Research Paper

Afr. J. Traditional, **Complementary and Alternative Medicines**
www.africanethnomedicines.net

ISSN 0189-6016©2008

GENETIC ANALYSIS OF *ARALIA CORDATA* THUNB BY RAPD

Fan Qu,[1*] Jue Zhou,[2] Zhou Zhou,[3] Huiyu LI,[4] and Elizabeth Burrows,[5]

[1] Room 604, B Building ,School of Medicine, Zhejiang University, 388 Yuhang Tang Road, Hangzhou, Zhejiang 310058, P. R. China, [2]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China, [3]Heilongjiang Institute of Water Resources and Hydropower, Harbin, Heilongjiang 150080, China, [4]National Key Laboratory of Forest Tree Genetics Breeding and Biotechnology, Northeast Forestry University, Harbin, Heilongjiang 150080, China, [5]Global College of Long Island University, Brooklyn, NY, 11201, U.S.A.
***Email:** qufan43@yahoo.com.cn

Abstract

In the research, genetic analysis of *Aralia cordata* Thunb. (Araliaceae) was conducted using randomly amplified polymorphism DNA (RAPD). 161 loci were detected with 12 RAPD primers. Percentage of Polymorphic Band (PPB) varied from 34.78% to 63.35%. All the samples were respectively collected from the eight provinces richest in *Aralia cordata* Thunb resources in China, including Hunan, Yunnan, Zhejiang, Sichuan, Jiangxi, Anhui, Shanxi and Gansu. The results showed that Hunan Province enjoyed the highest level of genetic differentiation and Gansu was the lowest. The total genetic diversity (H_T) of RAPD, intraspecific genetic diversity (H_S) and genetic diversity (D_{ST}) of the various places was respectively 26.33%, 11.14%, and 49.36%. The differentiation among the species accounted for 98.76% of total genetic diversity (G_{ST}). Based on the cluster results of genetic distance, the 8 samples were classified into three groups. It is concluded that Hunan Province enjoyed the highest level of genetic differentiation of *Aralia cordata* Thunb and Gansu was the lowest, which provides a basis for the taxonomic identification and germplasm resource research of *Aralia cordata* Thunb in the future.

Keywords: Genetic Analysis ; *Aralia cordata* Thunb.; RAPD

Abbreviations: RAPD: Randomly Amplified Polymorphism DNA; CTAB: Cetyltrimethylammonium Bromide; PPB: Percentage of Polymorphic Band; Shannon I: Shannon's Information index; Nei H: Nei's gene diversity; H_T: Total genetic diversity; H_S: Intraspecific genetic diversity; D_{ST}: Genetic diversity

Introduction

Aralia cordata Thunb.(Araliaceae), is a traditional Chinese medicinal herb, which grows in woodland garden, dappled shade and shady edge. The root of *Aralia cordata* Thunb. has often been used to treat rheumatism, lumbago, common cold, migraines and lameness clinically(Kim and Kang, 1998). Diterpenes isolated from the plant have it has analgesic and anti-inflammatory properties (Han et al.,1983; Okuyama et al.,1991). In addition to medical uses, *Aralia cordata* Thunb is also widely used in food. The edible parts of *Aralia cordata* Thunb. include leaves, roots and stems. The root is sometimes used in China as a substitute for ginseng (Panax species). They are usually up to 1.5 metres long and have a mild and agreeable flavour. They are crisp and tender with a unique lemon-like flavour.

They can also be sliced and added to salads, soups etc. The shoots contain about 1.1% protein, 0.42% fat, 0.8% soluble carbohydrate and 0.55% ash (Peng et al., 2005).

The root of *Aralia cordata* Thunb. contains essential oil, saponins, sesquiterpenes and diterpene acids, seven diterpenes, four polyacetylenes, a lipid glycerol, and two sterols were isolated from the methylene chloride fraction of the root (Dang et al., 2005) The chemical structures were determined as (-)-pimara-8 ,15-dien-19-oic acid, pimaric acid, (-)-kaur-16-en-19-oic acid, 17-hydroxy-ent-kaur-15-en-19-oic acid, 7alpha-hydroxy-(-)-pimara-8, 15-dien-19-oic acid, 16alpha, 17-dihydroxy-(-)-kauran-19-oic acid, 16-hydroxy-17-isovaleroyloxy-ent-kauran-19-oic acid, falcarindiol, dehydrofalcarindiol, dehydrofalcarindiol -8-acetate, falcarindiol-8-acetate, alpha-mono palmitin, stigmasterol, and daucosterol by the spectral evidences and these compounds have been tested with COX-1 and COX-2 inhibition assays (Dang et al., 2005). The analgesic principles from *Aralia cordata* Thunb were respectively identified as (ent)-kaur-16-en-19-oic acid (KA) and (ent)-pimara-8, 15-dien-19-oic acid (PA). Both compounds were significantly effective regarding analgesics, hypothermia, duration of pentobarbital-induced anesthesia, and depression of locomotor activity enhanced by methamphetamine at doses of 300 mg/kg (KA) and 500 mg/kg (PA) by oral administration (Dang et al., 2005; Peng et al., 2005).

Literature searches of MEDLINE, searching for keywords "Germplasm;" "RAPD" and "*Aralia cordata* Thunb.," found no previous study. We therefore decided to introduce RAPD marker technology into the analysis of *Aralia cordata* Thunb. All the samples in the present research were respectively collected from the eight provinces richest in *Aralia cordata* Thunb resources in China, including Hunan, Yunnan, Zhejiang, Sichuan, Jiangxi, Anhui, Shanxi and Gansu. They were classified into three groups based on the cluster results of genetic distance. The research may be a basis to improve the methods of taxonomic identification and germplasm of *Aralia cordata* Thunb. in the future.

Materials and methods

Plant Samples

The seeds of the samples were collected from the introduced areas (Table 1). The Plant material was authenticated in Zhejiang University (Hangzhou, China). Seeds without apparent damage were dipped in water overnight and cultured on damp filter papers at 25 °C for 2-3 days. Swollen seeds with small embryos were collected and sterilized with 70% ethanol and 10% $Ca(ClO)_2$ solution for 10 min. Small seedlings were aseptically taken out and inoculated on 3% agar with Murashige Skoog medium, and those about 5 cm long were used for the DNA extraction.

Table 1: The sources and amount of *Aralia cordata* Thunb.

Number	Sources	Amount of the individuals
1	Sichuan Province	17
2	Zhejiang Province	18
3	Yunnan Province	20
4	Anhui Province	18
5	Jiangxi Province	20
6	Shanxi Province	22
7	Hunan Province	20
8	Gansu Province	20

DNA Extraction

Total DNA was extracted from the young leaves of the tested samples by the cetyltrimethylammonium bromide (CTAB) method with some modifications. 1g of leaf tissue was frozen in liquid nitrogen and grounded to a fine powder and then added to 1ml of CTAB buffer (1.4M NaCl, 100M Tris-HCl pH 8.0, 0.20mM EDTA pH 8.0,

2% CTAB, 1% PVPP, 0.25% β-mercaptoethanol). Samples were incubated at 65°C for 30 min. 600 µl chloroform-isoamyl alcohol (24:1) was added and gently mixed for 15 min. After centrifugation at 15,000 rpm for 10 min, the aqueous layer was removed. 600 µl of CTAB precipitation buffer (100mM Tris-HCl pH 8.0, 0.20mM pH 8.0 2% CTAB, 0.25% β- mercaptoethanol) was added to the deposition, which was then allowed to incubate for 30 min at room temperature. The precipitated DNA was pelleted by centrifugation at 15,000 rpm for 15 min. The DNA was spooled, air-dried briefly, and resuspended in 30µl 0.1×TE. DNA samples for RAPD analysis were prepared at a concentration of 12.5ng/µl. To verify the concentration and quality of the DNA, 50 ng of each DNA sample was run on a 1.0% agarose gel and compared against a DNA standard of known concentration. DNA concentration was determined by absorbance at 260 nm.

RAPD Analysis

PCR amplification of *Aralia cordata* Thunb. was performed according to RAPD-PCR reaction system and reaction procedure. Total DNA of 8 samples were used as templates and 12 RAPD primers were used in the PCR reaction (Table 2). The 20-µl solution of reaction contained 40 ng template, 0.5 pmol·L^{-1} primer, 2 µl 10×PCR buffer (0.2 mmol·L^{-1}), 1.6 µl dNTP (2.5 mmol·L^{-1}), and 3 U *Taq* polymerase (TaKaRa, Dalian, China). The conditions of PCR amplification were: 94 ℃ for 4 min; 94 ℃ for 45 sec, 45 cycles; 37 ℃ for 2min; 72 ℃ for 2 min; and 72 ℃ for 10 min. PCR products were separated in 1.5% agarose gels. The sizes of amplified products were estimated in comparison with a 100 bp ladder standard (MBI). After electrophoresis, PCR products were detected by UVP Gel Documentation Systems (GDS7600).

Sequence Analysis

8 templates were prepared from seeds of *Aralia cordata* Thunb. from various provinces in China. 80 of 10-mer random primers (four series) were screened for polymorphism. The gel electrophoresis employed enabled the separation of 250-2,000 bp DNA fragments. 12 primers showed polymorphism, in which No. 10 primer was selected out because it caused the most distinct and reproducible polymorphism. As shown in Figure 1, an apparent 1,000 bp band was common in the seeds of 5, and 7, but was not present in 6. An apparent 1,200 bp band was in 7, but not present in 5. The bands from DNA templates of 5 (Jiangxi Province), 6 (Shanxi Province) and 7 (Hunan Province) with No. 10 as the primer were shown in Figure 1.

Table 2: Sequence of primers

Primer No.	Sequence
1	5'CAGGCCCTTC3'
2	5'TGCCGAGCTG3'
3	5'AGTCAGCCAC3'
4	5'GTGACGTAGG3'
5	5'GGGTAACGCC3'
6	5'CAGCACCCAC3'
7	5'TCTGTGCTGG3'
8	5'ACGAGAGGCA 3'
9	5'CATCCCCCTG3'
10	5'GTAGACCCGT3'
11	5'GAACGGACTC3'
12	5'ACGAGAGGCA3'

The DNA bands in the gels were recorded after electrophoresis. According to the corresponding place of Marker DNA ladders in the gels, the sizes of the target fragments can be easily estimated.

Data analysis

The data were analysed by an independent university-based statistician on the basis of hierarchical cluster analysis using the software of Popgen32 and Statistical Package for Social Sciences (SPSS 13.0 for Windows). RAPD bands were transformed into a binary character matrix ("1" for the presence and "0" for the absence of a band at a particular position) and similarity matches were calculated.

Results and Discussion

One hundred and fifty-five individuals of *Aralia cordata* Thunb from 8 places were analyzed by RAPD-PCR, and 161 loci were detected with 12 primers with a size ranged from 250 bp to 2,000 bp. The Percentage of Polymorphic Band (PPB) of the eight places presented a huge change, ranging from 34.78% to 63.35% (Table 3). The sequence of the provinces (from the maximal PPB to the minimum) was: Hunan, Yunnan, Zhejiang, Sichuan, Jiangxi, Anhui, Shanxi and Gansu. Hunan had the highest PPB(63.35%), while Gansu had the lowest(34.78%).

The genetic diversities of *Aralia cordata* Thunb. were estimated by Shannon's Information index (I) and Nei's gene diversity (H) (Nei,1973). The results showed that Shonnon index varied from 0.1168 to 0.2771 (Table 3). The sequence of the provinces (from the maximum to the minimum) was: Yunnan, Hunan, Zhejiang, Sichuan, Jiangxi, Anhui, Shanxi and Gansu. Nei index varied from 0.0692 to 0.1798 (Table 3). The sequence of the provinces (from the maximum to the minimum) was: Hunan, Yunnan, Zhejiang, Sichuan, Jiangxi, Anhui, Shanxi and Gansu. These results showed that in the eight places, the genetic variation of Hunan Province was the highest, while Gansu Province was the lowest.

Table 3: Comparison of the genetic variation

Sources	Total sites	Number of polymorphic loci	PPB %	Nei H	Shannon I
Sichuan	161	82	50.93%	0.1483	0.2300
Zhejiang	161	85	52.80%	0.1347	0.2155
Yunnan	161	88	54.66%	0.1610	0.2486
Anhui	161	70	43.48%	0.1153	0.1809
Jiangxi	161	79	49.07%	0.1355	0.2111
Shanxi	161	64	39.75%	0.1173	0.1811
Hunan	161	102	63.35%	0.1798	0.2771
Gansu	161	56	34.78%	0.0692	0.1168

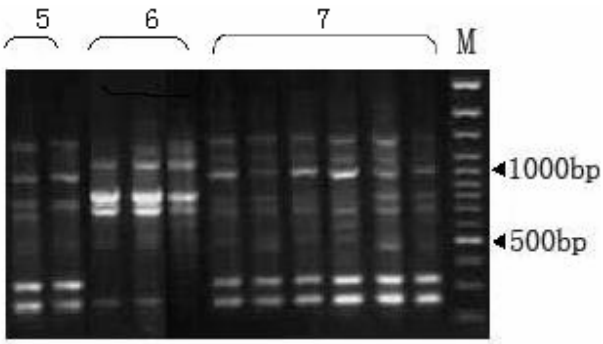

Figure 1: The bands amplified by primer No.10

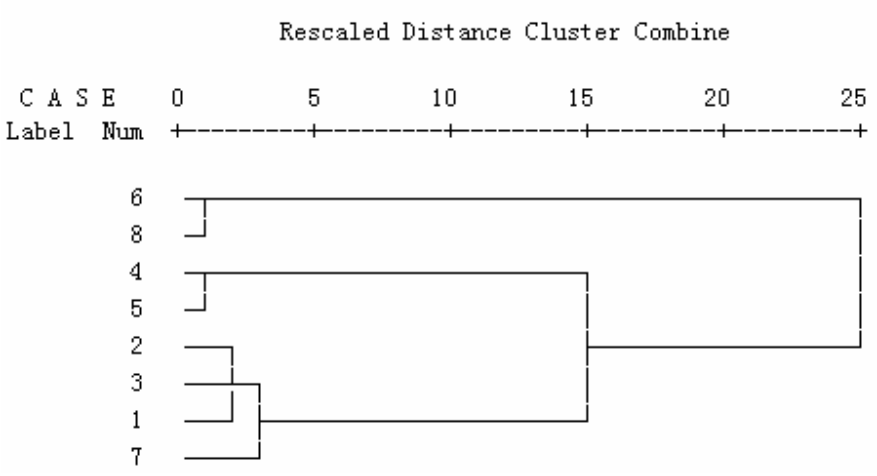

Figure 2: Dendrogram of the genetic distance

The total genetic diversity (H_T) of RAPD, intraspecific genetic diversity (H_S) and genetic diversity (D_{ST}) of the various places was respectively 26.33%, 11.14%, and 49.36%. The differentiation among species accounted for 98.76% of total genetic diversity (G_{ST}). The matrix of the genetic distance of the samples was computed based on Jaccard's similarity coefficient using SPSS13.0. The dendrogram of the genetic distance is shown in Figure 2. The tested species were clustered into three groups. The first group included Yunnan, Hunan, Zhejiang and Sichuan Provinces. The second group included those of Jiangxi and Anhui Provinces. The third group included Shanxi and Gansu Provinces.

Analysis of well-characterized marker compounds has now become the most popular method for identifying the herbal materials and for the quality control of traditional Chinese medicinal herbs. However, in the research of germplasm, the chemical composition of the plant often changes with the external environment and processing conditions, which lowers the reliability of these authentication methods. Since the 1990s, molecular methods have been developed for the identification of plant species. RAPD has been employed as a DNA fingerprinting technique for the identification and the determination of phylogenetic correlations and intraspecific diversity at the molecular genetic level. The genetic tools are now considered to provide more standardized and reliable methods for the germplasm research of herbal materials at the DNA level.

Although some research has been conducted on the phytochemical characterization and pharmacological activity of *Aralia cordata* Thunb (Peng et al., 2005), few has been done on the genetic diversity present in the germplasm of *Aralia cordata* Thunb. The methods used in the present research is applicable to seeds, seedlings, leaf, stem, root and callus with its simple-manipulation and high efficiency. The research may be a basis to improve the methods of taxonomic identification in the researches on the germplasm resourses of *Aralia cordata* Thunb. in the future.

Conclusion

Hunan Province enjoyed the highest level of genetic differentiation of *Aralia cordata* Thunb and Gansu was the lowest, which provides a basis for the taxonomic identification and germplasm resourse research of *Aralia cordata* Thunb in the future.

Acknowledgements

Dr. Fan QU was funded by China Postdoctoral Science Foundation (No.20070421188), Outstanding Young Medical Scientist Foundation of Zhejiang Province (No. 2008QN022) and Zhejiang Traditional Chinese Medicine Foundation(No. 2008YB010). Dr. Jue Zhou was funded by Zhejiang Traditional Chinese Medicine Foundation (No. 2008YA015).

References

1. Dang, N.H., Zhang, X., Zheng, M., Son, K.H., Chang, H.W., Kim, H.P., Bae, K. and Kang, S.S. (2005). Inhibitory constituents against cyclooxygenases from *Aralia cordata* Thunb. Arch. Pharm. Res. **1:**28-33.
2. Han, B. H., Han, Y. N., Han, L. A., Park, M. H., and Lee, E. O. (1983). Studies on the anti-inflammatory activity of Aralia continentalis (I). Arch. Pharm. Res. **6:**17-23.
3. Kim, J. S. and Kang, S. S. (1998). Saponins from the aerial parts of Aralia continentalis. *Nat. Pro. Sci.* **4:** 45-50.
4. Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. **70:** 3321-3323.
5. Okuyama, E., Nishimura, S. and Yamazaki, M.(1991). Analgesic principles from *Aralia cordata* Thunb.Chem. Pharm. Bull. **2:**405-407.
6. Peng, T., Dong, X., Deng, Y., Tu, Y. and Li, X. (2005). Research on chemical ingredients of the root from cultivar *Aralia cordata* Thunb.Zhong Yao Cai. **11:** 996-998.