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Full Length Research Paper

Genetic variability of *Artemisia capillaris* (Wormwood capillary) by random amplified polymorphic DNA (RAPD) in Terengganu State, Malaysia

Sayed M. Zain Hasan, Mohammad Shafie B. Shafie* and Ramisah M. Shah

Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu, 21030 Mengabang Telipot, Kuala Terengganu, Malaysia.

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The genetic variability among individuals of *Artemisia capillaris* from state of Terengganu, Malaysia was examined by using the random amplified polymorphic DNA (RAPD) technique. The samples were collected from differences regional in Terengganu State. The genomic DNA was extracted from the samples leaves. Fifthty-seven oligonucleotide primers were screened and ten primers were selected (OPA 04, OPA 09, OPA 16, OPA 17, OPA 18, OPG 03, OPG 05, OPG 09, OPG 15 and 391) to amplify DNA from five samples of *A. capillaris*. A total of 335 RAPD fragments (RAPDs) with all polymorphic fragments (100%) with size ranging from 150 – 3000 bp were scored from the population. Genetic distance for samples ranges from 0.0000 to 0.2600.

Key words: *Artemisia capillaris*, genomic DNA, genetic variability, RAPD.

INTRODUCTION

Artemisia capillaris is a species from class Magnoliopsida and family Asteraceae. *A. capillaris* is also known as wormwood or wormwood capillary in Europe (ShamanShop.net, 2002), Yin Chen Hao in China (ShamanShop.net, 2002; Dharmananda, 2002) and the common names are Pokok Ru Nyamuk, Pokok Daun Ru, Pokok Halau Nyamuk and Pokok Jata Hitam in Terengganu. *A. capillaris* is a member of the parsley family; it is a strong-smelling, fennel-like, annual plant reaching a height of about 4 feet or more. *A. capillaris* was introduced to this country from Asia (Duane and Martha, 2006; Plant for Future, 2006), America and Europe (Duane and Martha, 2006). It is cultivated in China, Japan, Taiwan (ShamShop.net, 2002) and to some extent in this country. Small acreages of *A. capillaris* have been grown successfully as a commercial crop.

Artemisia plants, particularly *Artemisia iwayomogi*, *Artemisia capillaris*, *Artemisia princeps* and *Artemisia argyi* are important medicinal materials that are utilized in traditional Asian medicines (The Korean Herbal Pharmacopoeia, 2002). *Artemsia* herbs are used for various purposes, such as medicine, food, spices, and ornamenttation (Lee et al., 2006). This *A. capillaris* considered to be a bitter and cooling herb, clearing "damp heat" from the liver and gall ducts and relieving fevers (Chevallier, 1996). *A. capillaris* widely used in Asia to prevent and treat neonatal jaundice, also effective remedy for liver problems, works on stomach and spleen (Chevallier, 1996; Huang et al*.,* 2003; Abestmall, 2006). Modern research has confirmed that the plant has a tonic and strengthening effect upon the liver, gallbladder and digestive system (Chevallier, 1996). The studies from Hong et al. (2004) suggest that *A. capillaris* can be a useful therapeutic agent for endotoxin-induced inflammation and injuries of the liver.

Owing to recent innovation in molecular biological techniques, such as polymerase chain reaction (PCR) and DNA automated sequencing, nucleic acid data are becoming more and more important in biology (Hillis et al*.,* 1996). One of the modern marker techniques for studying genetic variability is random amplified polymorphic DNA, RAPD (Williams et al*.,* 1990). The technique requires no prior knowledge of the genome and it needs only a small amount of DNA (Hadrys et al*.,* 1992). Using this technique polymorphism can be detected in closely related organism.

*Corresponding author. E-mail: msshafie@yahoo.com.my.

MATERIALS AND METHODS

Sample collections

The samples of *A. capillaris* were collected from the area in state of Terengganu. 5 samples were collected randomly, each sample was collected from differences regional. Figure 1 had shown the sampling sites.

DNA extraction

Sarkosyl nitrogen method was used for DNA extraction method. Fresh and healthy leaves were placed in a mortar. Then, liquid nitrogen was added and the material were crushed to a fine powder with a pestle. The powders were added to 3 ml of DNA extraction buffer in a fresh mortar and were homogenized. Then, 1 ml phenol was added and homogenized again. The mixed power was transferred to a test tube (with cap), 2 ml phenol was added again and was centrifuged for 5 min to separate phase. The upper aqueous phase was transferred into new tube. Then, two volume of ice-cool 95% ethanol was added to the aqueous phase and was centrifuged for 5 min at 12,000 rpm. Then, the ethanol was poured from tube. Precipitated DNA was washed with ice-cool 70% ethanol. DNA was dissolved in 0.5 ml of TE and 2 µg RNAase was added. It was incubated at 37°C for 15 to 30 min. 0.25 ml phenol and 0.25 ml chloroform was added and shaking well. Then, it was centrifuged and the upper aqueous phase was transferred into new tube. Then, two volume of ice-cool 95% ethanol was added to the aqueous phase for ethanol precipitated DNA and was centrifuged for 5 min at 12,000 rpm. Then, the ethanol was poured from tube. Precipitated DNA was washed with ice-cool 70% ethanol. Finally, it was dissolved in 0.2 to 0.5 ml of TE.

Gel electrophoresis

Product was separated by agarose gel electrophoresis through a 1.0% of agarose gel in 1.0 X TBE (10 mM Tris, 1mM EDTA pH 8.0, 1 µl EtBr). Electrophoresis was started at 75 volts for 1 h for genomic DNA and 55 volts for 1 to 2 h for PCR amplifications. Then the gels were photographing with Image Master VDS.

Screening of RAPD primers

Operon and UBC (University of British Columbia, Canada) 10mers were used in this study. 57 RAPD primers were screened from a single individual. Primers that have the basic of sharpness, clarity of the profile and the existence of polymorphism were chosen for further study (D'Amato and Corach, 1997).

DNA amplification of selected primers

The selected primers were used to amplify the genomic DNA for five samples of *A. capillaris*. The total reaction volume of 25 µl was used with the final concentration containing 1.0 × of reaction buffer, 50 ng genomic DNA, 4.0 mM magnesium chloride (Fermentas), 2 U *Taq* DNA polymerase (Fermentas), 0.4 mM dNTP-mixture (Fermentas) and primer 10 pM. The DNA was amplified by using a DNA Engine Thermal Cycler with Dual Alpha Unit (BIO-RAD). The amplification was programmed at 45 cycles for 30 s of denaturation at 94°C, 30 s of annealing temperature at 36°C, 1 min of primers extension at 72°C and final extension of 2 min at 72°C.

PCR product was electrophoreses on 1.5% (w/v) agarose gel in 1.0 × TBE buffer at 55 V for 1 to 2 h depending on the size of amplified fragment from each primer. The gel was stained in 1 µg/mL ethidium bromide (EtBr) for 20 to 30 min. Then the gel was washed with distilled water for 5 to 10 min and photographed with Image Master VDS. A 100 ladder plus maker (Fermentas) was used as a molecular weight standard. Each set of PCR products included with the negative control to ensure that the observed banding pattern was reproducible, repeatable and uncontaminated before scoring.

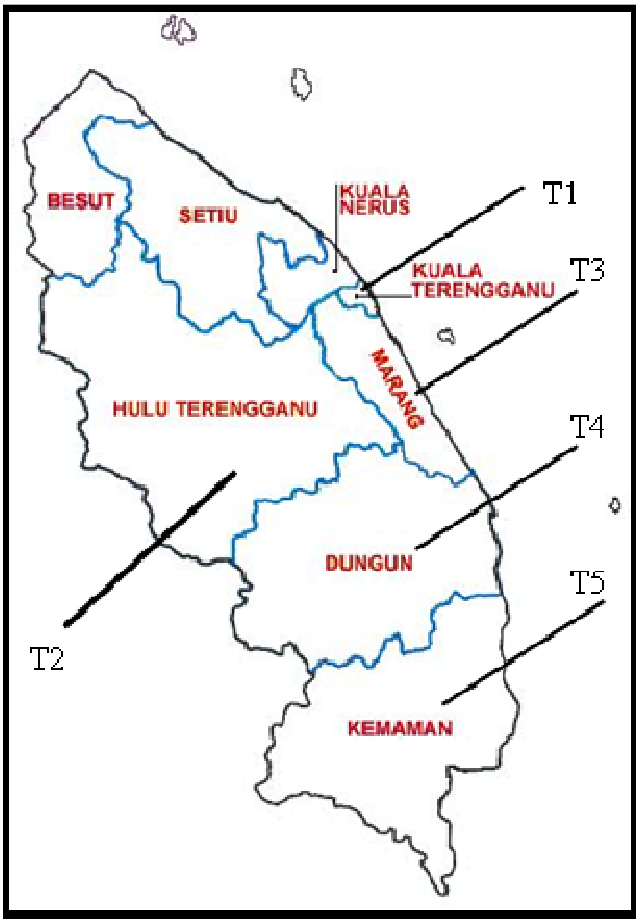

Figure 1. Sampling site in state of Terengganu, Malaysia.

Data analysis

The RAPDistance Package Software Version 1.04 (Armstrong et al., 1994) and Numerical taxonomy and Multivariate Analysis System (NTSYS-pc) were used in this study. The molecular weights of band were estimated based on the standard bands from Gene Ruler DNA Ladder Marker. The presence of band was scored from the photograph. Only clear and reproducible bands were given consideration. The bands were considered as polymorphic when they were absent in some sample in frequency greater than 1% (Jorde, 1995) and change in band intensity were not considered as polymorphism. Clear bands were scored as present (1) or absent (0) at particular position or distance migrated on the gel. The data matrix of 1's and 0's was prepared from the scorable bands and was entered into the data analysis package (Armstrong et al., 1994). The indices of similarity were calculated across all possible pair wise comparisons of individual within and among population following the method of Nei and Li (1979). The formula is:

$$SI = 2NXY/(NX + NY)$$

Table 1. Number of fragments, size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic fragments of *Artemisia capillaris* generated by the primers.

Primer	Primer sequence (5' to 3')	No. of fragments	Size of fragments (bp)	Total no. of fragments	No. of polymorphic fragments	Polymorphic fragments (%)
OPA 04	AATCGGGCTG	0 – 7	300 – 1750	9	9	100.00
OPA 09	GGGTAACGCC	0 – 11	350 – 2500	14	14	100.00
OPA 16	AGCCAGCGAA	0 – 11	650 – 3000	11	11	100.00
OPA 17	GACCGCTTGT	0 – 10	450 – 2000	12	12	100.00
OPA 18	AGGTGACCGT	0 – 10	750 – 2500	12	12	100.00
OPG 03	GAGCCCTCCA	0 – 7	300 – 1200	10	10	100.00
OPG 05	CTGAGACGGA	0 – 14	250 – 2500	20	20	100.00
OPG 09	CTGACGTCAC	0 - 11	250 – 1350	13	13	100.00
OPA 13	CTCTCCGCCA	0 – 14	250 – 1750	17	17	100.00
391	GCGAACCTCG	0 - 12	150 - 2500	12	12	100.00
Total				**335**	**335**	**100.00**

Table 2. Similarity Index of *Artemisia capillaris* samples.

Sample	T1	T2	T3	T4	T5
T1					
T2	0.749881				
T3	0.000000	0.000000			
T4	0.514594	0.695298	0.000000		
T5	0.524602	0.685656	0.000000	0.756743	

See Figure 1 for the sampling sites, T1 to T5.

NXY is the number of RAPD bands shared in common between individuals X and Y, NX and NY are the total number of bands scored in X and Y, respectively.

The index similarity was used to calculate the genetic distance values and to construct the dendrogarm. The dendrogarm provides a visual representation of the relationship of difference population of *Artemisia capillaris*. The dendrograms were constructed using the Unweighted Pair-Group Method of Arithmetic (UPGMA) employing Sequential, Agglomerative, Hierarchical, and Nested Clustering (SAHN) from NTSYS-pc program (Rohlf, 1994).

RESULTS AND DISCUSSION

Screening of RAPD primers

Chosen suitable primers are very important process for PCR-RAPD to get clear and good band. Fifty-seven primers from the Operon and UBC (University of British Columbia, Canada) 10mers were used during the screening of the RAPD primers. The banding patterns which were clear and reproducible bands selected. The primers selected are OPA 04, OPA 09, OPA 16, OPA 17, OPA 18, OPG 03, OPG 05, OPG 09, OPG 15 and 391. These primers were selected to generate RAPD pattern of genomic DNA for all individuals of *A. capillaris* samples.

RAPD Profiles

RAPD is one of the genetic marker studies that had been used in genetic study. RAPD technique had been used in genetic study for wheat (Devos and Gale, 1992) and also had used for filogenetic relations for rice species (Ishii, 1996). Ten primers were applied on five individuals of *A. capillaris* for DNA amplification. The results showed that different primers generated different fragment numbers and length of DNA amplification products as shown in Table 1. 335 fragments were generated by the ten primers. The percentage of polymorphism generated for all primer was 100%. The size of bands generated by the primers ranged from 150 to 3000 bp.

Dendrogram analysis

The dendrogram produced of *A. capillaris* (Figure 2) shows two main clusters. The first cluster consisted of individual's number T1, T2, and T3. The second cluster consisted of individual number T4 and T5. Genetic distance levels of the *A. capillaris* samples ranged from 0.000 to 0.260. The UPGMA cluster analysis of *A. capilla-*

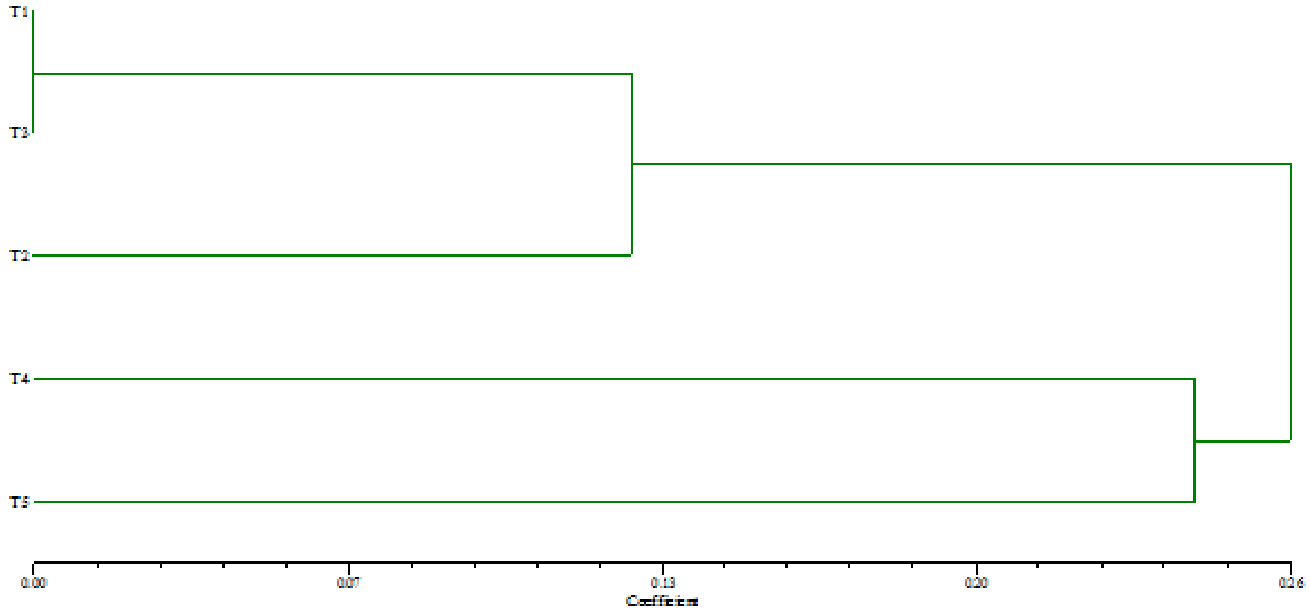

Figure 2. Dendrogram based on the genetic distance generated from Nei and Li's indices of *Artemisia capillaris* from Terengganu State. Data of RAPD generated by primer OPA 04, OPA 09, OPA 16, OPA 17, OPA 18, OPG 03, OPG 05, OPG 09, OPA 13 and 391. See Figure 1 for the sampling sites, T1 to T5.

ris was based on the genetic distance generated from Nei and Li's. The average similarity index for *A. capillaris* was from 0.000000 to 0.756743. The similarity indices among individuals in each sample of *A. capillaris* are represented in Table 2. This high similarity in samples indicated low variability between individuals in that area. The similarity indices show the relationship of the individual in each sample. Higher similarity indices suggest that the individuals in the population have closer genetic relation among them, while lower similarity indices suggest that the individuals in the population have farther genetics relation.

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REFERENCES

Abestmall (2006). Artemisia Capillaris Herb (Yin Chen Hao) C/S 16 oz: V. online. http://www.abestmall.com/best/1862375.html Accessed on 22sd November 2006.

Armstrong JS, Gibbs AJ, Peackall R, Weiller G (1994). The RAPDistance Package, Australian National University, Camberra, Australia, http://life.anu.au/molcular/software/rapd.html.

Chevallier A (1996). The Encyclopedia of Medicinal Plants Dorling Kindersley. London. ISBN 9-780751-303148. An excellent guide to over 500 of the more well known medicinal herbs from around the world.

D'amato ME, Corach D (1997) Population genetic structure in the fresh water *anomuran aesla jujuyana* by RAPD analysis.

Devos KM, Gale MD (1992). The Random Amplified Polymorphic DNA markers in wheat. Theor. Appl. Genet. 84: 567-572.

Dharmananda S (2002). CHING-HAO and the Artemisias Used in Chinese Medicine Ph.D., Director, Institute for Traditional Medicine, Portland, Oregon.

Duane M, Martha AD (2006). Instituto de Alternativas para Sobresalientes. Lulu, Inc. Wormwoods: Botanical and Common Names online. http://www.innvista.com/health/herbs/wormwood.htm.

Hadrys H, Balick M, Shierwater B (1992). Aplications of Random Amplified Polymorphic DNA (RAPD) in Mol. Ecol. 1: 55-63.

Hillis DM, Moritz C, Mable BK (1996). Molecular Systematics, 2nd edn. Sinauer Associates, Sunderland.

Hong SH, Seo SH, Lee JH, Choi BT (2004). The aqueous extract from *Artemisia capillaris* Thunb. inhibits lipopolysaccharide-induced inflammatory response through preventing NF-κB activation in human hepatoma cell line and rat liver. Intl. J. Mol. Med. 13: 717-720.

Huang W, Zhang J, Moore DD (2003). A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. J. Clin. Invest. 113: 137-143 (2004). doi:10.1172/JCI200418385.

Ishii T (1996). Philogenetic relationship in A-genome species as revealed by RAPD analysis. Genes Genet. Syst. 71:195-201.

Jorde LB (1995). Population specific genetic markers and diseases In Biology and Biotechnology: A comprehensive desk reference, ed. Meyesr RA, New York: VCH Publisher, Inc. pp 724-728.

Lee MY, Doh EJ, Park CH, KIM YH, KIM ES, Ko BS, Oh SE (2006). Development of SCAR Marker for Discrimination of *Artemisia princes* and *A. argyi* from Other *Artemisia* Herbs. Biol. Pharm. Bull. 29(4): 629-633.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Acad. Sci. USA 7: 5269-5273.

Plants For A Future, (2004). Edible, medicinal, and useful plants for a healthier word: *Artemisia capillaris* - Thunb. Yin Chen Hao online. http://www.pfaf.org/database/plants.php?Artemisia+capillaris.

Rohlf FJ (1994). NTSYS-pc Numerical taxonomy and multivariate analysis system. Exeter Software, New York.

ShamShop.net (2002). Artemisia Capillaris Herb (Yin Chen Hao) Cut &

Sifted 500 gm (1.17 lb): V online. http://www.shamanshop.net/store/proddetail.cfm/ItemID/160526.0/CategoryID/15500.0/SubCatID/3770.0/file.htm.
The Korean Herbal Pharmacopoeia (2002). Korea Food & Drug Administration, Seoul, p 307, 383, 461, 505.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res. 18: 6531-6535.