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Genetic variation and relationship of *Artemisia capillaris* Thunb. (Compositae) by RAPD analysis

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Abstract - Randomly Amplified Polymorphic DNA (RAPD) was performed to define the genetic variation and relationships of *Artemisia capillaris*. Fifteen populations by the distributions and habitat were collected to conduct RAPD analysis. RAPD markers were observed mainly between 300bp and 1600bp. Total 72 scorable markers from 7 primers were applied to generate the genetic matrix, and 69 bands were polymorphic and only 3 bands were monomorphic. The genetic dissimilarity matrix by Nei's genetic distance (1972) and UPGMA phenogram were produced from the data matrix. Populations of *Artemisia capillaris* were clustered with high genetic affinities and cluster patterns were correlated with distributional patterns. Two big groups were clustered as southern area group and middle area group. The closest OTUs were GW2 and GG1 in middle area group, and GB1 from southern area group was clustered with OTUs in middle area group. RAPD data was useful to define the genetic variations and relationships of *A. capillaris*.

Key words - *Artemisia capillaris*, RAPD, variations, genetic relationship, molecular marker

Introduction

Artemisia capillaris Thunb. is distributed in East Asia including Korea, Japan, China, Taiwan, and Philipines (Yook, 1977; Lee, 1980; Ohwi, 1984; Mata *et al*. 1985). It is known as a medicinal plants which is effective for various kinds of disease traditionally (Jung, 1956). Generally, what we call 'In-Jin-Ssook' which is reported as good for anti-liver disease (Lee, 1996) includes *A. iwayomogi*, *A. capillaris*, *A. sacrorum* subsp. *manshuric*, *A. sacrorum* var. *vestita*, *A. scroparia*, and *A. angustssia* (Kim, 1992). Among those species, *A. capillaris* recognised as 'In-Jin-Ho' or 'Jeong-gyeong-ssook'is a perennial plant which lives in sand area of steram side and grows up to 30-100 cm height (Lim, 1992). 'In-Jin' which is dried whole plants with young leaves and flowers includes oils and aromatic components such as capilin, 6,7-dimethylaescletin, benzaldehyde, pinene, myrcene, cineole, 2-pyrrolidinone, camphor, thujone, 1-acethlpiperidine, caryophyllene, coumarine, farnesol and etc. (Cho and Chiang, 2001). Saccharides of *A. capillaris* include mainly glucose (71%), xylose (25.4%), manose (2.8%), ramnose (0.6%), and 1.4% of amino acids components(Song, 1996). And the main flavonoid components of *A. capillaris* contain artemisidin, atemicapins, coumarins, and etc. (Wu, 2001).

There are many previous studies on *A. capillaris*, but most of them are mainly for the constituent analysis, phamacological effect and cultivation techniques including allelopathy effect (Kil *et al*., 1999), growth characteristics and component(Rho and Seo, 1993), pathological effect (Ahn, 2000; Choi *et al*., 2008a; Choi *et al*., 2008b; Seo and Yun, 2008), immunological toxicity of extracts (Lee *et al*., 2004), and characteristics of germination and cultivation (Lim *et al*., 2004; Choi *et al*., 2007). Also Rho and Seo (1993) mentioned the morphological variation patterns of collected samples, and Park *et al*. (2005) sugeted that RAPD analysis showed the higher resolution than cluster analysis based on morphology. It is because of the differences of the collected sample numbers as well as huge morphological variations of leaves in interspecies and even in infraspecific individuals. As the difficulties of classification and identification according to the growing stages and environments, the investigation for precise identification method of in *A. capillaris* has been needed (Rho and Seo, 1993).

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Table 1. Materials and collection data of *Artemisia capillaris*

Taxon	Collection date	Symb.
Artemisia capillaris Thunb.	GW: Ganghyeon-myeon, Yangyang-gun (Kim *et al*. 070415-001)	GW1
	GW: Mangsang-dong, Donghae-si (Kim *et al*. 070414-001)	GW2
	GG: Maehyang-ri, Ujeong-eup, Hwaseong-si (Kim *et al*. 070414-002)	GG1
	CN: Uihang2-ri, Sowon-myeon, Taean-gun (Kim *et al*. 070414-003)	CN1
	CB: Cheondong-ri, Danyang-eup, Danyang-gun (Kim *et al*. 070503-001)	CB1
	GN: Sicheon-myeon, Sancheong-gun (Kim *et al*. 070408-001)	GN1
	GN: Galgot-ri, Nambu-myeon, Geoje-si (Kim *et al*. 070407-001)	GN2
	GB: Samsan-ri, Iseo-myeon, Cheongdo-gun (Kim *et al*. 070420-001)	GB1
	GB: Deoksin-ri, Wonnam-myeon, Uljin-gun (Kim *et al*. 070414-004)	GB2
	GB: Hwajin-ri, Songna-myeon, Buk-gu, Pohang-si (Kim *et al*. 070414-005)	GB3
	JB: Bieungdo-dong, Gunsan-si (Kim *et al*. 070413-001)	JB1
	JN: Yeonsan-dong, Mokpo-si (Kim *et al*. 070406-001)	JN1
	JN: Pungnam-ri, Pungnam-myeon, Goheung-gun (Kim *et al*. 070407-002)	JN2
	JJ: Gosan-ri, Hangyeong-myeon, Jeju-si (Kim *et al*. 070330-001)	JJ1
	JJ: Jungeom-ri, Aewol-eup, Jeju-si (Kim *et al*. 070330-002)	JJ2

*GW: Gangwon prov., GG: Gyeonggi prov., CN: Chungnam prov., CB: Chungbuk prov., GN: Gyeongnam prov., GB: Gyeongbuk prov., JB: Jeonbuk prov., JN: Jeonnam prov., JJ: Jeju prov.

There are many molecular genetic protocols to classify the low level taxa such as interspecific, intergeneric levels and forms besides of cultivation environment and morphological characters (Park *et al*., 2005). Especially RAPD and AFLP are very useful to trace the origin on especially low level taxa. Also RAPD method overcomes many of the technical limitations of RFLP and has been used in many genetic analyses with very small amount of sample concentration including population genetics studies(Williams *et al*., 1990) It showed consistent results and merits even in various growing stages of samples and even in the variants from huge environmental differences.

Generally 'Sook' which designates several species of *Artemisia* is traditionally used as local name instead of scientific name, and mixed plants from unknown species of Artemisia are used because of difficult identification when they prepared the samples for folk medicine. And the taxa of Artemisia show huge variations according to the growth conditions and environments from cross-fertilization mechanism. The investigation to establish the identification system among inter- and infraspecific taxa of *Artemisia* has been needed (Park *et al*., 2005).

Therefore, this study was performed to determine the genetic diversity of *A. capillaris* Thunb. by distributions and habitats in Korea. And we conducted RAPD analyses of 15 regional populations, and we discussed the genetic variations and relationships among the taxa by RAPD data.

Materials and Methods

Plant Materials

The plant materials used in this study and their collection data are given in Table 1. They were collected from March to May in 2007 from 15 regional populations in South Korea. Leaves used as sources of DNA were collected from natural populations. And the voucher specimens were deposited in TUT. All plant materials were kept in vinyl zipper bag with silica-gel until returned to the laboratory and stored at -70℃ in the laboratory until use.

DNA extraction and PCR for RAPD

Total genomic DNA was extracted from fresh leaf tissue pulverized in liquid nitrogen using the 2X CTAB buffer (Doyle and Doyle, 1987). DNA samples were stored at -20℃ until use.

Randomly amplified polymorphic DNA in each genomic DNA was amplified by 35 cycles with No. 1-200 oligo primers

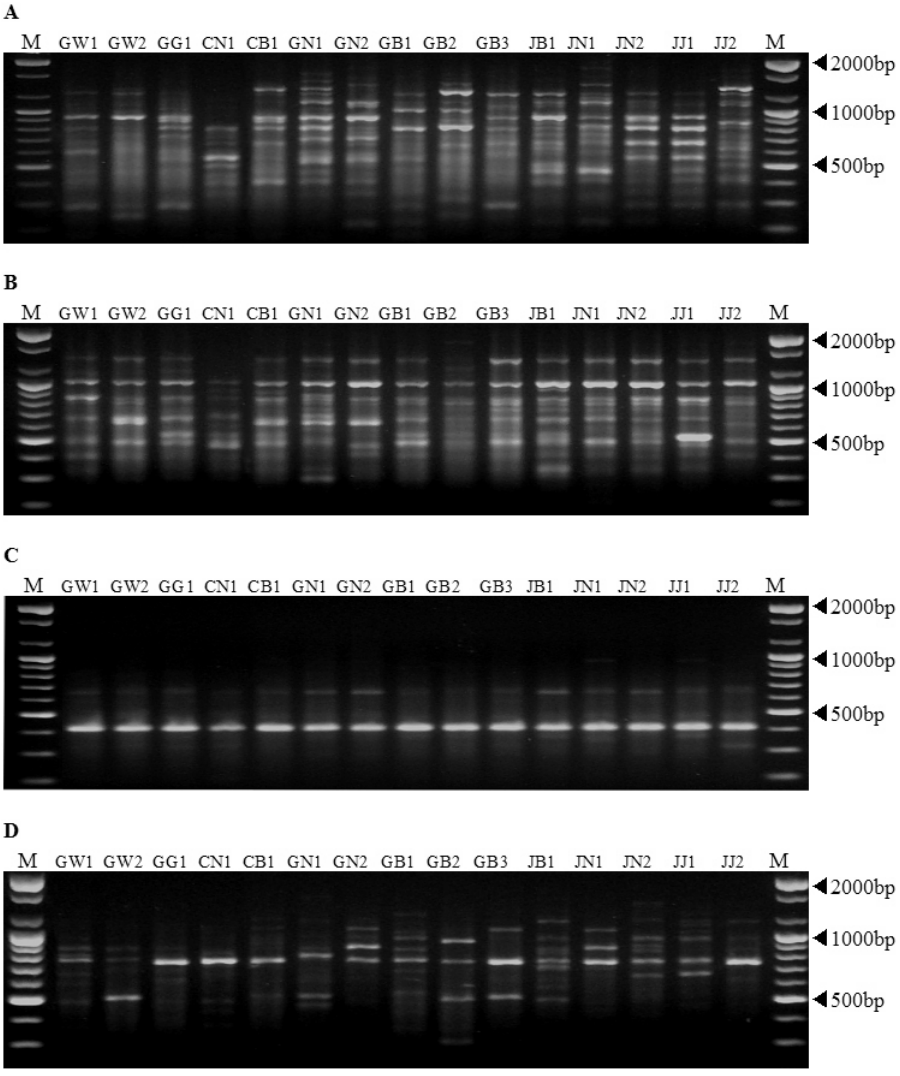

Fig. 1. RAPD electrophoresis profiles for *Artemisia capillaris*.
(M: molecular markers; A, primer No. 101; B, primer No. 105; C, primer No. 125; D, primer No. 155).

by NAPS (Univ. of British Columbia). Amplifications were perfomed in 25 μl reactions containing 10-50 ng DNA, 200 μM dNTP (equimolar), 0.5 units AmpliTaq DNA polymerase (Perkin & Elmer, Cetus), 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, and primers at 1.0 μM. Before the PCR cycles, PCR mixture was predenatured at 92℃ for 3 minutes. The PCR cycle consisted of 30 seconds at 95℃ for denaturation, 30 seconds at 50℃ for annealing, and 1 minutes at 72℃ for extension. After 35 cycles the PCR reactions were incubated at 72℃ for 7 minutes to complete final extension. After the useful primers were screened, we conducted 3 times repetitive reactions with same primers to confirm their availability. Agarose gel electrophoresis were employed to check the amplified DNA products by 1.5% agarose gel with 10^{-4}% EtBr. And the correct band positions were determined with 1D-PCR program by Universal Software (AAB, 2000) and photographed (Fig. 1)

Data Analysis

Each RAPD band was assigned a number and treated as a unit character coded as 0(absent) or 1(present) by the operational taxonomic units (OTUs), and the data matrix was conducted. The genetic variations were calculated by Nei and Li's genetic distance (1979) with NTSYS program (Applied Biostatistics, 2000, v2.1). The UPGMA phenogram was produced by the RAPD results and it was used to investigate the relationships

among OTUs (Fig. 2).

Results and Discussion

We conducted to select the proper primers after screening PCR reactions with 200 primers. And we investigated the reproducibility of selected primers by 3 times repetitive reaction. The considered RAPD markers from 15 OTUs which showed consistent amplification were found between 300bp 1600bp. Total 72 scorable markers from 7 primers were applied to generate the genetic matrix, and 69 bands were polymorphic and only 3 bands were monomorphic. This result showed the highly related genetic similarity among the taxa of *A. capillaris* (Fig. 1). We found 3-16 polymorphic bands according to primers and the average number of polymorphic bands was 10 (Table 2). The genetic dissimilarity matrix by Nei's genetic distance (1972) and UPGMA phenogram were produced from the data matrix (Table 3, Fig. 2).

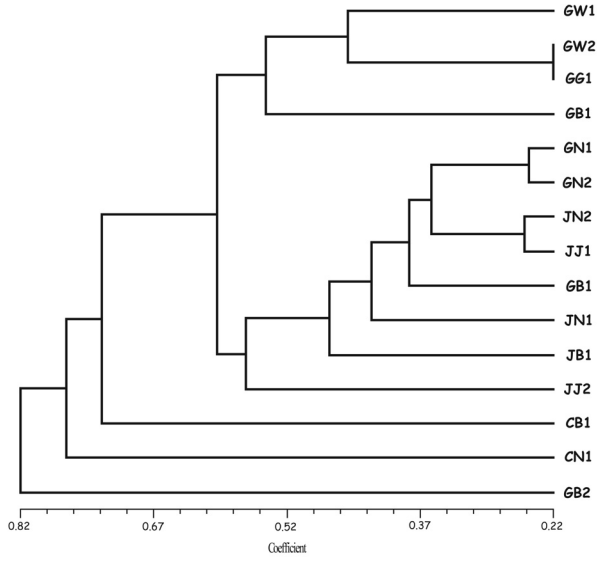

Fig. 2. UPGMA phenogram of 15 populations of *Artemisia capillaris* based on RAPD analysis.

Fifteen populations of *A. capillaris* showed various genetic differences ranging from 0.223 to 1.137, and it was 0.223-0.250 between the nearest distributed populations and 0.390-0.532 between regionally isolated populations (Table 3). Based on the UPGMA phenogram, two big groups were clustered as southern area group and middle area group. The closest OTUs were GW2 and GG1 in middle area group, and GB1 from southern area group was clustered with OTUs in middle area group. In the southern area group, GN1 and GN2 from Gyeongnam Province were clustered firstly, and populations from Jeonbuk Prov. and Jeju Prov. with GB3 formed a big cluster with CB1 and CB2 (Fig. 2, Table 3).

A. capillaris showed various genetic diversity without any relations of geographical isolation and habitat similarity based on RAPD analyses. This result supported partly the previous data which showed the morphological variations of leaves by the distributional regions and habitat environments (Rho and Seo, 1993). Park *et al*. (2005) classified *Artemisia* mixture collections into 5 groups by morphology and RAPD, but there was no obvious evidence or suggestion for identification of *Artemisia*. The reason is supposed as follows : *A. capillaris* and related species including *A. feddei*, *A. argyi*, and *A. princeps* var. *orientalis* showed similar patterns in morphology, and the collection numbers were very different by taxa. And especially leaf variations among the above Artemisia

Table 2. The code and sequences of primer analysed, total number of bands and fragment size which were used for *Artemisia capillaris*

Primer	Sequnence(5'→3')	polymorphic bands	monomorphic bands	Total no. of bands	Fragment size range (bp)
101	GCG GCT GGA G	13		13	300-1600
105	CTC GGG TGG G	7	1	8	300-1500
125	GCG GTT GAG G	2	1	3	400-1000
147	GTG CGT CCT C	11		11	300-1400
149	AGC AGC GTG G	14		14	300-1600
155	CTG GCG GCT G	10	1	11	300-1500
157	CGT GGG CAG G	12		12	400-1600
Total		57	3	72	
Mean/primer		9.9	0.4	10.3	

Table 3. Genetic dissimilarity matrix of *Artemisia capillaris* by Nei's (1972) based on RAPD analysis

	GW1	GW2	GG1	CN1	CB1	GN1	GN2	GB1	GB2	GB3	JB1	JN1	JN2	JJ1	JJ2
GW1	-														
GW2	0.390	-													
GG1	0.515	0.223	-												
CN1	0.645	0.718	0.718	-											
CB1	0.859	0.815	0.815	1.127	-										
GN1	0.720	0.631	0.691	0.685	0.472	-									
GN2	0.568	0.575	0.629	0.573	0.670	0.250	-								
GB1	0.658	0.568	0.405	0.823	0.815	0.825	0.811	-							
GB2	0.397	0.659	0.594	1.040	1.137	0.790	0.595	0.594	-						
GB3	0.416	0.322	0.476	0.683	0.685	0.434	0.349	0.533	0.767	-					
JB1	0.481	0.448	0.602	0.809	0.723	0.559	0.382	0.720	0.750	0.413	-				
JN1	0.577	0.519	0.631	0.780	0.782	0.482	0.446	0.825	0.944	0.385	0.464	-			
JN2	0.594	0.501	0.601	0.679	0.696	0.389	0.331	0.501	0.802	0.419	0.417	0.389	-		
JJ1	0.594	0.549	0.501	0.679	0.776	0.350	0.367	0.454	0.802	0.332	0.592	0.430	0.255	-	
JJ2	0.721	0.676	0.676	1.057	0.615	0.556	0.558	0.676	0.577	0.577	0.703	0.502	0.532	0.532	-

*GW: Gangwon prov., GG: Gyeonggi prov., CN: Chungnam prov., CB: Chungbuk prov., GN: Gyeongnam prov., GB: Gyeongbuk prov., JB: Jeonbuk prov., JN: Jeonnam prov., JJ: Jeju prov.

complex and even within the same species were extremely various. And there was no clear criteria to identify the mixture collections even they suggested the independent differentiation process of gene pool among the populations of *Artemisia* sp.

This study showed the high genetic diversity of *A. capillaris* and various genetic distances and patterns among the populations. Various range of genetic diversity would be the results of the adaptation to different environment. It showed the similar diverse patterns of leaf and inflorescense variations as well as genetic diversity of *A. capillaris*. RAPD data was useful to define the genetic variations and relationships of *A. capillaris*.

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Literature Cited

Ahn, B.M. 2000. What is In-Jin-Sook? *Artemisia capillaris*, *Artemisia iwayomogi*, and *Artemisia annua*, The Korean Journal of Hepatology. 16(4): 548-551. (in Korean)

Cho, Y.H and M.H Chiang. (2001). Research papers : Essential Oil Composition and Antibacterial Activity of *Artemisia capillaries*, *Artemisia argyi*, and *Artemisia princeps*. Kor. J. Intl. Agri. 13(4): 313-320. (in Korean)

Choi, S.R., I.O. Ju, D.H. You, Y.E. Song, I. Jang and J. Ryu. 2007. Changes of Major Components and Growth Characteristics According to Harvesting Times of *Artemisia capillaris* Thunberg. Kor. J. Medicinal Crop Sci. 15(3): 189-193 (in Korean)

Choi, S.R., D.H. You, I.O. Ju, I. Jang, J.Y. Kim, C.B. Park and J. Ryu. 2008a. Changes of Pharmacological Components and Growth Characteristics According to Cultivation Years of *Artemisia capillaris* Thunb. Kor. J. Medicinal Crop Sci. 16(1): 57-61. (in Korean)

Choi, S.R., D.H. You, J.Y. Kim, C.B. Park, J. Ryu, D.H. Kim and J.S. Eun. 2008b. Antioxidant and Antimicrobial Activities of *Artemisia capillaris* Thunberg. Kor. J. Medicinal Crop Sci. 16(2): 112-117. (in Korean)

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation methods for small quantities of fresh tissues. Phytochemical Bulletin. 17: 144-163.

Jung, T.H. 1956. Korean Flora. vol. II. Shinjisa, Seoul. (in Korean)

Kil, B.S. 1999. Allelopathic Effects of *Artemisia capillaris* on the Selected Species. Korean J. Ecol. 22(1): 59-63. (in Korean)

Kim, J.K. 1992. Illustrated natural drugs encyclopedia. Namsandang, Seoul. Pp. 64-79. (in Korean)

Lee, M.K., G.P. Choi, L.H. Ryu, G.Y. Lee, C.Y. Yu and H.Y. Lee. 2004. Enhanced Immune Activity and Cytotoxicity if *Artemisia capillaris* Thunb. Extracts against Human Cell

Lines. Kor. J. Medicinal Crop Sci. 12(1): 36-42. (in Korean)

Lee, S.B. 1996. Hepatoproteinactive effect of polysaccaride fraction from *Artemisia iwayomogi* in rat. Sungkyunkwan Univ. Ph. D. Dissertation.

Lee, T.B. 1980. Illustrated Flora of Korea. Hyangmunsa, Seoul. Pp. 755. (in Korean).

Lim, J.R., B.K. Choo, C.B. Park, D.H. Kim, J.S. Choi and Y.G. Choi. 2004. Germination and Growth Characteristics in Different Sowing Date of *Artemisia capillaris* Thunb. Kor. J. Medicinal Crop Sci. 12(4): 295-299. (in Korean)

Lim, S.N. 1992. Studies on the biological activities of Mugwort (*Artemisia asiatica Nakai*) Leaves. Yonsei Univ. Master thesis.

Mata, R., G. Delgado and A.R. De Vivar. 1985. Sesquiterpene lactones of *Artemisia klotzchiana*. Phytochemistry. 24: 1515-1519.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106: 283-292.

Ohwi, J. 1984. Flora of Japan. 2nd printing, Smithsonian Inst. Washinton, D.C.

Park, S.K., B.H. Chung, H.S. Kim and Y.G. Cho. 2005. Classification of *Artemisia spp*. Collections Based on Morphological Characters and RAPD Analysis. Kor. J. Medicinal Crop Sci. 13(6): 278-286. (in Korean)

Rho, T.H and G.S. Seo. 1993. Growth Characteristics and Chemical Components in Local Collections of *Artemisia sp*. Kor. J. Medicinal Crop Sci. 1(2): 171-177. (in Korean)

Seo, K.S and K.W. Yun. 2008. Antioxidant Activities of Extracts from *Artemisia capillaris* Thunb. and *Artemisia iwayomogi* Kitam. used as Injin. Kor. J. Plant Res. 21(4): 292-298. (in Korean)

Song, K.W. 1996. Phytochemical study of polysaccaride fraction from *Artemisia species*. Sungkyunkwan Univ. Master thesis.

Williams, J., A. Kubelik, J. Rafalski and S. Tingey. 1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucleic Acids Research 18: 6231-6239.

Wu, T.S., Z.J. Tsang, P.L. Wu, F.W. Lin, C.Y. Li, C.M. Teng and K.H. Lee. 2001. New constituents and antiplatelet aggregation and anti-HIV principles of *Artemisia capillaris*. Bioorganic & Medicinal Chemistry. 9: 77-83.

Yook, C.S. 1977. Medicinal Plants, Jinmyung Publishing Co. Pp. 293-298. (in Korean)

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