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Genetic Diversity of Common Reed in Korea Based on Morphological Characteristics and Random Amplified Polymorphic DNA Markers

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Abstract - To elucidate genetic diversity of common reed in Korea, we collected a total of 674 common reed plants from 27 regions in South Korea. Hierarchical clustering using 7 morphological traits divided the 27 common reed populations into 7 groups. Random amplified polymorphic DNA (RAPD) results identified three distinct groups of common reed. Common reed accessions in group I mostly inhabit coastal areas. Group II includes reeds mostly collected from inland areas. Group III consists of common reed accessions collected from inland and coastal areas, suggesting that this group might contain hybrids. In summary, we suggest that parapatric speciation might be an important factor in the genetic diversity of common reed and geographical speciation of common reed that might be also affected by environmental gradients.

Key words - Marker, Morphology, RAPD, Reed, Variation

Introduction

Common reed (*Phragmites australis*) is a tall, perennial grass growing to 16 feet or more in height. This plant can be found almost everywhere, ranging from the tropics to cold-temperate regions around the world, and it grows mainly in wetlands, such as coastal regions and lakeshores (Brix, 1999; Engloner, 2009; Kim *et al*., 2009). Common reed can propagate both by seeds and by vegetative spread through pieces of rhizome (League *et al*., 2006). Because of its vigorous growth characteristic, common reed is regarded as an invasive plant that can destruct ecosystems by expelling native plants after it is introduced into new marsh communities (Chun and Choi, 2009). As a result, the marsh hydrology and wildlife habitat can be changed with a high possibility for fires (Meyerson *et al*., 2000).

In spite of the ecological problem, this common reed has significant ecological merits (Kozłowska *et al*., 2009; Peruzzi *et al*., 2009). For example, it diminishes contamination of waste water produced from industry, agriculture and private residences and is also essential for wildlife and conservation, especially in Asia and Europe (Cui *et al*., 2009; Kozłowska *et al*., 2009). Furthermore, the reed has been traditionally used as a material for making mats, boater hats, furniture, baskets and handicrafts (Dogan *et al*., 2008). Genetic diversity of various common reed in the world has been studied using several molecular markers including AFLP, RAPD (Lambertini *et al*., 2006; Lambertini *et al*., 2008; Oh *et al*., 2006) and RFLP (Saltonstall, 2003).

In order to investigate the genetic diversity and certain factors affect the morphological traits of common reed, we collected a total of 674 common reed plants from 27 regions in Korea and determined their morphological and genetic diversity by RAPD markers.

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Materials and Methods

Collection of common reed

We collected a total of 674 common reed plants from 27 regions of South Korea (Fig. 1). Detailed information on the sampling regions is available in Table 1. The samples were

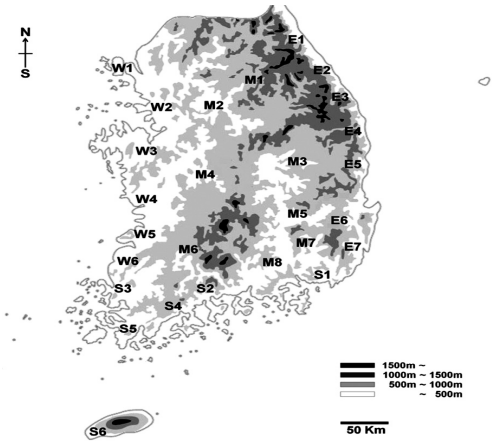

Fig. 1. A map of South Korea showing the 27 sampling regions. The individual code indicates the region of sampling. Each code was based on a direction and latitude, like seven regions in the eastern part of the country (named E1 to E7), eight inland regions (from M1 to M8), six southern regions (from S1 to S6) and six western regions (from W1 to W6). Detailed information for the sampling regions is listed in Table 1.

collected in November 2008. A majority of the sampling regions are located along the coast.

Measurement of morphological characteristics

In November 2008, at the end of the growing season, the shoot length, number of nodes, bottom outer diameter, bottom inner diameter, top outer diameter, top inner diameter and dry weight for each collected reed were determined. Detailed information for morphological characteristics was drawn in Fig. 2.

Genomic DNA isolation

For Random Amplified Polymorphism DNA (RAPD) analysis, we selected one representative sample from each common reed population resulting in 28 accessions. Harvested leaf materials were immediately frozen in liquid nitrogen and were kept at -80℃. Total genomic DNA was prepared

Table 1. Detailed information for sampling regions of common reed in Korea.

Code	Region	Latitude	Longitude
E1	Yangyang-gun, Gangwon-Do	38°04′	128°40′
E2	Gangneung Si, Gangwon-Do	37°45′	128°53′
E3	Samcheok Si, Gangwon-Do	37°30′	129°07′
E4	Uljin-gun, Gyeongsangbuk-Do	36°59′	129°25′
E5	Yeongdeok-gun, Gyeongsangbuk-Do	36°32′	129°25′
E6	Gyeongju Si, Gyeongsangbuk-Do	35°59′	129°25′
E7	Ulsan	35°33′	129°19′
M1	Wonju Si, Gangwon-Do	37°20′	127°57′
M2	Icheon Si, Gyeonggi-Do	37°16′	127°29′
M3	Andong Si, Gyeongsangbuk-Do	36°34′	128°42′
M4	Daejeon	36°22′	127°22′
M5	Daegu	35°54′	128°40′
M6	Namwon Si, Jeollabuk-Do	35°24′	127°20′
M7	Miryang Si, Gyeongsangnam-Do	35°29′	128°45′
M8	Haman-gun, Gyeongsangnam-Do	35°10′	128°34′
S1	Busan	35°06′	129°02′
S2	Suncheon Si, Jeollanam-Do	35°04′	127°14′
S3	Muan-gun, Jeollanam-Do	34°49′	126°23′
S4	Boseong-gun, Jeollanam-Do	34°41′	126°55′
S5	Haenam-gun, Jeollanam-Do	34°33′	126°34′
S6	Jeju Si, Jeju-Do	33°31′	126°30′
W1	Incheon	37°42′	126°27′
W2	Ansan Si, Gyeonggi-Do	37°16′	126°59′
W3	Hongseong-gun, Chungcheongnam-Do	36°20′	126°33′
W4	Gunsan Si, Jeollabuk-Do	36°00′	126°45′
W5	Yeonggwang-gun, Jeollanam-Do	35°08′	126°49′
W6	Buan-gun, Jeollabuk-Do	35°43′	126°42′

The code, regional name latitude and longitude for each common reed population are described.

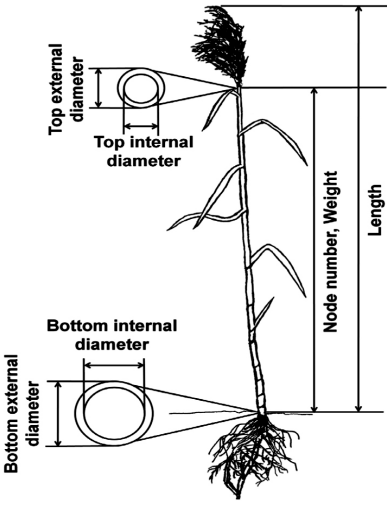

Fig. 2. Illustration of seven morphological traits of *P. australis*. All collected *P. australis* were analyzed based on seven morphological traits: shoot length, number of nodes, bottom outer diameter, bottom inner diameter, top outer diameter, top inner diameter and dry weight.

using a DNeasy® Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions.

RAPD analysis

For RAPD experiments, a set of random primers was obtained from Operon's RAPD 10 mer Kits (Operon Technologies, Inc., Alameda, CA.). A total of 20 random primers (OPC1-OPC20) were tested. Among them, OPC1, 2, 5, 18, 19 and OPC20 were selected for RAPD analysis. The PCR mixture used contained genomic DNA, diluted to a final concentration of 2 ng/μl, 0.1 μl (10 mM dNTP mix), 2 μl of 10-mer random primer (10 pmole/μl), 0.5 μl of Taq DNA polymerase (2.5 Units/μl), 2 μl of 10X Taq buffer (SolGent Co., Ltd., Daejeon, South Korea) and distilled water to a final volume of 20 μl. PCR for RAPD analysis was performed in a MJ Research-Peltier Thermal Cycler PTC-200 programmed for an initial step of 4 min at 94℃, then 40 repeats of 30 s at 94℃, 60 s at 35℃ and 2 min at 72℃ followed by a final termination step of 5 min at 72℃. RAPD experiments were repeated three times and amplified major DNA fragments were selected for statistical analysis using Excel (Microsoft Excel 2007, Redmond, USA).

Statistical analyses

Statistical Package for the Social Sciences (SPSS) was used for the statistical analysis (One-way analysis of variation). For the use of RAPD results in the MEGA4 program, the data was converted as follows: the presence of a fragment was noted by a T, whereas its absence was noted by an A. A phylogenetic tree was created using the MEGA4 program with the UPGMA method.

Hierarchical clustering

To divide common reed populations based on morphological traits, each obtained value was normalized by 7 morphological traits and 27 populations. Normalized values were subjected to hierarchical clustering using the Genesis program (Sturn *et al*., 2002).

Results

Morphological characteristics of Korean *Phragmites australis*

The S3 population had the greatest length and number of nodes, the M8 population had the largest bottom inner and outer diameters, the E5 population had the largest top inner diameter and the S5 population had the largest top outer diameter and the highest weight, according to the analysis of morphological traits (Table 2). Interestingly, it was very difficult to find correlations among the morphological traits. For instance, the S3 population was the tallest and had the highest number of nodes, but its other traits did not have as high values as those of other populations.

For each morphological trait, the 27 populations could be divided into 11 to 13 different subgroups (Table 1). For example, the E2, S3 and S5 populations belong to only one subgroup based on length, node number and weight. The E7 and M7 populations belong to at least two subgroups based on all traits. Based on node number, top outer diameter and top inner diameter, a majority of populations belonged to several subgroups with a maximum of seven groups (Table 1). As a result, morphological traits of common reed could not distinguish all 27 populations clearly. Therefore, we performed hierarchical clustering as an alternative. Hierarchical clustering based on morphological traits identified seven subgroups of common reed (Fig. 3). For example, group A, which includes the S2, E1, S3, S4 and S6 populations, has relatively higher values for five of the morphological traits

(all except the top outer and inner diameters) than other populations. The group C showed a totally opposite pattern compared to those in group A. Moreover, group G, containing five populations, had the highest values only for the number of nodes (Fig. 3).

Previously, it has been shown that the amount of solar radiation is an important environmental factor that promotes growth of *P. australis* (Charles-Edwards, 1982). Normally, the amount of solar radiation is dependent on latitude. To test for a correlation between the amount of solar radiation and the length of plants, information for the amount of solar radiation (from December 2007 to November 2008) at each location was obtained from the Korea Meteorological Administration (http://www.kma.go.kr). Unexpectedly, we could not find a correlation between the length of common reed plants and the amount of solar radiation (data not shown).

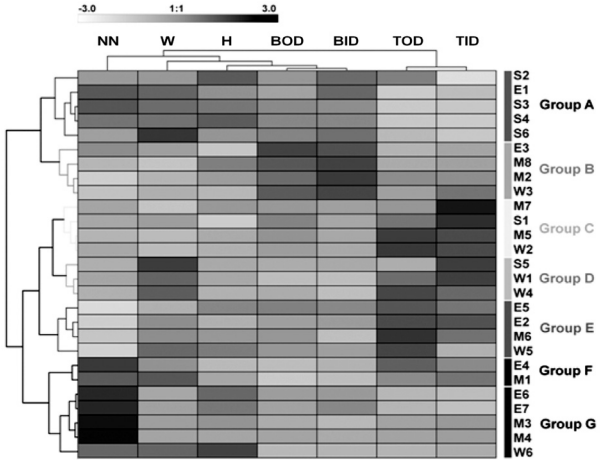

Fig. 3. Hierarchical clustering to group 27 common reed populations based on the 7 morphological traits. For the hierarchical clustering, average values for the 7 morphological traits in the 27 populations were used. First, values were normalized twice by 7 morphological traits and 27 populations. Using the complete linkage method with the default parameters implemented in the Genesis program, 7 morphological traits and 27 common reed populations were hierarchically clustered. Black and white indicate relatively high and low values, respectively. The seven clustered groups are indicated by bars (Group A to G). Abbreviations used for the seven morphological traits are: 'W' Weight, 'NN' Node number, 'BOD' Bottom outer diameter, 'BID' Bottom inner diameter, 'L' Length, 'TOD' Top outer diameter and 'TID' Top inner diameter.

RAPD analysis

Molecular markers have been powerful tools to analyze genetic divergence of various plant species. Among them, RAPD is the simplest and least expensive technique for detecting polymorphisms using arbitrary primers (Kim *et al*., 2009; Lambertini *et al*., 2008). To investigate the genetic diversity of common reed, we performed RAPD analysis. We selected 6 primers amplifying a total of 129 polymorphic bands from 28 accessions (Fig. 4). Using RAPD results, we

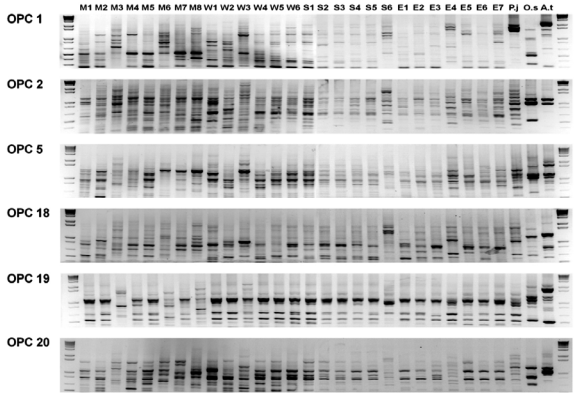

Fig. 4. Electrophoresis image of RAPD results. Amplified PCR products were separated on 1.5% agarose gels in 0.5X TBE buffer with two lanes of 1 Kb DNA ladder (SolGent Co., Ltd., Daejeon, South Korea) and three lanes for out-groups (*Phragmites japonica*, *Arabidopsis thaliana* and *Oryza sativa*). A total of 129 RAPD polymorphisms were detected from the six primers (OPC1, 2, 5, 18, 19 and OPC20).

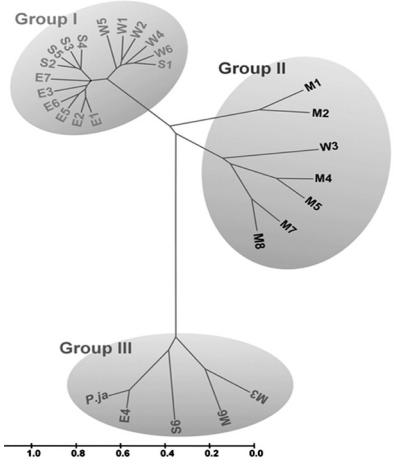

Fig. 5. A phylogenetic tree based on RAPD results identified three different groups of common reed. The 27 common reed accessions were divided into 3 distinct groups (Groups I, II and III). The scale bar indicates genetic distances.

analyzed pairwise distances and created phylogenetic tree for 28 accessions of common reeds (Table 3). In the phylogenetic tree based on the identified polymorphisms, common reed are divided into three distinct groups (Groups I, II and III) (Fig. 5). Group I contains mostly collected from coastal regions or near river mouths, whereas group III includes the S6, M6, M3 and E4 accessions as well as *P. japonica* as a control. Group II covers *P. australis* derived from inlands or middle rivers. This data supports the idea that genetic characteristics of common reeds are closely related to environmental factors that are often caused by geographical locations.

The results of the RAPD analysis showed that the common reeds seem to be diverged according to their geographical locations, such as coastal areas (Group I) and riversides (Group II). Group III identified by the RAPD analysis contains accessions of common reed growing in inland areas, such as M3 and M6, the S6 (Jeju island) accession, the coastal E4 accession and *P. japonica*.

Discussion

Characteristics of the representative common reed

RAPD results identified three distinct groups of common reed that might be good examples to explain the speciation of Korean *P. australis*. The representative features of common reed might be those of the common reed accessions in group I based on their natural habitats of mostly coastal areas. Moreover, *P. australis* in group I might be more tolerant of salinity than common reed. This salt-tolerance is caused by the unusual geographical feature of South Korea that it is surrounded by seas.

Geographical speciation of common reed

Group II from the RAPD analysis includes common reed mostly collected from inland areas, such as riversides. They might have originated from *P. australis* adapted to coastal regions that gradually expanded inland, inhabiting areas near rivers. Moreover, accessions in group II could not be detected in eastern areas, in which the area inhabited by common reed is physically restricted by geographical barriers, such as the Taebaek Ridge Mountains. This mountain range runs along the East Sea from Hwangnyong Mountain in North Korea to Busan in South Korea. In contrast, the mountain ranges of the western and southern regions of South Korea are gentler than those of the east. Thus, the biggest two rivers of South Korea, the Han and the Nakdong, can stretch to the southwest. We propose that common reed inhabiting coastal areas could expand inland along with rivers. Similarly, the previous study has shown ecological role of mountain ridges to conserve biodiversity within restricted area in Korea (Cho *et al*., 2008). As a result, populations of group II in different environmental conditions, such as fresh water, can be explained by parapatric speciation. Parapatric speciation refers to organisms that are separated by environmental conditions (Antonovics, 1971; Caisse and Antonovics, 1978; Doebeli and Dieckmann, 2003).

Group III in the RAPD analysis includes common reed populations collected from inland and coastal areas. Interestingly, some *P. australis* populations belong to group III with *P. japonica* based on RAPD analysis. These data indicate that these *P. australis*, along with *P. japonica*, might have similar genetic characteristics with which they might adapt well to certain environmental conditions.

Morphological diversity of Korean *P. australis*

Although we divided the 27 *P. australis* populations into 7 groups based on morphological traits, it was hard to explain their diversity by molecular markers. Numerous studies have shown that several factors can influence the morphology of common reed species. For instance, radiation, geographical location, salinity, biotic attacks and ploidy level can affect growth of *P. australis* (Brix, 1999; Hansen *et al*., 2007).

Previous studies suggested that ploidy level might play an important role in determining the morphologies of *P. australis*. *P. australis* is known to be an allopolyploid with a wide range of euploidy levels (Raicu *et al*., 1972). Among them, tetraploids (2n = 48) and octoploids (2n = 96) are commonly found (Clevering and Lissner, 1999). In general, octoploids had larger leaves, shoots and cells than hexaploids and tetraploids (Paucã-Comãnescu *et al*., 1999).

Pollen fertility or sterility was also caused by ploidy levels. For example, hexaploids tend to be sterile, unlike plants with other ploidy levels, as shown in previous studies (Björk, 1967; Gorenflot *et al*., 1990). Despite the lack of information related to the ploidy level of Korean *P. australis*, we found

that common reed in the M4 population did not have seeds, suggesting that this population might be hexaploid. Thus, we suppose that Korean *P. australis* is composed of various polyploids.

Although we attempted to use genetic markers to explain the diverse morphological traits of common reed, but there was not enough data from genetic markers to explain the morphological diversity, suggesting that additional experimental approaches, such as investigation of the ploidy level, should be performed. Similarly, morphological characteristics and RAPD markers have been used to reveal genetic diversity of Korean *Calanthe* (Cho *et al*., 2010). Thus, combination of phenotypes and molecular markers could be a useful tool for plant genetic diversity.

In this study, we studied genetic diversity of common reed based on morphological traits and RAPD markers. The results suggest that the various morphological traits of *P. australis* populations in Korea are caused by parapatric speciation. Furthermore, our results explain geographical speciation of common reed that might be also affected by environmental gradients.

Acknowledgement

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Table 2. One-way ANOVA analysis for seven morphological traits of collected *Phragmites*.

Region	Code	Length (cm)	Node number	Bottomouter diameter (mm)	Bottominner diameter (mm)	Topouter diameter (mm)	Topinner diameter (mm)	Weight (g)
Yangyang	E1	$220.16 \pm 13.50^{a,b}$	$17.92 \pm 1.66^{b,c,d}$	$5.50 \pm 0.68^{a,b,c,d}$	$3.32 \pm 0.44^{a,b,c,d}$	1.74 ± 0.36^{a}	0.88 ± 0.23^{a}	$10.22 \pm 2.74^{a,b}$
Samcheok	E3	$213.76 \pm 28.73^{a,b}$	$17.96 \pm 3.03^{b,c,d}$	$6.92 \pm 1.10^{e,f}$	$3.83 \pm 0.81^{c,d,e}$	$2.10 \pm 0.56^{a,b,c}$	$1.20 \pm 0.44^{a,b}$	$9.24 \pm 2.87^{a,b}$
Uljin	E4	$205.04 \pm 15.80^{a,b}$	$20.32 \pm 1.18^{d,e,f,g,h}$	$5.08 \pm 0.51^{a,b,c}$	$2.76 \pm 1.20^{a,b}$	$2.56 \pm 0.63^{a,b,c,d,e}$	$1.30 \pm 0.33^{a,b,c}$	$9.80 \pm 2.33^{a,b}$
Yeongdeok	E5	$358.52 \pm 18.51^{j,k}$	$17.28 \pm 1.77^{b,c}$	10.91 ± 1.07^{k}	$6.66 \pm 0.84^{j,k}$	4.81 ± 0.64^{j}	3.14 ± 0.54^{j}	40.94 ± 8.22^{i}
Gyeongju	E6	$306.72 \pm 27.95^{g,h}$	$25.84 \pm 2.01^{k,l}$	$7.49 \pm 1.08^{f,g,h}$	$4.41 \pm 1.02^{e,f,g}$	$2.40 \pm 0.90^{a,b,c,d}$	$1.32 \pm 0.60^{a,b,c}$	$22.70 \pm 7.69^{d,e,f}$
Ulsan	E7	$287.60 \pm 26.69^{e,f,g}$	$25.36 \pm 3.29^{j,k,l}$	$6.96 \pm 1.05^{e,f}$	$4.53 \pm 0.79^{e,f,g,h}$	$2.29 \pm 0.81^{a,b,c,d}$	$1.13 \pm 0.49^{a,b}$	$21.33 \pm 5.65^{c,d,e,f}$
Wonju	M1	$206.16 \pm 16.37^{a,b}$	$17.52 \pm 1.39^{b,c,d}$	4.65 ± 0.91^{a}	2.39 ± 0.47^{a}	$2.03 \pm 0.41^{a,b}$	$1.14 \pm 0.31^{a,b}$	$9.64 \pm 2.41^{a,b}$
Icheon	M2	$271.12 \pm 19.39^{d,e,f}$	$16.56 \pm 2.08^{b,c}$	$8.82 \pm 1.03^{h,i}$	$6.08 \pm 1.05^{i,j}$	$3.02 \pm 0.70^{d,e,f}$	$1.92 \pm 0.63^{c,d,e,f,g,h,i}$	$16.09 \pm 3.90^{a,b,c,d}$
Andong	M3	257.28 ± 25.95^{d}	$24.16 \pm 2.62^{i,j,k}$	$6.36 \pm 1.27^{c,d,e,f}$	$3.22 \pm 0.63^{a,b,c,d}$	$2.44 \pm 0.73^{a,b,c,d,e}$	$1.54 \pm 0.68^{a,b,c,d,e,f}$	$17.81 \pm 5.42^{a,b,c,d,e}$
Daejeon	M4	$246.72 \pm 19.21^{c,d}$	27.08 ± 1.61^{l}	$6.76 \pm 0.89^{d,e,f}$	$3.59 \pm 0.53^{b,c,d,e}$	$2.52 \pm 0.56^{a,b,c,d,e}$	$1.57 \pm 0.56^{a,b,c,d,e,f}$	$17.65 \pm 5.10^{a,b,c,d,e}$
Daegu	M5	$289.88 \pm 28.29^{f,g,h}$	$20.96 \pm 2.26^{e,f,g,h}$	$9.34 \pm 1.46^{i,j}$	$5.00 \pm 1.04^{f,g,h,i}$	$4.57 \pm 0.88^{i,j}$	3.20 ± 0.89^{j}	$26.61 \pm 5.83^{d,e,f,g}$
Namwon	M6	$352.72 \pm 29.29^{j,k}$	$24.20 \pm 4.05^{i,j,k}$	$10.51 \pm 2.10^{j,k}$	$5.80 \pm 1.44^{i,j}$	$4.60 \pm 1.31^{i,j}$	3.04 ± 1.16^{j}	66.66 ± 18.35^{j}
Miryang	M7	$262.96 \pm 18.35^{d,e}$	$19.32 \pm 4.67^{c,d,e,f}$	$7.24 \pm 0.74^{e,f,g}$	$4.03 \pm 0.69^{d,e,f}$	$2.72 \pm 0.47^{b,c,d,e,f}$	$2.30 \pm 0.50^{g,h,i}$	$11.38 \pm 2.54^{a,b,c}$
Haman	M8	374.28 ± 45.05^{k}	$23.08 \pm 3.29^{h,i,j,k}$	12.53 ± 1.80^{l}	8.51 ± 1.14^{l}	$3.56 \pm 0.97^{f,g,h}$	$2.61 \pm 0.75^{i,j}$	$33.64 \pm 8.53^{g,h,i}$
Busan	S1	$224.84 \pm 29.63^{b,c}$	$19.04 \pm 4.62^{c,d,e}$	$7.54 \pm 1.38^{f,g,h}$	$3.85 \pm 0.83^{c,d,e}$	$2.91 \pm 0.84^{c,d,e,f}$	$2.21 \pm 0.79^{f,g,h,i}$	$21.54 \pm 7.08^{c,d,e,f}$
Suncheon	S2	$314.48 \pm 16.23^{h,i}$	$22.12 \pm 1.45^{f,g,h,i}$	$8.42 \pm 1.05^{g,h,i}$	$5.38 \pm 0.89^{g,h,i}$	$3.28 \pm 0.83^{e,f,g}$	$1.58 \pm 0.62^{a,b,c,d,e,f,g}$	$38.26 \pm 6.83^{h,i}$
Muan	S3	406.60 ± 27.26^{l}	33.76 ± 2.24^{m}	$11.41 \pm 1.49^{k,l}$	7.07 ± 1.36^{k}	$2.39 \pm 0.46^{a,b,c,d}$	$1.46 \pm 0.41^{a,b,c,d,e}$	95.72 ± 17.56^{k}
Boseong	S4	$354.76 \pm 18.36^{j,k}$	$25.24 \pm 2.17^{j,k,l}$	$9.32 \pm 1.52^{i,j}$	$5.67 \pm 1.33^{i,j}$	$2.42 \pm 0.87^{a,b,c,d,e}$	$1.35 \pm 0.58^{a,b,c}$	57.35 ± 15.11^{j}
Haenam	S5	375.60 ± 25.71^{k}	27.72 ± 2.05^{l}	$11.24 \pm 1.33^{k,l}$	7.12 ± 1.24^{k}	$4.28 \pm 0.73^{h,i,j}$	4.11 ± 0.84^{k}	109.61 ± 20.45^{l}
Jeju	S6	$307.79 \pm 32.93^{g,h,i}$	$22.68 \pm 3.99^{g,h,i,j}$	9.15 ± 1.37^{i}	$5.78 \pm 1.30^{i,j}$	$2.98 \pm 1.24^{d,e,f}$	$1.77 \pm 0.88^{b,c,d,e,f,g,h}$	62.64 ± 16.89^{j}
Incheon	W1	$204.68 \pm 19.27^{a,b}$	15.80 ± 1.50^{a}	$4.73 \pm 0.55^{a,b}$	2.29 ± 0.50^{a}	$2.22 \pm 0.61^{a,b,c,d}$	$1.43 \pm 0.53^{a,b,c,d}$	$10.63 \pm 2.41^{a,b}$
Ansan	W2	259.44 ± 22.88^{d}	$20.12 \pm 2.22^{d,e,f,g}$	$7.64 \pm 1.40^{f,g,h}$	$4.22 \pm 1.07^{d,e,f}$	$3.54 \pm 0.82^{f,g,h}$	$2.31 \pm 0.74^{h,i}$	$19.00 \pm 5.34^{b,c,d,e}$
Hongseong	W3	$265.56 \pm 33.34^{d,e,f}$	$19.60 \pm 3.25^{c,d,e,f}$	9.01 ± 1.28^{i}	$5.65 \pm 1.17^{i,j}$	$2.96 \pm 0.64^{c,d,e,f}$	$2.16 \pm 0.61^{e,f,g,h,i}$	$27.67 \pm 10.70^{e,f,g}$
Gunsan	W4	$287.71 \pm 21.79^{e,f,g}$	$21.83 \pm 2.22^{e,f,g,h,i}$	$8.30 \pm 1.40^{g,h,i}$	$4.54 \pm 1.29^{e,f,g,h}$	$3.93 \pm 0.88^{g,h,i}$	$2.55 \pm 0.92^{i,j}$	57.82 ± 12.05^{j}
Yeonggwang	W5	$333.16 \pm 16.96^{i,j}$	$20.88 \pm 2.74^{e,f,g,h}$	$9.33 \pm 1.25^{i,j}$	$5.52 \pm 1.03^{h,i}$	$4.07 \pm 1.27^{g,h,i,j}$	$2.15 \pm 1.07^{d,e,f,g,h,i}$	59.65 ± 12.75^{j}
Buan	W6	$290.76 \pm 17.43^{f,g,h}$	$20.84 \pm 2.29^{e,f,g,h}$	$5.98 \pm 1.44^{b,c,d,e}$	$3.30 \pm 0.89^{a,b,c,d}$	$2.23 \pm 1.30^{a,b,c,d}$	$1.32 \pm 0.85^{a,b,c}$	$30.05 \pm 10.60^{f,g,h}$

Average values and standard deviations for seven morphological traits in each population containing 24~25 individuals were listed. Values for a total of 674 individuals were subjected to ANOVA analysis. Tukey's Honestly Significant Differences (HSD) test was used to identify differences between populations. Within each morphological trait, the 27 populations can be divided into several subgroups. Different letters (a–m) within columns indicate significant differences ($P<0.05$) between populations.

Table 3. Pairwise distances for 28 accessions of *P. australis*.

	M1	M2	M3	M4	M5	M6	M7	M8	W1	W2	W3	W4	W5	W6	S1	S2	S3	S4	S5	S6	E1	E2	E3	E4	E5	E6	E7	*P.j*
M1																												
M2	0.406																											
M3	0.907	1.284																										
M4	0.975	0.848	1.056																									
M5	0.750	0.406	1.284	0.301																								
M6	1.468	1.468	0.426	0.975	1.155																							
M7	0.797	0.709	0.848	0.636	0.518	0.709																						
M8	1.468	1.468	5.825	0.671	0.848	0.603	0.317																					
W1	1.468	0.545	0.797	0.671	0.406	4.840	0.573	4.840																				
W2	0.848	0.750	0.797	0.750	0.603	1.155	0.573	1.155	0.243																			
W3	1.056	0.797	6.106	0.709	0.797	1.056	0.750	0.709	0.636	0.709																		
W4	1.056	0.636	0.975	0.709	0.470	0.907	0.494	0.907	0.286	0.257	0.671																	
W5	1.056	0.709	1.155	5.825	0.797	6.276	0.975	6.276	0.350	0.387	0.671	0.271																
W6	1.155	0.750	0.797	0.975	0.494	6.704	0.797	0.848	0.271	0.368	1.056	0.257	0.204															
S1	0.636	0.636	1.155	0.636	0.709	6.276	0.603	1.056	0.317	0.257	0.975	0.243	0.243	0.180														
S2	0.797	0.797	0.750	0.573	0.573	1.796	0.671	6.496	0.350	0.350	0.603	0.545	0.368	0.317	0.368													
S3	0.848	0.603	0.797	0.750	0.545	4.840	0.518	1.468	0.333	0.301	0.797	0.426	0.426	0.333	0.286	0.204												
S4	0.671	0.671	0.636	0.848	0.603	0.975	0.518	1.468	0.448	0.368	0.709	0.426	0.350	0.301	0.286	0.180	0.192											
S5	1.155	0.545	0.709	0.603	0.448	4.840	0.518	1.155	0.333	0.406	0.636	0.518	0.350	0.333	0.426	0.157	0.192	0.217										
S6	0.975	0.750	0.709	1.468	1.468	0.603	0.907	0.750	1.468	1.468	0.907	0.797	0.797	0.750	0.907	0.907	0.848	0.545	0.848									
E1	1.155	0.750	0.573	0.848	0.750	0.975	0.573	0.848	0.406	0.494	0.797	0.518	0.470	0.333	0.470	0.317	0.368	0.301	0.301	0.671								
E2	0.797	0.709	0.545	0.907	0.709	0.709	0.448	0.518	0.518	0.709	0.848	0.603	0.603	0.470	0.603	0.406	0.350	0.286	0.387	0.573	0.157							
E3	0.848	0.603	0.907	0.750	0.603	1.468	0.518	0.848	0.448	0.603	0.709	0.573	0.387	0.406	0.518	0.350	0.406	0.406	0.301	0.671	0.271	0.317						
E4	6.496	1.056	0.848	6.276	5.825	0.797	0.975	0.797	1.284	0.797	0.975	0.750	0.603	0.797	0.750	0.671	0.709	0.573	0.636	0.709	0.797	0.603	0.709					
E5	1.056	0.797	0.975	0.636	0.573	0.907	0.406	0.709	0.573	0.797	1.155	0.545	0.494	0.470	0.406	0.301	0.350	0.257	0.257	0.573	0.257	0.243	0.257	0.603				
E6	0.709	0.573	0.975	0.797	0.518	0.797	0.448	0.573	0.387	0.573	0.750	0.368	0.545	0.350	0.333	0.333	0.257	0.230	0.350	0.518	0.230	0.217	0.257	0.448	0.168			
E7	0.907	0.907	1.155	0.907	0.907	0.907	0.750	1.056	0.470	0.636	0.750	0.671	0.545	0.426	0.406	0.406	0.317	0.350	0.350	0.636	0.317	0.301	0.426	0.494	0.333	0.243		
P.j	1.468	1.155	0.907	1.155	4.840	0.750	1.796	0.848	6.106	0.975	0.907	5.825	1.056	1.468	1.056	0.636	0.750	0.671	0.848	0.603	0.975	0.709	0.750	0.180	0.797	0.573	0.709	

Analysis of maximum composite likelihood/ pattern of lineages: same/ rates among sites: uniform rates/ overall average is 0.912.