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Analysis of Genetic Diversity of Korean Accessions of the Genus *Acorus* Using RAPD Markers and NIR Spectroscopy

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Abstract. The genus *Acorus* is known as an indigenous medicinal plant. Genetic diversity of thirteen accessions of *A. calamus* and eight of *A. gramineus*, with an accession of *Colocasia antiquorum* and two of *Iris pseudacorus* as outgroups, were evaluated using RAPD markers for cluster analysis and principal coordinate analysis, and NIR spectroscopic profiles for principal component analysis.A total of 371 polymorphic bands were obtained by using the selected 12 random primers. The genetic distances were estimated from 0.03 to 0.31 within *A. calamus* and from 0.03 to 0.51 within *A. gramineus*. The dendrogram and three-dimensional plot separated the accessions into four distinct groups (*A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus*). Moreover, for the diversity among genus *Acorus*, eleven *A. calamus* accessions, one *A. gramineus* accession, and two *I. pseudacorus* accessions were non-destructively analyzed from their leaves by NIR spectroscopy, which discriminated *Acorus* accessions like the RAPD analysis. Interestingly, thirteen accessions of *A. calamus* were clustered into two groups based on RAPD and NIR analyses, which indicates that there are two ecotypes of *A. calamus* in Korea. An accession (CZ) of *A. calamus* with yellow stripe on leaves was closely grouped with another (CX) at a genetic distance (GD) of 0.03, which shows that the stripe trait might be generated by chimeric mutation. The genetic distance between *A. calamus* and *A. gramineus* was revealed to be farthest from 0.80 to 0.88 GD. In genus *Acorus* the genetic diversity and genetic variation were identified by using RAPD marker technique and non-destructive NIRs.

***Additional key words*:** *Acoraceae*, cluster analysis, near infrared spectroscopy (NIRs), principal component analysis, principal coordinate analysis

Introduction

Genus *Acorus* is a monocot and a perennial aquatic herb. This genus initially belonged to the Araceae family, but recently separated from this family and is placed in its own family Acoraceae (APG II, 2003; Grayum, 1987; Thompson, 1995). There are two *Acorus* species, *A. calamus* and *A. gramineus* in Korea. *A. calamus* grows gregariously in marsh or shallow water. Its rhizome creeps beneath the land surface and its leaf grows erect. *A. gramineus* has thin rhizome and its narrow and short leaf is curved compared with *A. calamus*. *A. gramineus* grows in the crannies of the rock and is an evergreen plant but *A. calamus* is a deciduous plant.

The genus *Acorus* is one of the best oldest plants including two species. They are different in the morphological and ecological characteristics for adaptation to environments during the evolution. RAPD analysis using the genomic DNA showed a clear separation between the *A. calamus* and *A. gramineus*, but the analysis of chloroplast showed very close apomitic habit of genus *Acorus* (APG II, 2003; Duvall et al., 2008).

There have been some recent studies on the anti-cancer effect (Oh et al., 2007), antispasmodic effect (Gilani et al., 2006), anti-Alzheimer effect (Han, 2003), antioxidative activity (Ka et al., 2005), antimicrobial activity (Ghosh, 2006, Phongpaichit, 2005), insecticidal effect (Park et al., 2003), volatile flavor components (Choi, 2004), and hair protective effect (Hwang, 2004) of *Acorus*.

The knowledge of genetic variation and genetic similarities

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within individuals or populations are useful for estimating the practical value of genetic resources in a breeding program (Yűzbaşıoğlu et al., 2008). The genetic diversity study of genus *Acorus* is rather restricted on the study of *A. gramineus* in Taiwan (Liao and Hsiao, 1998) and study of *A. calamus* in southeast Ohio (Pai, 2005) using DNA marker system.

Near infrared spectroscopy (NIRs) is an effective method, in particular, for non-destructive analysis of sample without pre-treatment. It has been applied to cultivar identification (Li et al., 2007; Seregélya et al., 2004; Shao et al., 2007), origin discrimination (Galtier et al., 2007), viable and nonviable discrimination of seeds (Min and Kang, 2003; Mulualem, 2003), and differentiation and quantitative determination of various components (Bramble et al., 2006; Jaillaisa et al., 2005; Kim et al., 2007; Liu et al., 2007).

The objective of present study was to explore the genetic diversity of genus *Acorus* (*A. calamus* and *A. gramineus*) by using RAPD marker system and to apply analysis of inter- and intraspecific genetic relationship. Moreover, samples were non-destructively analyzed using NIR spectroscopy for estimating diversity and relationship of genus *Acorus*. This study will provide fundamental information on *Acorus* breeding program in Korea.

Materials and Methods

Plant Materials

Thirteen *A. calamus* and eight *A. gramineus*, together with two *Iris pseudacorus* and one *Colocasia antiquorum* as outgroups were collected from various areas in Korea (Fig. 1). All collected accessions were maintained in a greenhouse at Chonnam National University.

DNA Extraction

Young leaves of 24 collected accessions were prepared for DNA extraction. They were washed with distilled water, and surface water was removed. Fifty mg of fresh leaves of each accession were collected in 2 mL tube after they were pulverized with liquid nitrogen in pestle. Genomic DNA was isolated by using Gentra Puregene Cell Kit for Plant (Qiagen Inc., USA) with minor modifications. The DNA extract was quantified by using a spectrophotometer, ND-100 (Nanodrop Tech., USA).

DNA Amplification

DNA amplifications were conducted with 10-mer primers from Bioneer Inc. (Korea) and Operon Inc. (USA). All amplification reactions were performed as reported by Williams et al. (1990) with minor modification. Controls were made by replacing DNA with water. The reaction mixtures (20 μL) contained DNA 30 ng·μL^{-1}, dNTPs 1 mM, 10 X reaction buffer 2 μL, 10 bp random primer 10 pM, and *Taq* polymerase 1 unit.

A PTC-200 Peltier thermal cycler (MJ research, USA) was used with the following temperature profile: 94℃ for 2 min; 45 cycles of 94℃ for 20 sec, 36℃ for 20 sec, 72℃ for 2 min; 72℃ for 7 min; 4℃ end. PCR products were separated by electrophoresis in a 1% agarose gel made from 1 X TAE buffer included EtBr. The 1 kb DNA ladder (Bioneer Inc., Korea) was used as molecular size marker to estimate sizes of amplified PCR products. The electrophoresis was run for 1 h at 150 V. After electrophoresis, DNA band patterns were observed with a UV transilluminator and photographed with a digital camera under UV light.

NIR Spectroscopy Measurement

A total of 14 accessions, 11 principal accessions (CHA, CHB, CHC, CHD, CHE, CC1, CC2, CN, CS, CX, and CZ) of *A. calamus*, 1 accession (GH) of *A. gramineus*, and 2 accessions (IPH and IPN) of *I. pseudacorus* were selected to estimate the relationship of accessions by using NIRs as non-destructive analysis. Their fresh leaves were harvested, washed, and removed surface water. Then, they were cut at an interval of 1 × 1 cm. They were analyzed by using an IRPrestige-21 (Shimadzu co., Japan) attaching IntegratIR.

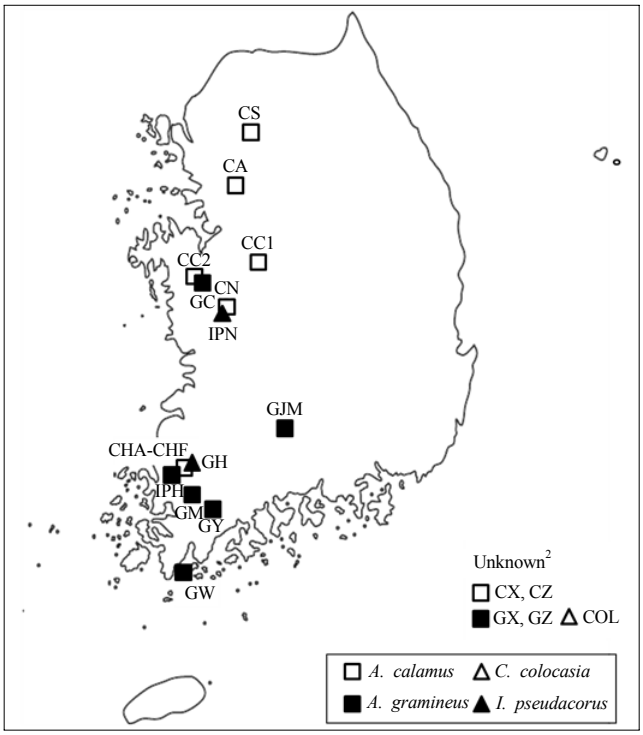

Fig. 1. Accessions of *Acorus calamus*, *A. gramineus*, *Colocasia antiquorum* and *Iris pseudacorus* used in RAPD and NIR analysis. [z]These were purchased from a market as in Gwangju.

Absorption spectra were obtained from each accession slice. Each spectrum was created by 25 scans for 3 times with 4 cm^{-1} of spectral resolution.

Data Analysis

The RAPD fingerprints were scored by assigning '1' for the presence and '0' for the absence for each genotype. Excluding indistinguishable or low intensity bands, unambiguously visible and polymorphic RAPD bands were scored, and converted into a binary matrix as a raw data. The genetic distances between species were based on pair-wise comparisons and calculated according to the equation: $GDxy = 1 - [2N_{xy} / (N_x + N_y)]$, where N_{xy} is the number of fragments shared in lanes x and y, N_x the number of fragments in lane x, and N_y is the number of fragments in lane y (Nei and Li, 1979). The cluster analysis (Sneath and Sokal, 1973) was performed to construct dendrogram of genus *Acorus* using UPGMA (the unweighted pair-group method with arithmetical averages) with 1000 bootstrap sub-samples based on Nei's genetic distances by using the software package TREECON (ver. 1.3b) (Van De Peer and De Wachter, 1993). The bootstrap analysis was used to measure the reliability of each branch in a dendrogram (Efron and Gong, 1983; Felsenstein, 1985). The cophenetic correlation based on genetic distances of cluster analysis was computed as a measure of goodness of fit (Rohlf and Sokal, 1981). The principal coordinate analysis according to Gower (1966) was performed to estimate both interspecific and intraspecific relationships of genus *Acorus* based on the genetic distance matrix by using the procedures of the NTSYS-pc (Rohlf, 1997).

The original NIR spectral points were pre-processed 3 point baseline and smooth including program package IRsolution of IRPrestige-21 (Shimadzu co., Japan). They were transformed into numerical values for data analysis. The principal componentanalysis (PCA) was performed by using Unscrambler® 9.6 trial version (CAMO Inc., India).

Results

RAPD Analysis

In order to obtain reproducible and clear banding patterns which could discriminate accessions and determine genetic relationship among genus *Acorus*, we tested 45 random 10-mer primers. Twelve primers, N8002, N8006, N8007, N8009, N8010, N8012, N8028, N8021, OPB15, OPC6, OPC11, and OPD13 showed polymorphism and a number of bands were scored by using primer screening (Table 1 and Fig. 2). The fragment size of RAPD products of *A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus* were observed from 300 to 4,000 bp, 200 to 4,000 bp, 300 to 6,000 bp, and 300 to 4,000, respectively. The number of scored bands for each primer was various from 23 to 43, with an average of 31 bands per primer.

A total of 371 reproducible and polymorphic bands were scored by using 12 primers and used for statistical analysis (Table 1). *A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus* showed distinctive band patterns. In 24 accessions used in this study, 70-92 (on average 83) bands were scored. In *A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus*, 83-92 (on average 87), 70-82 (on average 77), 84, and 74-76 (on average 75) bands were scored, respectively.

Genetic Diversity Analysis

In order to evaluate the genetic relationship of genus *Acorus*, the UPGMA cluster analysis and principal coordinate analysis were performed based on genetic distance using Nei's coeffi-

Table 1. Selected primers for RAPD analysis in *Acorus calamus*, *A. gramineus*, *Colocasia antiquorum*, and *Iris pseudacorus*.

Primer	Sequencem (5'→3')	GC content (%)	Total band number
N8002	CAATCGCCGT	60	42
N8006	AGCCAGCGAA	60	28
N8007	GTGACGTAGG	60	32
N8009	GGGTAACGCC	70	25
N8010	CTGAGACGGA	60	28
N8012	TACAACGAGG	50	27
N8021	AATCGGGCTG	60	32
N8028	CCCGCCGTTG	80	43
OPB15	GGAGGGTGTT	60	23
OPC6	GAACGGACTC	60	26
OPC11	AAAGCTGCGG	60	42
OPD13	GGGGTGACGA	70	23
Total			371

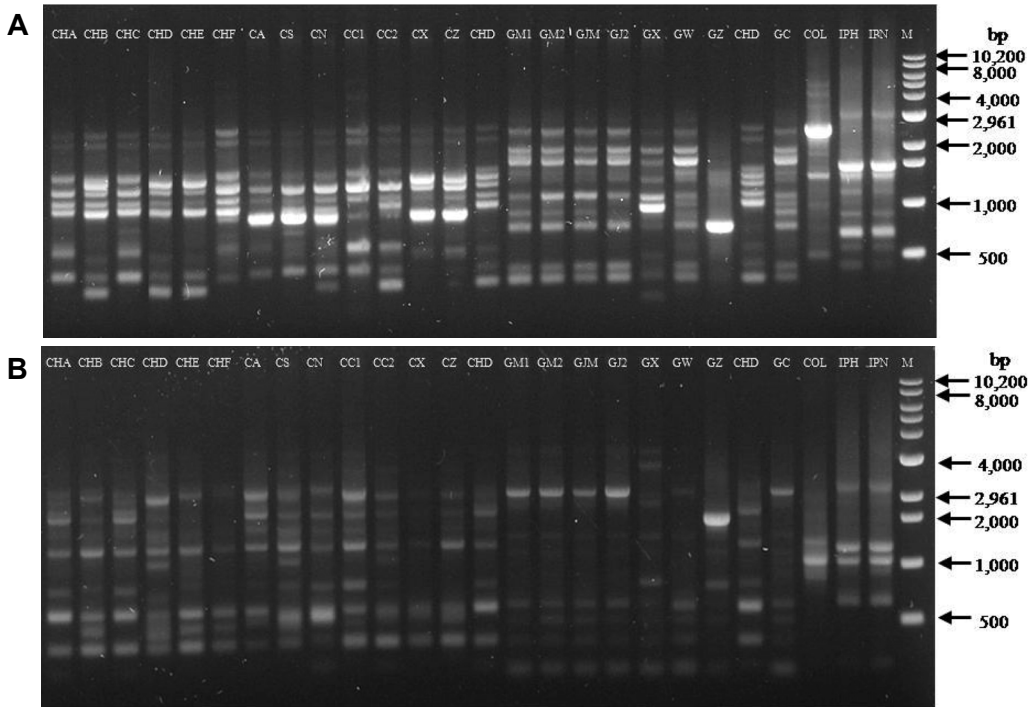

Fig. 2. RAPD profiles obtained from *Acorus calamus*, *A. gramineus*, *Colocasia antiquorum* and *Iris pseudacorus* with the primers, OPC11 (A) and N8009 (B). CHA, CHB, CHC, CHD, CHE, CHF, CN, CA, CC1, CC2, CS, CX, and CZ: A. calamus; GC, GH, GM, GW, GX, GY, and GZ: *A. gramineus*; COL: *C. antiquorum*; IPH and IPN: *I. pseudacorus*; M: size marker.

Table 2. Genetic distance matrix of 24 accessions of *Acorus calamus*, *A. gramineus*, *Colocasia antiquorum*, and *Iris pseudacorus* based on Nei's coefficients calculated from RAPD analysis.

	CHA	CHB	CHC	CHD	CHE	CHF	CA	CS	CN	CC1	CC2	CX	CZ	GM	GY	GJM	GH	GX	GW	GZ	GC	COL	IPH	IPN
CHA	0.00																							
CHB	0.24	0.00																						
CHC	0.05	0.26	0.00																					
CHD	0.26	0.08	0.28	0.00																				
CHE	0.25	0.10	0.25	0.16	0.00																			
CHF	0.26	0.27	0.24	0.31	0.25	0.00																		
CA	0.26	0.26	0.27	0.26	0.27	0.27	0.00																	
CS	0.25	0.28	0.25	0.31	0.28	0.27	0.31	0.00																
CN	0.22	0.25	0.24	0.28	0.26	0.30	0.26	0.22	0.00															
CC1	0.25	0.30	0.24	0.30	0.31	0.26	0.29	0.24	0.18	0.00														
CC2	0.26	0.27	0.27	0.28	0.25	0.22	0.26	0.27	0.22	0.21	0.00													
CX	0.21	0.23	0.24	0.27	0.21	0.24	0.27	0.20	0.20	0.26	0.28	0.00												
CZ	0.21	0.23	0.23	0.27	0.22	0.24	0.28	0.20	0.19	0.25	0.25	0.03	0.00											
GM	0.86	0.86	0.86	0.84	0.84	0.84	0.86	0.84	0.87	0.83	0.85	0.85	0.86	0.00										
GY	0.85	0.84	0.85	0.83	0.82	0.83	0.85	0.83	0.85	0.81	0.83	0.84	0.84	0.03	0.00									
GJM	0.83	0.83	0.84	0.81	0.81	0.81	0.83	0.81	0.84	0.80	0.82	0.82	0.83	0.07	0.06	0.00								
GH	0.85	0.85	0.85	0.83	0.83	0.83	0.85	0.83	0.85	0.82	0.84	0.84	0.85	0.06	0.09	0.09	0.00							
GX	0.82	0.81	0.81	0.80	0.81	0.81	0.85	0.81	0.85	0.82	0.85	0.81	0.81	0.49	0.48	0.51	0.46	0.00						
GW	0.88	0.84	0.88	0.82	0.83	0.83	0.85	0.81	0.84	0.83	0.84	0.84	0.85	0.17	0.17	0.18	0.15	0.47	0.00					
GZ	0.84	0.88	0.85	0.85	0.87	0.86	0.86	0.86	0.87	0.85	0.86	0.86	0.85	0.36	0.35	0.37	0.34	0.50	0.36	0.00				
GC	0.85	0.83	0.86	0.81	0.81	0.81	0.84	0.80	0.82	0.81	0.83	0.83	0.84	0.15	0.17	0.16	0.13	0.51	0.09	0.37	0.00			
COL	0.83	0.79	0.82	0.80	0.82	0.86	0.87	0.86	0.82	0.82	0.82	0.80	0.80	0.90	0.90	0.89	0.90	0.87	0.91	0.90	0.92	0.00		
IPH	0.90	0.90	0.89	0.88	0.91	0.91	0.87	0.86	0.90	0.89	0.89	0.89	0.89	0.85	0.85	0.86	0.87	0.91	0.87	0.88	0.87	0.89	0.00	
IPN	0.91	0.92	0.90	0.89	0.93	0.93	0.89	0.88	0.91	0.90	0.90	0.90	0.90	0.86	0.85	0.87	0.87	0.92	0.87	0.88	0.89	0.88	0.11	0.00

cients calculated from RAPD data (Table 2, Figs. 3, 4, and 5). The genetic distances (GD) were calculated from 0.03 to 0.31 GD within *A. calamus*, from 0.03 to 0.51 GD within *A. gramineus*, and 0.11 GD within *I. pseudacorus*. The genetic distance between *A. calamus* and *A. gramineus* was revealed to be farthest from 0.80 to 0.88 GD. The genetic distances

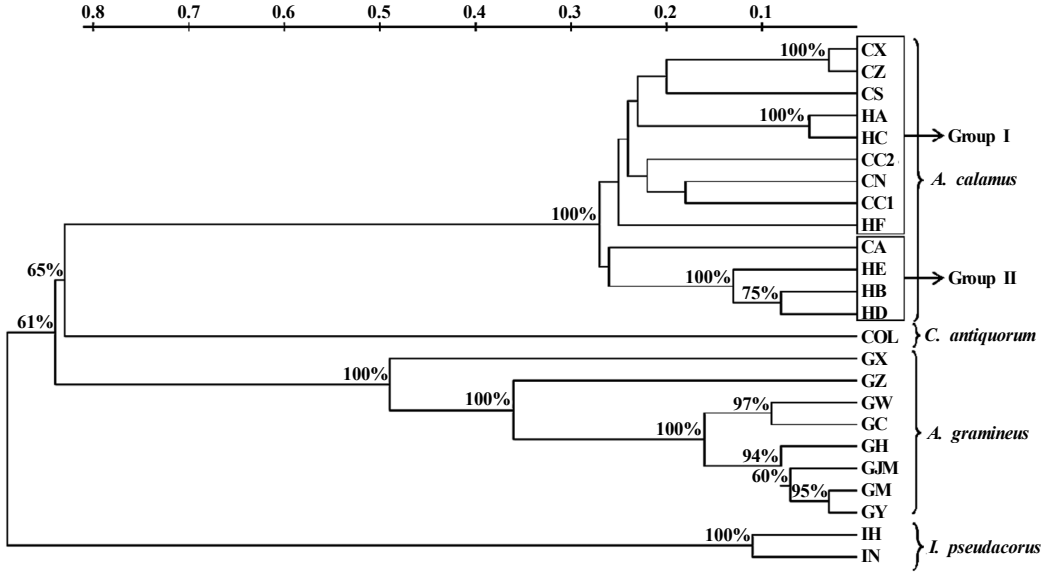

Fig. 3. Dendrogram of 24 accessions of *Acorus calamus*, *A. gramineus*, *Colocasia antiquorum*, and *Iris pseudacorus* resulting from a UPGMA cluster analysis based on Nei's genetic distances calculated from RAPD data. The bootstrap analysis was conducted using TREECON (ver. 1.3b) with 1000 bootstrap subsamples of the data matrix. Bootstrap values for those branches greater than 60% of the topologies are reported.

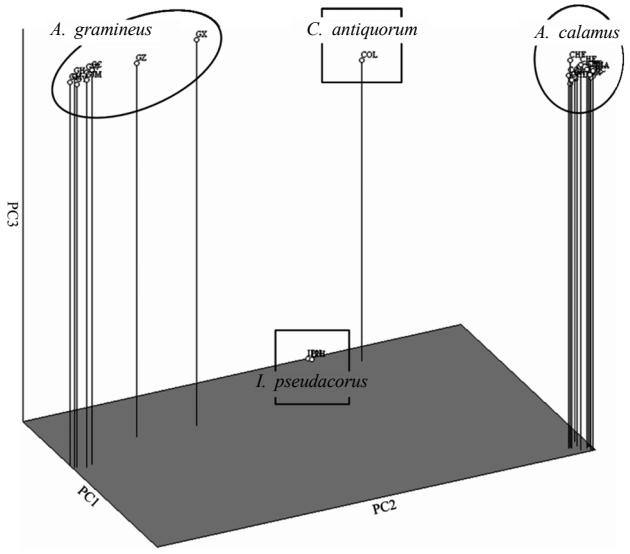

Fig. 4. Relationships among 24 accessions of *Acorus calamus*, *A. gramineus*, *Colocasia antiquorum* and *Iris pseudacorus* by principal coordinate analysis based on Nei's genetic distances calculated from RAPD data. The three principal coordinate accounted for 76.33% of the total variation. PC1, PC2, and PC3: 45.04% on the first principal coordinate axis, 23.67 % on the second, and 7.66% on the third.

between *A. calamus* and *C. antiquorum* and between *A. gramineus* and *C. antiquorum*, were 0.79 to 0.87 GD and 0.87 to 0.92 GD, respectively. *I. pseudacorus* was separated from *A. calamus* and *A. gramineus* with 0.86 to 0.93 GD and 0.85 to 0.92 GD, respectively. The genetic distance between *C. antiquorum* and *I. pseudacorus* were 0.88 to 0.89 GD.

In the result of cluster analysis, the dendrogram clearly separated the accessions into four distinct groups by their taxonomic classification with a bootstrap value of 100%: *A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus* (Fig. 3). As a result of principal coordinate analysis, three-dimensional plot was also obviously divided into *A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus* (Fig. 4). In the cluster analysis using the genetic distance, the matrix correlation coefficients (r) was 0.9973 (data not shown).

A. calamus group was subdivided into two subgroups with 100% bootstrap value in the dendrogram and three-dimensional plot (Figs. 3 and 5). On average, the genetic distance of two sub-groups was estimated around 0.27 GD (Table 2). Interestingly, one of *A. calamus* accession (CZ) with yellow stripeof leaves was tightly grouped with another *A. calamus* accession, CX with 0.03 GD (Table 2, Figs. 3 and 5). On the other hand, one accession (GZ) with yellow stripeleaves within *A. gramineus* was separated from other accessions with on average 0.36 GD (Table 2, Figs. 3 and 5). Moreover, analysis of eight accessions of *A. gramineus* by RAPD markers, one accession (GX) was isolated from other *A. gramineus* accessions (Figs. 3 and 4). The genetic distances between GX and other *A. gramineus* accessions were from 0.46 to 0.51 GD (Table 2).

The variance of the first principal coordinate accounted for 76.33% of the total variation. PC1, PC2, and PC3, accounted for 45.04%, 23.67%, and 7.66% of the total variation, respectively (Fig. 4). The first and second coordinates could resolve relationship of genus *Acorus*, *A. calamus* and

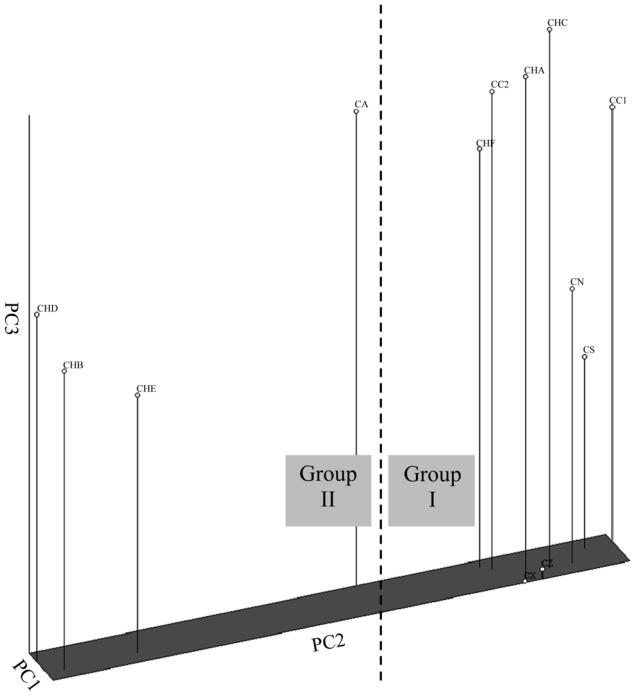

Fig. 5. Relationships among 13 accessions of *Acorus calamus* by principal coordinate analysis based on Nei's genetic distances calculated from RAPD data. The three principal coordinate accounted for 85.48% of the total variation. PC1, PC2 and PC3: 77.6% on the first principal coordinate axis, 4.50% on the second, and 3.38% on the third.

A. gramineus, *C. antiquorum* was closely allocated with *A. gramineus* in the first coordinate and with *A. calamus* in the second coordinate, respectively. Especially, the third coordinate could differentiate *I. pseudacorus* from other groups.

Non-destructive analysis by using NIR spectroscopy was applied to show its power in the diversity study of genus *Acorus* comparing with the result of RAPD analysis. Fourteen accessions were used for principal component analysis obtained from NIR spectral data. The two-dimensional plot was clearly divided into *A. calamus*, *A. gramineus*, and *I. pseudacorus* (Fig. 6). Two variances of principal components accounted for 98% of the total variation. PC1 and PC2 accounted for 84% and 14%, respectively. *A. calamus* accessions were separated from outgroups, *A. gramineus* and *I. pseudacorus*, by the first component (PC1). In the second component, *A. calamus* could be subdivided into two subgroups, and outgroups could be also divided from *A. gramineus* and *I. pseudacorus*. Interestingly, one accession (CZ) with yellow stripe leaves of *A. calamus* was allocated on the same position of CX in PC1. However, two accessions were allocated into PC2. *A. gramineus* was positioned in the similar point with *I. pseudacorus* in PC1, but they was clearly differentiated each other.

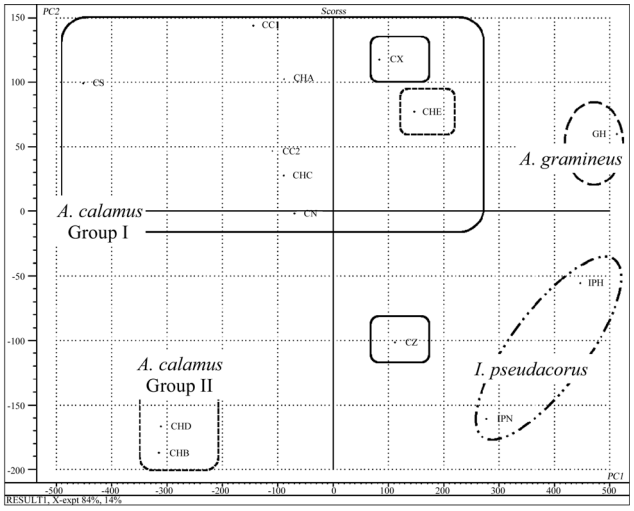

Fig. 6. Plot of 14 accessions of *Acorus calamus*, *A. gramineus*, and *Iris pseudacorus* resulting from principal component analysis obtained from NIR.

Discussion

The genus *Acorus* including *A. calamus* and *A. gramineus*, was previously placed within the family Araceae. But it is recently separated from the family and placed in its own family Acoraceae in order Acorales (APG II, 2003; Grayum, 1987). Molecular phylogenetic analysis suggested that *Acorus* is importantly sole genus of the oldest surviving line as original extant monocotyledon by sequencing chloroplast *rbcL* gene (Duvall et al., 1993). Duvall et al. (2008) discovered that *A. calamus* and *A. gramineuswere* sister to each other in analysis of chloroplast, selected nuclear sequences and muligene studies.

In the present study, we investigated the genetic diversity among accessions of genus *Acorus* in Korea by using RAPD markers. In the dendrogram and three-dimensional plot derived from cluster analysis and principal coordinate analysis, the results showed that interspecific relationship between *A. calamus* and *A. gramineus* was obviously elucidated with 0.80-0.88 GD (Table 2).

The intraspecific genetic distances of *A. calamus* and *A. gramineus* were GD 0.03-0.31 (on average 0.21) and 0.03-0.51 (on average 0.20), respectively (Table 2). The genetic distance showed relatively narrow variation below 0.3 GD on average except GX and GZ in *A. gramineus*. In Taiwan *A. gramineus* showed also less than 0.3 GD on average based on RAPD markers (Liao and Hsiao, 1998). Pai (2005) reported that *A. calamus* populations in southeast Ohio showed narrow genetic variation with 0.06-0.13 GD and homozygous. He suggested that *A. calamus* populations in southeast Ohio were primarily clonal in nature. Our results also supported this evidence. It seems that apomitic plants, intraspecies of genus

Acorus, resulted in a narrow genetic variation.

In the dendrogram of cluster analysis, *A. calamus* was remarkably subdivided into two subgroups (Group I and II) on average of GD 0.27 (Figs. 3 and 5). Group II had tall plant and narrow leaf width compared with Group I. The specific and distinct primers differentiated two groups in this study. As a result, we suggest that there are two ecotypes of *A. calamus* in Korea. It is worthwhile to study botanical, chemical, and chromosomal characteristics of two subgroups. One accession (CZ) in *A. calamus* was located within this group, but the green leaves contained yellow stripe. This accession was closest to CX as a genetic distance of 0.03 (Table 2 and Fig. 3). The two accessions were not different in plant height, leaf width, and rhizome size except for yellow stripe of leaves. It gives an evidence that stripe trait (CZ) was probably generated by chimera from the normal type (CX).

On the other hand, one accession (GZ) with yellow stripe leaves within *A. gramineus* was separated from other accessions on average 0.36 GD. It seems that GZ might be a chimera obtained from an uncollected accession of *A. gramineus*. In addition, analysis of 8 accessions of *A. gramineus* by RAPD markers, one accession (GX) was distinctively isolated from other *A. gramineus* accessions. The genetic distances of GX were from 0.46 to 0.51 GD (Table 2, Figs. 3 and 4). The GX has short plant height and narrow leaf width compared with other accessions. Accessions including *A. gramineus* generally grow to 30-40 cm in height and 0.3-0.7 cm of leaf width. But GX is shorter than other accessions as about 10 cm tall and 0.1-0.2 cm wide. It verified clear separation compared to other lines. Further study like chloroplast DNA sequencing of GX and GZ might identify the origin of the accessions.

As a result of RAPD analysis, the cluster and principal coordinate analysis distinctly divided into four groups, *A. calamus*, *A. gramineus*, *C. antiquorum*, and *I. pseudacorus* (Fig. 4). Remarkably, non-destructive NIR analysis separated the accessions as the result using RAPD data (Fig. 6). Lee and Kim (2005) reported that *A. calamus* and *C. antiquorum* were close with 0.37 GD. In this study, *C. antiquorum* and *I. pseudacorus* were used as outgroups. *C. antiquorum* was separated from *A. calamus* and *A. gramineus* with 0.79 to 0.87 GD and 0.87 to 0.92 GD, respectively. However, their study does not make a comparison with the present study due to the use of different primers. The genus *Acorus* is recently placed in its own family Acoraceae, separated from family Araceae (APG II, 2003; Grayum, 1987). The spathe protecting the spadix is not in existence, which supported the modification that *Acorus* is removed from Araceae (Ray, 1987). In addition, the genetic distances between *A. calamus* and *I. pseudacorus* and between *A. gramineus* and *I. pseudacorus*, were 0.86 to 0.93 GD and 0.85 to 0.92 GD, respectively (Table 2). Meanwhile, Seregélya et al. (2004) computed were computed genetic distances of different melon genotypes in Hungary by DNA marker technique such as RAPD or SSR, but cultivar identification was limited. He reported that it was possible for NIRs to distinguish and identify several representative cultivars.

We conclude that the RAPD marker technique appears to be a reliable tool for identifying the genetic diversity and genetic variation in genus *Acorus*. Furthermore, NIR spectroscopy using non-destructive sample analysis could be adequate to identify the diversity and relationship. This study will provide fundamental information on *Acorus* breeding program in Korea.

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