Natural Medicines 52 (2), 130-134 (1998)

Random Amplified Polymorphic DNA Analysis of *Angelica acutiloba* and Its Varieties

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(Received July 30, 1997)

Total DNAs from fresh leaves of *Angelica acutiloba* KITAGAWA and its varieties were studied by random amplified polymorphic DNA analysis. The marker band at 1.1 kb generated by primer No. 21 (5'-TGG TCG CTG A-3') was found to be useful to distinguish between *A. acutiloba* var. *acutiloba* and *A. acutiloba* var. *sugiyamae*. The data showed that *A. acutiloba* var. *sugiyamae* was genetically not closely related to *A. anomala*, which had been considered a parent plant and that *A. acutiloba* var. *sugiyamae* was rather close to *A. acutiloba* var. *lanceolate*. It was also found that *A. acutiloba* var. *iwatensis* was genetically very close to *A. acutiloba* var. *acutiloba*, and that geographic variations existed in *A. acutiloba* var *iwatensis*.

Keywords: *Angelica acutiloba* ; Umbelliferae ; RAPD ; PCR

Dried roots of *Angelica acutiloba* var. *acutiloba* KITAGAWA (Umbelliferae) and its varieties have been used as a crude drug in Kampo medicine.[1] The crude drug is derived from *A. acutiloba* var. *acutiloba* cultivated in Nara or Wakayama Prefecture in Japan (called "Yamato-Toki"), and from *A. acutiloba* KITAGAWA var. *sugiyamae* HIKINO mainly cultivated in Hokkaido (called "Hokkai-Toki"). These two species show some different characters in their external shape and taste of root.[2]

The relation between *A. acutiloba* var. *acutiloba* and *A. acutiloba* var. *sugiyamae*, and their origins have not been cleared yet. It is said that *A. acutiloba* var. *sugiyamae* is a cross between *A. acutiloba* var. *acutiloba*, probably from Nara, and *A. anomala* LALLEM (Ezono-yoroigusa), growing wild in Hokkaido.[3, 4] However, the crossing experiment between these two varieties failed to produce the hybrid seeds.[4] Furthermore, *A. acutiloba* KITAGAWA var. *lanceolate* OHWI (Hosoba-Toki), *Ligsticum hultenii* FERNALAND (Maruba-Toki) and the other related *Angelica* plants grow natively in Hokkaido without producing crosses.[4]

A. acutiloba KITAGAWA var. *iwatensis* HIKINO (Miyama-Toki) and *A. acutiloba* KITAGAWA var. *iwatensis* forma *tsukubana* HIKINO (Tsukuba-Toki) grow wild in nature in Honshu[4-6] as related species of *A. acutiloba* and *A. acutiloba* var. *acutiloba* (Yamato-Toki) is considered to have originated from these native species, though the relation among them is still not clear.

To clarify the relation between the related plants

of the original plants of *Angelicae* Radix, which is indispensable for the breeding of cultivar line as Toki, recently developed DNA analysis techniques for gene diagnosis was used to obtain molecular genetic information. The random amplified polymorphic DNA (RAPD) analysis [7, 8] using PCR provides a powerful tool to detect genetic differences, and by applying this analysis phylogenetic relationship among *Angelica* plants was partly established.

MATERIALS AND METHODS

Plant Materials Plant materials collected from different habitats and several botanical institutes listed in Table Ⅰ were cultivated in The Experimental Station of Medicinal Plants, Hiroshima University School of Medicine, Japan (Table Ⅰ). Two plants were used for each sample

Table Ⅰ. Plant Materials used in the Present Experiment

Plant specied	Source	
Angelica acutiloba KITAGAWA var. *acutiloba*	Wakayama (Fuki), Japan	① ②
	Nara (Tenkawa), Japan	① ②
	Nara (Shimoichi), Japan	① ②
A. acutiloba KITAGAWA var. *iwatensis* HIKINO	Gifu (Mt. Ibuki), Japan	① ②
	Toyama (Toga), Japan	① ②
	Aomori (Shirikkapu), Japan	① ②
	Niigata (Yoneyama), Japan	① ②
A. acutiloba KITAGAWA var. *sugiyamae* HIKINO	Hokkaido (Hikimi), Japan	① ②
A. gigas NAKAI	Goharado (Mt. Godai) Korea	① ②
A. acutiloba KITAGAWA var. *lanceolate* OHWI	Hokkaido (Shizunai), Japan	① ②
Ligsticum hultenii FERNALAND	Hokkaido (Samani), Japan	① ②
A. anomala LALL.	Hokkaido (Atsuta), Japan	① ②

"①" and "②" are of the same strain but different individuals.

which are numbered "①" and "②" in Table Ⅰ. Identification of plant species was done on the basis of the morphological characters.

Preparation of total DNA Total DNA was extracted from fresh leaves by the modified cetyltrimetylammonium bromide (CTAB) method. [9] Sampling of each leaf was made in August, 1996. The DNA solution for template of PCR was adjusted to 0.5 ng/ μ L with sterile distilled water.

RAPD Analysis The reaction mixture (10 μ L) for PCR was composed of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001 % gelatin (Sigma), 2.5 mM $MgCl_2$, 200 μ M dNTPs (Perkin Elmer), 0.4 μ M Primer (Toyobo), 0.75 units AmpliTaq DNA polymerase and 0.5 ng template DNA. Thermal cycling was carried out for 45 cycles of 1 minute at 94 ℃, 2 minutes at 45 ℃ and 2 minutes at 72 ℃, followed by one cycle of 7 minutes at 72 ℃. 20 decamer primers, custom synthesized (Toyobo), were used for PCR amplification (Table Ⅱ). The amplified products were subjected to electrophoresis at 75 V in 2 % agarose gels with Tris-acetate-EDTA (TAE) buffer. After staining with ethidium bromide, the gels were examined under UV light.

Table Ⅱ. Primers Used in the Present Experiment

Code of Primer	Sequence	GC content(%)
No.02	5'-ATT TGA TCG C -3'	40
No.03	5'-CAG CTC AAG T -3'	50
No.05	5'-ATG AGT CCA C -3'	50
No.07	5'-ACG CTG ATC A -3'	50
No.08	5'-TGG ACA CTG A -3'	50
No.09	5'-TGG TCA GTG A -3'	50
No.12	5'-GCA GAG CAT C -3'	50
No.15	5'-ACT CCG CAG T -3'	60
No.16	5'-CCG ACA GCT T -3'	60
No.20	5'-TGG TCC CTG A -3'	60
No.21	5'-TGG TCG CTG A -3'	60
No.22	5'-TGG CCA CTG A -3'	60
No.23	5'-TGG GCA CTG A -3'	60
No.25	5'-TGC GGA CGT C -3'	70
No.29	5'-GAG TGC GCA C -3'	70
No.30	5'-CCG GAC TGA G -3'	70
No.31	5'-CGC GGA CGA T -3'	70
No.34	5'-CGG GAA CCG A -3'	70
No.36	5'-GTC ACT CCC C -3'	70
No.37	5'-GGT GGG CAG A -3'	70

RESULTS AND DISCUSSION

All twenty primers used in the present experiment generated clear fragment patterns.

In the present experiment, intraspecific variation was not found among the *A. acutiloba* var. *acutiloba* plants.

The fragment patterns of *A. acutiloba* var. *acutiloba*, *A. acutiloba* var. *iwatensis*, *A. acutiloba* var. *sugiyamae* and *A. acutiloba* var. *lanceolate* generated by the primer No. 7 or No. 9 were generally similar to those of the rest of the species tested. However, the bands at 0.45 kb and 1.6 kb observed in the fragment profiles of the above four species by primer No. 7 were not seen in those of *A. gigas*, *L. hultenii* and *A. anomala*. (Fig. 1, arrows). When primer No. 9 was used as in the case of No. 7 primer, the above 4 varieties, *A. acutiloba* var. *acutiloba*, *A. acutiloba* var. *iwatensis*, *A. acutiloba* var. *sugiyamae* and *A. acutiloba* var. *lanceolate* showed the characteristic bands, 0.9 kb and 1.05 kb which were not detected in other varieties (Fig. 2).

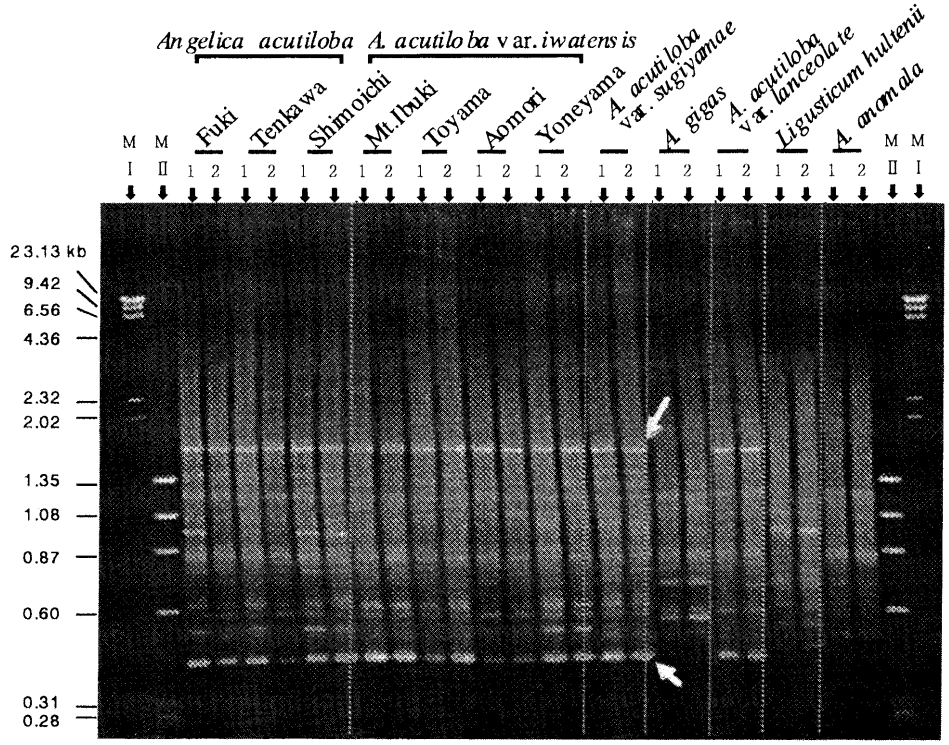

Fig. 1. RAPD profiles generated by primer No. 7.
M I : λ/*Hin*d III digest, M II : φ X174/*Hae* III digest
"1" and "2" are from different individual of the same strain.

Fig. 3 shows the fragment patterns of amplified DNAs produced by using the primer No. 21. The three strains of *A. acutiloba* var. *acutiloba* showed almost the same fragment patterns.

The major band at 1.1 kb present in the fragment pattern of *A. acutiloba* var. *acutiloba* was not observed in that of *A. acutiloba* var. *sugiyamae*. This band may be a useful marker band for distinguishing *A. acutiloba* var. *acutiloba* from *A. acutiloba* var. *sugiyamae*. The fragment patterns of *A. anomala* which had been considered a parent plant of *A. acutiloba* var. *sugiyamae* were different from these of *A. acutiloba* var. *acutiloba* and *A. acutiloba* var. *sugiyamae*. So the results suggested

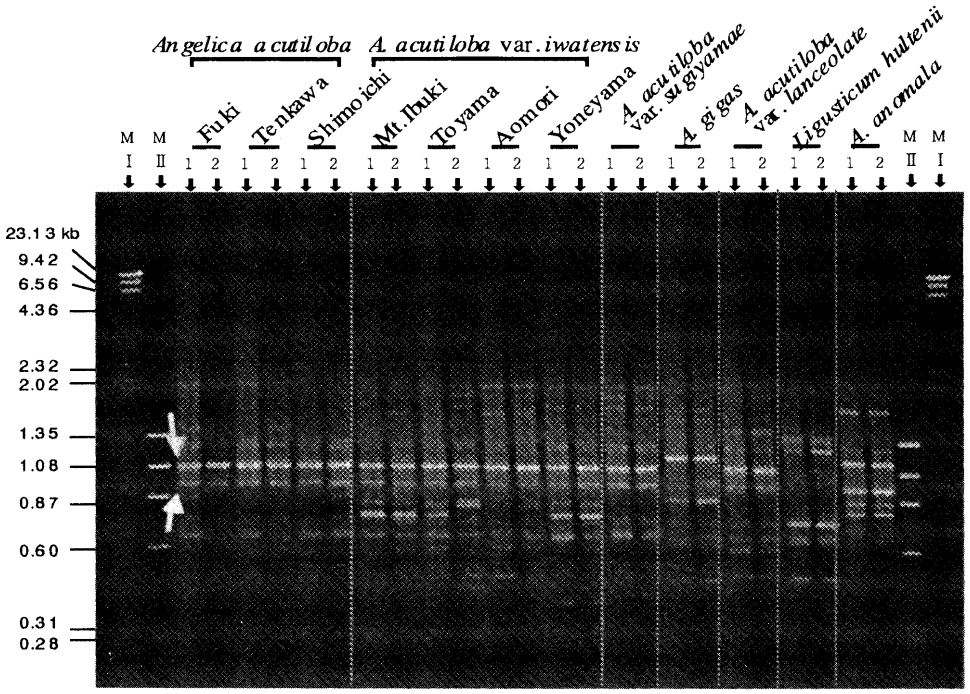

Fig. 2. RAPD profiles generated by primer No. 9.
M I : λ/*Hin*d Ⅲ digest, M Ⅱ : φ X174/*Hae* Ⅲ digest
"1" and "2" are from different individual each other.

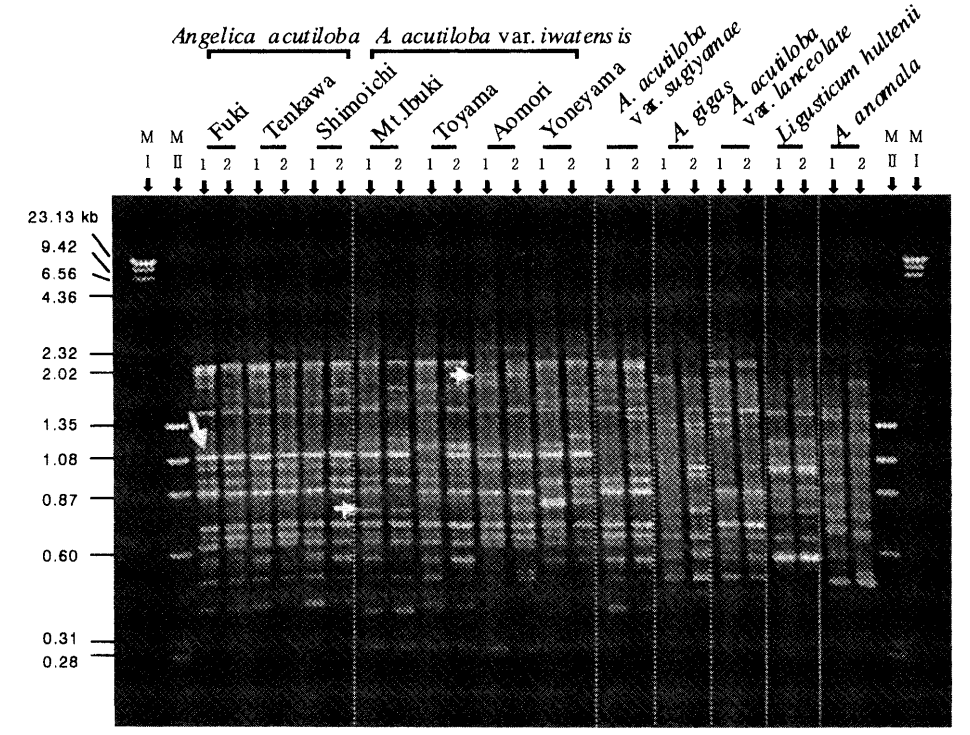

Fig. 3. RAPD profiles generated by primer No. 21.
M I : λ/*Hin*d Ⅲ digest, M Ⅱ : φ X174/*Hae* Ⅲ digest
"1" and "2" are from different individual each other.

that *A. anomala* did not take part in the formation of *A. acutiloba* var. *sugiyamae*. On the other hand, the fragment patterns of *A. acutiloba* var. *lanceolate* were quite similar to those of *A. acutiloba* var.*sugiyamae*, implying that *A. acutiloba* var. *sugiyamae* was closely related to *A. acutiloba* var. *lanceolate*.

A. acutiloba var. *iwatensis* and *A. acutiloba* var. *acutiloba* showed generally the same fragment patterns implying very close genetic relations between *A. acutiloba* var. *iwatensis* and *A. acutiloba* var. *acutiloba*. Partial differences in the pattern among the four strains of *A. acutiloba* var. *iwatensis*, such as the band at 0.75 kb of strain Mt. Ibuki or the band at 1.85 kb of strain Aomori might be due to polymorphisms among the geographic variations of *A. acutiloba* var. *iwatensis*.

The fragment patterns of *A. gigas* were different from those of other *Angelica* plants implying that *A. gigas* was of a different taxon according to the RAPD profile.

The results of RAPD analysis obtained in this study may provide a useful means for the classification of *A. acutiloba* var. *acutiloba* and its varieties.

Acknowledgments The authors thank Mr. T. Watanabe, Medicinal Plant Garden, School of Pharmaceutical Sciences, Kitasato University, Dr. S. Isoda, School of Pharmaceutical Sciences, Showa University, Mr. H. Fujino, Medicinal Plant Garden, Toyama Medical and Pharmaceutical University, Mr. S. Fukuda, Fukuda Shoten, and Mr. Y. Shimada, Mitsuboshi Seiyakusyo, for their kindly gift of plant materials. We are also grateful to the Research Center for Molecular Medicine, Hiroshima University School of Medicine for the use of their facilites. This research was supported, in part, by the Ministry of Health and Welfare, Science Research Fund Subsidy granted to Japan Health Science Foundation.

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