3	1062	42.7	381	30.2
P. yedoensis native 4	1398	44.3	1062	42.6	381	30.4
Subgenus *Amygdalus*						
P. persica	1398	44.5	1062	42.0	384	29.7
P. persicar for. *rubroplena*	1398	44.5	1062	42.2	384	31.3
Subgenus *Padus*						
P. buergeriana 1	1398	43.8	1062	42.8	358	30.7
P. buergeriana 2	1398	43.8	1062	43.0	358	30.4
P. grayana	1398	43.5	1062	42.6	361	30.7
P. padus	1398	43.7	1062	42.4	358	30.7
Subgenus *Prunophora*						
P. armeniaca var. *ansu*	1398	44.3	1062	42.1	385	31.7
P. mume	1398	44.3	1062	41.8	384	31.5
P. salicina	1398	44.1	1062	42.6	384	31.5
Subgenus Laurocerasus						
P. spinulosa	1398	43.9	1062	42.2	387	33.3

mutation was a 21-bp deletion in *P. padus* and two taxa of *P. buergeriana*. In *P. grayana,* 18-bp deletion was also detected (Fig. 3). Interestingly, these four accessions were grouped the subgenus *Padus*.

The GC content ranged from 43.5 (*P. grayana*) to 44.8% (*P. glandulosa* for. *sinensis* and *P. japonica* var. *nakaii*) for the *rbc*L gene, from 41.4 (*P. tomentosa*) to 43.0% (*P. apetala*, two taxa of *P. pendula* for. *ascendens*, two taxa of *P. serrulata* var. *spontanea*, and *P. buergeriana* 2) for the *psb*A gene, and from 29.7 (*P. persica*) to 33.6% (*P. leveilleana* 1) for the *trn*L(UAA)-*trn*F(GAA) intergenic spacer. In the subgenus *Padus*, the GC content of the *rbc*L gene was slightly lower than that of the subgenera *Amygdalus*, *Cerasus*, *Prunophora*, and *Laurocerasus*. However, the GC contents of the *psb*A gene and the *trn*L(UAA)-*trn*F(GAA) intergenic spacer were similar among all five subgenera of *Prunus*.

As found in the ITS sequence analysis, the *rbc*L and *psb*A gene sequences from *P. yedoensis* natives and cultivars were also distinct. In case of the *rbc*L gene, total 26 of amino acid substitutions were found among the natives and the cultivars of *P. yedoensis*. Five of the substitutions were completely changed among *P. yedoensis* cultivars and natives used in this study: N (AAT, asparagine) → Y (TAT, tysosine), V (GTC, valine) → I (ATC, isoleucine), H (CAT, histidine) → L (CTT, leucine), S (TCT, serine) → F (TTT, phenylalanine), and H (CAT, histidine) → L (CTT, leucine), at amino acid positions 19, 145, 187, 335, and 427, respectively (Table 7). In the *psb*A gene, 4 of 15 amino acid substitutions were also distinct: N (AAT, asparagine) → D (GAT,



Lane 1:	P.gray	**AACAAT**----------**TGAAACTT**
Lane 2:	P.padu	**AACAAT**----------**AACTT**
Lane 3:	P.buer1	**AACAAT**----------**AACTT**
Lane 4:	P.buer2	**AACAAT**----------**AACTT**
Lane 5:	P.pers	**AACAAT**ATATACCATTATTTGTACTGA**AACTT**

Figure 3. Comparison of the nucleotide length and deletion patterns of the *trn*L(UAA)-*trn*F(GAA) intergenic spacer among the subgenus *Padus* and other species. Bold letters indicate that the same nucleotide is found in five species; dashes (-) indicate gaps (deletion). Complete species names are given in Table 4.

Table 7. Amino acid substitutions of *rbc*L gene from 10 taxa of *P. yedoensis*. Abbreviations of taxa were listed in Table 4

Taxa	Coordinative Positions of Amino Acids																											
	19	25	68	78	84	96	97	109	117	138	140	145	187	216	231	259	282	288	295	296	314	335	380	388	414	427
P.yedo.C1	N*	D	E	E	E	D	L	S	I	F	G	V	H	Y	N	Y	H	H	K	N	G	S	L	S	L	H
P.yedo.C2	N	D	E	E	E	D	L	P	F	F	G	V	H	Y	N	Y	H	H	K	N	G	S	L	S	L	H
P.yedo.C3	N	D	E	E	E	D	L	S	I	F	G	V	H	Y	N	Y	H	H	K	N	G	S	L	S	L	H
P.yedo.C4	N	D	E	E	E	D	L	S	F	F	G	V	H	Y	N	Y	H	H	K	N	G	S	L	S	L	H
P.yedo.C5	N	D	E	E	G	D	L	S	F	F	G	V	H	Y	N	Y	H	H	K	N	G	S	L	S	L	H
P.yedo.C6	N	D	E	E	G	D	L	S	F	F	G	V	H	Y	N	C	H	H	K	N	G	S	L	S	L	H
P.yedo.N1	Y	D	E	G	E	D	L	P	F	Y	A	I	L	C	D	Y	K	N	R	T	G	F	V	S	Q	L
P.yedo.N2	Y	D	D	E	E	D	L	P	F	F	G	I	L	Y	N	Y	H	H	K	N	D	F	L	P	L	L
P.yedo.N3	Y	A	D	E	E	D	P	P	F	Y	A	I	L	C	N	Y	K	N	R	T	G	F	V	S	Q	L
P.yedo.N4	Y	D	D	G	E	G	L	P	F	F	A	I	L	C	D	Y	K	N	R	T	G	F	V	S	Q	L

*The symbols of Amino acids as follows: A, Alanine; C, Cysteine; D, Aspartic acid; E, Glutamic acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; Y, Tyrosine.

Table 8. Amino acid substitutions of *psb*A gene from 10 taxa of *P. yedoensis*. Abbreviations of taxa were listed in Table 4

Taxa	Coordinative Positions of Amino Acids															
	50	52	91	108	127	136	148	224	227	232	235	259	266	296	297
P.yedo.C1	I*	F	L	N	R	P	P	I	T	S	E	T	N	F	K
P.yedo.C2	M	F	S	N	M	P	P	I	T	S	E	T	N	F	K
P.yedo.C3	I	F	S	N	M	P	P	I	T	S	E	T	N	F	K
P.yedo.C4	I	L	S	N	R	P	S	I	T	S	E	T	N	F	K
P.yedo.C5	I	L	S	N	R	P	S	I	T	S	E	T	K	F	K
P.yedo.C6	I	L	S	N	R	P	S	I	T	S	E	T	K	F	K
P.yedo.N1	M	L	S	D	M	R	S	T	P	T	D	F	N	N	L
P.yedo.N2	M	L	S	D	M	R	P	T	T	S	E	I	N	N	L
P.yedo.N3	M	L	L	D	R	R	S	I	P	T	D	F	N	N	L
P.yedo.N4	M	L	L	D	R	R	S	I	T	S	D	F	N	N	L

*The symbols of Amino acids as follows: D, Aspartic acid; E, Glutamic acid; F, Phenylalanine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; R, Arginine; S, Serine; T, Threonin.

aspartic acid), P (CCT, proline) → R (CGT, arginine), F (TTC, phenylalanine) → N (AAC, asparagine), and (AAA, lysine) → L (TTA, leucine), at positions 108, 136, 296, and 297, respectively (Table 8).

2. Secondary Folding Structure Analysis

As mentioned above, sites in the nrDNA ITSs separated the native and cultivar *P. yedoenisis*. Namely, a five-bp deletion in ITS1 was found in the natives and a five-bp deletion in ITS2 in the cultivars. However, both the natives and cultivars had a highly conserved sequence motif in ITS1. The conserved sequence motif, 5′-GGCGCGAATTGCGCCAAGGAA-3′, was located in the center of ITS1 (correspond to 167-189 position in Appendix I). Two taxa of *P. buergeriana*, subgenus *Padus*, had the conserved sequence motif with an additional 2 bp, 5′-GGCGCGAAATTTGCGCCAAGGAA-3′. Four conserved sequence motifs were identified in the 45 accessions of *Prunus* (Fig. 4).

A difference in the genetic structure of *P. yedoensis* natives and cultivars was also seen in a comparative analysis of the ITS1 region. The secondary folding structure of ITS1 sequences including the conserved sequence motifs in native and cultivar *P. yedoensis* was examined (Fig. 5). The intra-taxon secondary folding structure of each population was also examined using ITS1 sequences (Figs. 6, 7). The significant structural variation was found in ITS1 region excluding conserved sequence motif between *P. yedoensis* native and cultivar

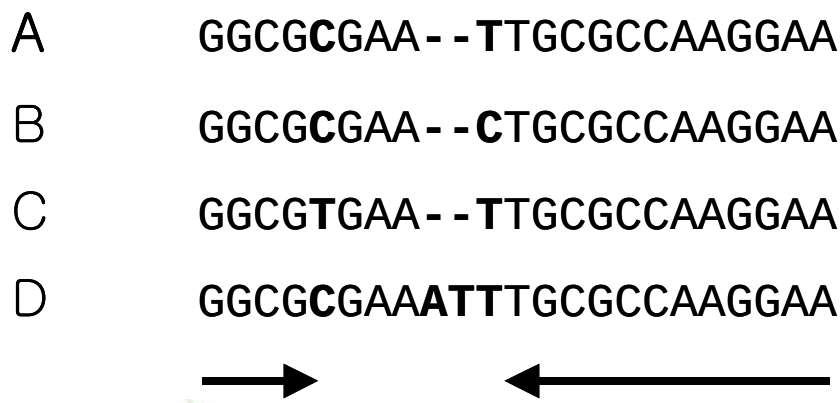


Figure 4. The four types of conserved sequence motifs in the nrDNA ITS1 region in 45 taxa of *Prunus*. The plant taxa belong to the following four types: *P. persica* and *P. armeniaca* var. *ansu* (B), *P. choreiana* and *P. japonica* var. *nakaii* (C), two taxa of *P. buergeriana* (D), and the others (A). The arrows indicate the same sequence in all *Prunus* taxa.

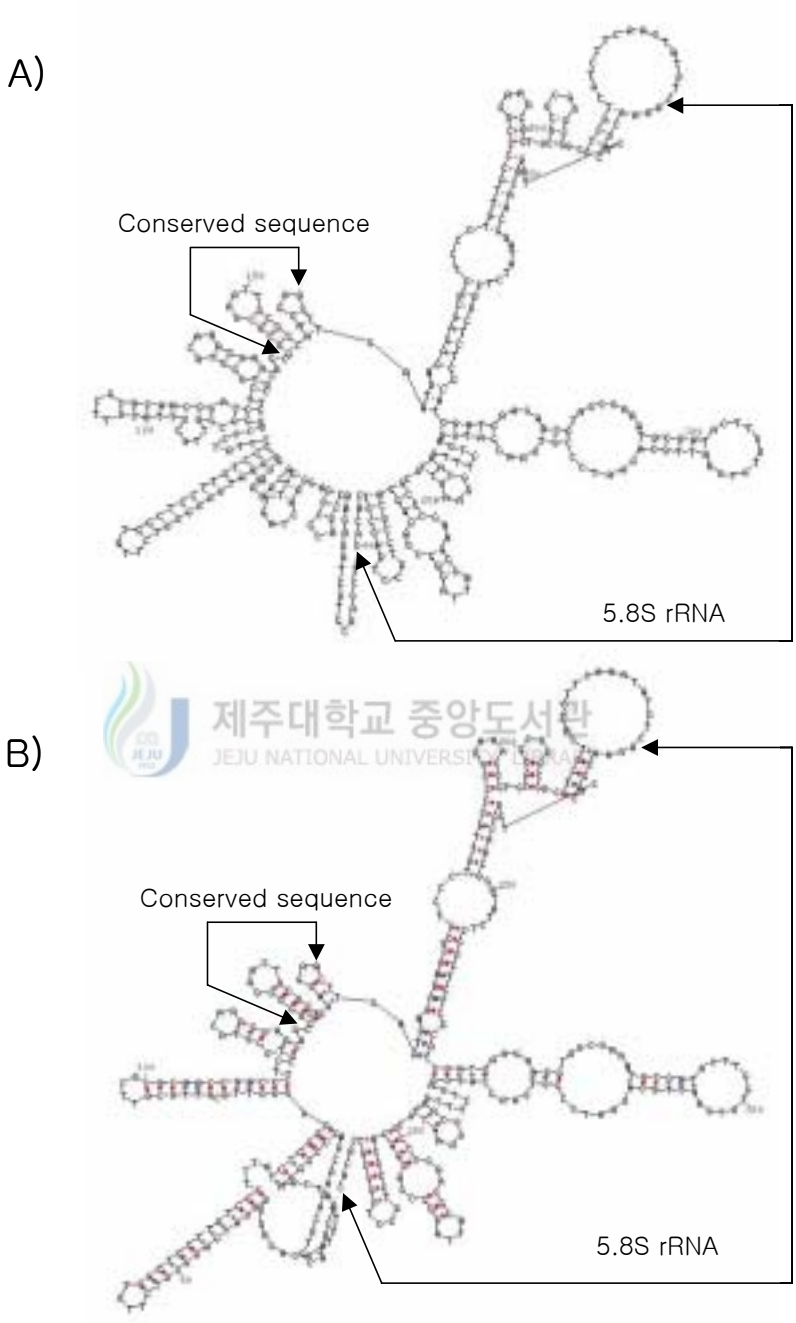


Figure 5. Secondary folding structure of the ITS1 and 5.8S rRNA region in cultivar (A) and native (B) *P. yedoensis*.

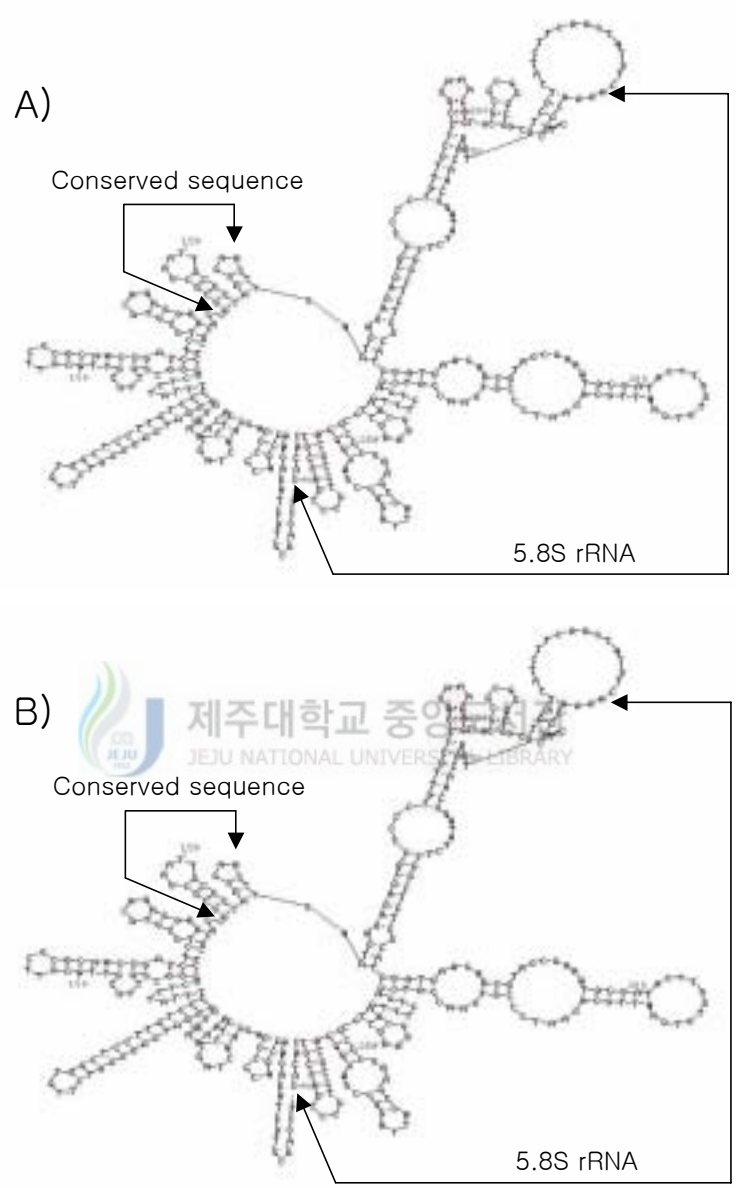


Figure 6. Secondary folding structure of the ITS1 region containing the 5.8S rRNA gene in *P. yedoensis* cultivars. A) *P. yedoensis* cultivar 2 from Haenam, Korea; B) *P. yedoensis* cultivar 4 from Tokyo, Japan.

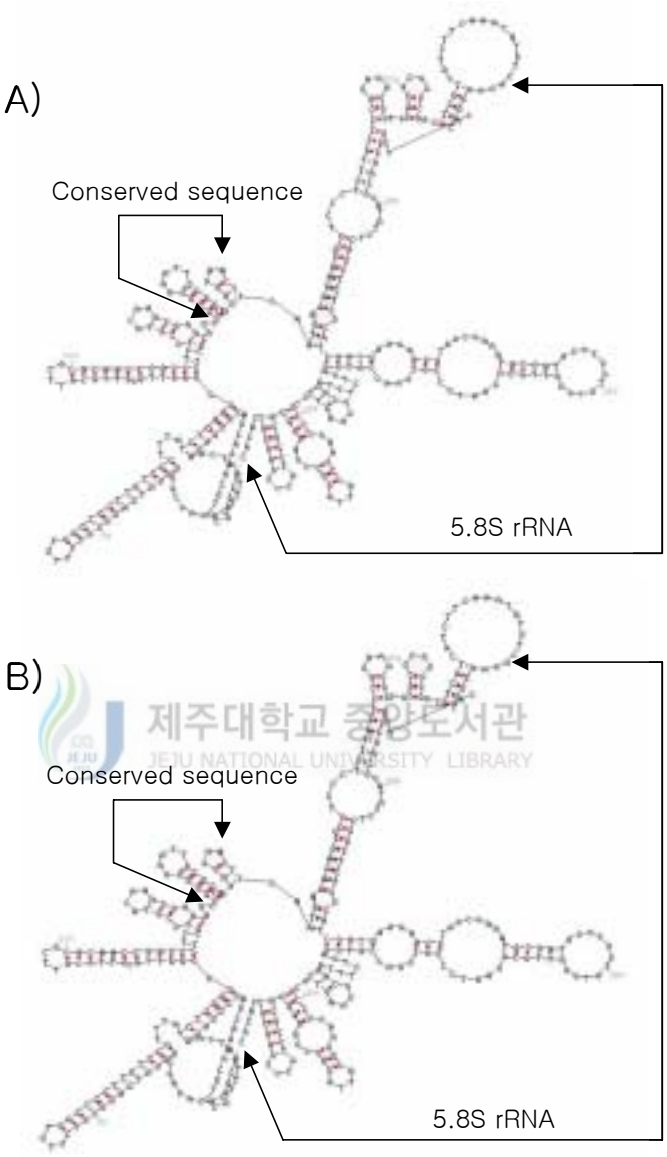


Figure 7. Secondary folding structure of the ITS1 region containing the 5.8S rRNA gene in *P. yedoensis* natives. A) *P. yedoensis* native 2; B) *P. yedoensis* native 3.

(Fig. 5). On the other hand, no structural variation was identified in intra-taxon (Fings. 6, 7). These results indicated that native *P. yedoensis* in Jeju is a different taxon from other cultivar.

3. Sequence Divergence

Sequence divergences were calculated using the combined data set for the nrDNA ITSs (ITS1, 5.8S rRNA, and ITS2) and the three cpDNA [*rbc*L gene, *psb*A gene, and *trn*L(UAA)-*trn*F(GAA) intergenic spacer] regions for the 45 accessions of *Prunus* distributed in Korea and Japan (Table 9). Pairwise sequence divergences ranged from 0.1 to 6.2% among *Prunus* species, and to 10.4% compared with the outgroup taxa (*Magnolia pyramidata*). The sequence divergences between the outgroup and the *Prunus* species is at least twice that among the ingroup species. The greatest sequence divergence (6.2%) was between *P. leveilleana* var. *pendula* and *P. armeniaca* var. *ansu*.

Pairwise intraspecies divergences (*P. lannesiana*, *P. leveilleana*, *P. maximowiczii*, *P. pendular* for. *ascendens*, *P. sargentii*, *P. serrulata* var. *spontanea*, and *P. buergeriana*) were similar, 0.1%. However, the divergence among 6 of *P. yedoensis* cultivars varied from 0.1 to 0.6%, and from 0.4 to 1.0% among the 4 of *P. yedoensis* natives. The divergence between *P. yedoensis* natives and cultivars ranged from 1.2 to 1.9%, and was two to three times greater than that within each *P. yedoensis* group (Table 9). When calculated separately, the divergences

for the ITSs and *rbc*L gene were greater than for the combined data (Table 9). Sequence divergences in the ITSs ranged from 0.0 to 1.0% among the 6 *P. yedoensis* cultivars, and from 0.0 to 0.5% among the 4 native *P. yedoensis*. Divergences of 2.4 to 3.4% were observed between *P. yedoensis* natives and cultivars (Table 10). In addition, the divergence values of the *rbc*L for multiple substitutions ranged from 0.1 to 0.4% among the 6 *P. yedoensis* cultivars, and from 0.2 to 1.5% among the native taxa. Divergences of 0.8 to 1.9% were observed between the native and cultivar *P. yedoensis* (Table 10).

Table 9. Sequence divergences in the combined data set of *Prunus* species and outgroups. Numbers below the diagonal are divergence values corrected for multiple substitutions using the two parameter model (Kimura, 1980)

Taxon*	1	2	3	4	5	6	7	8	9	10	11	12
1. P.apet	–											
2. P.camp	0.018	–										
3. P.chor	0.035	0.034	–									
4. P.glan.sine	0.046	0.046	0.029	–								
5. P.inci	0.013	0.012	0.031	0.044	–							
6. P.inci.kink	0.013	0.015	0.033	0.046	0.008	–						
7. P.japo.naka	0.043	0.042	0.035	0.020	0.039	0.041	–					
8. P.lann1	0.020	0.018	0.034	0.046	0.014	0.018	0.042	–				
9. P.lann2	0.021	0.019	0.034	0.046	0.015	0.018	0.042	0.001	–			
10. P.leve1	0.034	0.029	0.040	0.047	0.028	0.031	0.046	0.030	0.031	–		
11. P.leve2	0.034	0.030	0.040	0.048	0.028	0.031	0.046	0.030	0.031	0.001	–	
12. P.leve.pend	0.033	0.030	0.041	0.051	0.027	0.029	0.049	0.028	0.030	0.011	0.012	–
13. P.maxi1	0.027	0.024	0.031	0.040	0.023	0.024	0.039	0.025	0.026	0.030	0.030	0.032
14. P.maxi2	0.028	0.025	0.031	0.041	0.024	0.026	0.040	0.027	0.027	0.031	0.031	0.033
15. P.pend.asce1	0.019	0.021	0.032	0.042	0.019	0.020	0.042	0.023	0.024	0.034	0.035	0.033
16. P.pend.asce2	0.021	0.022	0.034	0.043	0.020	0.022	0.043	0.024	0.024	0.036	0.036	0.035
17. P.sarg1	0.024	0.024	0.031	0.042	0.021	0.022	0.043	0.015	0.016	0.033	0.034	0.031
18. P.sarg2	0.025	0.024	0.032	0.042	0.021	0.023	0.044	0.016	0.016	0.034	0.034	0.032
19. P.serr.pube	0.025	0.023	0.029	0.037	0.021	0.024	0.038	0.023	0.024	0.028	0.028	0.031
20. P.serr.quel	0.024	0.023	0.030	0.042	0.020	0.021	0.042	0.017	0.018	0.032	0.033	0.031
21. P.serr.spon1	0.021	0.021	0.027	0.038	0.017	0.020	0.038	0.020	0.021	0.027	0.027	0.028
22. P.serr.spon2	0.023	0.022	0.028	0.039	0.019	0.021	0.039	0.021	0.022	0.028	0.029	0.030
23. P.serr.tome	0.026	0.024	0.027	0.038	0.022	0.024	0.040	0.023	0.024	0.028	0.029	0.029
24. P.take	0.025	0.025	0.029	0.041	0.021	0.023	0.042	0.022	0.023	0.032	0.033	0.030
25. P.tome	0.042	0.042	0.020	0.026	0.039	0.041	0.032	0.041	0.042	0.048	0.049	0.049
26. P.yedo.C1	0.024	0.026	0.034	0.041	0.023	0.024	0.044	0.024	0.025	0.036	0.037	0.035
27. P.yedo.C2	0.025	0.026	0.033	0.041	0.024	0.025	0.044	0.025	0.026	0.036	0.037	0.035
28. P.yedo.C3	0.025	0.026	0.034	0.041	0.023	0.025	0.044	0.024	0.025	0.036	0.036	0.034
29. P.yedo.C4	0.025	0.026	0.034	0.042	0.023	0.025	0.044	0.025	0.026	0.036	0.037	0.035
30. P.yedo.C5	0.025	0.026	0.032	0.043	0.023	0.024	0.044	0.025	0.026	0.036	0.036	0.034
31. P.yedo.C6	0.027	0.027	0.035	0.044	0.024	0.026	0.046	0.026	0.026	0.037	0.037	0.036
32. P.yedo.N1	0.024	0.026	0.031	0.041	0.024	0.025	0.041	0.024	0.024	0.036	0.036	0.036
33. P.yedo.N2	0.019	0.019	0.028	0.038	0.018	0.019	0.038	0.019	0.020	0.031	0.031	0.031
34. P.yedo.N3	0.024	0.026	0.033	0.043	0.024	0.025	0.043	0.024	0.025	0.037	0.038	0.036
35. P.yedo.N4	0.023	0.025	0.031	0.041	0.022	0.024	0.041	0.023	0.024	0.035	0.036	0.033
36. P.pers	0.045	0.044	0.029	0.032	0.042	0.044	0.033	0.044	0.045	0.051	0.051	0.051
37. P.pers.rubu	0.041	0.041	0.025	0.029	0.038	0.040	0.031	0.040	0.041	0.047	0.048	0.047
38. P.buel1	0.038	0.040	0.037	0.043	0.038	0.040	0.040	0.040	0.041	0.046	0.047	0.049
39. P.buel2	0.040	0.042	0.038	0.045	0.040	0.042	0.042	0.041	0.042	0.048	0.048	0.050
40. P.gray	0.040	0.041	0.038	0.044	0.039	0.040	0.043	0.041	0.042	0.049	0.050	0.051
41. P.padu	0.040	0.043	0.042	0.045	0.039	0.041	0.044	0.043	0.044	0.050	0.050	0.050
42. P.arme.ans	0.056	0.055	0.048	0.042	0.054	0.056	0.044	0.055	0.056	0.058	0.059	0.062
43. P.mume	0.050	0.049	0.039	0.035	0.047	0.049	0.037	0.048	0.049	0.050	0.051	0.053
44. P.sali	0.046	0.045	0.034	0.034	0.042	0.045	0.036	0.045	0.045	0.047	0.047	0.048
45. P.spin	0.041	0.040	0.029	0.029	0.037	0.040	0.030	0.040	0.040	0.041	0.042	0.043
46. Outgroup	0.093	0.094	0.090	0.094	0.092	0.095	0.095	0.093	0.094	0.103	0.104	0.103

Table 9. (Continued)

Taxon	13	14	15	16	17	18	19	20	21	22	23	24
1. P.apet												
2. P.camp												
3. P.chor												
4. P.glan.sine												
5. P.inci												
6. P.inci.kink												
7. P.japo.naka												
8. P.lann1												
9. P.lann2												
10. P.leve1												
11. P.leve2												
12. P.leve.pend												
13. P.maxi1	-											
14. P.maxi2	0.001	-										
15. P.pend.asce1	0.022	0.023	-									
16. P.pend.asce2	0.023	0.024	0.001	-								
17. P.sarg1	0.019	0.020	0.017	0.018	-							
18. P.sarg2	0.019	0.020	0.018	0.018	0.001	-						
19. P.serr.pube	0.016	0.017	0.022	0.023	0.020	0.021	-					
20. P.serr.quel	0.019	0.021	0.014	0.015	0.010	0.011	0.019	-				
21. P.serr.spon1	0.016	0.018	0.017	0.018	0.016	0.017	0.015	0.015	-			
22. P.serr.spon2	0.018	0.018	0.018	0.018	0.017	0.018	0.016	0.015	0.001	-		
23. P.serr.tome	0.018	0.019	0.021	0.023	0.020	0.021	0.007	0.019	0.015	0.017	-	
24. P.take	0.020	0.021	0.020	0.021	0.018	0.019	0.018	0.018	0.015	0.016	0.018	-
25. P.tome	0.039	0.040	0.038	0.040	0.039	0.040	0.036	0.038	0.034	0.036	0.036	0.037
26. P.yedo.C1	0.024	0.025	0.016	0.017	0.016	0.017	0.023	0.016	0.019	0.020	0.023	0.022
27. P.yedo.C2	0.024	0.024	0.018	0.018	0.017	0.018	0.023	0.017	0.019	0.020	0.022	0.022
28. P.yedo.C3	0.024	0.024	0.018	0.018	0.016	0.017	0.023	0.016	0.019	0.020	0.023	0.022
29. P.yedo.C4	0.024	0.024	0.017	0.018	0.016	0.018	0.023	0.016	0.019	0.020	0.023	0.022
30. P.yedo.C5	0.024	0.024	0.018	0.019	0.017	0.018	0.024	0.017	0.020	0.021	0.022	0.023
31. P.yedo.C6	0.025	0.025	0.019	0.019	0.018	0.018	0.025	0.018	0.021	0.021	0.024	0.024
32. P.yedo.N1	0.024	0.025	0.018	0.019	0.022	0.022	0.023	0.019	0.019	0.021	0.022	0.020
33. P.yedo.N2	0.019	0.020	0.013	0.014	0.016	0.017	0.018	0.015	0.013	0.015	0.017	0.017
34. P.yedo.N3	0.025	0.026	0.018	0.019	0.022	0.022	0.024	0.018	0.019	0.021	0.024	0.020
35. P.yedo.N4	0.023	0.023	0.017	0.018	0.021	0.021	0.021	0.018	0.019	0.020	0.021	0.018
36. P.pers	0.040	0.041	0.040	0.041	0.041	0.041	0.037	0.040	0.036	0.038	0.037	0.038
37. P.pers.rubu	0.036	0.037	0.037	0.039	0.037	0.038	0.034	0.036	0.033	0.035	0.034	0.036
38. P.buel1	0.035	0.035	0.031	0.033	0.036	0.037	0.034	0.035	0.033	0.034	0.034	0.037
39. P.buel2	0.036	0.037	0.033	0.034	0.038	0.038	0.035	0.036	0.034	0.036	0.036	0.038
40. P.gray	0.036	0.037	0.034	0.036	0.038	0.039	0.035	0.036	0.035	0.036	0.036	0.037
41. P.padu	0.037	0.037	0.037	0.038	0.038	0.039	0.036	0.037	0.034	0.035	0.038	0.036
42. P.arme.ans	0.050	0.051	0.052	0.054	0.053	0.054	0.046	0.052	0.048	0.049	0.049	0.053
43. P.mume	0.044	0.045	0.045	0.047	0.046	0.046	0.039	0.045	0.041	0.043	0.040	0.045
44. P.sali	0.038	0.039	0.040	0.041	0.040	0.041	0.037	0.039	0.036	0.037	0.037	0.038
45. P.spin	0.035	0.036	0.037	0.038	0.037	0.038	0.034	0.036	0.033	0.034	0.034	0.035
46. Outgroup	0.089	0.091	0.088	0.089	0.087	0.088	0.090	0.088	0.085	0.086	0.088	0.088

Table 9. (Continued)

Taxon	25	26	27	28	29	30	31	32	33	34	35	36
1. P.apet												
2. P.camp												
3. P.chor												
4. P.glan.sine												
5. P.inci												
6. P.inci.kink												
7. P.japo.naka												
8. P.lann1												
9. P.lann2												
10. P.leve1												
11. P.leve2												
12. P.leve.pend												
13. P.maxi1												
14. P.maxi2												
15. P.pend.asce1												
16. P.pend.asce2												
17. P.sarg1												
18. P.sarg2												
19. P.serr.pube												
20. P.serr.quel												
21. P.serr.spon1												
22. P.serr.spon2												
23. P.serr.tome												
24. P.take												
25. P.tome	-											
26. P.yedo.C1	0.040	-										
27. P.yedo.C2	0.040	0.002	-									
28. P.yedo.C3	0.040	0.001	0.002	-								
29. P.yedo.C4	0.040	0.002	0.003	0.002	-							
30. P.yedo.C5	0.040	0.004	0.004	0.004	0.003	-						
31. P.yedo.C6	0.042	0.006	0.006	0.005	0.004	0.002	-					
32. P.yedo.N1	0.038	0.016	0.014	0.016	0.016	0.017	0.019	-				
33. P.yedo.N2	0.034	0.013	0.012	0.012	0.012	0.014	0.015	0.009	-			
34. P.yedo.N3	0.039	0.016	0.016	0.017	0.016	0.018	0.019	0.004	0.010	-		
35. P.yedo.N4	0.038	0.015	0.014	0.016	0.015	0.016	0.018	0.004	0.008	0.005	-	
36. P.pers	0.023	0.042	0.042	0.042	0.042	0.043	0.045	0.040	0.036	0.041	0.039	-
37. P.pers.rubu	0.018	0.037	0.037	0.037	0.037	0.039	0.040	0.036	0.032	0.037	0.035	0.010
38. P.buel1	0.042	0.035	0.034	0.035	0.035	0.035	0.037	0.032	0.030	0.034	0.033	0.042
39. P.buel2	0.044	0.036	0.036	0.037	0.037	0.036	0.039	0.034	0.032	0.036	0.034	0.044
40. P.gray	0.043	0.038	0.039	0.039	0.039	0.039	0.040	0.034	0.032	0.035	0.034	0.042
41. P.padu	0.044	0.039	0.039	0.039	0.039	0.040	0.041	0.037	0.034	0.038	0.036	0.041
42. P.arme.ans	0.040	0.052	0.052	0.051	0.052	0.053	0.054	0.050	0.046	0.051	0.050	0.032
43. P.mume	0.032	0.045	0.045	0.044	0.045	0.046	0.047	0.043	0.039	0.044	0.043	0.034
44. P.sali	0.032	0.041	0.042	0.040	0.041	0.041	0.042	0.040	0.036	0.041	0.039	0.031
45. P.spin	0.029	0.039	0.040	0.037	0.038	0.038	0.039	0.037	0.033	0.037	0.036	0.028
46. Outgroup	0.094	0.089	0.090	0.089	0.089	0.091	0.092	0.089	0.083	0.088	0.089	0.092

Table 9. (Continued)

Taxon	37	38	39	40	41	42	43	44	45	46
1. P.apet										
2. P.camp										
3. P.chor										
4. P.glan.sine										
5. P.inci										
6. P.inci.kink										
7. P.japo.naka										
8. P.lann1										
9. P.lann2										
10. P.leve1										
11. P.leve2										
12. P.leve.pend										
13. P.maxi1										
14. P.maxi2										
15. P.pend.asce1										
16. P.pend.asce2										
17. P.sarg1										
18. P.sarg2										
19. P.serr.pube										
20. P.serr.quel										
21. P.serr.spon1										
22. P.serr.spon2										
23. P.serr.tome										
24. P.take										
25. P.tome										
26. P.yedo.C1										
27. P.yedo.C2										
28. P.yedo.C3										
29. P.yedo.C4										
30. P.yedo.C5										
31. P.yedo.C6										
32. P.yedo.N1										
33. P.yedo.N2										
34. P.yedo.N3										
35. P.yedo.N4										
36. P.pers										
37. P.pers.rubu	-									
38. P.buel1	0.040	-								
39. P.buel2	0.041	0.001	-							
40. P.gray	0.040	0.014	0.015	-						
41. P.padu	0.038	0.026	0.027	0.016	-					
42. P.arme.ans	0.032	0.054	0.056	0.054	0.053	-				
43. P.mume	0.031	0.049	0.050	0.049	0.048	0.021	-			
44. P.sali	0.029	0.045	0.047	0.046	0.043	0.031	0.026	-		
45. P.spin	0.024	0.026	0.028	0.027	0.027	0.029	0.023	0.025	-	
46. Outgroup	0.091	0.092	0.094	0.095	0.093	0.104	0.098	0.094	0.092	-

*Species abbreviations are given in Table 4

Table 10. Sequence divergences among *P. yedoensis* native and the cultivars. Numbers above the diagonal are divergence values of ITSs for mulltiple substitutions using the two parameter model (Kimura, 1980). Calculated divergence values of *rbc*L gene in pairwise comparison are shown below diagonal. Species abbreviations are given in Table 4.

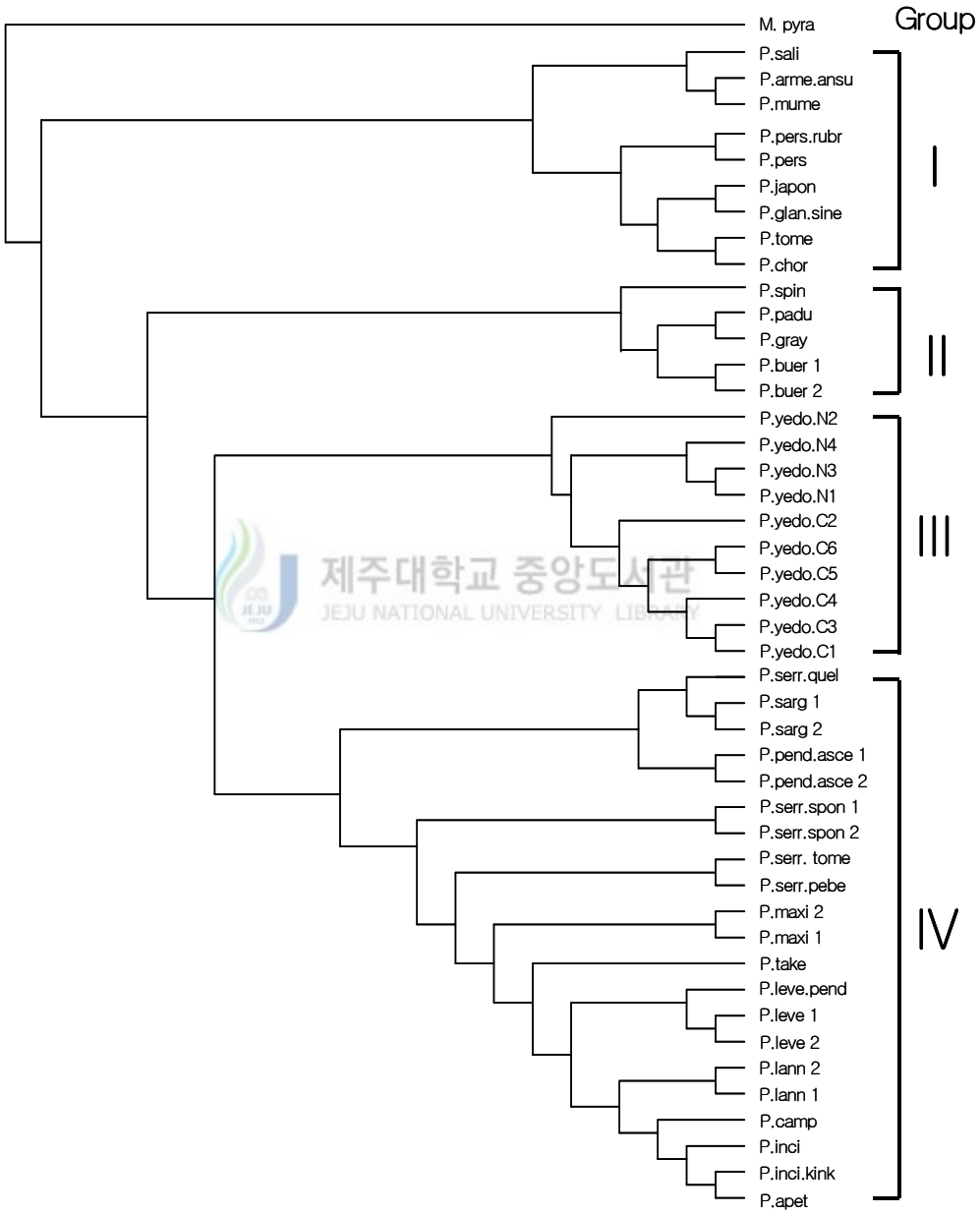
Taxa	1	2	3	4	5	6	7	8	9	10
1. P.yedo.C1	-	0.000	0.002	0.002	0.008	0.010	0.026	0.028	0.026	0.024
2. P.yedo.C2	0.001	-	0.002	0.002	0.008	0.010	0.026	0.027	0.026	0.024
3. P.yedo.C3	0.001	0.002	-	0.000	0.006	0.008	0.028	0.026	0.028	0.026
4. P.yedo.C4	0.001	0.001	0.001	-	0.006	0.008	0.028	0.026	0.028	0.026
5. P.yedo.C5	0.002	0.002	0.001	0.001	-	0.002	0.031	0.031	0.032	0.031
6. P.yedo.C6	0.004	0.004	0.003	0.002	0.001	-	0.032	0.032	0.034	0.032
7. P.yedo.N1	0.014	0.013	0.015	0.014	0.015	0.016	-	0.005	0.000	0.002
8. P.yedo.N2	0.009	0.008	0.009	0.009	0.009	0.011	0.014	-	0.005	0.003
9. P.yedo.N3	0.017	0.016	0.017	0.016	0.017	0.019	0.006	0.015	-	0.002
10. P.yedo.N4	0.015	0.014	0.015	0.014	0.014	0.016	0.002	0.014	0.007	-

4. Phylogenetic Analyses of the Genus *Prunus*

A parsimony analysis for the unambiguously aligned combined sequences [ITSs, *rbc*L, *psb*A, and *trn*L(UAA)-*trn*F(GAA)] from 46 accessions including outgroup taxon produced 7 equally most parsimonious trees, when the coded gaps were excluded from the data. One of the 7 most parsimonious trees is shown in Figure 8. The tree had a length of 513 steps. The consistency index (CI) were 0.642 or 0.609 with uninformative characters excluded (Fig. 8). The retention index (RI) was 0.745; therefore, the rescaled consistency index (RC) was 0.604 without uninformative characters (Fig. 8). The most parsimonious trees had four large, independent groups: group I (subgenera *Prunophora*, *Amygdalus*, and section *Microcerasus* of subgenus *Cerasus*), group II (subgenera *Padus* and *Laurocerasus*), group III (subgenus *Cerasus* II, *P. yedoensis*), and group IV (subgenus *Cerasus* III). Interestingly, the subgenus *Cerasus* was split among three subgroups (Fig. 8), and *P. yedoensis* was separated from the other *Cerasus* species. The four specimens of *P. yedoensis* native to Mt. Halla, Jeju, Korea, were clearly distinguished from the cultivar plants (Group III in Fig. 8).

A neighbor-joining dendrogram based on the combined nrDNA ITSs and cpDNA data was similar to the parsimony trees (Fig. 9). However, *P. pendula* for. *ascendens* was grouped with *P. yedoensis* in the neighbor-joining tree, while it was grouped with *P. serrulata* var. *quelpaertensis* and 2 taxa of *P. sargentii* in the most parsimonious trees

Figure 8. One of the 7 most parsimonious trees generated with PAUP in a heuristic search using simple taxon addition, TBR, and MULPARS. The tree was 513 steps long. Excluding uninformative characters, the consistency index was 0.609 and the retention index 0.745. M.pyra (*Magnolia pyramidata*) was used as the outgroup taxon. Complete taxon names are given in Table 4.



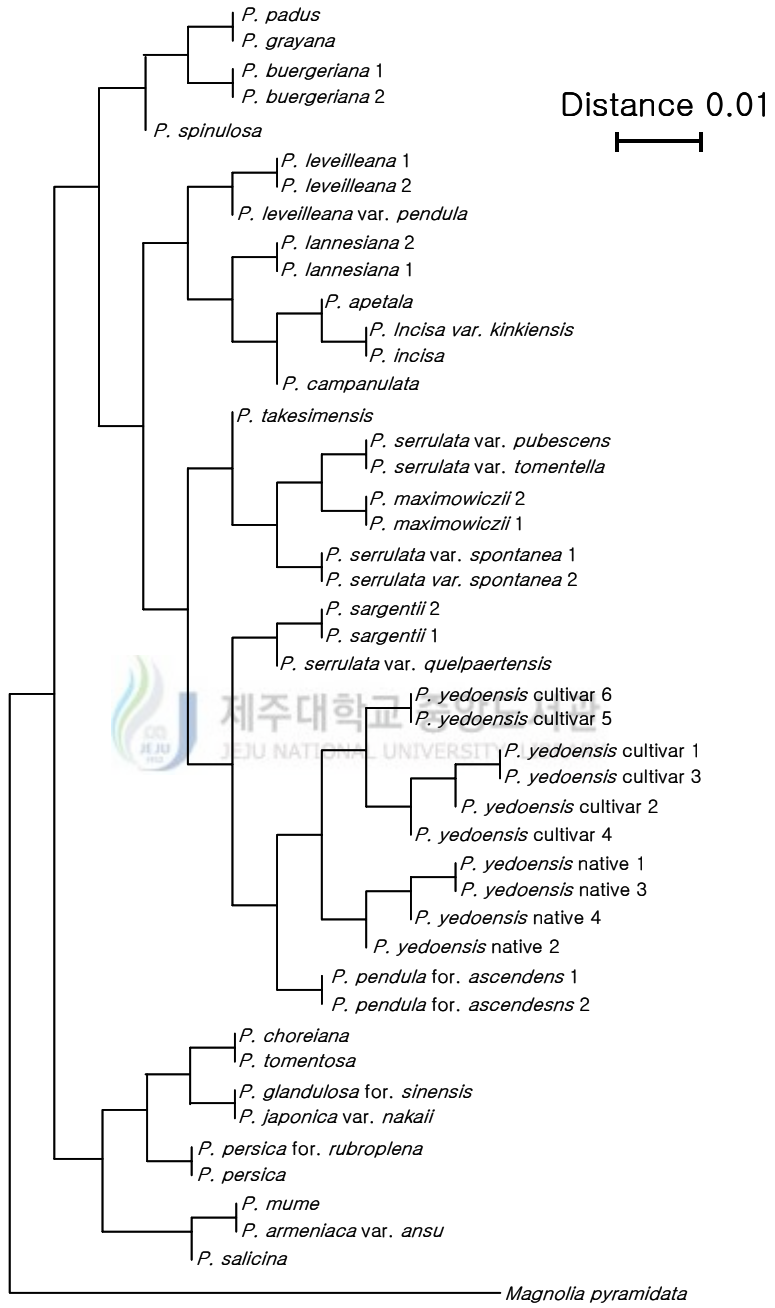
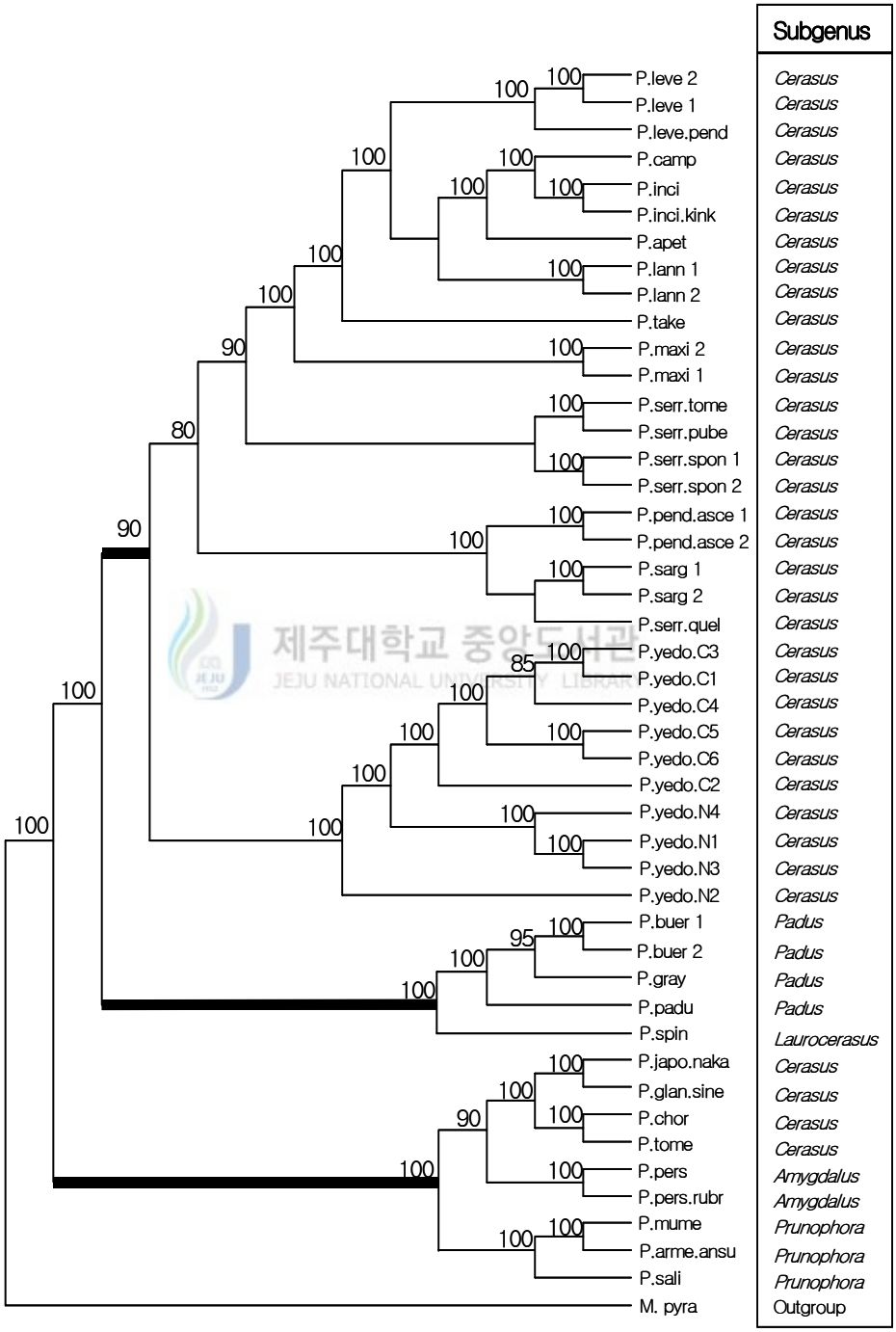


Figure 9. Neighbor-joining dendrogram based on the combined sequence data for the 45 taxa of *Prunus*. *Magnolia pyramidata* was used as the outgroup.

(Figs. 8, 9). In addtion, *P. choreiana*, *P. tomentosa*, *P. glandulosa* for. *sinensis*, and *P. japonica* var. *nakaii*, section *Microcerasus* of subgenus *Cerasus*, were related to the subgenus *Amygdalus*, *P. persica,* and *P. persica* for. *rubroplena,* as in the parsimony trees (Fig. 9).

The majority-rule bootstrap consensus tree based on the most parsimonious trees is shown in Figure 10. The bootstrap values for individual clades based on 1000 replicates ranged from 80 to 100%. The strict consensus tree, constructed using the SEQBOOT, DNAPARS, and CONSENSUS programs in PHYLIP (version 3.572), was not especially informative, since it was basically the average of the two general tree topologies obtained previously (Fig. 10). The strict consensus trees had monophyletic groups consistent with the five subgenera *Cerasus*, *Padus*, *Amygdalus*, *Prunophora*, and *Laurocerasus*, with a common ancestral divergence point with a bootstrap value of 100%. In addition, the subgenera *Prunophora* (*P. salicina*, *P. mume*, and *P. armeniaca* var. *ansu*) and *Amygdalus* (*P. persica* and *P. persica* for. *rubroplena*) had a well-supported sister group relationship with a bootstrap value of 100% for their divergence, as did the subgenera *Cerasus, Laurocerasus,* and *Padus* (Fig. 10). However, the phylogenetic positions of *P. glandulosa* for. *sinensis*, *P. tomentosa*, *P. japonica* var. *nakaii*, and *P. choreiana* in the subgenus *Cerasus* were noticeable. These taxa joined with the subgenus *Amygdalus*, *P. persica,* and *P. persica* for. *rubroplena*, with 100% bootstrap support for their divergence (Fig. 10).

Figure 10. A strict consensus tree for the seven equally most parsimonious trees based on the combined sequence data for the 45 taxa of *Prunus*. Bootstrap values (1000 replicates) are shown on the consensus trees above the nodes. Node in bold indicate well-supported major clades in *Prunus*. *Magnolia pyramidata* (M.pyra) was used as the outgroup. Complete taxon names are provided in Table 4.



5. Origin and Evolution of *P. yedoensis*

From the most parsimonious trees based on the ITSs and *rbc*L sequence data, *P. yedoensis* natives and cultivars were separated from the other *Prunus* species. In addition, 4 of *P. yedoensis* native to Mt. Halla, Jeju, Korea, were clearly distinguished from the six cultivars (Figs. 11, 12). However, in the parsimony trees using *rbc*L sequences, one of *P. yedoensis* natives (P.yedo.N2 in Fig. 12) was separated from the other three native taxa. *P. yedoensis* native 2 was grouped with *P. pendula* for. *ascendens* and *P. serrulata* var. *spontanea* (Fig. 12).

The majority-rule bootstrap consensus trees based on these most parsimonious trees are shown in Figs. 13 and 14. The bootstrap values for individual clades based on 1000 replicates ranged from 56 to 100% for the ITS tree, and from 50 to 100% for the *rbc*L tree (Figs. 13, 14). The majority consensus tree, found using the SEQBOOT, DNAPARS, and CONSENSUS programs in PHYLIP (version 3.572), was similar to the most parsimonious trees. Both the most parsimonious and majority-rule consensus trees based on the nrDNA ITS sequences had monophyletic groups consisting of the 6 cultivars and 4 natives taxa of *P. yedoensis* and *P. pendula* for. *ascendens*, with 100% bootstrap value. However, in the trees based on the *rbc*L sequences, the 10 *P. yedoensis* taxa formed monophyletic groups with two *Prunus* species (*P. serrulata* var. *spontanea*, *P. pendula* for. *ascendens*), although *P. yedoensis* native 2 was more closely related to the two species.

From the independent parsimony analyses, each parsimonious tree

had similarly topology. Therefore, the *rbc*L and ITS data sets were combined. The combined analysis using 15 taxa yielded a 116-step parsimonious tree with CI=0.742 and RI=0.887 (Fig. 15). The majority-rule consensus tree based on the combined data set was similar to the trees produced by the ITS analyses. However, the support for the clades was much stronger; all these clades had 100% bootstrap value (Fig. 15).

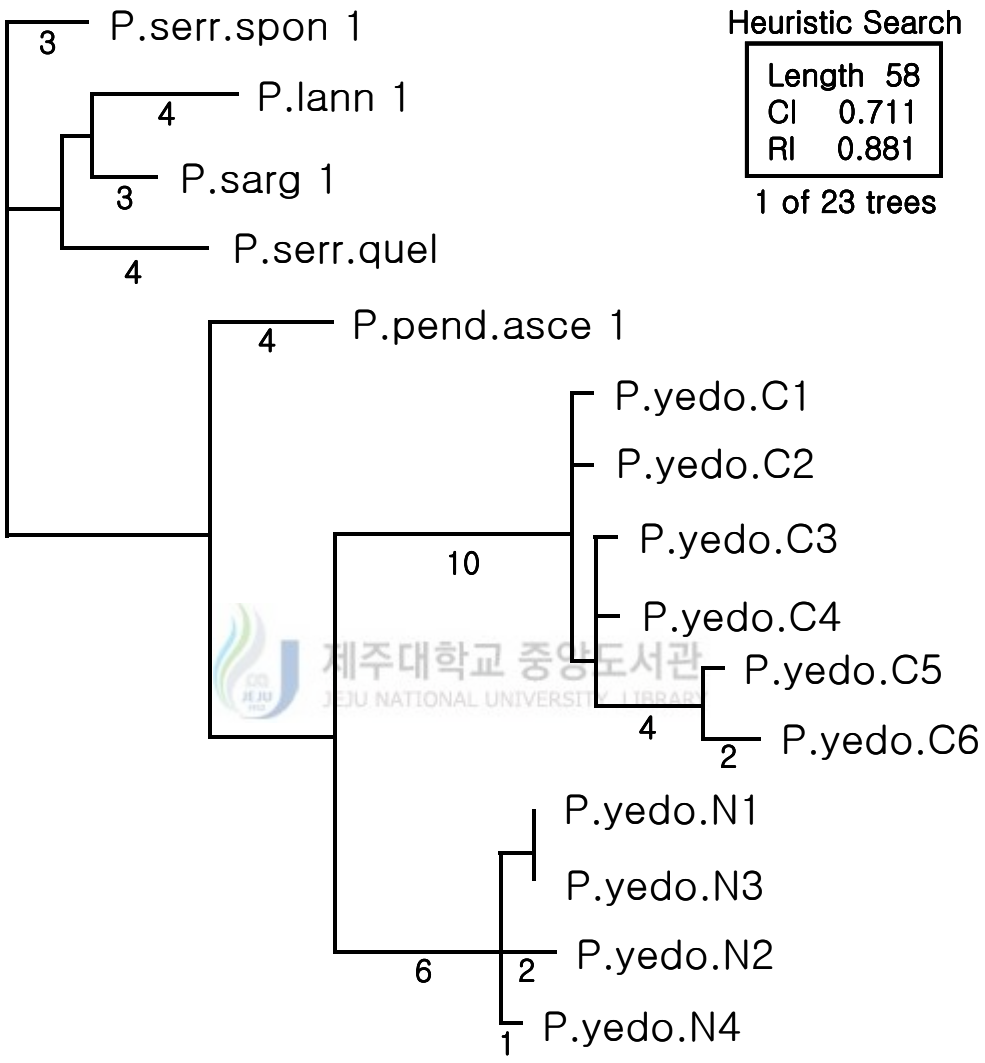


Figure 11. One of the 23 equally most parsimonious trees (CI=0.711, RI=0.881) based on the nrDNA ITS sequences for 15 taxa of *Prunus*. The tree was generated through a heuristic search with MULPARS, TBR branch swapping, and simple addition. Numbers below the branches indicate branch length. Complete taxon names are provided in Table 4.

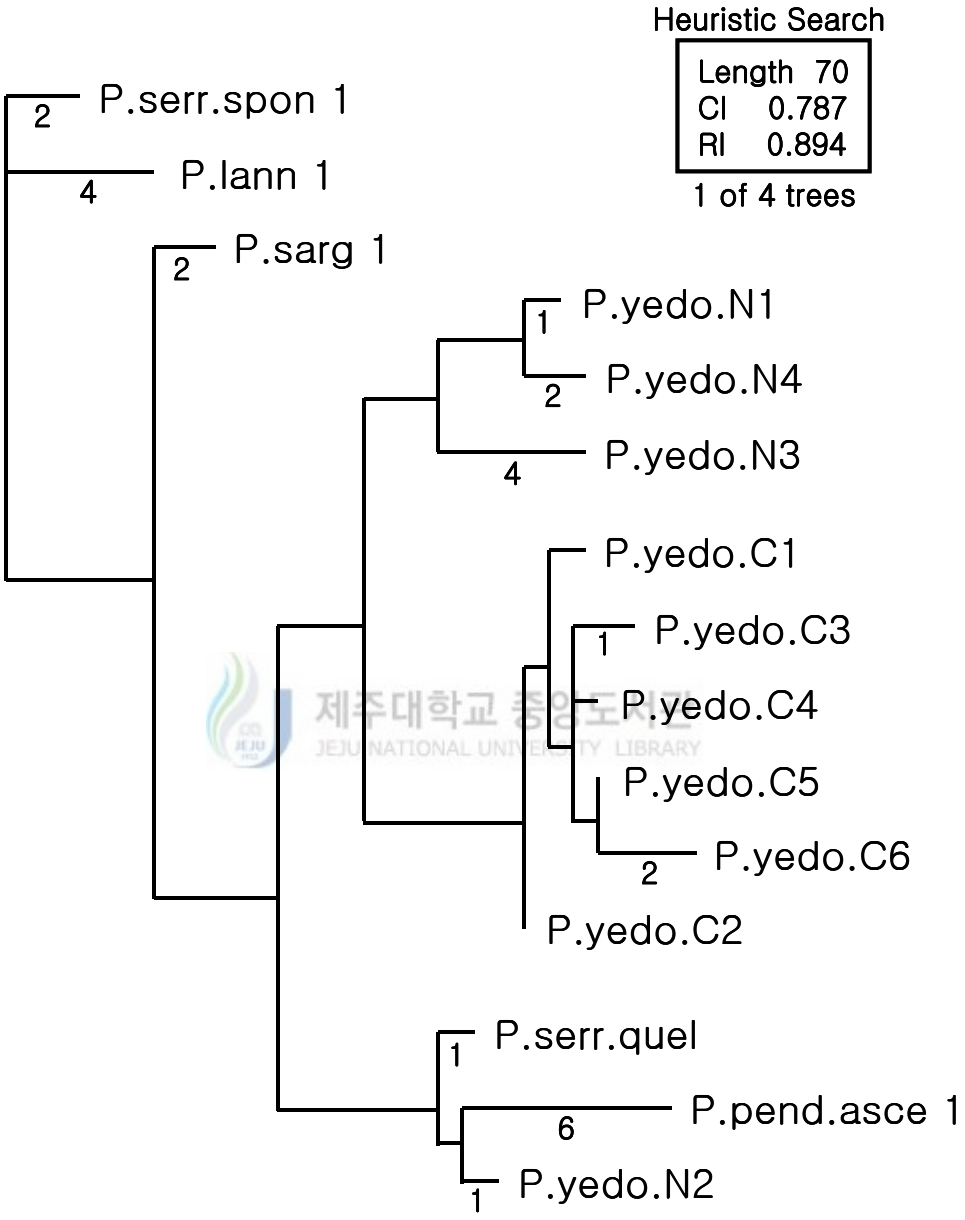


Figure 12. One of the 4 equally most parsimonious trees (CI=0.787, RI=0.894) based on the cpDNA *rbc*L gene sequences for the 15 taxa of *Prunus*. The tree was generated through a heuristic search with MULPARS, TBR branch swapping, and simple addition. Numbers below the branches indicate branch length. Complete taxon names are provided in Table 4.

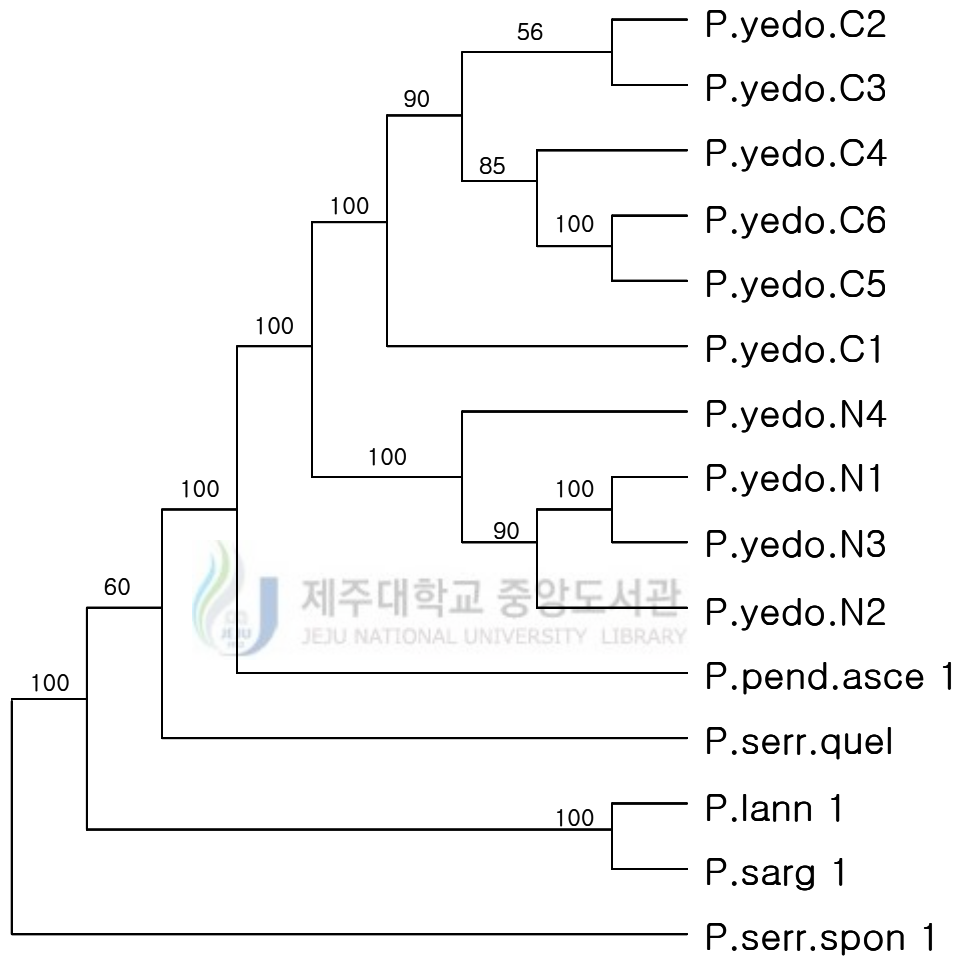


Figure 13. The majority-rule strict consensus tree derived from the most parsimonious trees based the nrDNA ITSs sequences of *P. yedoensis* and five related taxa. Bootstrap values (1000 replicates) are shown on the consensus trees above each nodes. Complete taxon names are provided in Table 4.

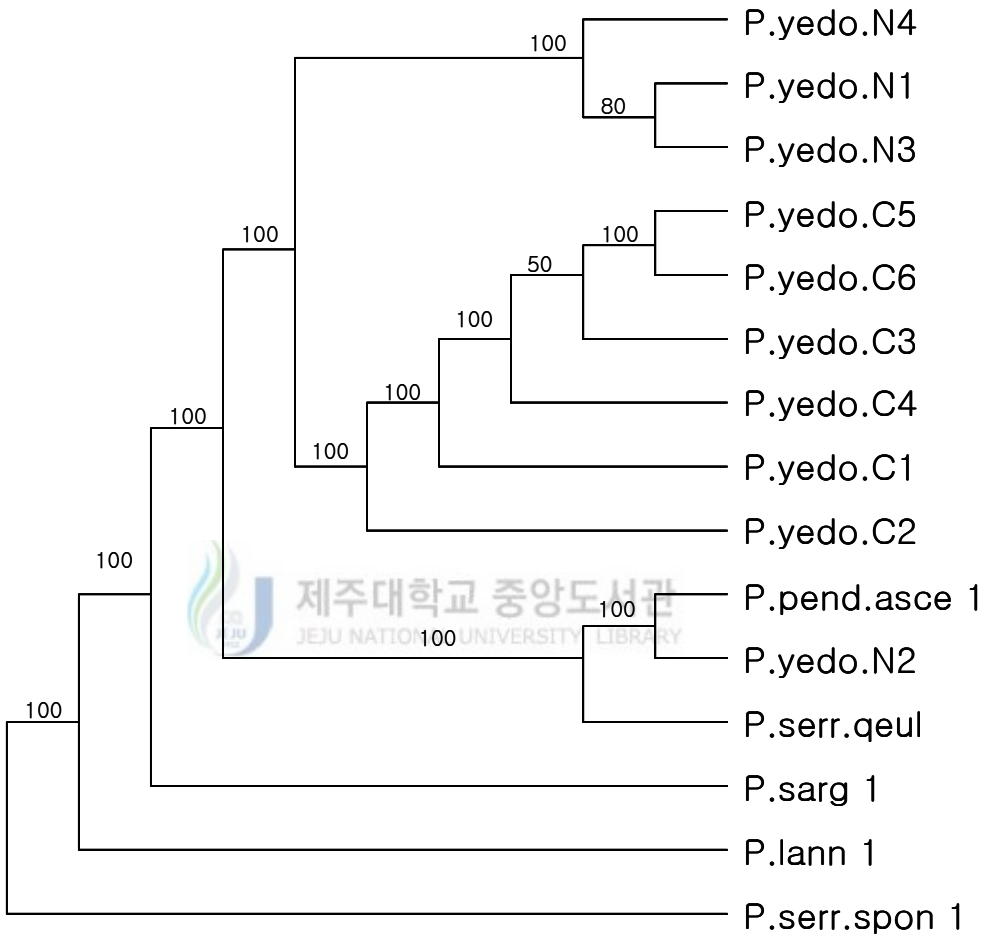


Figure 14. The majority-rule strict consensus tree derived from the most parsimonious trees based on cpDNA *rbc*L gene sequences for *P. yedoensis* and five related taxa. Bootstrap values (1000 replicates) are shown on the consensus trees above each nodes. Complete taxon names are provided in Table 4.

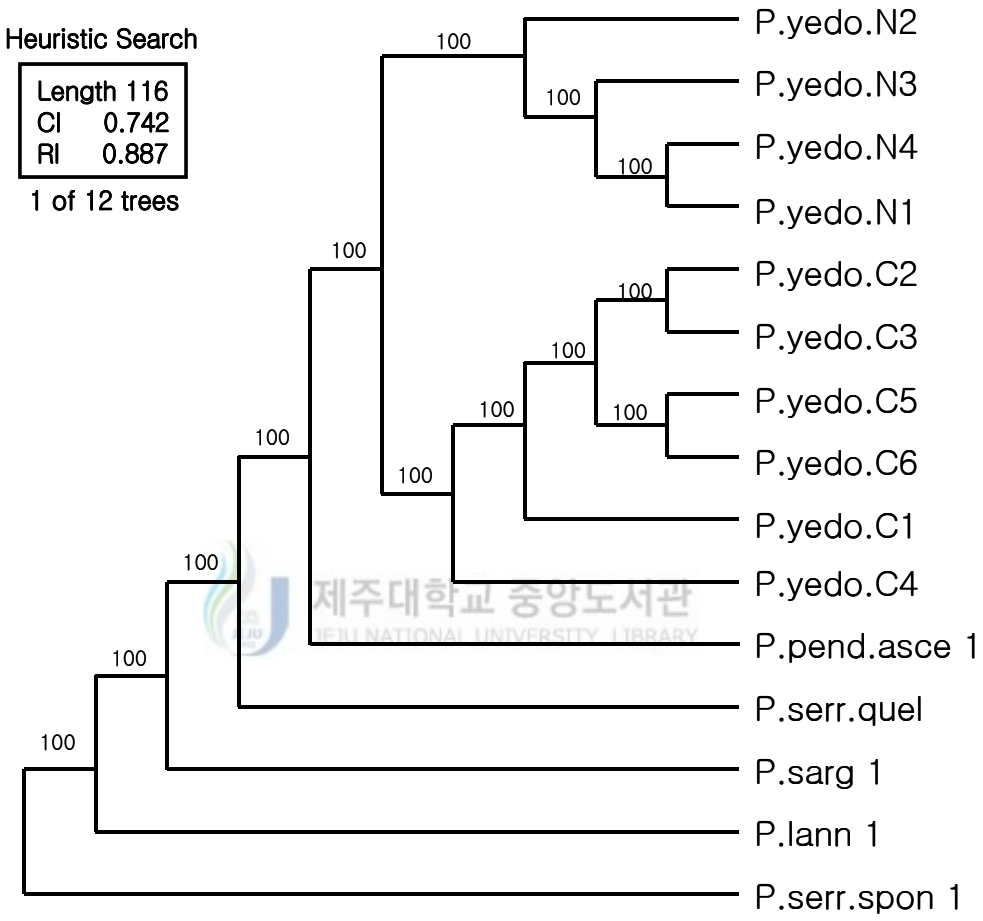


Figure 15. A strict consensus tree derived from the combined data (ITSs and *rbc*L gene) for 15 taxa of *Prunus*. The strict consensus tree was calculated with the CONSENSE program in PHYLIP. Bootstrap values (based on 1000 replicates) are shown on the consensus tree above each node. Complete taxon names are provided in Table 4.

DISCUSSION

1. Sequence Variation among *Prunus* species

The length of ITS1 (222-243 bp) for 45 accessions of *Prunus* was similar to that for other angiosperms (187-298 bp; reviewed in Baldwin, 1992; Kim and Jansen, 1994; Baldwin *et al.*, 1995; Downie and Katz-Downie, 1996). On the other hand, ITS2 was somewhat longer (230-245 bp) than previously reported (187-252 bp; reviewed in Hamby and Zimmer, 1992; Baldwin *et al.*, 1995; Downie and Katz-Downie, 1996). In the five subgenera of *Prunus* (*Amygdalus*, *Padus*, *Cerasus*, *Laurocerasus* and *Prunophora*), ITS2 is slightly longer than ITS1 (by 2 to 18 bp), except for in the 6 *P. yedoensis* cultivars, *P. tomentosa*, and *P. salicina*. Similar length variation has also been reported for the two genera in the subfamily Cucurbitoideae (Cucurbitaceae), the genus (*Oryza*) in the Poaceae, the Maloideae, and the genus (*Vauquelinia*) in the Rosaceae (Takaiwa *et al.*, 1985; Campbell *et al.*, 1995; Jobst *et al.*, 1998). The relative lengths of ITS1 and ITS2 vary considerably among groups of angiosperms (Kim and Jansen, 1994; Baldwin *et al.*, 1995). For example, ITS1 (235 bp) is 27 bp longer than ITS2 (208) in *Vicia faba* (Fabaceae), while ITS2 (220) is 15 bp longer than ITS1 (205) in *Vigna radiata* in the same family (Schiebel and Hemleben, 1989; Yokota *et al.*, 1989). Both ITSs have similar sizes in two genera (*Nicotiana* and *Lycopersicon*) of Solanaceae, two genera (*Avena* and *Hordeum*) of

Poaceae, and genus *Acer* of Aceraceae (Kiss *et al.*, 1988; Venkateswarlu and Nazar, 1991; Chatterton *et al.*, 1992; Suh *et al.*, 1996). However, ITS1 (194 bp) is 39 bp shorter than ITS2 in *Oryza sativa* of the Poaceae (Takaiwa *et al.*, 1988). The most extreme length difference (77 bp) is reported from *Sinapsis alba* of the Brassicaceae (Rathgeber and Capesius, 1989).

The GC content ranged from 58.1 to 65.3% in ITS1 and from 62.0 to 68.3% in ITS2 (Table 5). These values are comparable to those from most angiosperms (Baldwin *et al.*, 1995). In all taxa of *Prunus*, the GC content of ITS2 was slightly higher than that of ITS1, except for *P. tomentosa*. The difference in the GC content among the taxa ranged from 0.7 to 5.2%. This balance of GC content between ITS1 and ITS2 is obviously a common feature, as already described by Torres *et al.* (1990). However, the GC content of ITS2 in *P. persica* (subgenus *Amygdalus*) appeared to be almost identical to that of ITS1 (Table 5).

Short indel mutations of ITSs may be the primary source of length variation in *Prunus*, but not in other genera. Length variation of up to 200 bp was reported from nine genera of *Fabaceae* (Jorgensen and Cluster, 1988). Differences of up to 100 bp were also reported among individuals within the population of *Lisianthus skinneri* (Gentianaceae; Sytsma and Schaal, 1990). These large indels might be due to unequal crossing-over of rDNA repeat units (Smith, 1976).

Unlike ITSs analyses, no sequence length variation was found for coding genes (*rbc*L and *psb*A) sequences of the 45 accessions. However, the length of the *trn*L(UAA)-*trn*F(GAA) intergenic spacer

ranged from 358 (two taxa of *P. buergeriana* and *P. padus*) to 387 bp (two taxa of *P. serrulata* var. *spontanea* and *P. spinulosa*). The nucleotide sequence of the *trn*L(UAA)-*trn*F(GAA) intergenic spacer in *Prunus* was slightly longer than that of the genera *Betula* (*Betula pubescens*, 124 bp), *Hordeum* (*Hordeum vulgare*, 279 bp), *Puccinellia* (*Puccinellia distans*, 246 bp), *Quercus* (*Quercus rubra*, 168 bp), and others (Gielly and Taberlet, 1994). However, the length in the palm family (Palmae) were similar to that of *Prunus*, 307-378 bp (Baker *et al.*, 1999).

The percentage of variable nucleotide sites was 21.4% (299/1398) and 19.1% (203/1062) in the *rbc*L and *psb*A genes, respectively (Fig. 2). On the other hand, that for the noncoding region [*trn*L(UAA)-*trn*F(GAA) intergenic spacer] was 27.3% (107/392). The nucleotide variation was 1.3 to 1.43 times greater in the noncoding region than in the coding regions. The results clearly show that noncoding regions evolve faster than do coding genes, as reported previously (Curtis and Clegg, 1984; Wolfe *et al.*, 1987; Zurawski and Clegg, 1987; Clegg and Zurawski, 1991; Gielly and Taberlet, 1994). They evolve between 1.93 (*Avena sativa-Oryza sativa*) and 11.72 (*Triticum aestivum-Aegilops* spp.) times faster than the *rbc*L gene. However, the rates of variable sites (27.3%) were significantly lower than in several groups of plants (Gielly and Taberlet, 1994, 1996; Kita *et al.*, 1995; Gielly *et al.*, 1996; Mcdade and Moody, 1999). In addition, the nucleotide sequence of the *trn*L(UAA)-*trn*F(GAA) intergenic spacer needed 14 indels to align the 45 accessions of *Prunus*. Of note, 4 taxa in the subgenus *Padus* had long

deletion mutations (18 to 21 bp) in the noncoding region (Fig. 3). The long mutations play an important role in the sequence divergence observed at the intra- and intergeneric levels, as previously reported (Taberlet *et al.*, 1991; Kim *et al.*, 1996; Mes *et al.*, 1996; Mcdade and Moody, 1999). Long deletion mutations occur at least as frequently as nucleotide substitutions, as reported elsewhere (Curtis and Clegg, 1984; Zurawski and Clegg, 1987; Clegg and Zurawski, 1991).

2. Phylogenetic Analyses of Korean and Japanese *Prunus*

The most parsimonious trees were 513 steps long, with a CI of 0.609 and an RI of 0.745, excluding uninformative characters. This is considerably lower than the CI value (0.96) calculated by Badenes and Parfiff (1995) in a chloroplast DNA study of 8 species representing 3 subgenera, *Amygdalus* (*P. persica* and *P. dulcis*), *Cerasus* (*P. avium*, *P. cerasus*, and *P. fruticosa*), and *Prunophora* (*P. domestica*, *P. salicina*, and *P. armeniaca*), of *Prunus*. In addition, the most parsimomious trees contained four large independent groups: group I (subgenera *Prunophora*, *Amygdalus*, and section *Microcerasus* of subgenus *Cerasus*), group II (subgenera *Laurocerasus* and *Padus*), group III (subgenus *Cerasus* II), and group IV (subgenus *Cerasus* III) (Fig. 8).

Interestingly, group I in Fig. 8 (*P. tomentosa*, *P. glandulosa* for. *sinensis*, *P. japonica* var. *nakaii*, and *P. choreiana*) was distinct from the other subgenus *Cerasus* taxa. Three of these four taxa belong to

section *Microcerasus* of the subgenus *Cerasus* (Rehder, 1940; Kitamura and Murata, 1979); *P. choreiana* was previosly unclassified. Therefore, these results strongly suggest that taxonomic realignment of the delimitations and composition of the subgenus *Cerasus* is necessary. Moreover, the subgenus *Amygdalus* and section *Microcerasus* of subgenus *Cerasus* formed one group with taxa of subgenus *Prunophora*. This suggests that *Amygdalus* is relatively closer to *Prunophora* than to any other subgenus. Watkins (1976) also postulated that *Amygdalus* and *Prunophora* were genetically monophyletic, with the caveat mentioned above, and Badenes and Parfitt (1995) suggested that *Amygdalus* is closely related to *Prunophora* and that *Cerasus* evolved more extensively and earlier, in agreement with Lersten and Horner's (2000) results based on calcium oxalate crystal types and trends in their distribution patterns in leaves. Lersten and Horner (2000) concluded that *Amygdalus* and *Prunophora*, considered the most advanced subgenera, virtually all have druses that are almost always associated with veins. On the other hand, Kaneko *et al*. (1986) suggested that *P. mume* (subgenus *Prunophora*) is more closely related to the subgenus *Cerasus* than to any other subgenus based on restriction analyses using cpDNAs from only one species of each group. However, Kaneko *et al.*'s suggestions, resulting from studies carried out without *Amygdalus* species, are not rational.

The subgenus *Padus* formed one group with taxa of the subgenus *Laurocerasus* (Group II in Fig. 8). The combined nrDNA ITS and cpDNA data indicate that species from the two subgenera may be intermingled each other. Morphologically, these two taxa share several common

characteristics such elongated raceme with 12 or more flowers, and conduplicate vernation (Koehne, 1893). Kalkman (1965) also regarded *Padus* as the least advanced subgenus, with *Laurocerasus* next, and this result corresponds to the crystal pattern distribution reported in Lersten and Horner (2000). The major differences between the two subgenera are the deciduous habit for the subgenus *Padus* (vs. evergreen of subgenus *Laurocerasus*) and bracteate racemes for the subgenus *Padus* (vs. bractless racemes in subgenus *Laurocerasus*). The differences in the deciduous or persistent nature of leaves and the presence or absence of bracts within the *Padus*-*Laurocerasus* group may be resulted from the adaptation to temperate mesic vs. tropical humid environments (Webb, 1968; Davis and Taylor, 1980; Wilf, 1997; Lee and Wen, 2001).

The neighbor-joining tree and the strict and majority-rule consensus tree derived from the most parsimonious trees were very similar to the most parsimonious trees. These results suggest the earlier divergence of subgenus *Cerasus* from the genus *Prunus*, compared with previous reports on conventional taxonomic interpretations and chloroplast DNA variation (Watkins, 1976; Badenes and Parfitt, 1995).

In addition, all the taxa in *Cerasus* share a most recent common ancestor. Although the groupings obtained at the subgenus level agreed closely, they do not agree with the traditional classification. Interestingly, in subgenus *Cerasus*, the native *P. yedoensis* from Jeju (Korea) were completely separated from the cultivars, as suggested in previous morphological studies and a RAPD analysis (Jung *et al.*, 1997; Kim *et*

al., 1998). In the RAPD patterns, the genetic distance between the native and cultivated *P. yedoensis* was 0.430, which is very high compared to other pairs (Jung *et al.*, 1997). In addition, Kim *et al.* (1998) insisted that native *P. yedoensis* from Jeju is clearly distinguished from cultivated *P. yedoensis* based on the morphology of flowers, leaves, seeds, and trichomes. In particular, the calyx tube of cultivated *P. yedoensis* is cup-shaped, whereas it is wedge-shaped in native specimens (Kim *et al.*, 1998). Kim *et al.* (1990) classified that the 12 *Prunus* species distributed in Korea into four groups based on inflorescence shape: raceme bearing, corymbose or umbelliferous, corymbose, and umbelliferous.

3. Origin and Evolution of *P. yedoensis*

There have been morphological, cytological, RFLP, RAPD, and distribution studies of the native *P. yedoensis* plants on Mt. Halla, Jeju, Korea (Funazu, 1966; Park *et al.*, 1984; Iwasaki, 1981; Kaneko *et al.*, 1986; Innan *et al.*, 1995; Jung *et al.*, 1997). Nevertheless, the phylogeny and evolution of *P. yedoensis* are not clear. Takenaka (1963) argued that *P. yedoensis* originated from Oshima Island, Japan or Jeju Island, Korea. However, it has long been disputed whether *P. yedoensis* originated from Jeju Island and whether the taxon originating on Jeju Island is the same as that cultivated in Japan. Therefore, both nuclear (ITSs) and chloroplast (*rbc*L, *psb*A, and *trn*L-*trn*F) DNA from 10 taxa of

P. yedoensis (4 natives and 6 cultivars) were used as markers to clear their origin and evolution, because the former DNA has diploid inheritance and the latter has maternal inheritance. In addition, five species of *Prunus* (*P. lannesiana*, *P. pendula* for. *ascendens*, *P. sargentii*, *P. serrulata* var. *quelpaertensis*, and *P. serrulata* var. *spontanea*) that are putative parental plants for *P. yedoensis* were examined to obtain further data on the origin of *P. yedoensis*.

Previously, *P. yedoensis* was reported to be an interspecific hybrid of *Prunus*. Interestingly, within the subgenus *Cerasus*, both native and cultivar *P. yedoensis* are clearly distinguished from the other species. In the parsimony tree based on nrDNA ITSs sequences, *P. pendular* for. *ascendens* was closely related to *P. yedoensis* (Fig. 11). CpDNA analyses identify maternal inheritance patterns (Fig. 12), and the two groups of *P. yedoensis* showed different relationships on the ITS analyses (Fig. 11). It is noteworthy that *P. yedoensis* 2 was grouped with *P. pendula* for. *ascendens* (Fig. 12). These also formed a monophyletic group with *P. serrulata* var. *quelpaertensis*, with 100% bootstrap value (Fig. 14). These results was slightly different from previous morphological, palynological, cpDNA RFLP, DNA fingerpriting, and RAPD analyses (Funazu, 1966; Iwasaki, 1981; Park *et al.*, 1984; Kaneko *et al.*, 1986; Innan *et al.*, 1995; Jung *et al.*, 1997). Funazu (1966) reported that his grandfather noted that *P. yedoensis* was produced by crossing *P. lannesiana* as the maternal parent and *P. pendula* for. *ascendens* as the pollen parent. Iwasaki (1981) suggested that the maternal parent of *P. yedoensis* is *P. lannesiana* based on a

morphological comparison of pollen and bark, and the content of sakuranetin, a flavonoid. His conclusion was based on matroclinous inheritance, which was reported from some plants (Kakizaki, 1925; Tokumasu, 1965). Matroclinous inheritance is a phenomenon in which all progeny have the phenotypes of the mother. By contrast, the cpDNA RFLP analysis of Kaneko *et al*. (1986) showed that *P. yedoensis* and *P. pendula* for. *ascendens* shared the same restriction pattern, while *P. lannesiana* had a different one. If cpDNA is inherited maternally, this suggests that the maternal parent of *P. yedoensis* is *P. pendula* for. *ascendens*. Iwasaki (1986) argued against this suggestion, saying that there must be two types of *P. yedoensis,* which were derived from reciprocal crosses between *P. lannesiana* and *P. pendula* for. *ascendens*. Summarizing the previous studies, there are three hypotheses on the origin of *P. yedoensis*: 1) *P. lannesiana* is the maternal parent (the Funazu and Iwasaki hypothesis); 2) *P. pendula* for. *ascendens* is the maternal parent (the Kaneko hypothesis); 3) both *P. lannesiana* and *P. pendula* for. *ascendens* are maternal parents (the Iwasaki hypothesis). In addition, Innan *et al*. (1995) suggested that *P. yedoensis* was produced only once by hybridization between single plants of *P. lannesiana* and *P. pendula*, and that a clone of this particular plant has spread throughout Japan.

However, the argument that the *P. yedoensis* native to Mt. Halla is a hybrid between *P. pendula* for. *ascendens* and *P. lannesiana* is not persuasive, because *P. lannesiana* dose not occur in Jeju. In addition, Park *et al*. (1984) suggested that the striae pattern, along with other

pollen characters, shows that *P. yedoensis* is more closely related to *P. serrulata* var. *quelpaertensis*, implying that *P. serrulata* var. *quelpaertensis* is a putative parent of *P. yedoensis*. According to the RAPD anlaysis, the genetic distance between native *P. yedoensis* and *P. serrulata* var. *quelpaertensis* was 0.3863, which was the lowest value among all pairs (Jung *et al.*, 1997).

According to our results, *P. pendula* for. *ascendens* may be the maternal parent of *P. yedoensis,* if the chloroplast DNA is inherited maternally, and *P. serrulata* var. *quelpaertensis* may be the pollen parent of *P. yedoensis*.

As mentioned above, there were several differences between native and cultivar *P. yedoensis,* including the lengths of ITS1 and ITS2 (Table 5), the secondary folding structure of the nrDNA ITS1 (Fig. 5), and the amino acid sequences of the cpDNA coding genes, *rbc*L and *psb*A (Tables 7, 8). These data suggest that native *P. yedoensis* from Jeju is a different taxon from the cultivars. This argument also is supported by the results from the morphological studies and the RAPD analysis (Jung *et al.*, 1997; Kim *et al.*, 1998). According to the RAPD analysis, the genetic distance between the native and cultivated *P. yedoensis* was 0.430, which is very high compared to other pairs (Jung *et al.*, 1997). In addition, the native and cultivated *P. yedoensis* shared 61.5% (16/26) and 53.5% (16/30) identical bands, respectively. Kim *et al.* (1998) also insisted that native *P. yedoensis* from Jeju is clearly distinguished from cultivated *P. yedoensis,* based on the basis of the morphology of the flowers, leaves, fruits, seeds, and trichomes. In particular, the calyx tube

of cultivated *P. yedoensis* is cup-shaped, whereas it is wedge-shaped in native specimens from Jeju and the inflorescences are corymbose and umbelliferous in the cultivars and native *P. yedoensis*, respectively (Kim *et al.*, 1998).

In opposition to the arguments that *P. yedoensis* is not native to Jeju, there are much more genetic variations among *P. yedoensis* individuals native to Jeju compared to *P. yedoensis* cultivars and other species. Moreover, the hypothesis that *P. yedoensis* native to Mt. Halla is a hybrid between *P. pendula* for. *ascendens* and *P. lannesiana* is not persuasive, since *P. lannesiana* does not occur on Jeju. Accordingly, *P. yedoensis* should be considered as native species in Jeju and also independent species distinguishable from other *Prunus* species.

This study was limited to examining the phylogenetic relationships and evolution of *Prunus* natives or cultivars found in Korea and Japan, although studies on *Prunus* worldwide should produce better results. A firmer conclusion on the origin and evolution of *Prunus* native to Jeju should be obtained in future DNA fingerprint analyses with the development of DNA markers for *Prunus* worldwide, including Korean, Japanese, and Chinese species.

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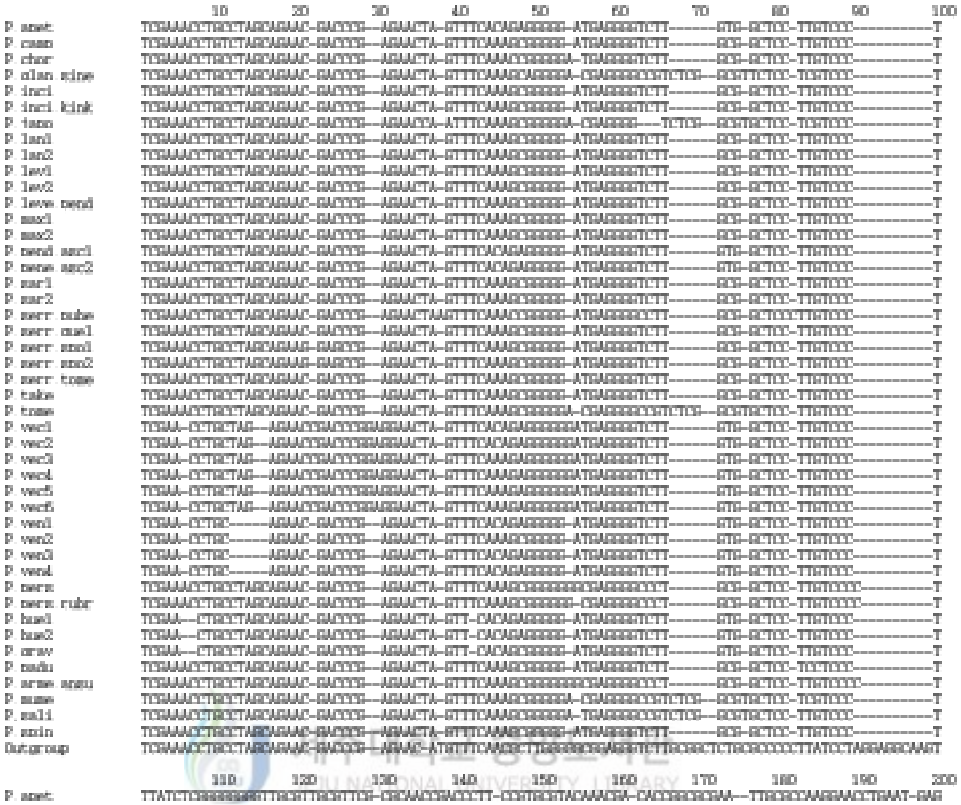
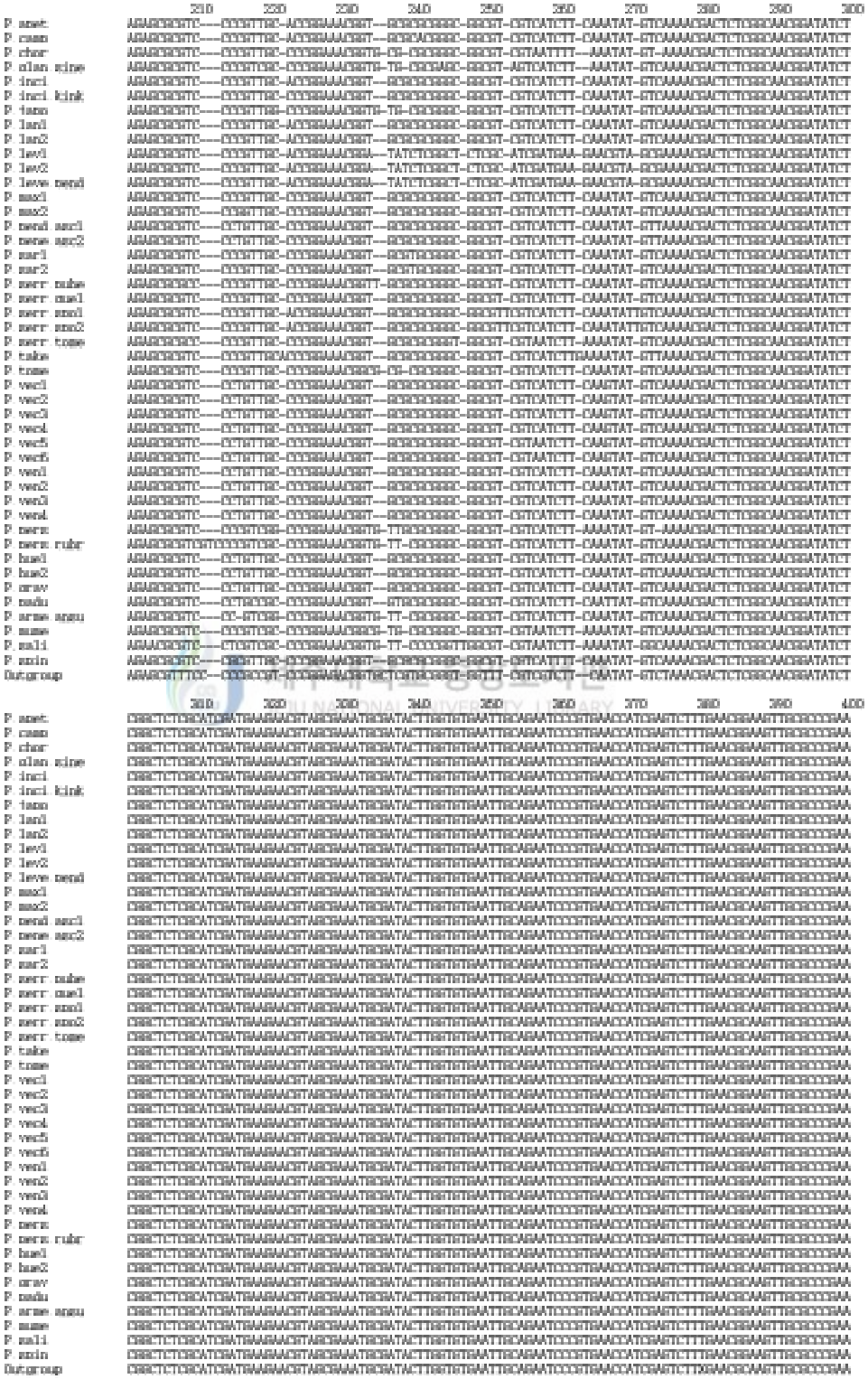
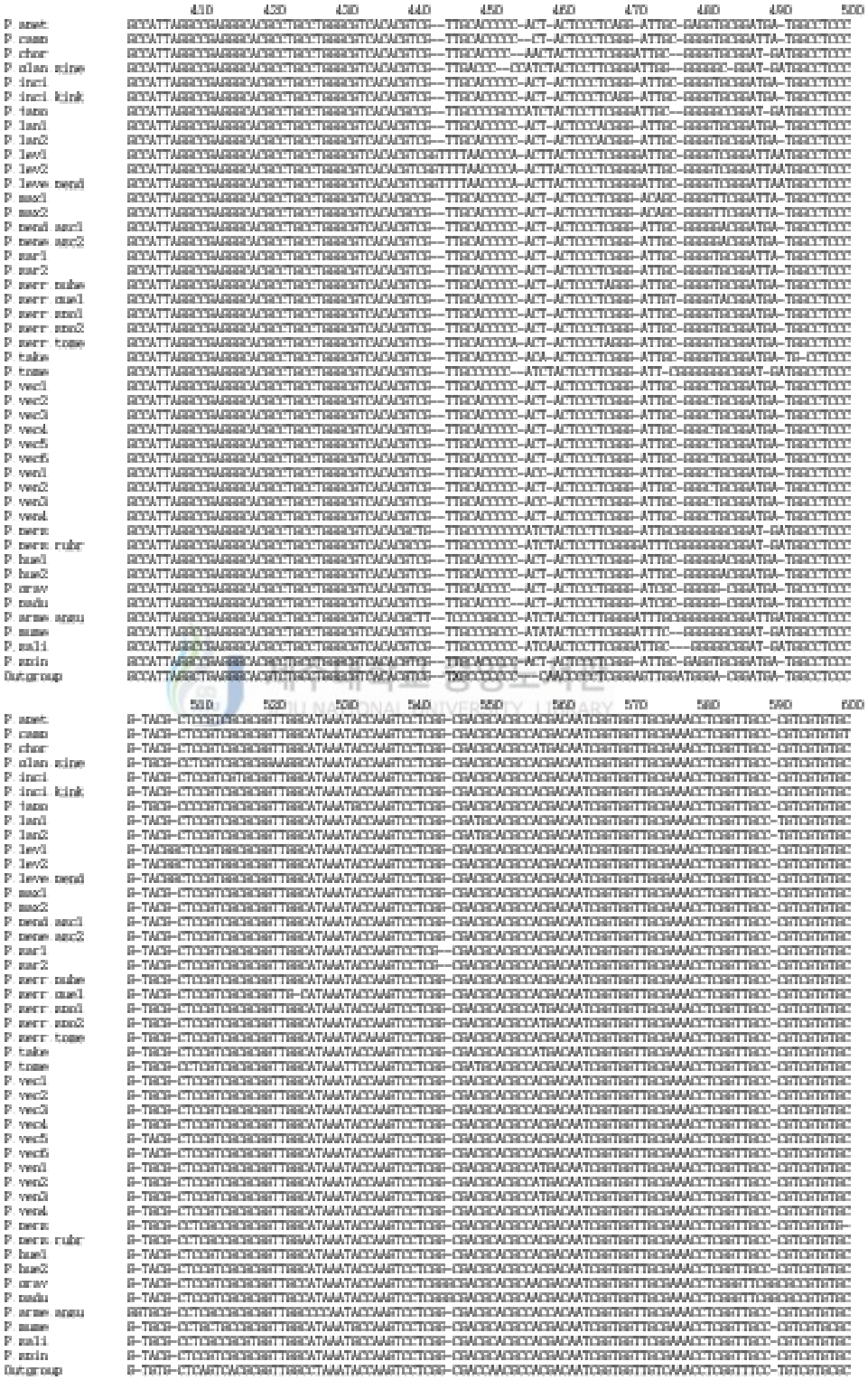
APPENDIX I

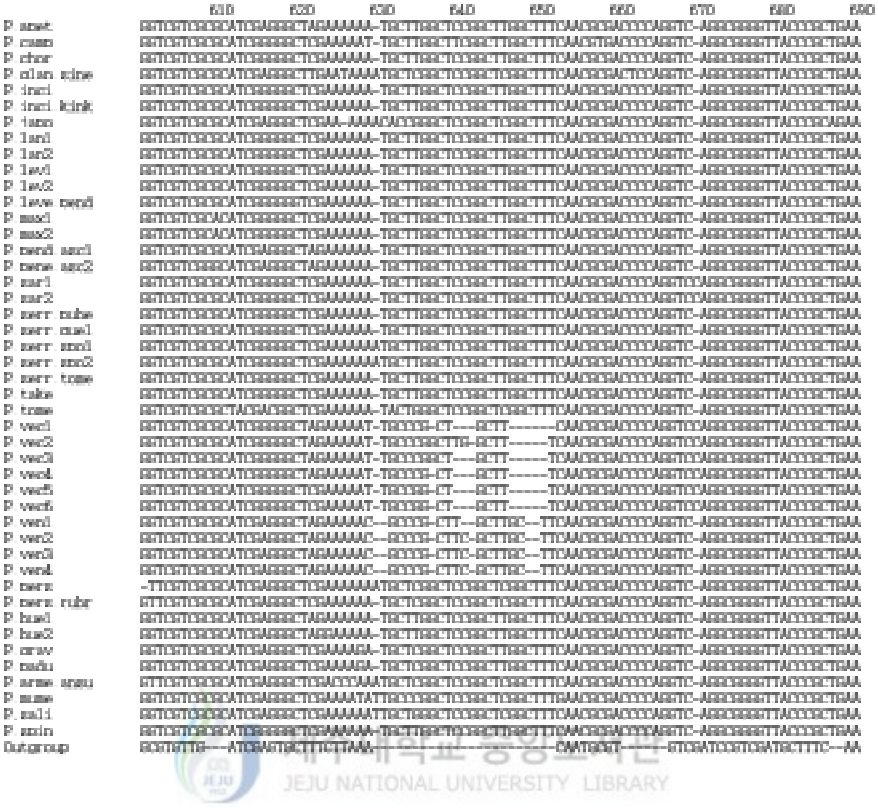
Aligned sequences of the nrDNA ITSs

for 45 accessions of genus *Prunus*



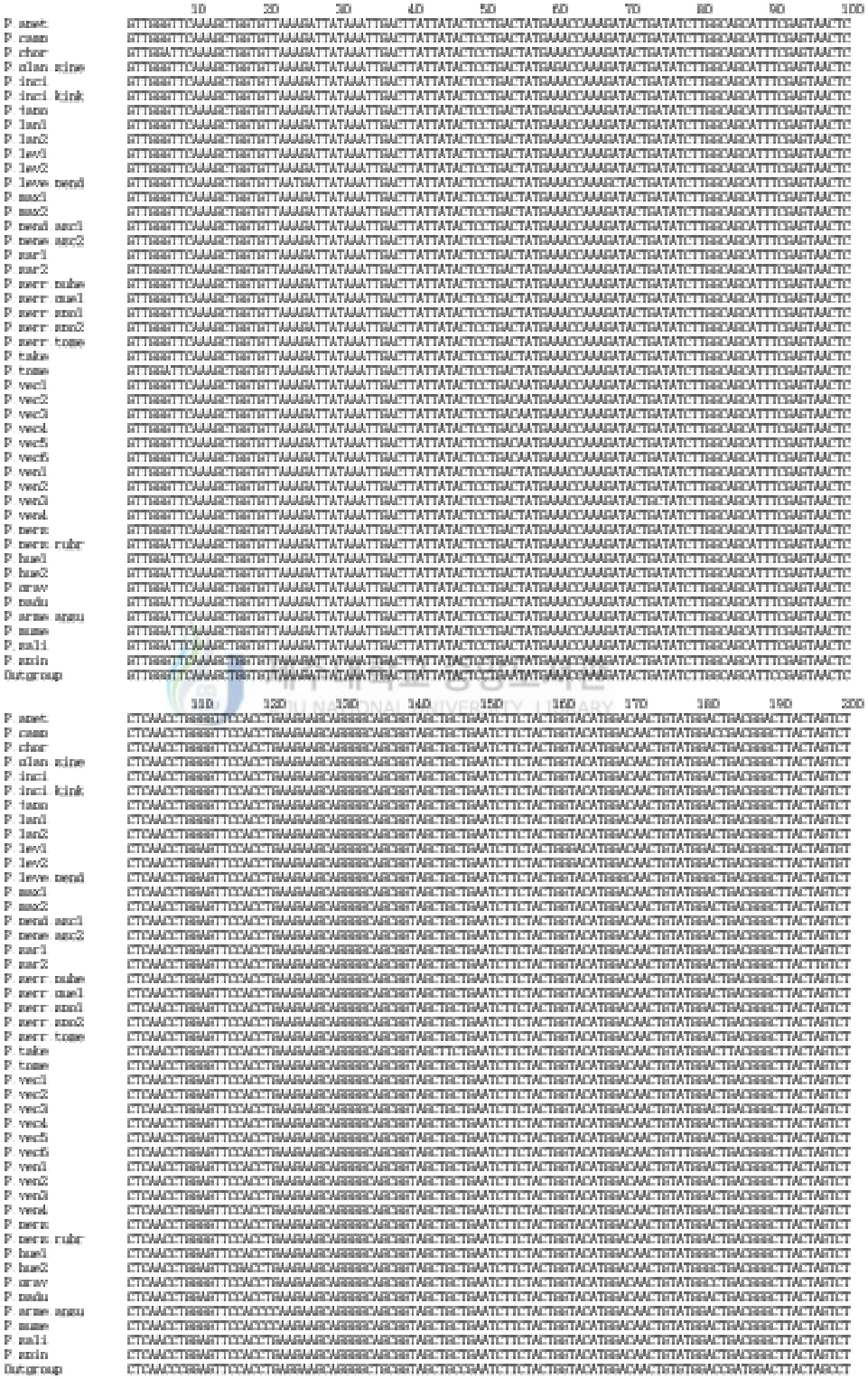






APPENDIX II

Aligned sequences of the cpDNA *rbc*L gene for 45 accessions of genus *Prunus*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P chor
P olan nine
P inci
P inci kink
P iann
P lanl
P lan2
P lev1
P lev2
P leve neod
P mac1
P mac2
P neod mic1
P neae mic2
P nar1
P nar2
P nerr nube
P nerr nue1
P nerr nno1
P nerr nno2
P nerr tone
P take
P tone
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
P nera
P nera rubr
P hue1
P hue2
P oray
P nadu
P arne aneu
P mume
P sali
P anin
Outgroup
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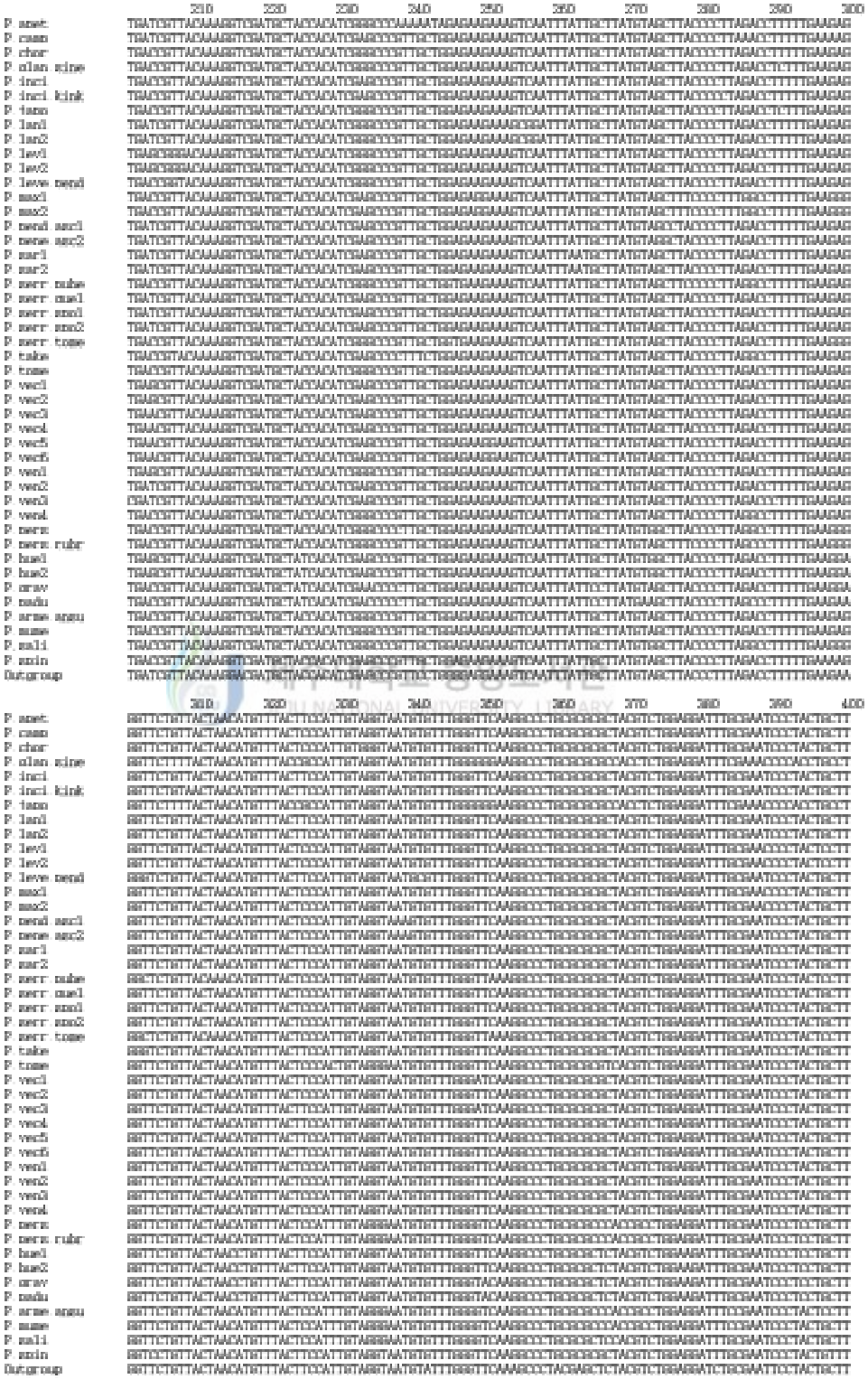
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P casn
P chor
P olan nine
P inci
P inci kink
P iasn
P lard
P lar2
P lev1
P lev2
P leve nend
P mac1
P mac2
P mend macl
P mene mac2
P mar1
P mar2
P merr nube
P merr nuel
P merr mno1
P merr mno2
P merr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
P mers
P mers rubr
P hue1
P hue2
P oray
P nadu
P arme angu
P mume
P sali
P anin
Outgroup
310 320 330 340 350 360 370 380 390 400
P aswt
P casn
P chor
P olan nine
P inci
P inci kink
P iasn
P lard
P lar2
P lev1
P lev2
P leve nend
P mac1
P mac2
P mend macl
P mene mac2
P mar1
P mar2
P merr nube
P merr nuel
P merr mno1
P merr mno2
P merr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
P mers
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P hue2
P oray
P nadu
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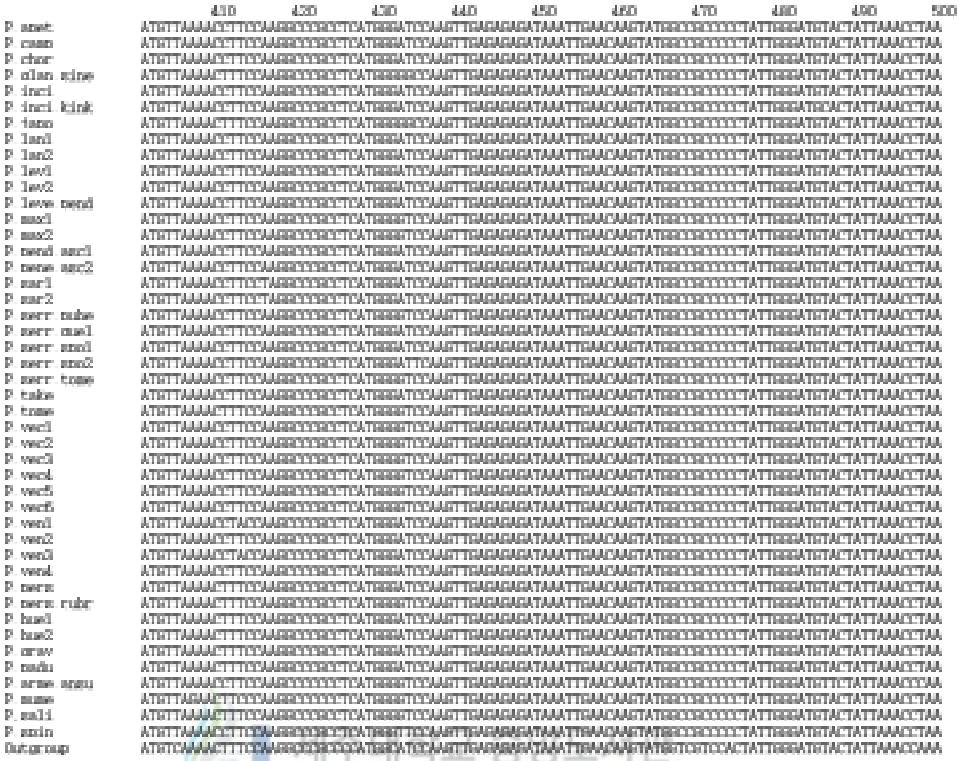
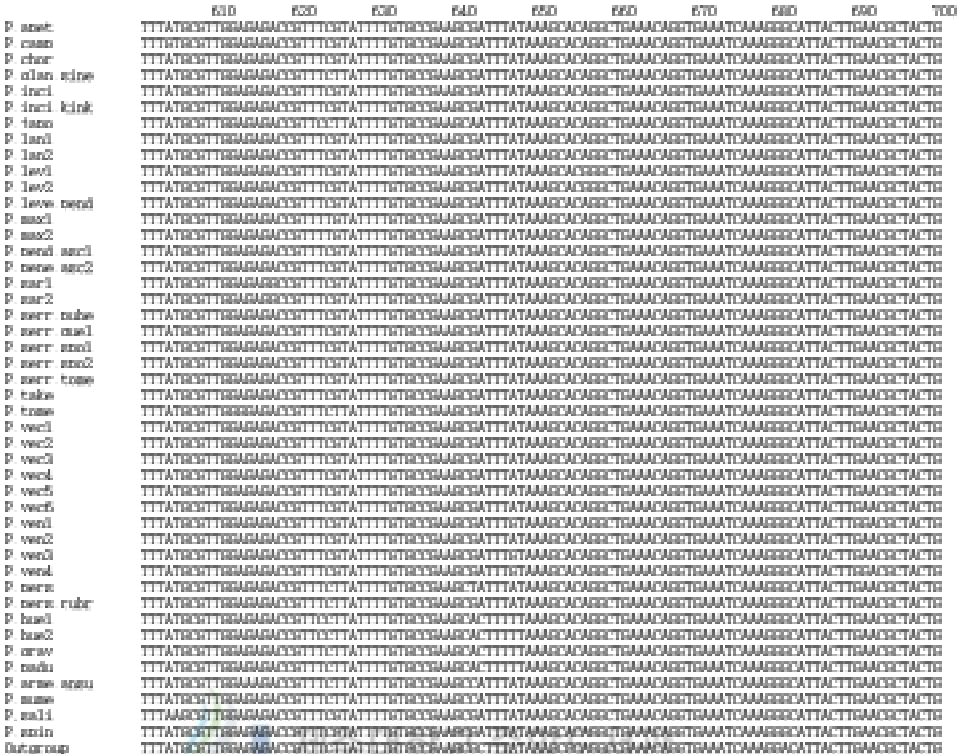
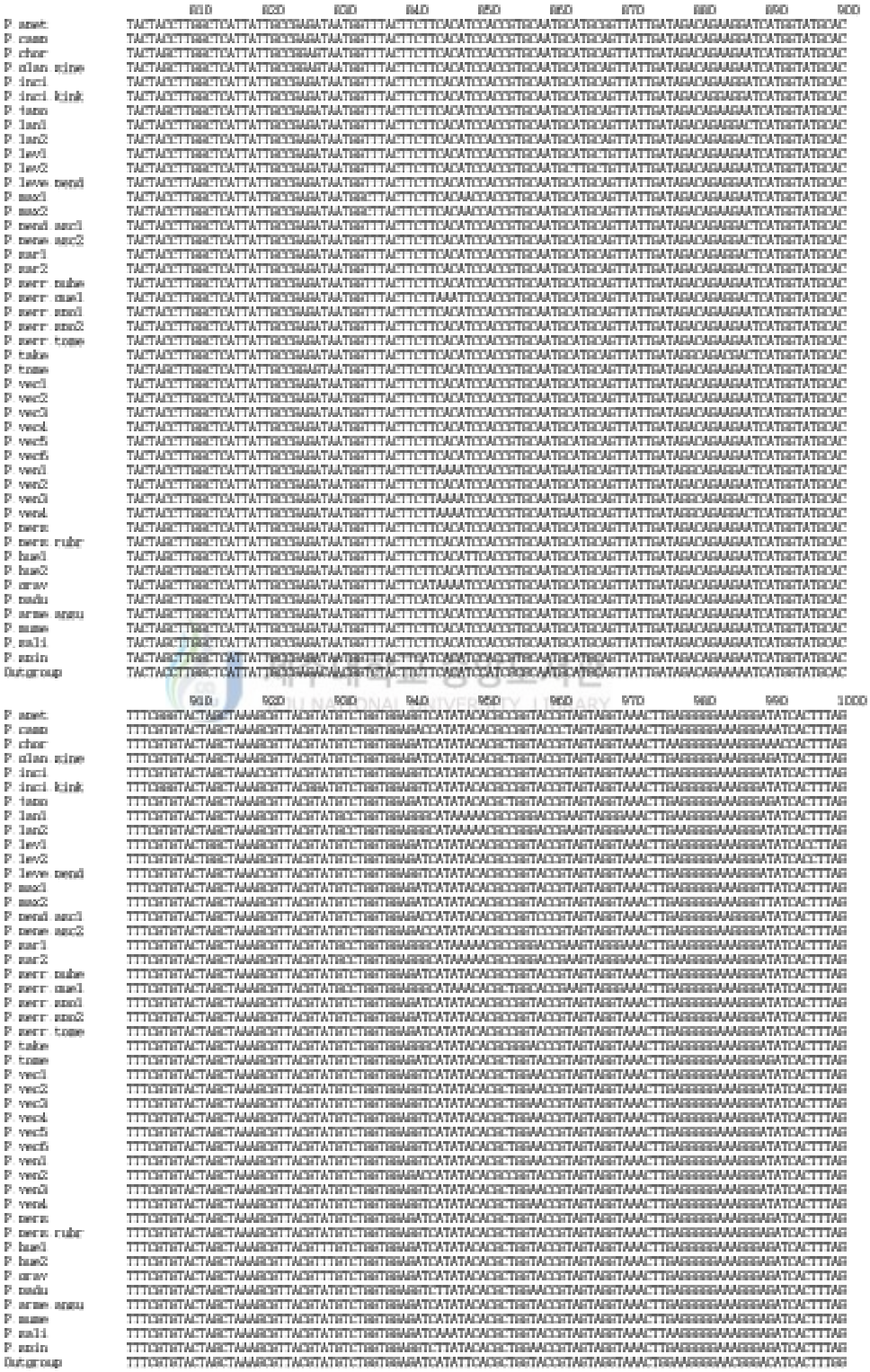
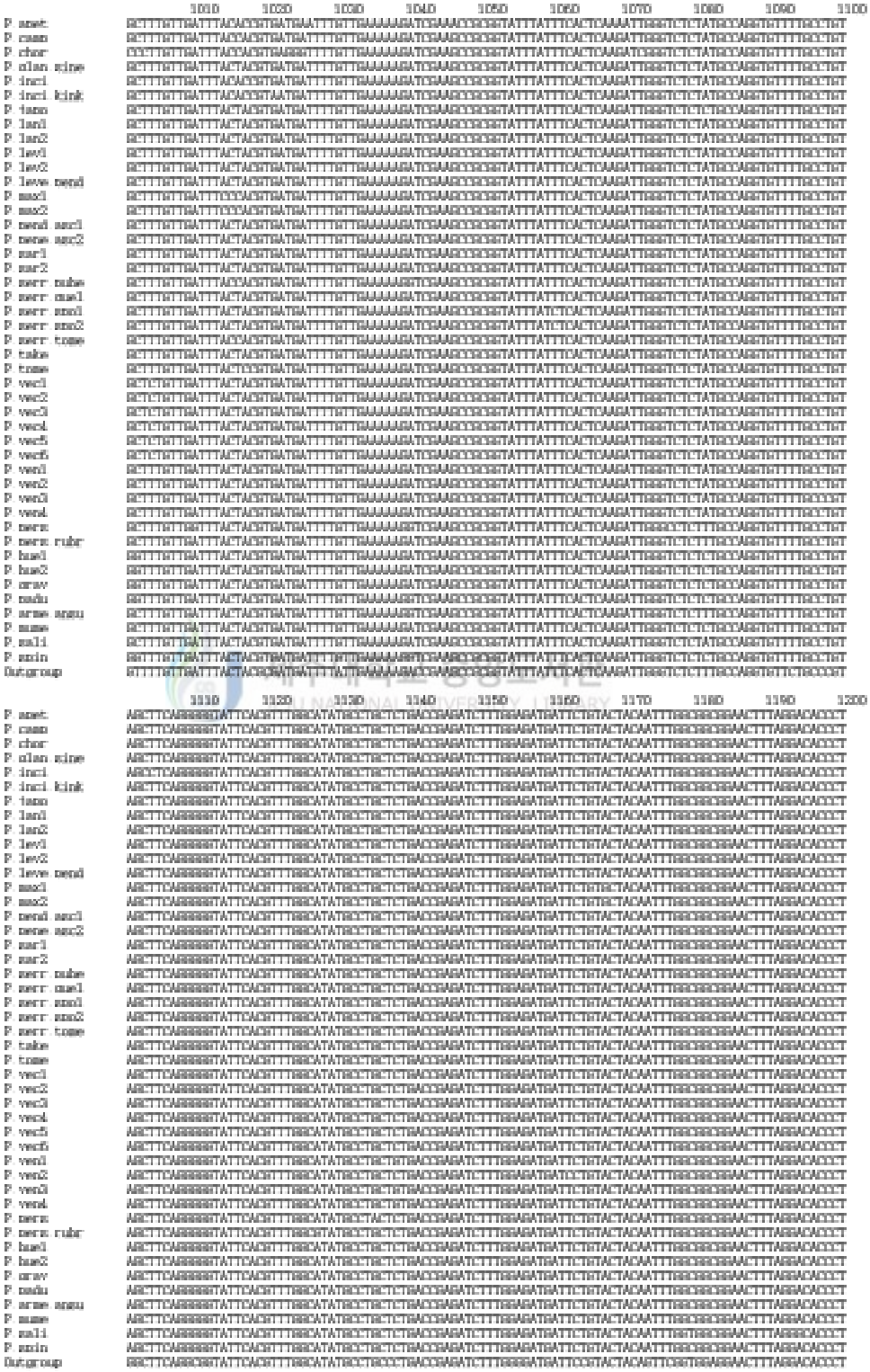
P mume
P sali
P anin
Outgroup





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P chor
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P inci
P inci kink
P iann
P lard
P lar2
P lev1
P lev2
P leve nend
P mac1
P mac2
P mend mac1
P mene mac2
P mar1
P mar2
P merr nube
P merr nue1
P merr mno1
P merr mno2
P merr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
P mere
P mere rubr
P hue1
P hue2
P oray
P nadu
P arme aneu
P mume
P sali
P anin
Outgroup
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
P aswt
P casn
P chor
P olan nine
P inci
P inci kink
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P lard
P lar2
P lev1
P lev2
P leve nend
P mac1
P mac2
P mend mac1
P mene mac2
P mar1
P mar2
P merr nube
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P merr mno1
P merr mno2
P merr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
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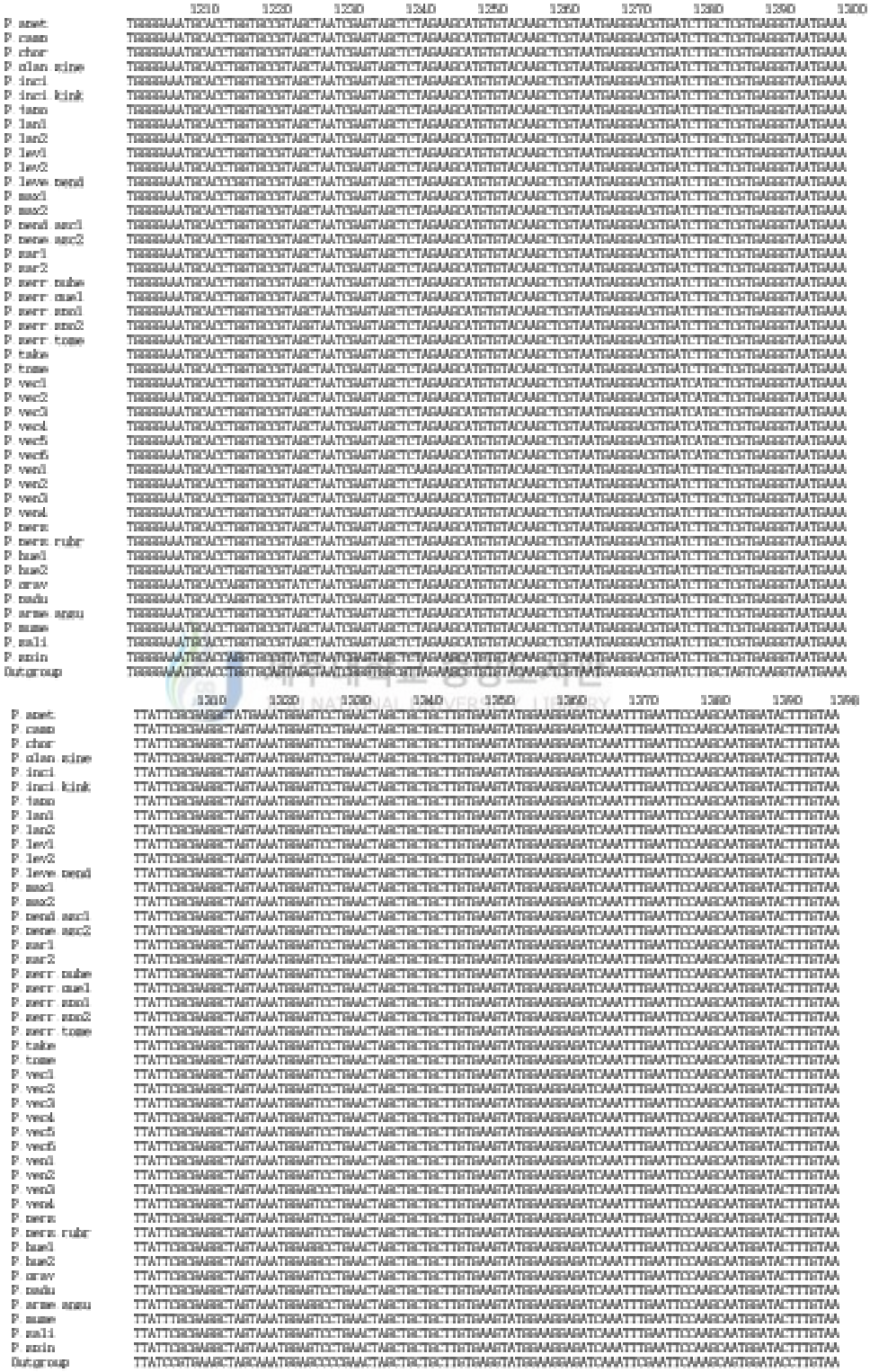
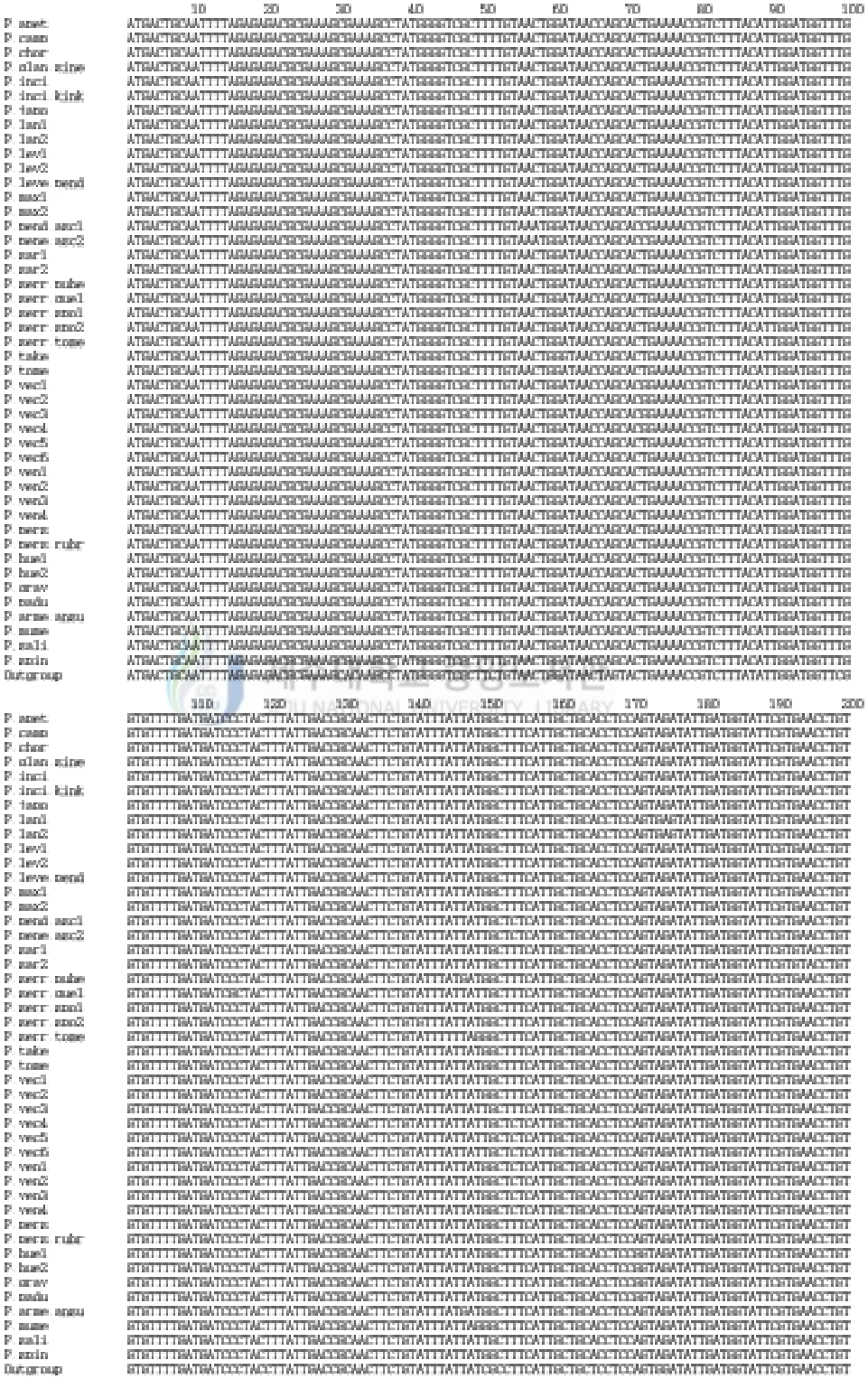
P hue1
P hue2
P oray
P nadu
P arme aneu
P mume
P sali
P anin
Outgroup



APPENDIX III

Aligned sequences of cpDNA *psb*A gene for 45 accessions of genus *Prunus*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P chor
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P inci kink
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P lan2
P lev1
P lev2
P leve nend
P mac1
P mac2
P mend macl
P mene mac2
P mar1
P mar2
P merr mube
P merr muel
P merr mmol
P merr mmo2
P merr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
P mern
P mern rubr
P hae1
P hae2
P oray
P nadu
P arme angu
P mume
P sali
P anin
Outgroup
110 120 130 140 150 160 170 180 190 200
P aswt
P casn
P chor
P olan nine
P inci
P inci kink
P iann
P lanl
P lan2
P lev1
P lev2
P leve nend
P mac1
P mac2
P mend macl
P mene mac2
P mar1
P mar2
P merr mube
P merr muel
P merr mmol
P merr mmo2
P merr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
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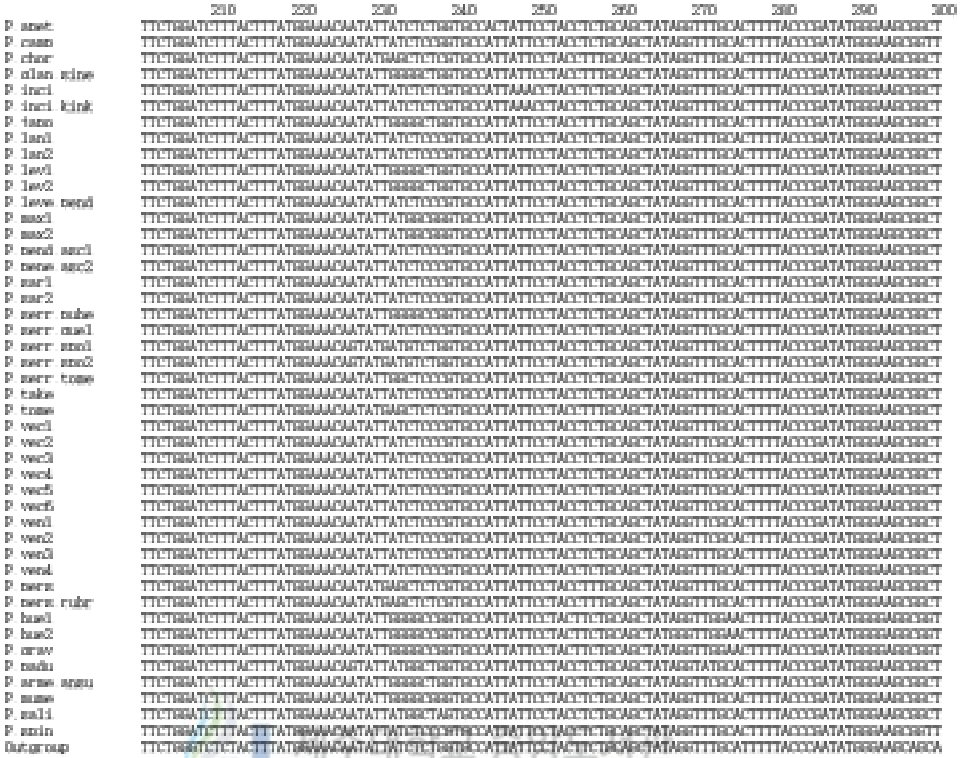
P nadu
P arme angu
P mume
P sali
P anin
Outgroup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P lev2
P leve neod
P mac1
P mac2
P neod mic1
P neoe mic2
P nar1
P nar2
P nerr nube
P nerr oue1
P nerr oue1
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P nerr tome
P take
P tome
P vec1
P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
P nera
P nera rubr
P hue1
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P orav
P nadu
P arne aqau
P mume
P sali
P anin
Outgroup
310 320 330 340 350 360 370 380 390 400
P aswt
P casn
P chor
P olan nine
P inci
P inci kink
P iann
P lard
P lar2
P lev1
P lev2
P leve neod
P mac1
P mac2
P neod mic1
P neoe mic2
P nar1
P nar2
P nerr nube
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P nerr oue1
P nerr oue2
P nerr tome
P take
P tome
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P vec2
P vec3
P vecA
P vec5
P vec6
P ven1
P ven2
P ven3
P venA
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P hue1
P hue2
P orav
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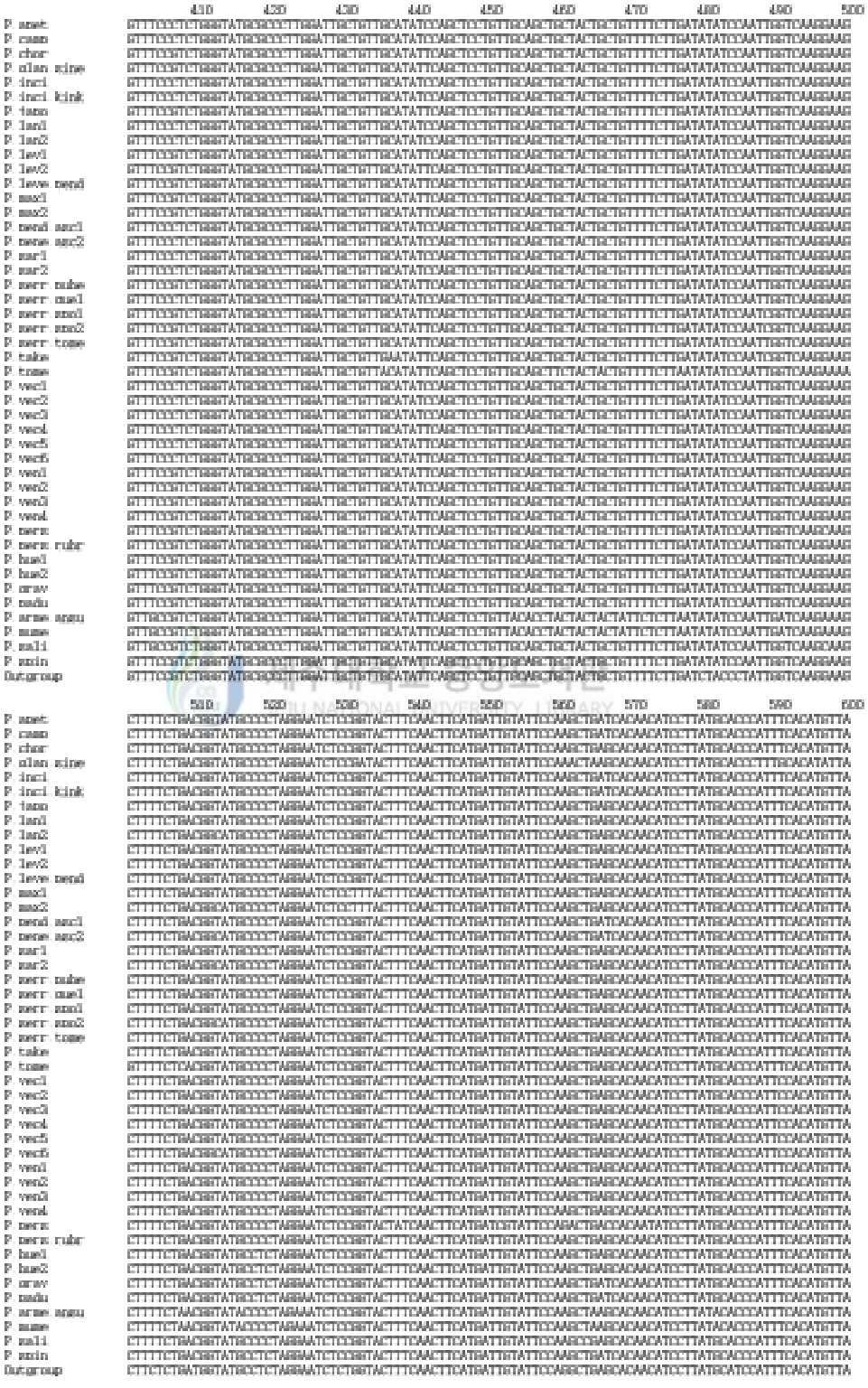
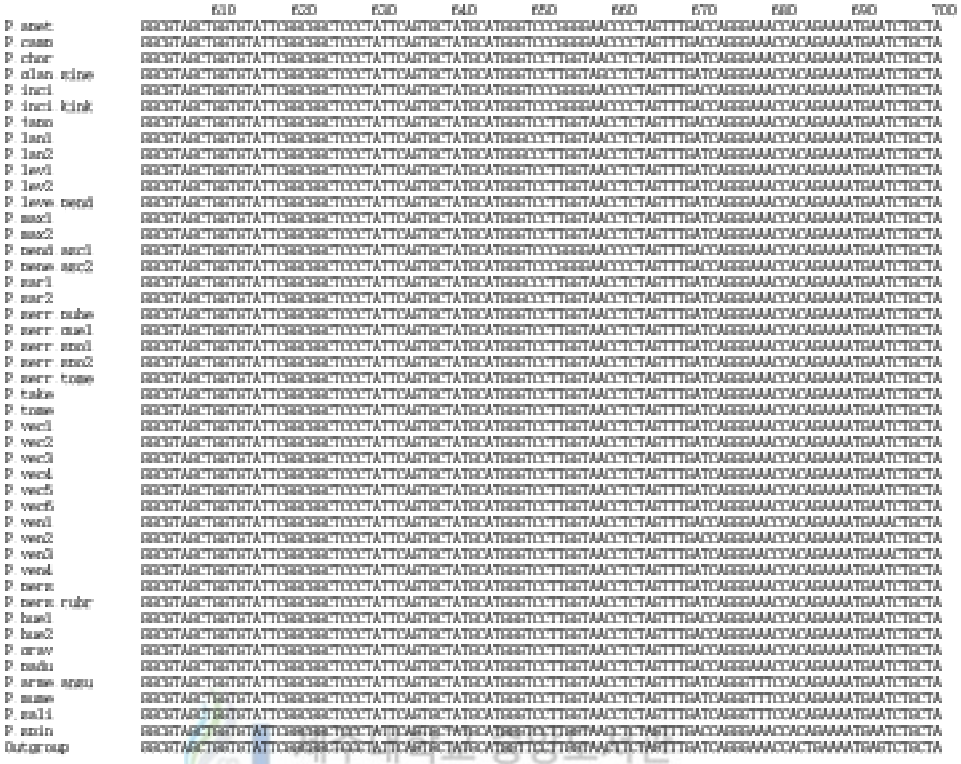
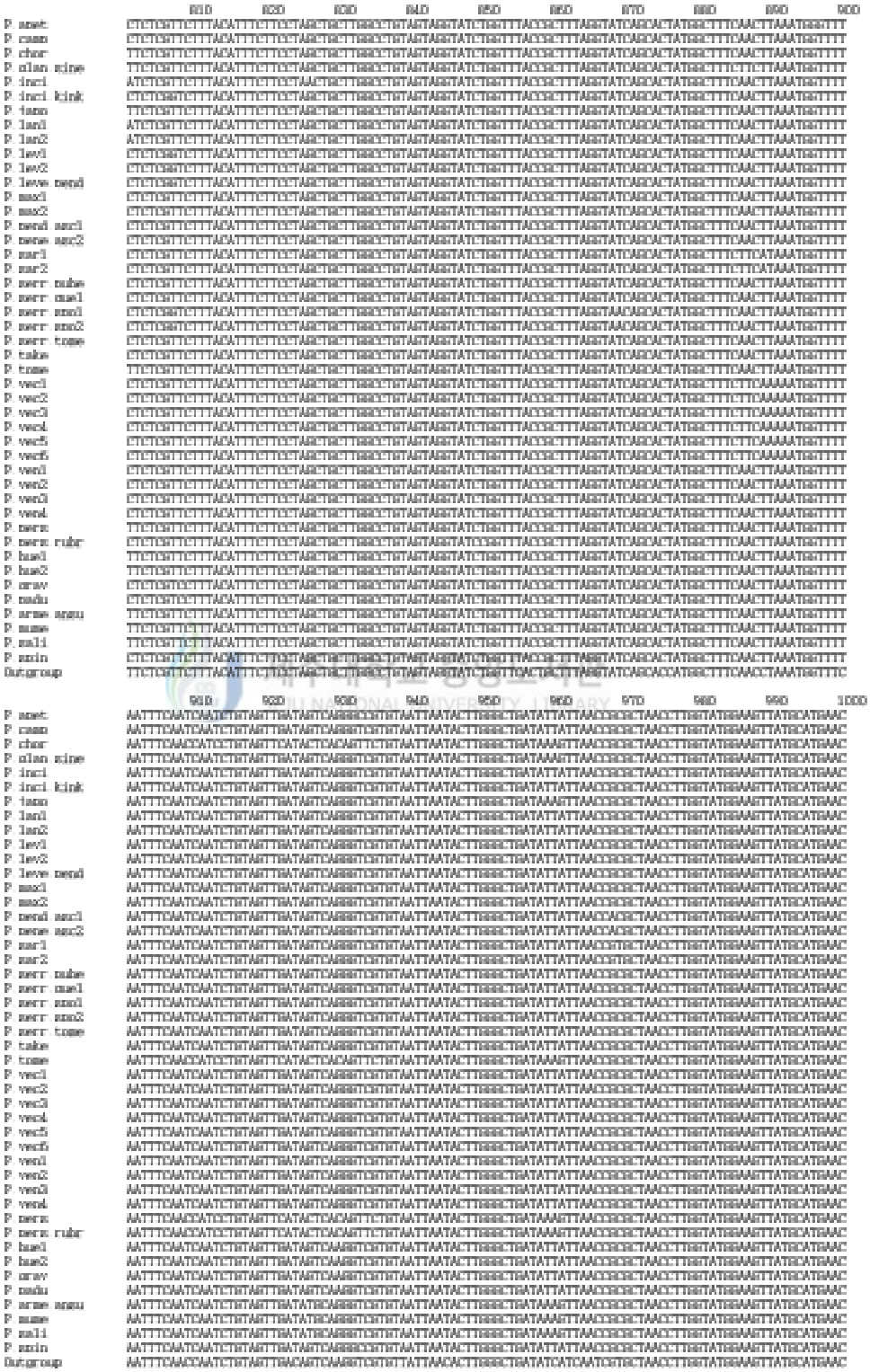
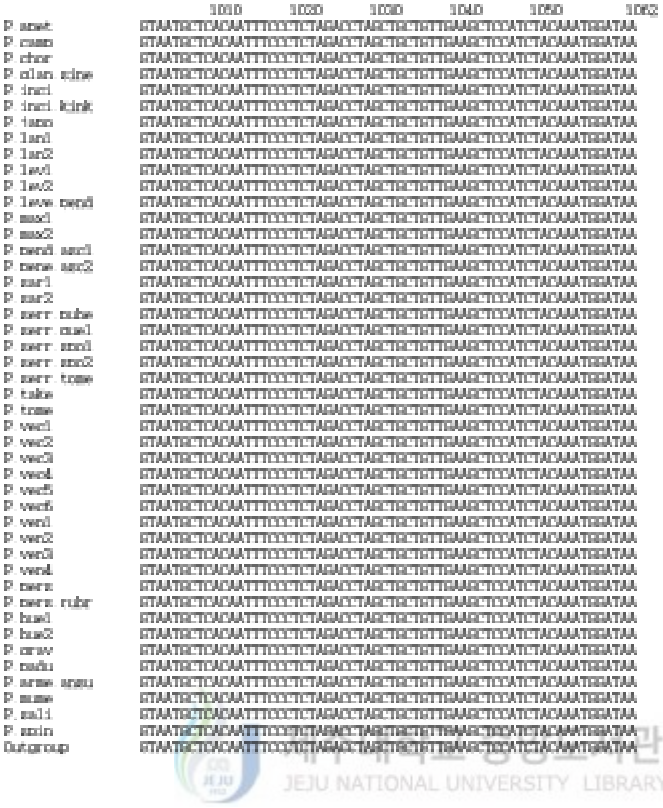
P arne aqau
P mume
P sali
P anin
Outgroup



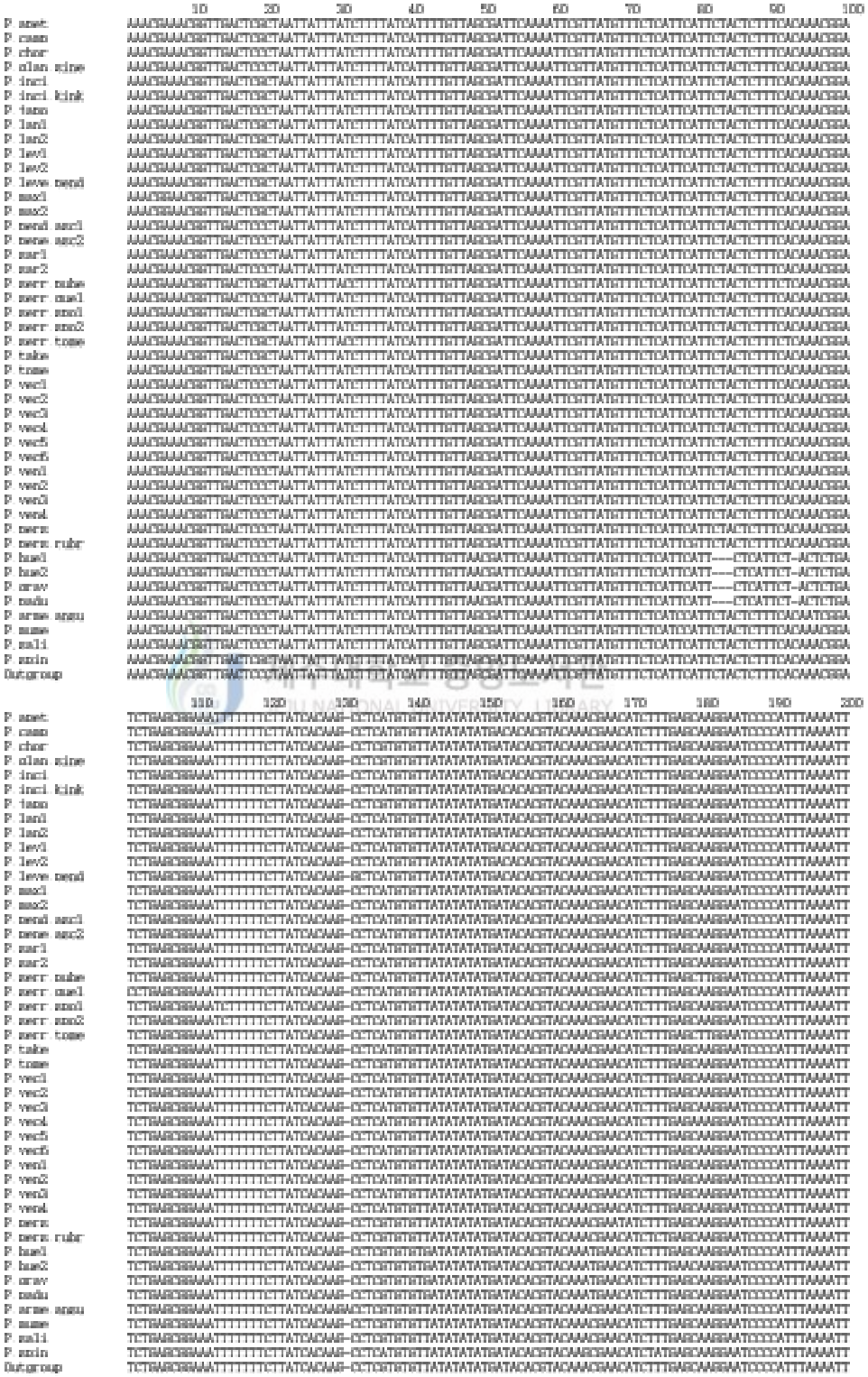


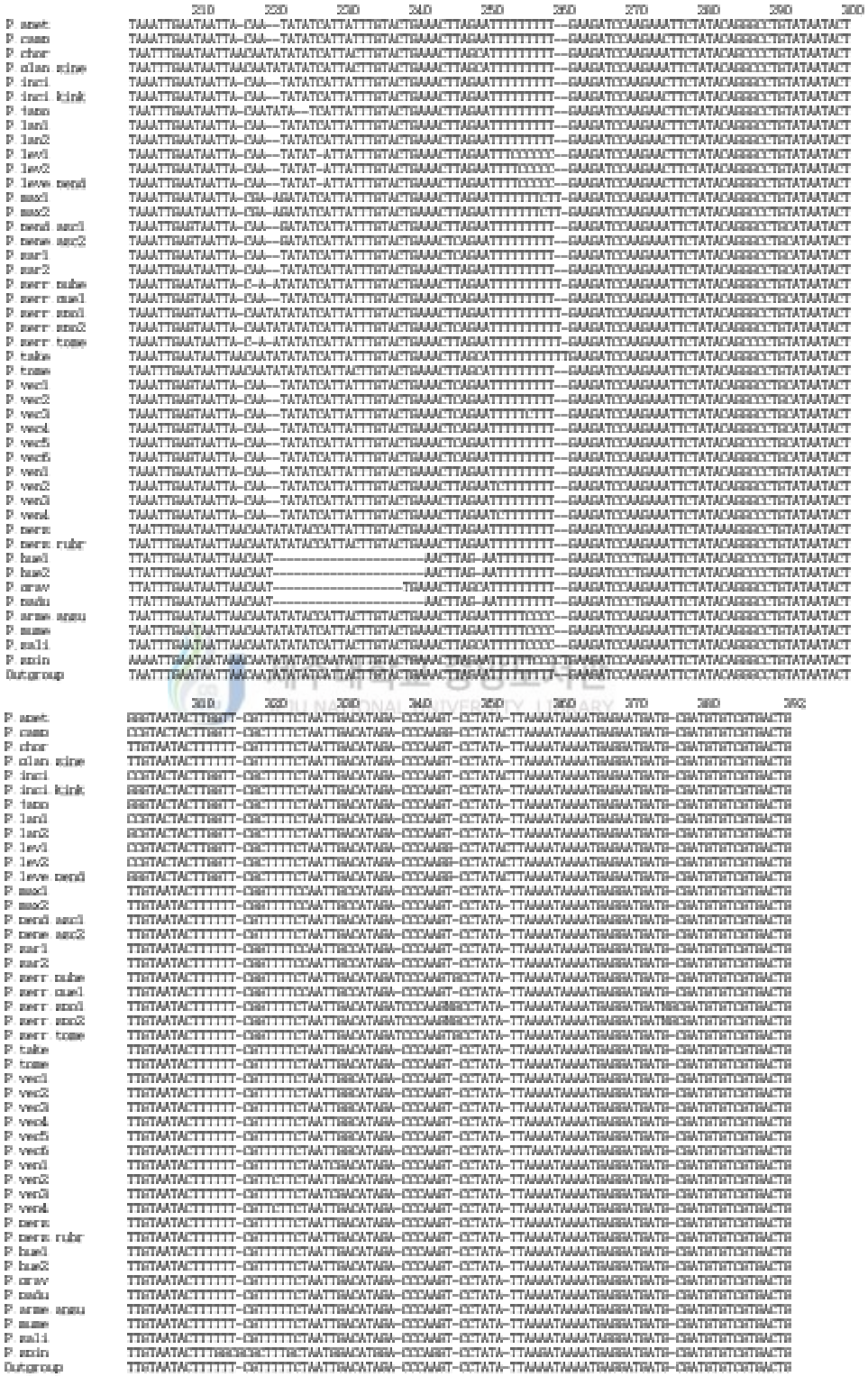




APPENDIX IV

Aligned sequences of the cpDNA *trn*L(UAA)-*trn*L(GAA) intergenic spacer for 45 accessions of genus *Prunus*





국 문 초 록

핵 리보솜 DNA와 엽록체 DNA 염기서열에 기초한 한국과 일본 벚나무속 식물의 분자계통학적 연구

정 용 환

본 연구는 한국과 일본에 분포하는 벚나무속 식물의 모든 아속을 대표하는 45 분류군을 대상으로 핵리보솜 DNA의 ITS 부위와 엽록체 3개 부위 [*rbc*L 유전자, *psb*A 유전자, *trn*L(UAA)-*trn*F(GAA) 부위]의 염기서열을 바탕으로 한 계통유전학적 분석을 통하여 제주도 자생왕벚나무의 기원과 특성을 규명하고, 벚나무속 식물의 분자계통과 진화를 밝히기 위하여 수행되었다. 특히, 종간잡종으로 추정되고 있는 자생왕벚나무의 기원을 밝히기 위해 왕벚나무의 모계 식물로 추정되는 5종 (올벚나무, 사옥, 산벚나무, *P. lannesiana*, 벚나무), 그리고 재배왕벚나무 6개체와 자생왕벚나무 4개체 등 왕벚나무 10개체를 대상으로 핵 리보솜 DNA의 ITS 부위와 엽록체 DNA의 *rbc*L 유전자를 분석하였다.

Parsimony 계통수 분석결과, 크게 독립된 4개의 집단을 형성하였다. 제1집단은 *Prunophora* 아속, *Amygdalus* 아속과 *Cerasus* 아속 I로 구성되었으며, 제2집단은 *Padus* 아속과 *Laurocerasus* 아속, 제3집단은 *Cerasus* 아속 II, 제4집단은 *Cerasus* 아속 III으로 각각 구성되었다. Parsimony 계통수 분석에서 특이한 점은 *Cerasus* 아속이 I, II, III등 세집단으로 나누어졌으며, 왕벚나무는 *Cerasus* 아속에서 분리되었고 자생왕벚나무 4개체는 재배왕벚나무 집단과 확연히 분리됨을 보였다.

Parsimony 계통수의 신뢰도를 높이기 위한 consensus 계통수 분석에서는 *Cerasus*, *Padus*, *Amygdalus*, *Prunophora* 및 *Laurocerasus* 아속들이 신뢰도 100%를 나타내면서 단일계통집단을 형성하는 것으로 나타났다. 또한 *Cerasus* 아속의 *Microcerasus*절, *Amygdalus* 아속과 *Prunophora* 아속들은 *Ceraus* 아속, *Laurocerasus* 아속 및 *Padus* 아속의 관계처럼 신뢰도 100%를 나타내면서 공통조상에서 분리된 하위집단을 형성하고 있었다. 또한 계통유연관계 분석에서 가장 두드러진 특징은 *Cerasus* 아속에 속하는 복사앵도나무, 앵도나무, 홍매 및 이스라지나무 등이 *Amygdalus* 아속과 아주 밀접한 분류관계를 나타내었다. 이러한 결과는 벚나무속 식물에서의 분류학적 위치가 재정립해야 할 것으로 보인다. 또한 엽록체 DNA는 모계유전을 하는 것으로 이미 알려져 있어서, 올벚나무가 왕벚나무의 모계 중 하나로 추정되며, 사옥은 왕벚나무의 기원에서 부계일 가능성을 제시해주고 있다. 그리고, 핵 리보솜 DNA의 ITS1과 ITS2 부위의 염기서열의 길이, ITS1 부위의 DNA 2차구조 및 엽록체 DNA의 유전자 염기서열의 치환으로 인한 아미노산 치환을 근거로 자생왕벚나무와 재배왕벚나무가 다른 분류군일 가능성을 강하게 뒷받침하는 것으로 보인다. 또한 염기변이율을 재배왕벚나무와 자생왕벚나무의 부모로 추정되는 종들과 비교하여, 한라산 자생왕벚나무간의 염기변이율은 형태학적 분석의 진행 결과와 동일하게 변이폭이 크게 나타남으로써 한라산 자생설을 부정하는 지금까지의 주장과는 정면으로 배치되는 것이다. 특히, 한라산 자생왕벚나무가 올벚나무와 *P. lannesiana*의 잡종이라는 주장은 *P. lannesiana*가 한라산에 분포한다는 보고가 없으므로 그 타당성을 뒷받침할 근거가 없어 보인다. 따라서, 왕벚나무는 한라산에 자생하며 다른 벚나무속 식물과 뚜렷이 구분되는 독립된 종으로 분류되어야 할 것으로 사료된다.

주요어: 벚나무속, 왕벚나무, ITS, *psb*A, *rbc*L, *trn*L(UAA)-*trn*F(GAA), 분자계통, 단일계통, 기원

감사의 글

지난 4년 동안의 박사학위연구가 이 논문으로 결실을 맺게 되었습니다. 그동안 제게 많은 도움을 주신 분들께 이 지면을 빌어 감사의 말씀을 드립니다.

우선, 본 연구를 수행하고 논문이 완성되기까지 부족한 저를 끊임없는 지도와 사랑으로 보살펴 주시며 학문에 임하는 기본자세를 일깨워주신 오문유 선생님께 진심으로 감사의 말씀을 드립니다. 학부시절부터 대학원 생활까지 십 여 년 동안 강직한 학자의 모습을 보여주신 점 제겐 커다란 힘이 되었습니다. 다시 한번 감사의 말씀을 드리며 항상 건강하시길 빕니다.

늘 격려와 사랑으로 바쁘신 중에도 기꺼이 심사를 맡아 미흡한 논문을 다듬어 주신 제주대 생물학과 김문홍 선생님, 고석찬 선생님, 김세재 선생님과 서울대 생명과학부 김호방 선생님께 깊은 감사를 드립니다. 그리고 평소에 생물학에 관한 많은 가르침을 주신 허인옥 선생님, 오덕철 선생님, 이용필 선생님, 김원택 선생님, 이화자 선생님께도 더불어 감사의 뜻을 전합니다.

본 연구의 성공적인 실험수행을 위한 재료 채집에 많은 도움을 주신 임업연구원 제주임업시험장 김찬수 박사님과 일본 벚나무속 식물의 실험재료를 채집하여 제공해주신 日本 森林綜合研究所 勝木俊雄 박사님께 감사드립니다. 유전자 분석에 필요한 실험 기자재 사용에 많은 도움을 주신 제주대 공동실험실습관 고정은 선생님, 현중선 선생님과 농촌진흥청 제주농업시험장 김성철 선배님께도 감사의 말씀을 드립니다. 또한 결과분석을 위한 통계프로그램을 제공해주신 서울대 원효식 박사님과 논문작성에 지적을 아끼지 않으셨던 강형일 박사님께도 감사의 마음을 전합니다.

7년 전 더운 여름 유전학실험실에서 무지한 제게 여러 가지 기초실험들에 대한 가르침과 수많은 밤들을 함께 지새며 엄하고 날카로운 충고를 아낌없이 해주면서 논문발표까지 지켜봐주신 고미희 박사님과 항상 제 학위과정에 관심을 가져주신 Washington State University에 계신 김기옥 박사님께도 큰 감사를 드립니다. 항상 미래를 향한 힘찬 전진을 하는 유전학실험실 가족

들인 오유성 선배와 한상현, 김재환, 송지훈, 정용욱, 김유경, 강민철, 홍효정, 송은아 후배 및 그동안 유전학실험실을 거쳐간 여러 선후배들에게도 감사를 드립니다. 특히 지난 4년동안 함께 연구를 수행하며 실험의 마무리와 결과해석에 많은 도움을 준 한상현 후배에게 고마움을 전합니다.

처음 대학생활을 시작할때부터 정신적으로 큰 도움이 되어주고 많은 조언을 준 이정배, 송관필, 김민규군 그리고 힘든 대학원 생활에 즐거움을 더해준 이동헌 선배님을 비롯한 대학원 선후배 여러분께도 감사드립니다. 또한 어릴적 꿈과 추억을 항상 간직할 수 있도록 곁에서 지켜봐 준 고명건 회장을 비롯한 물마루 회원들과 그외 고향 친구들에게도 고마움을 전하며 기쁨을 함께 나누고 싶습니다.

무엇보다도 지금까지 저를 낳아 길러 주시고 무한한 사랑과 인내로 뒷바라지 하면서 학문의 길로 정진할 수 있게 도와주신 사랑하는 부모님께 큰 고마움과 감사의 마음을 드립니다. 또한 어려울 때 힘이 되어준 형님가족, 동생가족들과 관심으로 지켜봐주신 친지분들께도 깊은 감사를 드립니다. 묵묵히 지켜보아 용기를 주신 장모님과 처남식구들 그리고 장애를 극복하기위해 노력하는 막내처남 운식에게 감사의 마음을 전합니다.

끝으로 박사학위과정 시절 결혼해서 지난 시간동안 어려운 생활을 참아내고 고생한 아내 오효정과 단 한번도 제대로 함께 놀아 주지 못해 늘 안스러웠던 귀여운 두 딸 재영이와 민영이에게 이 논문을 통해서나마 감사의 뜻을 기록합니다.

2001년 12월 마지막날에

정용환 拜上