Genetic Diversity of the *Thalictrum coreanum* LĚV, an Endangered Plant Species in South Korea, and of the Congener *Thalictrum ichangense* LECOYER: Implications for Conservation

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Abstract: *Thalictrum coreanum* (Ranunculaceae), an endangered herb in South Korea, and widespread *T. ichangense* are congeneric species with similar morphologies and life forms. Currently, there is a debate about the identification and distribution of these two species. We carried out a comparative analysis of the genetic variability of these two species using random amplified polymorphic DNA (RAPD) markers. Genetic diversity at species level was high, but lower at the population level. Analysis of molecular variance (AMOVA) appeared to moderate genetic differentiation among the species. In the total variance of *T. coreanum*, 54.53% was attributable to among populations and 45.66% to within populations. On the other hand, 35.24% of the total variance was due to among populations, while 64.76% was due to the within populations of *T. ichangense*. Our results for *T. coreanum* and *T. ichangense* showed a high commonality at the genetic level. *T. coreanum* appeared to have a genetic diversity at a similar level to *T. ichangense*. In the AMOVA and Principle Coordinate Analysis (PCoA), however, *T. coreanum* cleary distinguished with *T. ichangense*. To secure and preserve an endangered species, systematizing species information should be prioritized, according to the accurate criteria. Therefore, the use of a molecular marker such as RAPD could provide hints, diminishing the uncertainty of the distribution area and the identification of these two congeneric species.

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1. Introduction

Thalictrum coreanum (Ranunculaceae), a short-lived perennial herb, was reclassified as an endangered species (Grade II) by the Ministry of Environment (South Korea) (Ministry of Environment, 2005). Most of the literature has recorded that the *T. coreanum* is distributed at the northern part of the central area of the Korean Peninsula (Im, 1996; Lee, 2003; Lee, 1996). According to the Korea Biodiversity Information System (www.nature.go.kr) and Lee (1996), *T. coreanum* is an endemic plant and is found in most regions of South Korea, from islands such as Heuksando to Mt. Seorak in northern Gangwon-do. However, Oh et al. (2005), Kim et al., (1988), and Lee (2011) stated that it is found in northeastern China as well. Chang et al. (2005) suggested that *T. coreanum* should be considered to be data deficient (DD) and should be viewed as a rare and endangered plant, worldwide, based on the categorization of the IUCN Red List. They also suggested that it requires long-term accumulation of relevant data because it is difficult to determine the threat, exactly, with the current data.

T. ichangense is a congener species with *T. coreanum*. The characteristics of the *T. coreanum* are very similar to *T. ichangense*, excluding the tuberous or fibrous, which has a tuber-type underground structure and a sessile or stipitate-type of achenes, respectively (Jeon et al., 2007; Park and Park, 2008). *T. ichangense* is geographically distributed in Jeolla-do, Gangwon-do, Hwanghae-do, northeastern China, and Manchuria (Lee, 1996; Im, 1996; www.nature.go.kr), and is one of the major herbaceous species of *Quercus mongolica* wood in Liaoning Province, China (Kolbek et al. 2003).

The characteristics of the habitats of these two species are in the semi-shade at the edge of forests, or on steep slopes or cliffs. The distribution area is usually confused between the species because of the morphological similarities in many literatures. Jeon et al. (2007) identified specimens at Mt. Juwang in Gyungsangbuk-do, Danyang in Chungcheongbuk-do, and at Jungsun in Gangwon-do as *T. coreanum*, while samples taken from northern Mt. Seorak were identified as *T. ichangense*. These results were similar to those reached by Park and Park (2008).

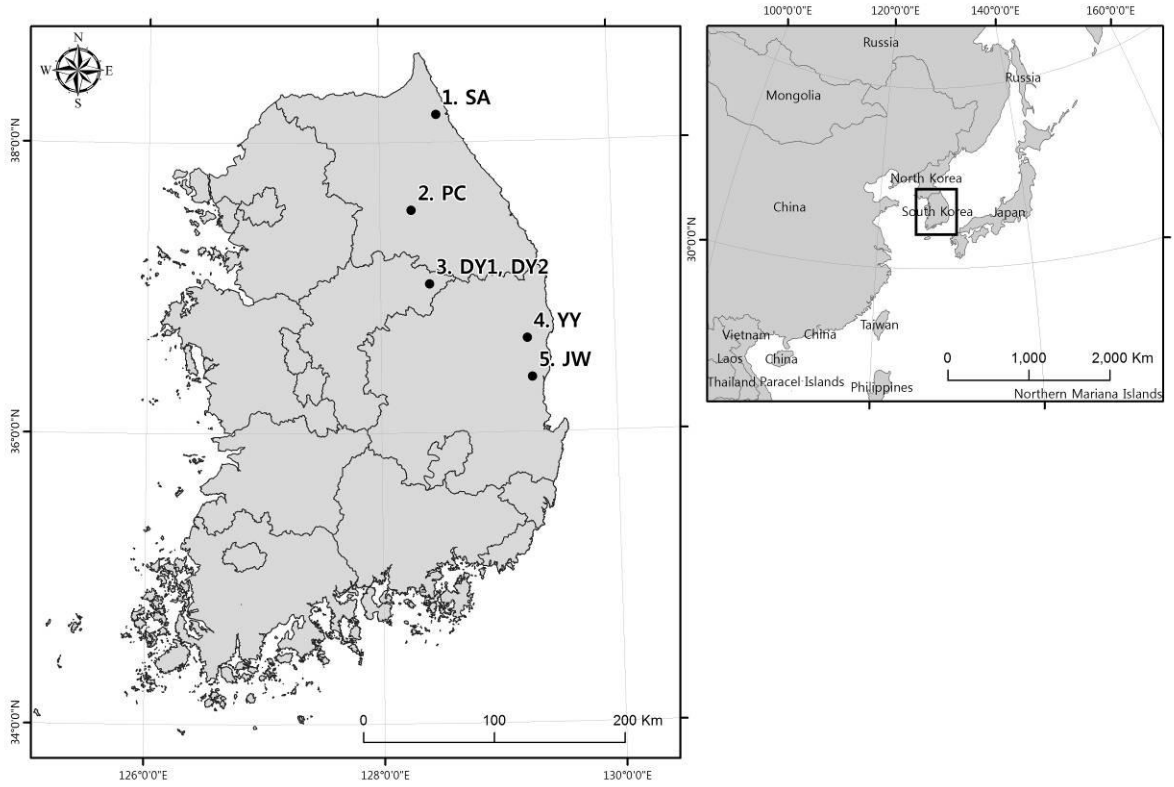

Figure 1. Map of the geographical position populations of *T. coreanum* and *T. ichangense* sampled in this study. 1, Mt. Sorak(SA); 2, Pyungchang(PC); 3, Danyang(DY1, DY2); 4, Youngyang(YY); 5, Mt. Juwang(JW).

The Mt. Seorak specimens were *T. coreanum*, while the samples from Danyang were *T. ichangense*.

However, the Pyeongchang region was found to be home to both species.

The survival of the species ultimately depends on the amount and distribution of genetic variation as well as the elements of the physical environment. The genetic diversity determines the fitness of the population for the short-term and long-term environmental changes, which can exterminate the species and population (Vida 1994). In addition, the genetic variability and partitioning are closely associated with the breeding system, seed dispersal, and geographic distribution range (Hamrick and Godt 1989). Therefore, the understanding of the genetic diversity of rare plants and endangered plants is essential for effective conservation and management (Ge et al. 2005; Huang et al. 2001; Allnutt et al. 2003).

The molecular marker is widely used for the genetic diversity and differentiation of the endangered species (Allnutt et al., 1999, 2003; Raina et al, 2001; He et al, 2000; Dai et al., 2013; Kim et al., 2008; Liu et al., 2012; Rodrigues et al., 2013). The RAPD marker has been used to determine the genetic parameters of rare species and endangered species by using a small amount of plant samples. The advantage of this technique is simplicity, efficiency, and needlessness of the prior sequence information (Williams et al. 1990, Stewart and Porter 1995).

We collected samples of *T. coreanum* and *T. ichangense* from its typical habitats in South Korea. Such areas included Mt. Seorak and Pyeongchang in Gangwon-do, Danyang in Chungcheongbuk-do, and Youngyang and Mt. Juwang in Gyeongsangbuk-do. We carried out RAPD PCR to identify the genetic diversity and genetic structure of these species. Using the information obtained through this experiment, we tried 1) to determine the amount and distribution of the genetic variation of *T. ichangense* and *T. coreanum*, 2) to distinguish whether these two species had a very similar morphology at the genetic level, and 3) to help the effective management and conservation of *T. coreanum*.

Table 1. Geographical location, population area and sample size of *T. coreanum* and *T. ichangense* population studied.

Species	Population	Area(m2)	Samplesize	Altitude(m)	Coordinates
T. coreanum	SA	270	4	860	N38°09'58" E128°29'06"
	PC	254	12	544	N37°27'24" E128°27'45"
T. ichangense	DY1	590	23	189	N37°03'28" E128°18'08"
	DY2	673	25		
	YY	943	23	292	N36°43'59" E129°08'55"
	JW	1614	17	350	N36°23'30" E129°09'43"

2. Material and Methods

2.1. Sampling and site characteristics

We collected the plant samples from five populations (Fig. 1, Table 1) and identified them based on the morphological characters, especially the tuberous or fibrous having a tuber-type underground structure, which corresponds with the morphological criteria set out by Jeon et al.(2007) and Park and Park (2008).

The SA and PC samples were identified as being *T. coreanum*, while the samples from DY and YY and JW were *T. ichangense*. Populations of *T. coreanum* were located at the northern area of Pyungchang (N37°27'24" E128°27'45"), while the *T. ichangense* populations were concentrated around the southern area of Dangyang(N37° 03'28" E128° 18'08"). Therefore, the PC population is considered to be the southern limit of the distribution of *T. coreanum* (Fig. 1). In case of the DY population, because the habitat was divided into two groups by road, We conducted an experiment, treating the populations as a separate entity(Table 1).

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In each population, 4–25 examples were randomly collected at an interval of 5m to minimize genetic relation. Young and complete leaves were collected and stored at -20℃, until DNA extraction.

2.2. DNA extraction and RAPD PCR

The leaves were powdered in liquid nitrogen and the genomic DNA was extracted using a DNeasy Plant Mini Kit (Operon co., Ltd). DNA concentration and purity was determined with a UV-spectrophotometer. DNA was diluted to 25ng/㎕ and then used at RAPD PCR.

One hundred primers (RAPD 10mer Kits: QIAGEN Operon Technologies) were screened, and ten primers were subsequently selected for their reproducible and distinct banding patterns (Table 1).

PCR reactions were performed in 20 ㎕, containing 1unit Taq DNA polymerase, 2.0 ㎕ PCR buffer (10×), 1.0 ㎕ dNTPs mixture (2.5mM each), and 2.0 ㎕ primer (20pmol), 1.0 ㎕ template DNA (25ng genomic DNA). Amplifications were conducted using a T Professional Basic(Biometra) under the following conditions: an initial cycle at 94℃ for 2min; 39 cycles of 30 sec at 94℃, 30 sec at 36℃, and 1 min at 72℃. A final cycle at 72℃ for 10 min was included. The amplification product was electrophoresed on 1.6% agarose gel in a 0.5×TAE buffer and stained with ethidium bromide. Images were photographed using the Gel Doc 2000 (Bio Rad) gel imaging system.

2.3. Data analysis

Each RAPD fragment was scored for presence (1) and absence (0), and the binary matrix was used for statistical analysis. Genetic variation was measured by the percentage of polymorphic band (PPB), Nei's gene diversity (*h*), and Shannon' diversity index (*I*), using the POPGENE 1.32 (Yeh and Boyle, 1997). In order to estimate the hierarchical genetic structure of *T. coreanum* and *T. ichangense*, a genetic differentiation coefficient (Φst) was estimated using the Arlequin program (ver 3.01 program) (Excoffier et al., 2005). The level of variation significance was obtained through tests, which included 2000 permutations per analysis. Gene flow was estimated using the following formula: Nm = 1/4(1-Φst)/Φst, where Φst is used for the estimator of Fst (Slatkin and Barton, 1989). The unweighted pair group method with arithmetic mean (UPGMA) cluster

analysis was performed to demonstrate the relationships among populations and species using FAMD 1.25 (Schlüter, 2006). The resulting clusters were represented as dendrograms and viewed using the program MEGA 5.05 (Tamura et al., 2011). A Principal Coordinate Analysis (PCoA) was conducted using genetic distance matrix obtained from the binary data set. GenAlex 6.5 software (Peakall and Smouse, 2012) was used to compute the Euclidean distances among all pair-wise individuals of *T. coreanum* and *T. ichangense*. The distance matrix was then directly plotted in a 3-dimensional plate. Genetic distance and geographic distances was calculated, and used to perform a Mantel test to investigate the correlation between these two distance matrices in both species. Mantel tests for isolation by distance (IBD) were performed using IBDWS (isolation by distance web service, at http://ibdws.sdsu.edu/～ibdws/) (Jensen et al., 2005).

Table 2. Primer sequence and amplified products of RAPD PCR.

Primer code	Primer sequence (5'-3')	No. of polymorphic fragments/total np. of fragments	
		T. coreanum	*T. ichangense*
OPA-15	TTCCGAACCC	5/6	9/9
OPAF-11	ACTGGGCCTC	4/4	5/5
OPAF-16	TCCCGGTGAG	6/6	17/17
OPN-15	CAGCGACTGT	11/11	10/10
OPN-08	ACCTCAGCTC	11/12	13/13
OPN-11	TCGCCGCAAA	4/5	7/9
OPO-03	CTGTTGCTAC	9/9	12/12
OPO-02	ACGTAGCGTC	13/13	18/18
OPP-06	GTGGGCTGAC	8/9	9/9
OPP-14	CCAGCCGAAC	10/11	10/10
Total		81/87	108/110

3. Results

3.1. Genetic diversity

10primers showing the best resolution in the PCR amplification profiles were selected, and 120 clearly identifiable bands were obtained for the two species under investigation. Of these, 87 bands were from *T. coreanum*, while 110 bands were from *T. ichangense* (Table. 2). 77 fragments were common to both species. Each primer yielded a form 3-18 bands. For *T. coreanum*, 81 of the 87 bands were polymorphic at the species level (93.1%), while the percentage of polymorphic loci for each population was 52.9% for the SA population, and 47.13% for PC population (Table 3). For *T. inchangense*, 108 of the 110 bands were polymorphic at the species level (98.2%), while around 60.0% of the loci were polymorphic at the population level.

There were slight differences in diversity index values at the species level between *T. coreanum* and *T. ichangense* (Table 3). However, the diversity indices showed some variations among populations. PC, of *T. coreanum*, exhibited the lowest value of diversity index among the observed populations (PPB; 47.1%, *I*; 0.232, *h*; 0.153). On the other hand, the DY1 showed the highest genetic diversity (PPB; 72.7%, *I*; 0.337, *h*; 0.220). SA, of *T. coreanum*, which has a very small population size and sample size, had a higher genetic diversity than PC (PPB; 52.9%, *I*; 0.303, *h*; 0.206).

3.2. Genetic differentiation among populations

A hierarchical AMOVA analysis was carried out to examine the distribution of genetic variation between the species, between the populations in the species, and between the individuals in the species. The percentage of total genetic variance between the two species was found to be 36.58%.

The genetic differentiation among the population in *T. coreanum* (54.34%) was slightly higher than that within population (45.66%). On the other hand, 35.24% and 64.76% of the total variance was found in the among populations and within populations of *T. ichangense*, respectively. *T. coreanum* (54.34%) exhibited more genetic differentiation in the among populations than *T. ichangense* (35.24%).

The gene flow (Nm), assumed by Φst value, was 0.165 between species, 0.210 among the *T. coreanum* populations, and 0.459 among the *T. ichangense* populations, respectively.

Table 3. Genetic variability statics of *T. coreanum* and *T. ichangense* (PPB= Percentage of polymorphic band; *I*= Shannon's information index; *h*= Nei's gene diversity).

Populations	PPB	*I*	*h*
T. coreanum			
SA	52.9	0.303	0.206
PC	47.1	0.232	0.153
mean	50.0	0.267	0.180
species level	93.1	0.399	0.254
T. ichangense			
JW	52.7	0.241	0.155
YY	60.0	0.259	0.166
DY1	72.7	0.337	0.220
DY2	54.6	0.257	0.167
mean	60.0	0.274	0.177
species level	98.2	0.383	0.240

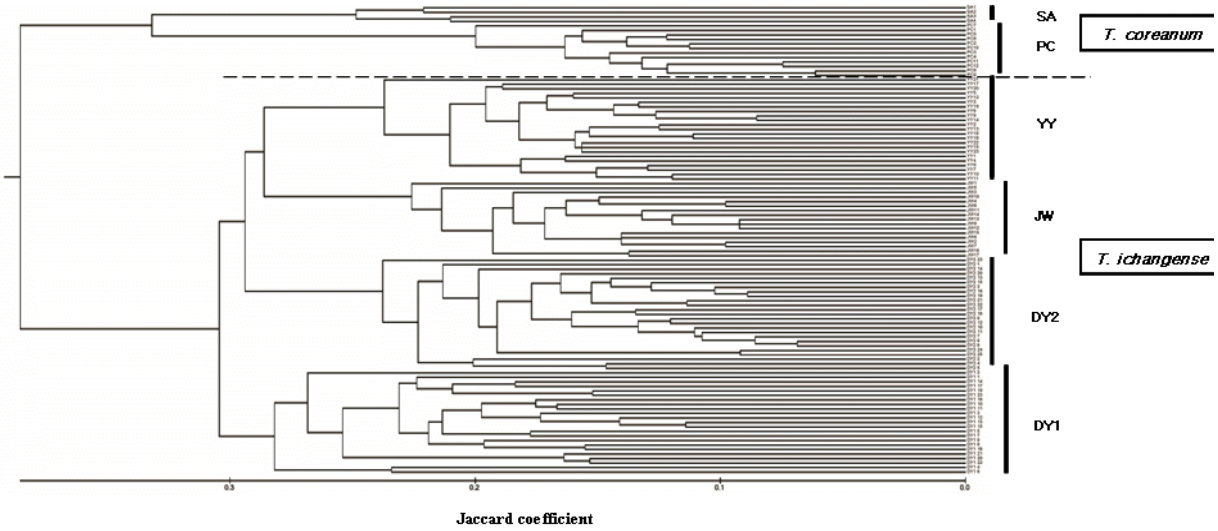

Figure 2. UPGMA dendrogram of 118 individuals from populations of *T . coreanum* and *T. ichangense* based on RAPD marker. The *x*-axis represents Jaccard coefficient.

For the cluster analysis, a UPGMA dendrogram was constructed using the Jaccard coefficient based on the frequency of RAPD band. *T. coreanum* was distinguished with *T. ichangense* at high level of Jaccard coefficient. This means that the two species are distinctly separated by genetic differences.

The SA and PC populations made a clade at a high level of the index, compared to populations of *T. ichangense*. On the other hand, each population of *T. ichangense* was separated at a similar index level, but DY2 made a clade with YY and JW, DY2 forming a clade with the others. DY1 and DY2 were found to be very similar populations, but demonstrated a comparatively high genetic variation than other populations in the species, showing a clade a higher level than other populations.

The plot of PCoA for each population of *T. coreanum* and *T. ichangense* can be seen in Fig. 3. Cumulatively, the first three vectors accounted for

73.0% of the total variance detected, comprised of 39.0%, 17.9%, and 16.1% from vectors, respectively. This was consistent with the results of the cluster analysis (Fig. 2).

An ordination plot (Fig. 3) of the first two vectors showed that the RAPD banding pattern was clearly separated into two clades, corresponding *to T. coreanum* and *T. ichangense*.

In order to determine the correlation between geographic distance and genetic distance, the Mantel test was performed using Nei's genetic distance and geographic distance between each of the populations (Table 5, Fig. 2). As the geographical distance departed, genetic distance showed an increasing tendency, but did not indicate a significant difference (r^2=0.204, p=0.803).

4. Discussion

4.1 Genetic diversity and differentiation

A genetic diversity comparison of endangered species and their congener widespread species has remarkable advantages when compared to comparisons between taxonomically distant species. This is because the recent common ancestors of closely related species share many life-history features, allowing us to easily identify the causes of variation in genetic diversity (Kruckeberg and Rabinowiz, 1985; Dodd and Helenurm 2002).

In this study, two species shared the majority (64.1%) of the band amplified by the RAPD analysis. In the case of *T. coreanum*, only 9 bands, out of 120, appeared to be unique. The result of AMOVA showed that only 36.58% of genetic variability could be attributed to the among species. When compared with other study results based on congeneric species, the genetic differences between *T. coreanum* and *T. ichangense* were still rather small. For example, Helena and Lovato (2010) compared the genetic diversity of endangered tree species, *Dimorphandra wilsonii*, and congeneric general species, *D. mollis*. Here, the genetic differentiation among these species was found to be 55.9%, a greater value than our result. Also, the genetic differentiation was 60.1% between *Sagittaria natans*, which is an endangered perennial marsh herb, and *S. trifolia*, a widespread congener. This value was also greater than ours (Chen et al., 2007). A high commonality of loci shows that the genetic differences of the two species is not large and that the morphological similarity of the between species seems to be related to the genetic commonality. In addition, it reflects a common evolutionary history or homoplasy (Ge et al., 2005).

In the case of taxonomically close species, the endangered species has a low genetic diversity compared to other species (Silva et al., 2007; Helena and Lovato, 2010; Maki and Horie, 1999). *T. coreanum* appeared to have a genetic diversity at a similar level with *T. ichangense* (Table 6). When compared to other populations, the genetic diversity of the SA population appeared to have high a value (I: 0.303, PPL: 52.9%, h: 0.206). Given that genetic diversity decreases as the sample number is reduced (Fahrig, 2003; Chang et al., 2004), the genetic diversity of the SA population appeared in an analysis of only four examples. This means that the amount of genetic variation in this population is significantly higher compared to other populations.

Table 4. Analysis of molecular variance for populations of *T. coreanum* and *T. ichangense*.

Source of variation	d.f.	Variance components	% of total variance	*Φst*	*P*-value
Among species	1	9.53985	36.58	0.6021	<0.001
Among populations/within species	4	6.16300	23.63		<0.001
Within populations	98	10.37568	39.79		<0.001
T. coreaum					
Among populations	1	10.04464	54.34	0.5434	<0.001
Within populations	14	8.44048	45.66		<0.001
T. ichangense					
Among populations	2	5.82268	35.24	0.3524	<0.001
Within populations	85	10.69821	64.76		<0.001

Table 5. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Population	SA	PC	JW	YY	DY1	DY2
SA	*****	0.8063	0.7295	0.7742	0.7785	0.7516
PC	0.2153	*****	0.6944	0.7290	0.7492	0.6944
JW	0.3154	0.3646	*****	0.8952	0.9158	0.9004
YY	0.2560	0.3161	0.1107	*****	0.9300	0.8961
DY1	0.2503	0.2887	0.0879	0.0726	*****	0.9169
DY2	0.2855	0.3648	0.1049	0.1097	0.0867	*****

Nymbom and Bartish (2000) reviewed studies on plant species carried out by an RAPD marker. They compared diverse genetic diversity measures based on life-history and sampling strategy. They found that when the life form is annual, short-lived perennial, or long-lived perennial, each average *Φ*st value is 0.70, 0.39, and 0.25. The *Φ*st value, according to the breeding systems of selfing species, mixed species, and outcrossing species, are 0.70, 0.27, and 0.28, respectively. This had a lower differentiation between groups, as they art more outcrossing species. The *Thalictrum* genus is a short-lived perennial, which has a diverse breeding system, according to the species (Pellmyr, 1995). The Φst value of *T. ichangense* was 0.3524, showing that there was little difference between genetically similar plants' mean values. However the *Φ*st value, based on the breeding system, was between selfing and mixed species. On the one hand, the *Φ*st value of *T. coreanum* was 0.5434, showing a much greater value than *T. ichangense*. We can assume that there are several possible reasons for such differences. It is possible that the differences between the two species' genetic structure stems from differences in the reproductive system. Reproductive biology is considered the most important factor in determining a plant population's genetic structure (Hamrick and Godt, 1989). *Thalictrum* species show differences in regards to breeding system, pollination mechanism, and ploidy level. Species can be hermaphroditic (all flowers have both male and female organs), dioecious (male and female individuals), or andromonoecious (male and hermaphroditic flowers on an individual), and are pollinated by wind, insects, or both (Steven and Waller, 2004; Kaplan and Mulcahy, 1971). It is assumed that the changes in reproductive system promoted differentiation in two species from a common ancestor, and became a cause of differences in genetic structure.

Genetic differentiation between populations is attributed to the heterozygous alleles, or genotypes, which are fixed in a population or common in populations (Chang et al., 2004). In this study, the *T. coreanum* population showed a higher *Φ*st value and lower gene flow than the *T. ichangense* population (Table 3). This means that the fractionalized populations have likely been isolated for considerably long periods of time, enough to experience genetic drift (Ellstrand and Elam, 1993).

The DY1 and DY2 among *T. ichangense* populations were in close geographic proximity, but were not closely tied in the cluster analysis. This difference between the two populations was also shown in genetic diversity (Table 3). This means that two populations have a different genetic structure. Two populations are physically disconnected by the road, which could be a factor interfering with gene flow. When considering that the DY2 population's genetic diversity is lower than the DY1 population, we should assume that, in the DY2 population, the loss of genetic resources decreased. This may be the reason why the YY and JW populations are more geographically distant than the DY1 population, which is genetically similar to the DY2 population. In the case of the DY1 population, the damage risk of the habitat is relatively small because it is adjacent to the protected areas containing Paleolithic relics. On the contrary, the DY2 population's habitat is unfavorable, adjacent to open-pit mines for limestone extraction, which have many rockfall slopes.

4.2 Confused distribution area and genetic evidence

As well as the Korean Peninsula, *T. ichangense* is found in northeastern China and China's southern Guangdong province (Jeon et al., 2007). On the other hand, literature pertaining to *T. coreanum* states that the species is endemic to Korea. However, other reports argue that the species has been observed in the northeastern part of China, as is the case with *T. ichangense*. The Korea Biodiversity Information System, established by South Korea's National Arboretum and the Forest Service, collected sample information and presented the distribution chart for domestic plant species from herbarium and botanical gardens across the country. According to this system, *T. coreanum* is an endemic species. However, according to "Endemic Vascular Plants in the Korean

Peninsula (Oh et al., 2005)", a report issued by the National Arboretum, *T. coreanum* cannot be classed as an endemic species because it is distributed in China. Lee (2011) and Chung (2005) proposed that the *T. coreanum* population would be distributed in Mt. Juwang and Mt. Ilwol, and claimed that these two species could be found in China. Kolbek et al. (2003) described that *T. ichangense* is common in China's northeast, but states that certain reports claim that *T. coreanum* is native only to North Korea. Even in the specimen data, which Jeon et al. (2007) studied, the distribution of *T. coreanum* in China was not confirmed, and verification of its distribution in China is still needed.

Through the identified results, using the specimens and collected samples, Jeon et al. (2007) and Park and Park (2008) noted frequent false identification from previous data. They established that Pyeongchang's northern population is a *T. coreanum* population, while the area's south population is a *T. ichangense* population. This is similar to Lee's (2003) belief that it is found on Mt Seorak, and also similar to Lee's (1996) belief that it is distributed throughout the north of Gangwon-do. Such facts support our results of genetic experiments using the RAPD marker. The UPGMA cluster analysis and the PCoA divided Pyeongchang's northern group and southern group into two different groups. This is consistent with the results of classification due to the morphological trait.

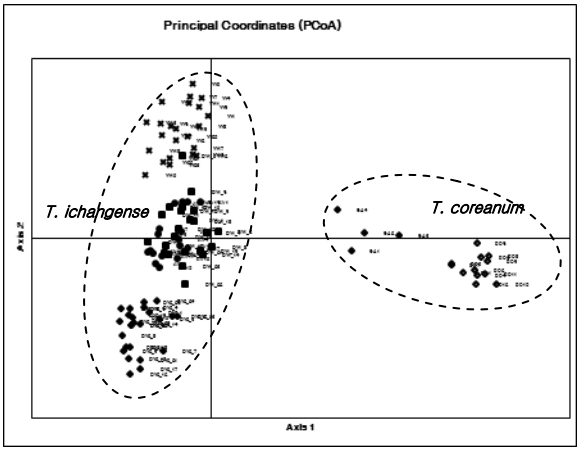

Figure 3. Principal coordinate analysis(PCoA) using RAPD data of 118 individuals of *T. coreanum T. ichangense*..

Therefore, the southern limit of the distribution of *T. coreanum*, the boreal plant (National Arboretum, 2011), is likely to be in Pyeongchang (N37°27'24" E128°27'45"). We believe that most of the central part of the peninsula and the southern region's *T. coreanum* population is actually *T. ichangense*.

4.3 Conservation Implication

Information on the amount and structure of genetic variation of the population in the area in which endangered species are present is an important tool in supporting the conservation and management of endangered species programs (Holsinger and Gottlieb, 1991; Kim et al., 2008). The results pertaining to the patterns of genetic variation of *T. coreanum* and *T. ichangense*, using the RAPD marker, can provide hints for the preservation of the species.

According to the AMOVA, PCoA, and cluster analysis, the genetic differences between the endangered species *T. coreanum* and congener *T. ichangense*, are not so great (77 common loci among 120 loci), but the two species have very distinct characteristics. Accurate identification serves as the basic for the designation and maintenance of protected species. If necessary, species-specific genetic markers need to be developed and utilized.

The most basic way to conserve endangered species is to preserve as many individuals as possible in the largest protected habitat. Protecting populations is the key to species conservation, because endangered species may consist of a small number of populations, or just one population (Primack, 2004; Lu et al., 2006). In the case of *T. coreanum*, only a small number of populations remain. This reinforces the necessity of providing protection. However, if conservation is necessary, populations with high levels of genetic diversity should be the priority (Lu et al., 2006; Kark et al., 1999; Kim et al., 2008). The SA population, despite having a small sample number, showed a relatively high level of genetic diversity. Typically, in the conservation of endangered species with a small number of individuals and a limited habitat, an appropriate level of genetic diversity is necessary for adapting to environmental change, and for the evolutionary maintenance of the species. Therefore, the SA population should be treated seriously in the conservation strategy of *T. coreanum*.

It is known that the smaller the size of the population, the higher the possibility of extinction of the local species (Fahrig, 2003). The rapid decline of individual numbers and the local extermination of the small population is due to the loss of genetic variability, the changes of the individual number, environmental changes, and natural disasters. Small *T. coreanum* populations have highly likely to lose genetic variation over generations, and, as a result, the local extermination of the population may occur. New genetic resources should be supplied through transplantation of the individuals between the populations. This is necessary to ensure that the size

of the small population is as large as possible. In addition, to promote the exchange of genes between populations, it is necessary to promote connectivity between habitats through new habitat construction.

As the recognition of the sovereign rights of biological resources grows (thanks to the establishment of the Convention on Biological Diversity in 1993), an awareness of the value of the country's biological resources is emerging. Moreover, the establishment of conservation strategies for endangered species to maintain as much biodiversity as possible is becoming more important. To secure and preserve the species' resources, systematizing species' information should be prioritized, according to the accurate criteria. Identifying the distribution and population size of a certain species, and the number of its objects, is the central concern of such efforts. In the case of *T. coreanum*, an endangered species, a basic overview of the species is not sufficient (Chang et al., 2005), and the existing data on the distribution and population size is difficult to trust due to the uncertainty of identification. Therefore, the most important goal in ensuring the conservation of this species is the precise identification of *T. coreanum* populations, population area, population size, and the ecological environment conditions of the species. The systematization of this information is also a highly important consideration.

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