# A genetic study of *Pinus parviflora* on Ulleung Island of Korea, Compared to *P. parviflora* of Japan and *P. armandii* of China

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**Abstract**

*Pinus parviflora* Siebold et Zucc. on Ulleung Island, Korea, has been proposed to be more closely related to *P. armandii* Franch. because both have long leaves and seeds that are either wingless or have very short wings. Randomly amplified polymorphic DNA (RAPD) markers using nine primers and sequence analysis of the *trn*G gene and the *mat*K gene and morphological characteristics of seeds and cones were used to assess the genetic relatedness of this taxon on Ulleung Island with *P. armandii* in China and *P. parviflora* in Japan. This current study showed that *Pinus armandii* from China, *P. parviflora* from Japan, and *P. parviflora* populations of Ulleung Island formed distinct groups that were separated from each other. *P. parviflora* from Ulleung Island grouped with *P. parviflora* from Japan, rather than *P. armandii* from China based on the RAPD dendrogram and SNPs in *mat*K. It is believed that *P. parviflora* on Ulleung Island is genetically well differentiated, indicating limited gene flow from Japan, although cones and seeds of *P. parviflora* on Ulleung Island are more similar to var. *parviflora* in southern Japan than *P. armandii* in central China. It seems that the entities that comprise *P. parviflora* exhibit widely overlapping ranges in morphological attributes except leaf length.

*Key words: Pinus parviflora*, *Pinus armandii*, RAPD, Single nucleotide polymorphisms, *mat*K gene, Ulleung Island.

## Introduction

The genus *Pinus* L. is generally divided into two subgenera, *Strobus* and *Pinus* (MIROV, 1967). Among the subgenus *Strobus*, whose common name is five-needle soft pines, native to eastern Asia, *P. armandii* Franch. and *P. koraiensis* Siebold & Zucc. are known to be important for timber production, while the rest of the five-needle pines, such as *P. parviflora* Siebold et Zucc. has rather limited economic importance due to their restricted gene resources (WANG and HONG, 2004). *P. parviflora*, commonly known as Japanese white pine, is distributed from southern Hokkaido to Kyushu including far eastern Russia and Ulleung Island of Korea (WILSON, 1916; KAWAMOTO, 1943; LEE, 1980; OHWI, 1984).

Ulleung Island, late Tertiary origin from three to five million years old, is located ca. 150 km off the coast of Korea at 37°30'N and 130°51'E. This island was then connected to the Korean peninsula and the west coast of Japan until the early Quaternary (YANG, 1996). Ulleung Island has a unique flora of ca. 180 woody species of which ten are endemic to the Island (LEE and JOO, 1958).

Information on the morphological variability of *P. parviflora* on Ulleung Island is fragmentary. Based on wing size of seeds, two different varieties of *P. parviflora* have been reported in Japan: var. *pentaphylla* (Mayr) Henry in southern Hokkaido and from north to central Honshu (mainly on the East side) and var. *parviflora* from central to western Honshu, Shikoku, and Kyushu (OHWI, 1984; YAMAZAKI, 1995; TANI et al., 2003).

More than 20 collections of seed cones with seeds of *P. parviflora* on Ulleung Island and specimens at the T.B. Lee Herbarium at Seoul National University and the Arnold Arboretum at Harvard University have been observed thus far. It was found that seed wings were ca. 3–5 mm in length with an appressed and vestigial structure. *Pinus armandii* is distributed in the southern provinces of China, i.e., Yunnan, Sichuan, Shaanxi, and Henan, and Taiwan, and southern Japan (MIROV, 1967; FU et al., 1999; YAMAZAKI, 1995; KANETANI et al., 2004) is known to have no seed wings which is one of the taxonomically important characters (WANG and HONG, 2004).

Limited observation from cultivated individuals of Japanese *P. parviflora* in Korea suggested that leaf length of *P. parviflora* from Japan is much shorter than that of *P. parviflora* on Ulleung Island. The individuals on Ulleung Island are delimited by discontinuities in the variation of leaf and seed wing characters and these patterns cannot be incorporated satisfactorily in the normal taxonomic framework. It needs confirmation since individuals on Ulleung Island can be classified as either *P. armandii* (WANG and HONG, 2004) or *P. parviflora* (WILSON, 1916; KAWAMOTO, 1943; LEE, 1980; OHWI, 1984).

Previous studies showed that *Fagus multinervis* Nakai and *Cotoneaster wilsonii* Nakai on Ulleung Island are more related to *F. engleriana* Seemen ex Diels and *C. multiflorus* Bunge of central China (CHANG and JEON, 2003), while *Tsuga* on Ulleung Island is more related to *T. diversifolia* (Maxim.) Mast. of northern Japan, rather than to *T. sieboldii* Carriere of southeastern Japan (HAVILL et al., 2008). On the other hand, *Acer takesimense* Nakai, *Prunus takesimensis* Nakai, and *Sambucus racemosa* subsp. *pendula* (Nakai) H. I. LIM and CHIN S. CHANG on Ulleung Island have been reported as closely related species of *Acer pseudosieboldianum* (Pax) Kom., *Prunus sargentii* Rehder, and *Sambucus racemosa*

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subsp. *kamtschatica* (E. L. Wolf) Hultén of the Korean peninsula (CHANG, 1992; CHANG et al., 2004; LIM et al., 2009). Most of the flora of Ulleung Island is considered to have originated from either the Asian mainland including the Korean peninsula and Central China or the Japanese archipelago (PFOSSER et al., 2002; PFOSSER et al., 2005; MAEKAWA and SHIDEI, 1974).

Molecular markers generated by RAPD (WILLIAMS et al., 1990; HICKS et al., 1998; LEIBENGUTH et al., 1998; ROH et al., 2007a; SCHEEPERS et al., 1997) and single nucleotide polymorphism (SNP) (BROOKES, 1999; RAFALSKI, 2002; HSU et al., 2008; ROH et al., 2007b; YOON et al., 2008) have been useful in studying genetic relationships, and very critical when genetic diversity or identification of certain germplasm cannot be effectively understood based on certain morphological characters. SNPs are the most abundant sequence variations in most genomes (BROOKES, 1999). The abundance, ubiquity, and interspersed nature of SNP throughout the genome make them ideal candidates as molecular markers (RAFALSKI, 2002). Information regarding the geographic distribution and genetic differentiation of *P. parviflora* in Japan and *P. armandii* in China including *P. parviflora* on Ulleung Island may offer major clues to possible relationships. Thus, it was desirable to determine if evidence from RAPD and DNA sequence variation from SNPs of two chloroplast genes [RNA-Gly (*trn*G) and maturase K (*mat*K)] corresponds to the current disposition of species based on morphology (WANG and HONG, 2004; LEE, 1980; OHWI, 1984).

## Materials and Methods

*Plant materials*

Most of the samples were collected from wild populations or known provenance as indicated in *Table 1*. Eight individuals of *Pinus armandii* were collected from the US National Arboretum (NA, Washington, DC, USA), the Arnold Arboretum (AA, Jamaica Plains, MA, USA), and Quarryhill Botanical Gardens (QHBG, Glen Allen, CA, USA). Also, 21 individuals of *P. parviflora* collected from Ulleung Island, Gyeongsangbuk-do, Korea were used in this study. Herbarium specimens from the material collected are stored at the T. B. Lee Herbarium of the Arboretum of Seoul National University (SNUA). Two individuals of *P. parviflora* from Niigata and Hokkaido were treated as var. pentaphylla here (TANI et al., 2003), but samples of unknown origin were recorded as *P. parviflora* including those from Ulleung Islands, not as *P. parviflora* var. *parviflora* here because of difficulty of assignment of an infraspecific rank. In addition, four samples of unknown wild origin of *P. parviflora* were assigned as *P. parviflora*. *Pinus lambertiana* Doug. and *P. armandii* × *P. lambertiana* were included in this study as outgroups (*Table 1*).

*Table 1.* – Individual number (NO.), location, and species names for 32 individuals of *Pinus parviflora*, *P. armandii*, and other taxa.

| No. | Species | General Location | GPS and collection information |
|---|---|---|---|
| 1 | *P. armandii* | US National Arboretum (NA) 64752.003 | Shaanxi, China, 2,400m |
| 2 | *P. armandii* | NA 64752.004 | Shaanxi, China, 2,400m |
| 3 | *P. armandii* | Quarryhill Botanical Garden (QHBG), 1991.134 | Sichuan, China |
| 4 | *P. armandii* | Arnold Arboretum (AA), Jamaica Plain, MS 729-83A | Cultivated plant of known wild (indirect) origin, Sichuan, China |
| 5 | *P. armandii* | AA 419-84A | Cultivated plant of known wild (indirect) origin, Mt. Taipei, Shanxii, China |
| 6 | *P. armandii* | AA 24-49A | Collected wild origin. Sichuan, China. |
| 7 | *P. armandii* | AA 472-54B | Cultivated plant of unknown wild origin. |
| 8 | *P. armandii* | AA 323-87B | Cultivated plant of known (indirect) wild origin. Sichuan, China. |
| 9 | *P. parviflora* var. *pentaphylla* | AA 1539-71B | Received as *P. pentaphylla*. Ofuna Bot. Garden, Kanagawa, Japan. Unknown origin. |
| 10 | *P. parviflora* | AA 893-49A | Yokohama Nursery, Japan via Larz Anderson, Brookline, MA, USA. Unknown origin. |



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*Table 1.* – Continued.

| No. | Species | General Location | GPS and collection information |
|---|---|---|---|
| 11 | *P. parviflora* var. *pentaphylla* | AA 192-90 | The same as 9A. |
| 12 | *P. parviflora* var. *pentaphylla* | AA 1732-77B | Collected directly from wild. Mt. Apoi, Hidaka Dist. Hokkaido Pref. Japan |
| 13 | *P. parviflora* | Korea 058 | 440m, Taeha, Ulleung Island, Korea. |
| 14 | *P. parviflora* | Korea 059-1 | 440m, Ulleung Island, Korea |
| 15 | *P. parviflora* | Korea 059-2 | 440m, Ulleung Island, Korea |
| 16 | *P. parviflora* | Korea 059-3 | 440m, Ulleung Island, Korea |
| 17 | *P. parviflora* | Korea 064-1 | 440m, Ulleung Island, Korea |
| 18 | *P. parviflora* | Korea 064-2 | 440m, Ulleung Island, Korea |
| 19 | *P. parviflora* | Korea 064-3 | 440m, Ulleung Island, Korea |
| 20 | *P. parviflora* | Korea 1 | 300m, Namseo, Ulleung Island, Korea |
| 21 | *P. parviflora* | Korea 4 | 300m, Namseo, Ulleung Island, Korea |
| 22 | *P. parviflora* | Korea 8 | 300m, Namseo, Ulleung Island, Korea |
| 23 | *P. parviflora* | Korea 16 | 300m, Namseo, Ulleung Island, Korea |
| 24 | *P. parviflora* | Korea 19 | 300m, Namseo, Ulleung Island, Korea |
| 25 | *P. parviflora* | Korea 21 | 300m, Namseo, Ulleung Island, Korea |
| 26 | *P. parviflora* | Korea 7 | 300m, Namseo, Ulleung Island, Korea |
| 27 | *P. parviflora* var. *pentaphylla* | Niigata, Japan 1 | 200m, Mt. Kakajo, Sanjo-Shi, Niigata, Japan. |
| 28 | *P. parviflora* var. *pentaphylla* | Niigata, Japan 3 | 200m, Mt. Kakajo, Sanjo-Shi, Niigata, Japan. |
| 29 | *P. parviflora* var. *pentaphylla* | AA 16568D | Received as *P. parviflora*. Sapporo, Japan |
| 30 | *P. parviflora* | AA 195-65A | Received as *P. parviflora* 'Brevifolia'. From US National Arboretum, Washington, DC. From a cultivated plant of unknown wild origin. |
| 31 | *P. lambertiana* | AA 16552D | Grafted plant from a cultivated plant of unknown wild origin. C. A. Dana, Glen Cove, NY. |
| 32 | *P. armandii* × *P. lambertiana* | AA 534-59A | Cultivated plant. Unknown wild origin. Crossed by Albert Johnson, 1952 from Bussey Institute. |

*DNA extraction, polymerase chain reaction and RAPD*

Total genomic DNA was extracted from dried needles (50 mg) using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). DNA was quantified with a NanoDrop D-1000 Spectrophotometer (Thermo Fisher Scientific Waltham, MA, USA). Nine informative primers (OPA4, OPA15, OPA16, OPB9, OPB11, OPC9, OPC14, OPC16, and OPC19) produced informative polymorphic bands were chosen from 23 random primers (kits A, B, and C; Operon Technologies, Alameda, CA) for final analysis.

Polymerase chain reaction (PCR) for RAPD was carried out in a 25 µl reaction volume using 10 ng/µl of template DNA, 5 pM of primers, and Ready-To-Go PCR Beads (PCR bead, GE Healthcare, Buckinghamshire, UK) using the PTC-100 Programmable Thermal Cycler (MJ Research, Watertown, MA, USA). PCR amplification profiles consisted of 94 °C for 3 min followed by 35 cycles of 94 °C for 30 sec, 37 °C for 30 sec, and 72 °C for 60 sec and a final extension at 72 °C for 3 min. Amplified products were separated on 1.5% agarose gels (0.5x TBE buffer) for 3 h at 120 volts, and stained with ethidium bromide. Gels were documented digitally using an Image Analyzer (AlphaImager 2000, Alpha Innotech Corp., San Leandro, CA, USA). Amplification products ranging in size from 300 bp to 1,400 bp were selected for scoring manually.



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*Table 2.* – Primer sets to amplify selected introns for *Pinus armandii* and *P. parviflora*.

| Primer sets | 5' to 3' Sequence | Product size (bp) /Tm (°C) [1] | NCBI acc number; definition |
|---|---|---|---|
| PA1 | Forward TGAATGCCCAGGAATAGCAT<br>Reverse AGCTCCTTCATGGGGGATAG | 741/57 | EF546745; *P. parviflora* isolate PARV10S3 tRNA-Gly (*trn* G) gene, intron; chloroplast |
| PA2 | Forward TCGGATGAACCCTCTTTTTG<br>Reverse TCCTGTATCTTTGCCAGGAA | 513/58 | EF546720; *P. parviflora* isolate PARV10S3 *maturase* K (*mat*K) gene, partial cds; chloroplast |

[1] Product size of amplifications and annealing temperature (Tm).

*Sequence analysis and single nucleotide polymorphism*

The *trn*G gene (intron, EF546745) and *mat*K gene (EF546720) of *P. parviflora* were obtained from GenBank (*Table 2*). Primers were constructed using Primer3 Input (v. 0.4.0) software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Amplification was performed using 10 ng/µl of template DNA, 10 pM of primers, and Ready-To-Go PCR Beads in a total volume of 25 µl, with a PTC-100 Programmable Thermal Cycler (MJ Research, Watertown, MA, USA). Amplification profiles were 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 1 min at 57 or 58 °C (*Table 2*) and 72 °C for 1 min, and a final extension of 5 min at 72 °C.

Sequencing reactions were performed with 0.5 µl of PCR product, 1 µl of BigDye Terminator (Version 3.1, Applied Biosystems, Foster City, CA), and 1 µl of PCR primers (1 pmol/µl). Amplification profiles were 94 °C for 2 min followed by 35 cycles of 94 °C for 15 sec, 50 °C for 15 sec, 60oC for 4 min. Amplified PCR products were then sequenced with an Applied Biosystems 3730 Genetic Analyzer (PE Applied Biosystems, Foster City, Calif., USA), and the consensus sequence was blasted using the NCBI nucleotide BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

*Data analysis*

Bands from 300 to 1, 400bp for RAPD were scored and the corresponding matrix was used to construct dendrograms using the Molecular Evolutionary Genetics Analysis (MEGA, version 3.1) program. Clusters were inferred with the neighbor-joining method (NJ) and genetic p-distances were obtained. Robustness of the tree was tested with interior-branch (IB). The numbers for each interior branch is the percentage bootstrap value (1000 resamplings), and only values higher that 50% are shown.

*Morphological Analysis*

Morphological variation was measured in four characters (leaf length, cone length, seed length, and seed wing length) of 20 individuals of *P. parviflora* collected from Ulleung Island to evaluate recognition of this taxon within the *P. parviflora* complex. Ten mature cones were collected from two localities of Ulleung Island on Sept. 14, 2007 and April 18, 2008 and preserved at SNUA (T. B. Lee Herbarium, Seoul National University). Due to insufficient collections on the island, additional ten individuals from specimens [Database, Nature (Korea Biodiversity Information System), http://www.nature.

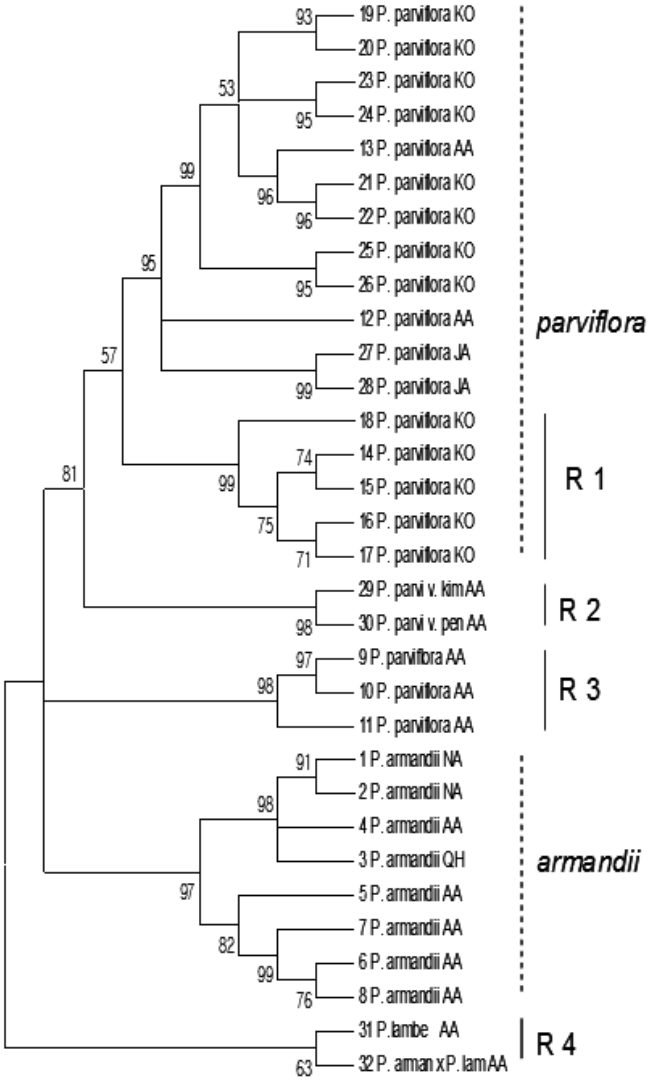


*Figure 1.* – The dendrogram constructed using RAPD markers by the neighbor-joining (NJ) method tested with Interior-Branch (IB) showing clustering of *P. armandii* (*armandii*) and *P. parviflora* (*parviflora*) with some exceptions as indicated by R1 through R4, and A1 through A4. Bootstrap values greater than 50 are indicated at each node.



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go.kr:9001/index.do) deposited at other institutes were measured for four characters. Morphological variation was assessed using univariate statistics (mean, maximum, minimum). Also, bivariate scatter diagrams were performed and characters associated with previous known data (BUSINSKY, 2004) were plotted.

## Results

*RAPD analysis*

In the dendrogram constructed using RAPD markers by the neighbor-joining (NJ) method tested with interior-branch (IB) (RAPD dendrogram), all individuals of *P. armandii* collected from the National Arboretum (1, 2), the Quarryhill Botanical Gardens (3) and the Arnold Arboretum (AA) (4, 5, 6, 7, 8) clustered together and separate from most individuals of *P. parviflora* (*Fig. 1*). All individuals of *P. parviflora* from Korea and from Japan formed one major cluster. Also, *P. parviflora* collected from Ulleung Island, Korea (14–18) formed a sub-group (R 1) and three individuals from the Arnold Arboretum and two individuals from Japan formed separate sub-groups (R 2 and R 3, respectively).

*Table 3.* – Single nucleotide polymorphisms for *Pinus parviflora* isolate PARV10S3 tRNA-Gly (*trn* G) gene, intron; chloroplast (EF546745). Sequence analysis of individuals number 10 of *P. parviflora*, number 31 of *P. lambertiana*, and number 32 of *P. armandii* × *P. lambertiana* did not yield good data, and are excluded.

| SNP position | Modification/ deletion | Samples |
|---|---|---|
| 103 | TGTGG | *P. armandii*: 1, 2, 3, 4, 5, 6, 7, 8 |
| | | *P. parviflora*: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 |
| | | *P. parviflora* var. *pentaphylla*: 27, 29, 30 |
| | ----- | *P. parviflora*: 9, 11, 28 |
| 127 | G | *P. armandii*: 2, 3, 5, 7 |
| | | *P. parviflora*: 12, 13, 14, 15, 16, 17, , 18, 19, 20, 21, 22, 23, 24, 25, 25, 26 |
| | | *P. parviflora* var. *pentaphylla*: 9, 11, 27, 29, 30 |
| | T | *P. armandii*: 1, 4, 6, 8 |

*Table 4.* – Single nucleotide polymorphisms for *Pinus parviflora* isolate PARV10S3 maturase K (*mat*K) gene, partial cds; chloroplast EF546720.

| SNP position | Modification/ deletion | Species and individual number (refer to Table 1) [1] |
|---|---|---|
| 116 | C | *P. armandii*: 1, 2, 3, 4, 5, 6, 7, 8 |
| | | *P. armandii* × *P. lambertiana*: 32 |
| | A | *P. parviflora*: 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 |
| | | *P. parviflora* var. *pentaphylla*: 9, 11, 27, 28, 29, 30 |
| | | *P. lambertiana*: 31 |
| 144 | TTTCTTCTCT | *P. parviflora*: 10 |
| | | *P. parviflora* var. *pentaphylla*: 9, 11, 27, 28, 29, 30 |
| | -TT------- | *P. armandii* × *P. lambertiana*: 32 |
| | TT-------- | *P. lambertiana*: 31 |
| | ---------- | *P. armandii*: 1, 2, 3, 4, 5, 6, 7, 8 |
| | | *P. parviflora*: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 |
| 537 | TCTT | *P. armandii*: 1, 2, 3, 4, 5, 6, 7, 8 |
| | | *P. lambertiana*: 31 |
| | | *P. armandii* × *P. lambertiana*: 32 |
| | ---- | *P. parviflora*: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 |
| | | *P. parviflora* var. *pentaphylla*: 9, 11, 27, 28, 29, 30 |

[1] Sequence analysis of individual numbers 10 and 12 of *P. parviflora* did not yield good data, and are excluded from the analysis here.



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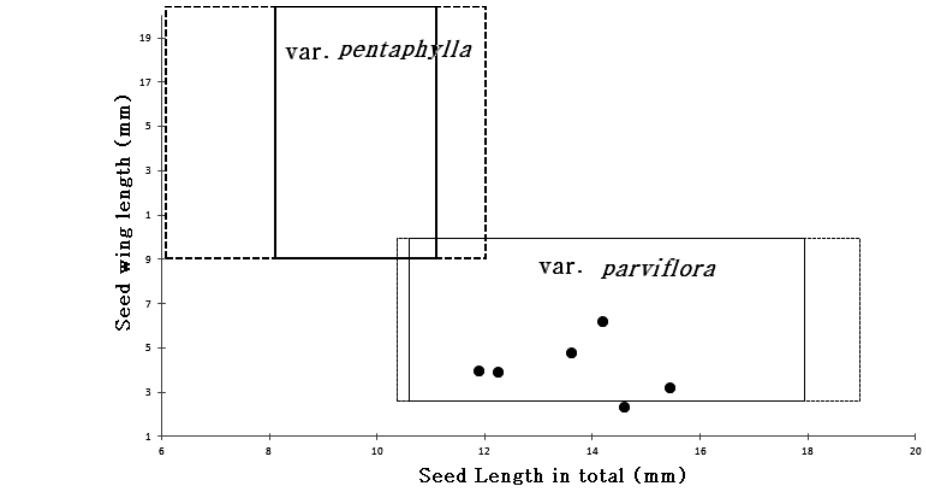
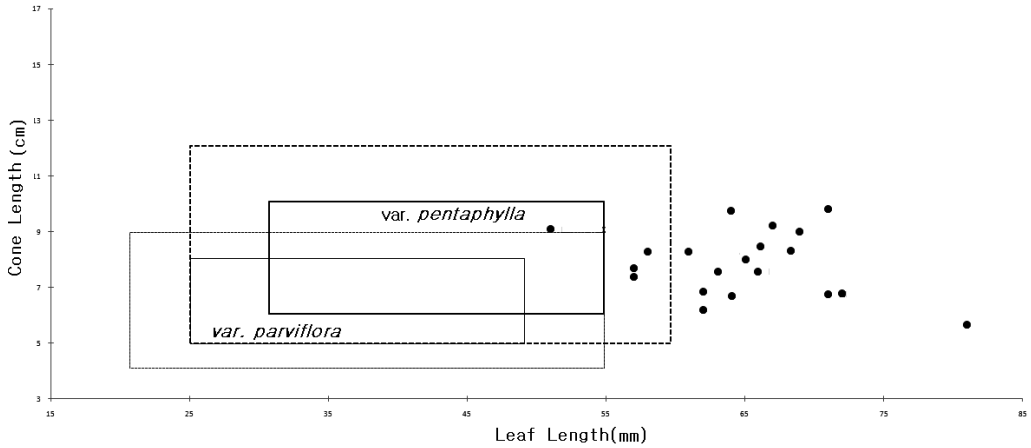



**A.**




**B.**

*Figure 2.* – The scatter plots of seed characteristics (*Fig. 2A*) and leaf length vs. cone length (*Fig. 2B*) produced by the Ulleung Island individuals and var. *pentaphylla* and var. *parviflora* in Japan. Plants are assumed to be spaces in a rectangular grid, separated by mean and variance values (BUSINSKY, 2004). Solid and dotted lines represent mean and variance range.

*Sequence analysis*

Sequence analysis of the *Pinus parviflora trn*G gene showed DNA sequence variation at positions 103–107 for most of *P. armandii*, *P. parviflora* var. *pentaphylla*, and *P. parviflora* individuals except for *P. parviflora* individuals (9, 11, and 28) (*Table 3*). At SNP position 127, only four individuals (1, 4, 6, and 8) of *P. armandii* showed modification T, while all others had G.

More DNA sequence variation from SNPs of *mat*K gene were noticed in *P. parviflora* and *P. armandii* (*Table 4*). At positions 116, all *P. armandii* (1, 2, 3, 4, 5, 6, 7, and 8) and *P. armandii* × *P. lambertiana* (32) had C, while all *P. parviflora* had A. At positions 144–153, two different SNPs were observed for *P. parviflora*: TTTCTTCTCT for individuals 9, 10, 11, 27, 28, 29 (Japanese origin samples) and variation for accessions 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26 (Ulleung Island samples), while all individuals of *P. armandii* showed variation at this SNP position. Moreover, at positions 537–540, all *P. armandii*, *P. lambertiana* (31), and *P. armandii* × *P. lambertiana* (32) showed TCTT, while all individuals of *P. parviflora* had deletions.

This analysis of seed characteristics showed that individuals collected from Ulleung Island are more similar to var. *pentaphylla* growing in the southern part of Japan (*Fig. 2A*). However, the scatter plots of leaf length produced by the Ulleung Island individuals are notably longer than those produced by both var. pentaphylla and var. *parviflora* of Japan (BUSINSKY, 2004; *Fig. 2B*).

## Discussion

The RAPD phenogram and single nucleotide polymorphisms for *mat*K gene showed that *P. parviflora* on Ulleung Island is more closely related to *P. parviflora* native to Japan than *P. armandii* of China, contrary to what would be expected based on the previous morphological observation.



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With the SNPs of *trn*G gene, *P. armandii* and *P. parviflora* were not differentiated well, since both species along with two varieties of *P. parviflora* had the same SNP at positions 103 and 127, although *P. armandii* was divided into two groups showing modifications G (accs 2, 3, 5, and 7) or T (accs 1, 4, 6, 8) at position 127. It was more useful to use *mat*K than *trn*G gene to effectively differentiate between *P. armandii* and *P. parviflora*, between *P. armandii* × *P. lambertiana* along with *P. armandii*, and also between *P. lambertiana* with *P. parviflora* and two of its varieties.

The RAPD dendrogram seems more reasonable than that with AFLP (not presented). Despite the potential limitation of using RAPD markers (JONES et al., 1997), they can be effectively used for characterization and identification of germplasm when they are supported by sequence variation in other markers such as the *mat*K gene. This study shows clearly that RAPD markers can be effectively utilized when the access to AFLP analysis system is limited and when low quality of DNA is amplified. When the dendrogram of RAPD was compared with AFLP dendrogram, the major clustering pattern was found to be similar, but changes in minor grouping were observed. In both RAPD and AFLP analysis clustering of samples did not show any correlation with this limited geographical area of collections such as Ulleung Island.

A similar genetic relationship among sea buckthorn varieties (14 individuals of *Hippophae rhamnoides* L.) from China was revealed effectively by RAPD (RYUN et al., 2004) as compared to AFLP (RYUN, 2006) which suggest that RAPD is a viable tool when supported by SNPs (JOUNG et al., 2010).

The seed wings of individuals in mature cones from Ulleung Island may be almost absent (AHN, 1976). BUSINSKY (2004) stated that subsp. *parviflora* (=var. *parviflora*) has rudimentary and frangible seed wings (2–9 mm) with a mostly lacerate distal margin. On the other hand, subsp. *pentaphylla* (=var. *pentaphylla*) possesses long seed wings (8–19 mm) with an entire margin. Cone length [(6)7–10 cm vs (4)5–10(12) cm] with leaf length from samples of Ulleung Island was determined by the univariate analysis to be significant in separating both var. *parviflora* and var. *pentaphylla* (*Fig. 2*). These individuals, however, can be consistently distinguished from the remainder of both varieties only in leaf length (6–8 cm vs 2.5–5.5 cm). ECKENWALDER (2009) assigned the Ulleung population to var. *parviflora* and describes the leaves as 3–6 cm long and the cones as (4-) 6–8 cm long, which are different from our results. It would be premature to suggest detailed relationships of the taxa here due to the limited samples of *P. parviflora* var. *pentaphylla* as well as individuals from Ulleung Island.

The several taxa on Ulleung island such as *Lonicera insularis* Nakai (currently treated as a synonym of *L. morrowii* A. Gray), *Tilia insularis* Nakai (treated as a synonym of *T. amurensis* Rupr.), *Acer takesimense* Nakai [treated as a synonym of *A. pseudosieboldianum* (Pax) Kom.], and *A. okamotoanum* Nakai (treated as a synonym of *A. pictum* Thunb.) were described as new species before by their rather large fruits and leaves (*Lonicer insularis* and *Acer okamotoanum*), pubescent leaves (*Tilia insularis*), or more dissected leaves (*Acer takesimense*) (CHANG, 2012). Previous studies (HAVILL et al., 2008; PFOSSER et al., 2002; PFOSSER et al., 2005; MAEKAWA and SHIDEI, 1974; CHANG, 1992; CHANG et al., 2004; LIM et al., 2009) speculated that stochasticity during early Quaternary on this island had a large effect at such population margins.

In the previous allozyme study, the genetic diversity of *P. parviflora* on Ulleung Island was moderate (average heterozygosity (He = 0.149) (CHUNG et al., 1998), and was a little lower (He = 0.259–0.272) than that of 16 populations examined in Japan. The extent of genetic differentiation between the Ulleung population and the Japanese populations as well as between two varieties in Japan seems to be high in terms of our current RAPD data and nuclear and mitochondrial genomes analysis (TANI et al., 2003). It seems that the majority of the island populations could be considered as one large metapopulation with geographically isolated populations as possible outliers. It seems that at the marginal population morphological variation is greatest across the geographical isolation, but the entities that comprise *P. parviflora* exhibit widely overlapping ranges in morphological attributes except leaf length.

In conclusion, based on the RAPD and DNA sequence variation from SNPs analysis including preliminary morphological analysis, it is clear that *P. parviflora* native to Ulleung Island is more related to *P. parviflora* in Japan, than *P. armandii* in central China. Furthermore, *P. parviflora* on Ulleung Island is closely related to var. *parviflora* in southern Japan based on the cones and seeds.

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