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Full Length Research Paper

Genetic variation in (*Artemisia capillaries)* using RAPD and ISSR markers

***Abdullah Krishnan, Robert Fernandes and Bernand Majid**

Department of Agrotechnology, Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu (UMT), 21030 Kuala Terengganu, Terengganu, Malaysia.

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Artemisia capillaris **(Thunb.) commonly known as wormwood or sagebussh is under-exploited plant. Genetic variation between *A. capillaris* was evaluated using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. Among these markers, RAPD primers generated 291 amplification products of which 108 were polymorphic and ISSR markers produced 283 amplification products, out of which 54 were polymorphic. RAPD fingerprinting detected more polymorphic loci (95.60%) than the ISSR fingerprinting (66.67%). Similarity, index values ranged from 0.1579 to 0.8189 (RAPD), 0.5686 to 0.9016 (ISSR) and 0.3933 to 0.8468 (combined RAPD and ISSR) and mean similarity index value of 0.4739, 0.7457 and 0.6174 for RAPD, ISSR and combined (RAPD and ISSR) data, respectively. RAPD and ISSR marker systems were found to be useful for the genetic diversity studies in *A. capillaris* and to identify the variation.**

Key words: *Artemisia capillaris*, genetic variation, polymorphism, random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR).

INTRODUCTION

Understanding of the genetic variation within and between populations is essential for the establishment of effective and efficient methods for conservation of the plants. *Artemisia capillaris* Thunb. (fam: Asteraceae) or vernacularly known as Pokok Ru Nyamuk is a perennial herb and commonly grown as an ornamental plant in pot or home garden Malaysia.

Artemisia is one of the larger genera in the family Asteraceae and the largest genus in the tribe Anthemideae, which comprises from 200 to more than 500 taxa at the specific or subspecific level (Bremer and Humphries, 1993), distributed in 5 sections or subgenera (Torrell et al., 1999). Many Artemisia species have a high economic value in several fields. Traditionally, *A. capillaris* has been used for various purposes including in medicine, food, spices, and ornamentation (Lee et al., 2006). It was reported useful as therapeutic agent for endotoxin-induced inflammation and injuries of the liver (Hong et al., 2004), as antiplatelet aggregation activity and against HIV replication in cells (Wu et al., 2001). *Artemisia* species are most commonly shrubs and more rarely annual or biennial herbs. Thus, perennial plants largely dominate the genus; only 5% of the taxa (approximately 10 species) are annual or biennial species. Leaves are alternate or sparse, usually more or less divided (exceptionally entire, such as in *Artemisia dracunculus* and *Artemisia cana*), with extremely variable shapes and dimensions (Valles and McArthur, 2001).

The high number of taxa and the usefulness of *A. capillaris* have attracted the interest of the researchers. Genetic diversity in crop species can be determined using morphological and agronomic characteristics, isozyme and DNA marker analysis (Koornneef, 1990; Reiter et al., 1993; Liu, 1997). However, morphological grouping may be based on characteristics which could be strongly affected by environmental factors (Liu, 1997). The morpho-agronomic markers may not reflect true genetic identities and diversities (Ferdinandez et al., 2001). The studies regarding genetic variation of *A. capillaris* are very scarce. There are only a few related reports, including the sequence characterized amplified regions (SCAR) markers of Lee et al. (2006).

*Corresponding author. E-mail: dr.krishnan1@yahoo.co.uk

Table 1. Details of *A. capillaris* samples used in the present study.

S/N	Accession No.	Origin
1	N1	Tampin, Negeri Sembilan
2	N2	Rembau, Negeri Semibilan
3	N3	Port Dickson, Negeri Sembilan
4	N4	Seremban, Negeri Sembilan
5	N5	Kuala Pilah, Negeri Sembilan

developed from RAPDs. Likewise, Lee et al. (2008) used multiplex PCR method for discrimination of *Artemisia iwayomogi* from other *Artemisia* herbs. Other reports described genetic fingerprinting studies in *Artemisia Annua* L. and *Artemisia herba-alba* by using RAPDs and inter simple sequence repeat (ISSRs), respectively (Sangwan et al., 1999; Haouari and Ferchichi, 2008).

Genetic studies are fundamental for the management and conservation of this species. The use of molecular markers is a powerful tool in the genetic study of populations. The use of DNA marker, such as RAPD and ISSR represents an alternative method in detection of polymorphism. Being a fast and sensitive method, RAPD can be quickly and efficiently applied to identify useful polymorphisms (Ko et al., 1998; Doldi et al., 1997). According to Pradeep et al. (2002) ISSR technique is also considered simple, fast, cost-effective, highly discriminative and reliable.

RAPD is preferred as an effective method for identification of genetic variation within and among populations in forest trees (Tsuda et al., 2004; Hardy et al., 2006). RAPD is also suitable for the analysis of genetic diversity in natural populations of dioecious species (Ferreira and Grattapaglia, 1996). Moreover, RAPDs show levels of polymorphism similar to isoenzyme markers and it can target amplifying a large number of loci (Lee et al., 2002). RAPD had been used in genetic variation between Pakistani wheat (*Triticum aestivum* L.) genotypes (Bhutta et al., 2006), in sweet potato (Lin et al., 2009) and in germplasms study in Rhizome Lotus (*Nelumbo nucifera* Gaertn. spp. *nucifera*) (An et al., 2009).

ISSRs are highly variable, require less investment in time, money and labor than other methods (Wolfe and Liston, 1998; Harris, 1999). ISSR can generate higher percentages of polymorphic loci than other PCR methods (Esselman et al., 1999). These can serve as an efficient tool for phylogenetic studies (Ajibade et al., 2000; Galvan et al., 2003). ISSRs had reported the used in the studies of cultivated species to produce genetic linkage maps (Kojima et al., 1998; Cekic et al., 2001) and to determine the relatedness of lines of agriculturally important species (Chowdhury et al., 2002; Mondal, 2002). ISSR analysis involves the PCR amplification of regions between adjacent, inversely oriented microsatellites, using a single simple sequence repeat (SSR) motifs (dinucleotide, trinucleotide, tetranucleotide or pentanucleotides) (Zietkiewicz et al., 1994). RAPD and ISSR markers have been used both for DNA fingerprinting (Martín and Sánchez-Yélamo, 2000) and population genetic studies (Wolfe et al., 1998). A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner. The objectives of this study are: (1) To study and compare genetic diversity among genotypes, using RAPD and ISSR markers and (2) to evaluate of the degree of polymorphism generated from each technique as a pre-requisite for their applicability to population genetics studies in *A. capillaris*.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of five *A. capillaris* samples were collected from five different areas in Negeri Sembilan, Malaysia through the root or rhizome cutting (Table 1). The collections were identified as N1, N2, N3, N4 and N5 and grown in pots under the black polythene net for use in the study. Genomic DNA was extracted by using Sarkosyl Method from the leaves of the five sample plants according to Jofuku and Goldberg (1988).

PCR analysis

A total of fifty seven 10-mer oligonucleotides with arbitrary sequence from Operon Technologies (kits A, B, D and G) and University British Columbia (UBC) were used in RAPD analysis (Table 2) and twenty five primers from UBC based on dinucleotide, tetranucleotide or pentanucleotide repeats were used in ISSR analysis (Table 3). The selected ten primers were used in both (RAPD and ISSR) PCR amplification.

RAPD analysis

The total reaction volume of 25 µl was used with the final concentration containing 1 × of reaction buffer included the concentration of genomic DNA 50 ng, Fermentas magnesium chloride 4.0 mM, Fermentas Taq DNA polymerase 2 units, Fermentas dNTP-mixture 0.4 mM 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.

Table 2. Code, sequence and nucleotide length of primers used in the RAPD analysis.

S/N	Primer code	Primer sequence 5' to 3'	Nucleotide length	S/N	Primer code	Primer sequence 5' to 3'	Nucleotide length
1	OPA 01	CAGGCCCTTC	10-mers	29	*OPG13	CTCTCCGCCA	10-mers
2	OPA 02	TGCCGAGCTG	10-mers	30	301	CGGTGGCGAA	10-mers
3	OPA 03	AGTCAGCCAC	10-mers	31	302	CGGCCCACGT	10-mers
4	*OPA 04	AATCGGGCTG	10-mers	32	308	AGCGGCTAGG	10-mers
5	OPA 05	AGGGGTCTTG	10-mers	33	309	ACATCCTGCG	10-mers
6	OPA 06	GGTCCCTGAC	10-mers	34	310	GAGCCAGAAG	10-mers
7	OPA 07	GAAACGGGTG	10-mers	35	311	GGTAACCGTA	10-mers
8	OPA 08	GTGACGTAGG	10-mers	36	312	ACGGCGTCAC	10-mers
9	*OPA 09	GGGTAACGCC	10-mers	37	313	ACGGCAGTGG	10-mers
10	OPA 10	GTGATCGCAG	10-mers	38	314	ACTTCCTCCA	10-mers
11	OPA 11	CAATCGCCGT	10-mers	39	315	GGTCTCCTAG	10-mers
12	OPA 12	TCGGCGATAG	10-mers	40	317	CTAGGGGCTG	10-mers
13	OPA 13	CAGCACCCAC	10-mers	41	319	GTGGCCGCGC	10-mers
14	OPA 14	TCTGTGCTGG	10-mers	42	321	ATCTAGGGAC	10-mers
15	OPA 15	TTCCGAACCC	10-mers	43	324	ACAGGGAACG	10-mers
16	*OPA 16	AGCCAGCGAA	10-mers	44	327	ATACGGCGTC	10-mers
17	*OPA 17	GACCGCTTGT	10-mers	45	328	ATGGCCTTAC	10-mers
18	*OPA 18	AGGTGACCGT	10-mers	46	328	ATGGCCTTAC	10-mers
19	OPA 19	CAAACGTCGG	10-mers	47	329	GCGAACCTCC	10-mers
20	OPA 20	GTTGCGATCC	10-mers	48	330	GGTGGTTTCC	10-mers
21	OPB07	GGTGACGCAG	10-mers	49	352	CACAACGGGT	10-mers
22	OPB15	GGAGGGTGTT	10-mers	50	353	TGGGCTCGCT	10-mers
23	OPD03	GTCGCCGTCA	10-mers	51	354	CTAGAGGCCG	10-mers
24	OPG02	GGCACTGAGG	10-mers	52	355	GTATGGGGCT	10-mers
25	*OPG03	GAGCCCTCCA	10-mers	53	356	GCGGCCCTCT	10-mers
26	*OPG05	CTGAGACGGA	10-mers	54	357	AGGCCAAATG	10-mers
27	OPG08	TCACGTCCAC	10-mers	55	360	CTCTCCAGGC	10-mers
28	*OPG09	CTGACGTCAC	10-mers	56	*391	GCGAACCTCG	10-mers
				57	392	CCTGGTGGTT	10-mers

ISSR analysis

The total reaction volume of 25 µl was used with the final concentration containing 1 × reaction buffer, 50 ng genomic DNA, magnesium chloride 3.0 mM, *Taq* DNA polymerase (2.5 units), 0.4 mM dNTPs and 10 pM primer.

The DNA was amplified by using a DNA Engine Thermal Cycler with Dual Alpha Unit (BIO-RAD). The amplification was programmed for 1 cycle for 2 min at 93°C, 2 min at 35°C and 2 min at 72°C. Then, 93°C at 1 min for denaturation, 2 min of annealing temperature at 36°C, 2 min of primers extension at 72°C with 38 cycles and final extension of 10 min at 72°C.

Agarose gel electrophoresis

PCR product was electrophoresed on 1.5% (w/v) agarose gel in 1 × TBE buffer at 55 V for about 1.5 h depending on the size of amplified fragment from each primer. The gel was stained in 1 µg/ml ethidium bromide for 20 to 30 min and photographed with Image Master VDS. The presence of band was scored from the photograph.

Data analysis

The data were analyzed by using the numerical taxonomy and multivariate analysis system (NTSYS-pc) version 2.1. The dendrogram was constructed using UPGMA cluster analysis based on the genetic distance of Nei and Li (1979).

RAPD and ISSR data were scored for presence (1), absence (0) or as a missing observation. These bands were considered as polymorphic when they were absent in some sample in a frequency greater than 1% (Jorde, 1995) and changes in band intensity were not considered as polymorphism. 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 similarity indices were calculated across all possible pair wise comparisons of individuals within and among the population, following the method of Nei and Li (1979). The formula was:

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

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 similarity index was used to calculate the genetic distance values and to construct

Table 3. Code, sequence and nucleotide length of primers used in the ISSR analysis.

S/N	Primer code	Primer sequence 5' to 3'	Nucleotide length
1	801	ATATATATATATATATT	17-mers
2*	807	AGAGAGAGAGAGAGAGT	17-mers
3*	809	AGAGAGAGAGAGAGAGG	17-mers
4	811	GAGAGAGAGAGAGAGAC	17-mers
5	818	CACACACACACACACAG	17-mers
6	820	GTGTGTGTGTGTGTGTC	17-mers
7*	825	ACACACACACACACACT	17-mers
8	830	TGTGTGTGTGTGTGTGG	17-mers
9*	834	AGAGAGAGAGAGAGAGYT	18-mers
10	836	AGAGAGAGAGAGAGAGYA	18-mers
11*	841	GAGAGAGAGAGAGAGAYC	18-mers
12	848	CACACACACACACACARG	18-mers
13	857	ACACACACACACACACYG	18-mers
14*	862	AGCAGCAGCAGCAGCAGC	18-mers
15*	866	CTCCTCCTCCTCCTCCTC	18-mers
16	870	TGCTGCTGCTGCTGCTGC	18-mers
17*	876	GATAGATAGACAGACA	16-mers
18	878	GGATGGATGGATGGAT	16-mers
19	881	GGGTGGGG TGGGG TG	15-mers
20	888	BDBCACACACACACACA	17-mers
21	890	VHVGTGTGTGTGTGTGT	17-mers
22*	nIssr1	GATAGATAGATAGATA	16-mers
23	nIssr2	GACAGACAGACAGACA	16-mers
24*	nIssr3	CAGCAGCAGCAGCAG	15-mers
25	nIssr4	CAACAACAACAACAA	15-mers

the dendrogarm. The dendrogarm provides a visual representation of the differences in the population of *A. 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

RAPD analysis

A total of 291 amplified fragments were generated by the ten primers from five populations of *A. capillaris* samples. OPA 04 generated 30 fragments, OPA 09 generated 30 fragments, OPA 16 generated 19 fragments, OPA 17 generated 29 fragments, OPA 18 generated 29 fragments, OPG 03 generated 29 fragments, OPG 05 generated 19 fragments, OPG 09 generated 37 fragments, OPG 13 generated 29 fragments and 391 generated 40 fragments respectively. A total of 113 fragments were scored having 108 polymorphic fragments. The percentage of polymorphism generated for all primers was 95.60%. The band size generated by ten primers ranged from 250 to 2500 bp. The results were presented in Table 4.

Dendrogram RAPD

The relationship among the samples of *A. capillaris* are shown by the dendrogram (Figure 1a). The dendrogram produced two main clusters. The first cluster consisted of 4 samples, respectively with two sub clusters (1A and 1B). Sub cluster 1A consisted of N1, N2 and N3 were linked at 0.3533 genetic distance level. Sub cluster 1B consisted of sample N4 at 0.5149 genetic distance level and were linked together with sub cluster 1A at 0.5149 genetic distance level. Second cluster only consisted of sample N5 and were joined together with cluster 1 at 1.3500 genetic distance level. The average similarity index within the samples was 0.4739 ± 0.2651. The similarity indices are shown in Table 7.

ISSR analysis

There were 283 fragments generated by the ten primers. Primer 807 generated 28 fragments, primer 809 generated 21 fragments, primer 825 generated 21 fragments, primer 834 generated 35 fragments, primer 841 generated 38 fragments, primer 862 generated 15 fragments, primer 866 generated 28 fragments, primer

Table 4. RAPD analysis of *A. capillaris*.

Primer	Range of fragment size (bp)	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphism (%)
OPA 04	350 – 1200	11	11	100
OPA 09	350 – 1350	11	11	100
OPA 16	600 – 2500	11	11	100
OPA 17	350 – 2000	12	11	91.67
OPA 18	250 – 1350	11	11	100
OPG 03	300 – 1350	10	8	80
OPG 05	300 – 1200	8	8	100
OPG 09	250 – 1350	14	13	92.86
OPG 13	300 – 966	11	11	100
391	350 – 2000	14	13	92.86
Total	-	113	108	95.60

876 generated 40 fragments, primer nIssr1 generated 30 fragments and primer nIssr3 generated 27 fragments. ISSR primers generated 81 fragments out of which 54 turned out to be polymorphic fragments. The percentage of polymorphism generated for all primer was 66.67%. The fragments were ranged from 250 to 2500 bp. The results are presented in Table 5.

Dendrogram ISSR

The dendrogram constructed for ISSRs also comprised two main clusters (Figure 1b). The first cluster with 3 samples consisted of N1, N2 and N3. These 3 samples were linked together at 0.1460 genetic distance level. Cluster 2 consisted of 2 samples (N4 and N5). These 2 samples were linked together at 0.2583 genetic distance level. Then, clusters 1 and 2 were joined together at 0.3900 genetic distance level. The average similarity index within the samples was 0.7457 ± 0.1164. The similarity indices are shown in Table 7.

Combine RAPD and ISSR

Combination data of both markers (RAPD and ISSR) generated a dendrogram that separated the samples into 2 distinct clusters (Figure 1c). This dendrogram were range from 0.1700 to 0.7400 genetic distance level. The first cluster consisted of two sub clusters (1A and 1B) with 4 samples respectively. Sub cluster 1A consisted of N1, N2 and N3 were linked at 0.254 genetic distance level. Sub cluster 1B consisted only sample N4 at 0.3960 genetic distance level and was linked together with sub cluster 1A at 0.3960 genetic distance level. Second cluster only consisted of sample N5 were linked together with cluster 1 at 0.7400 genetic distance level. The similarity index for combine markers RAPD and ISSR ranged from 0.3933 to 0.8468 (Table 7).

DISCUSSION

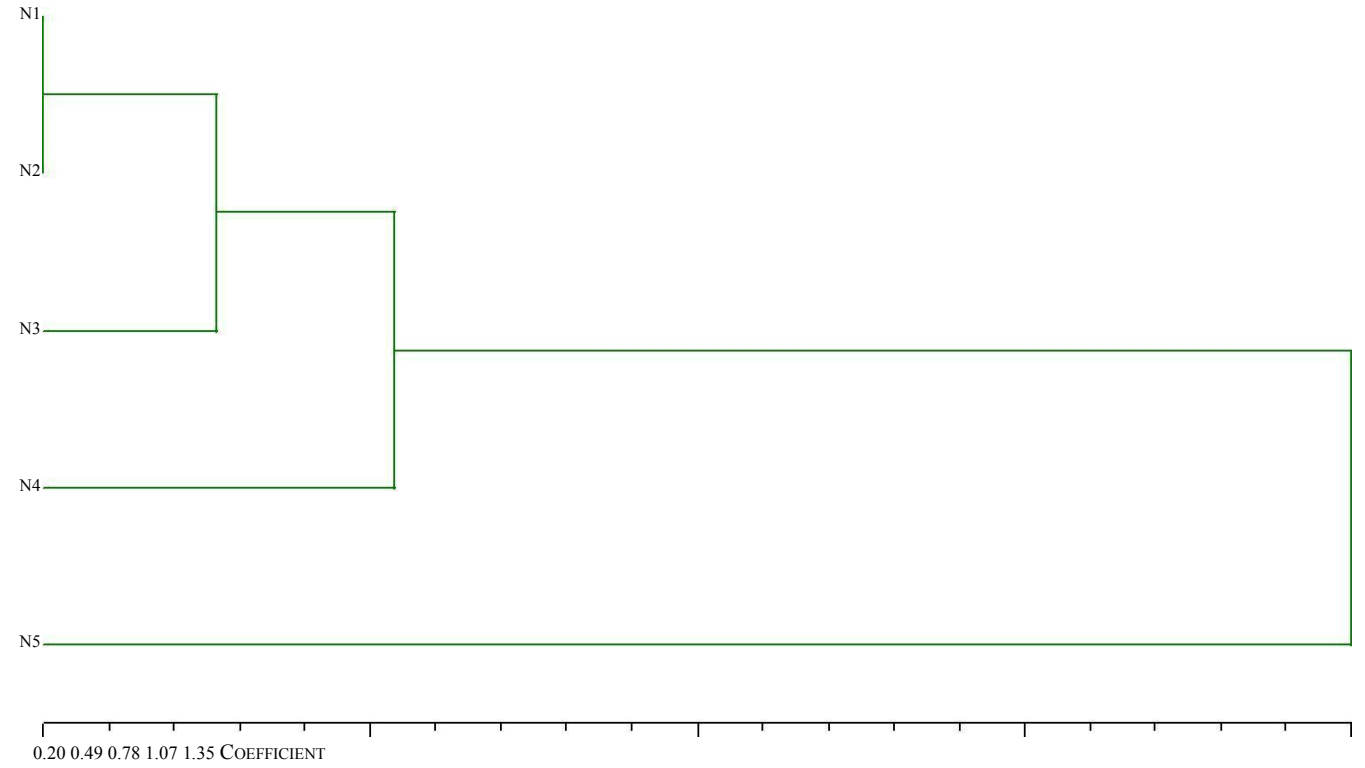
PCR analysis

The RAPD results indicated 95.60% polymorphic bands. This percentage of polymorphism is high. In comparison, RAPD study in *Dendrobium* species produced 323 polymorphic fragments out of 340, which leads to 95% polymorphism (Zha et al., 2009). Other studies reported 89.40% polymorphism in rice (Rabbani et al., 2008) and 70.07% polymorphism in *Erianthus arundinaceum (*Zhang et al., 2008) by using RAPDs.

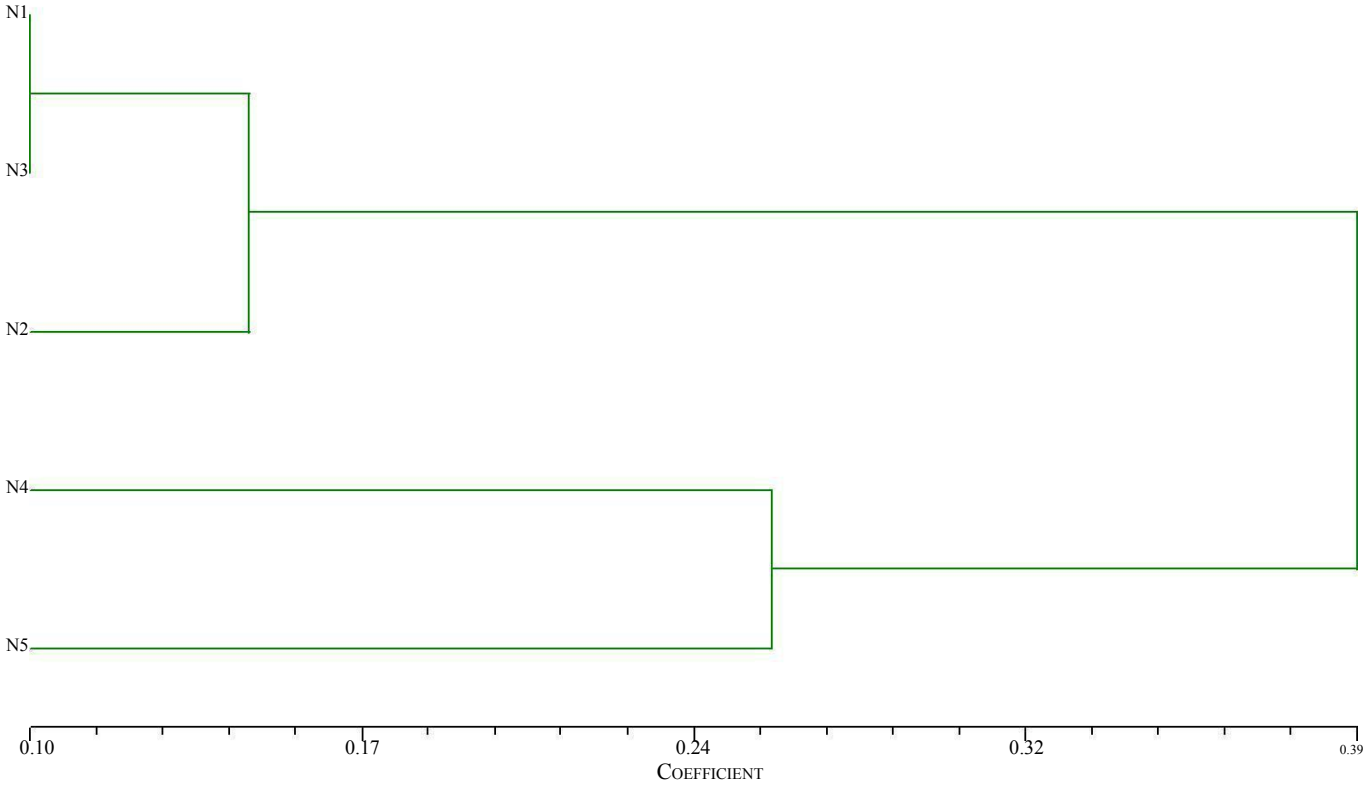
The percentage of polymorphic bands was recorded to be 66.67%, which is lower than RAPDs. The study also gave a low level of polymorphism, in which 55.61% of the 77 fragments were polymorphic in *Nelumbo nucifera* (Chen et al., 2008) and 39.61% of the 79 fragments were polymorphic in *Ctenopharyngodon idellus* (Chen et al., 2009). However, some studies showed high level of polymorphism, like *Hippophae* L. from China, while other countries gave 384 polymorphic fragments out of 385, in which case 99.74% polymorphism (Li et al., 2009) was given in barley from Tibet, and 79 polymorphic fragment showed 86.81% polymorphism (Wang et al., 2009a).

Phylogenetic analysis

The relationships within and between samples were estimated by UPGMA cluster analysis of genetic distance. The UPGMA cluster analysis of *A. capillaris* is based on the genetic distance generated from Nei and Li (1979). The dendrogram indicates the genetic relation between those samples. The dendrogram showed the relationship among the samples of *A. capillaris* that are presented in Figure 1a for RAPD marker. The RAPD dendrogram produced two main clusters and was in the range of 0.2000 to 1.3500 genetic distance level. Previous studies have reported phylogenetic similarity

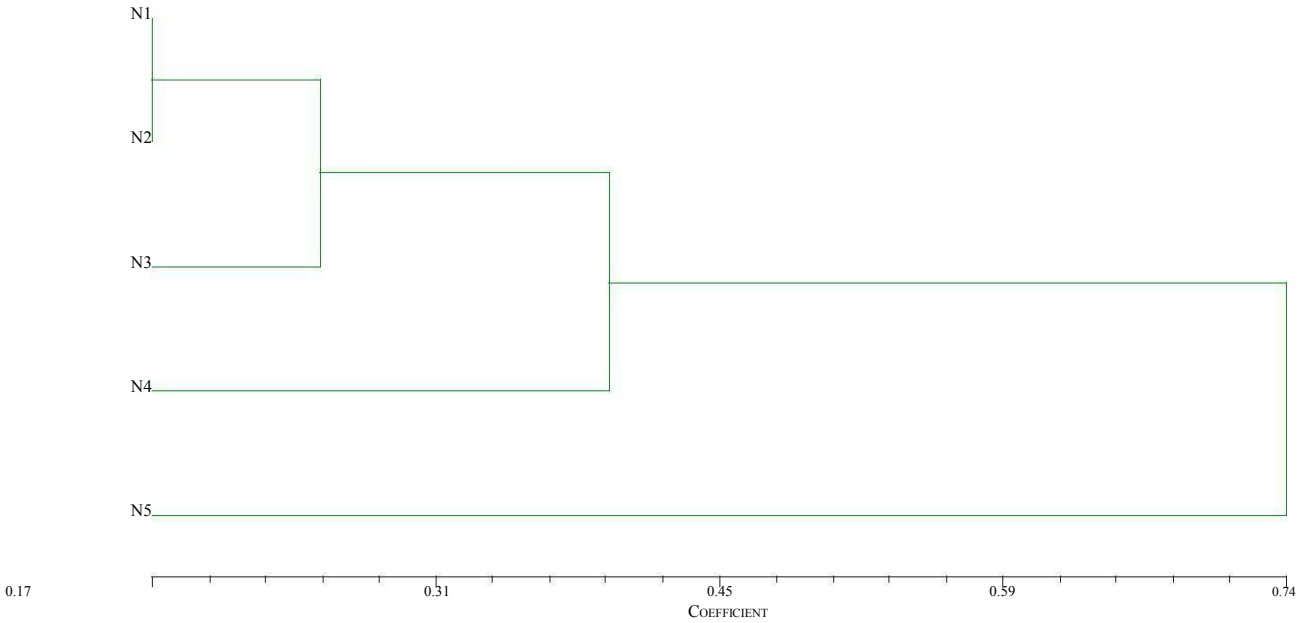


(a) RAPD



(b) ISSR

Figure 1. Dendrograms generated using unweighted pair of group method (UPGMA) with arithmetic average analysis, showing relationships between *A. capillaris* using (a) RAPD, (b) ISSR and (c) ISSR + RAPD data.



(c) RAPD + ISSR

Figure 1. Contd.

Table 5. ISSR analysis of *A. capillaris*.

Primer	Range of fragment size (bp)	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphism (%)
807	350 – 800	8	7	87.5
809	350 – 1031	7	5	71.43
825	400 – 1031	7	5	71.43
834	350 – 2000	9	5	55.56
841	250 – 900	9	3	33.33
862	350 – 800	4	2	50
866	350 – 1350	9	7	77.78
876	250 – 2000	10	8	80
nIssr1	350 – 2500	12	10	83.33
nIssr3	300 – 850	6	2	33.33
Total	-	81	54	66.67

Table 6. A comparative analysis of RAPD and ISSR used for diversity estimation of *A. capillaris*.

Primer	RAPD	ISSR
Number of primers used	10	10
Total number of polymorphic bands	108	54
Total number of bands	113	81
Total number of bands amplified	291	283
Percentage polymorphism (%)	95.60	66.67
Average number of bands amplified/primers	29.1	28.3
Average number of polymorphic bands/primer	10.8	5.4
Average number of bands/primers	11.3	8.1

Table 7. Similarity index of *A. capillaris* in Negeri Sembilan based on RAPD profile generated by the ten primers.

Samples	N1	N2	N3	N4	Range	Average	SD
a) RAPD							
N2	0.8189						
N3	0.7152	0.6757			0.1579 – 0.8189	0.4739	0.2651
N4	0.5910	0.5271	0.6928				
N5	0.1579	0.1644	0.1649	0.2308			
b) ISSR							
N2	0.8760						
N3	0.9016	0.8455			0.5686 – 0.9016	0.7457	0.1164
N4	0.7458	0.7059	0.8167				
N5	0.5686	0.6214	0.6154	0.7600			
c) RAPD + ISSR							
N2	0.8468						
N3	0.7985	0.7528			0.3933 – 0.8468	0.6174	0.1711
N4	0.6640	0.6129	0.7473				
N5	0.3933	0.4318	0.3980	0.5281			

between *A. capillaris* from Korea within a range of 0.644 to 0.840 (Lee et al., 2006). The dendrogram from this study indicated on geographic origin as seen in some other crops like *Erianthus arundinaceum* (Zhang et al., 2008) and *Hoottuynia* thunb. (Wu et al., 2005). The dendrogram with similar study also showed the genetic distriction between the Malaysia and Indonesia of the Barnyardgrass that showed genetic distance range of 0.32 to 1.00 (Tasrif et al., 2004). This variation could be due to the geographical isolation of the samples, which is similarly suggested to occur in the other crops (Paul et al., 1997; Zhang et al., 2006).

Present study gave genetic distance range of 0.2000 to 1.3500, which indicates that despite some degree of separation among the population, there is no considerably close genetic relationship among the population of *A. capillaris*. This might be due to the reason that the populations were derived and separated recently from a common stock. However, the existence of some level of differentiation among the populations might be due to different environmental effects including geographical, hydrographic connection, soil, climatic and biotic factors from different districts (Jayaram and Prasad, 2008).

The dendrogram produced by ISSR markers also showed 2 main clusters, with genetic distance level ranging between 0.1000 to 0.3900 (Figure 1b). The low levels of genetic distance owing to the origin of samples from the same ancestor, indicates close genetic distance for populations among regions, while, they were distinctly separated within clustering dendrogram. The results demonstrated very narrow genetic variations in some of the landraces, the reason being the long cultivation history of the species, as an adaptation to the local agroclimatic conditions (Seehalak et al., 2006). The similarity index is used to measure the similarity and variability in a population. In this study, the similarities between samples were assessed based on the presence and absence of the marker phenotype that were interpreted as the genetic distance. The average similarity index for *A. capillaris* by using RAPD marker was ranged from 0.1579 to 0.8189. The highest similarity of 0.8189 was observed in samples N1 and N2, as the lowest similarity of 0.1579 was observed between sample N1 with sample N5. The average similarity index within the samples was 0.4739 ± 0.2651 (Table 7).

While the similarity index of the ISSRs ranged between 05686 to 0.9016. The highest similarity of 0.9016 was observed in samples N1 and N3, while the lowest similarity of 0.5686 was observed between samples N1 and N5. The average similarity index value for each sample of *A. capillaris* are represented in Table 7. This high range of similarity in samples for both markers (RAPD and ISSR) indicated low genetic variability between individuals in this area. The average similarity index values for RAPD and ISSR were 0.4739 and 0.7457 respectively.

A similar study in RAPD analysis for the detected genetic similarity between nine Dendrobium species with the range of similarity index was 0.5614 to .07047 (Zha et al., 2009). The other study from Jayaram and Prasad (2008) also observed the similarity index in *Oroxylum indicum* (L.) *Vent.* range from 0.8077 to 0.9575.

The past ISSR analysis study from Wang et al. (2009b) had reported that the similarity index from *Iris lacteal var. chinensis,* range from 0.4000 to 0.9290. The similar study

of coconut (*Cocus nuciferal* L.) also gave similarity index in ISSR analysis, that range between 0.5260 to 0.8550 (Manimekalai and Nagarajan, 2006). 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. The high similarity indices slightly correlated with their close geographic locations. This also had been reported for RAPD study in barnyardgrass (Tasrif et al., 2004). The ISSR study from Ash et al. (2003) showed that there is distinct genetic variability within the *Cartahmus lanatus* population in Australia. Vellend and Waterway (1999) also observed the genetic diversity within *Carex rariflora* population from different habitat and locations in Canada.

Similarly, quantity and purity of extracted genomic DNA also plays crucial role for analysis of molecular diversity and optimization of different parameters for PCR (Staub et al., 1996). The previous study show that RAPD and ISSR markers have been successfully applied to detect the genetic similarities or dissimilarities in micro-propagated material in various plants (Ramage et al., 2004; Modgil et al., 2005).

According to Jain et al. (2003), it is possible that genotypes from different geographical regions can be genetically similar. The close similarity shown among these samples might be due to their close collection sites. It could also result from recent introduction of the samples in that area or the samples could be derived from the same original stock (Jayaram and Prasad, 2008).

Conforming results were obtained by the combined analysis of RAPD and ISSR data as revealed by the joint dendogram. However, combined dendogram was more conforming with the results of RAPD rather than ISSR results. ISSR primers are more specific as compared to RAPD primers, in that ISSR primers are the specific sequences target. The ISSR primers sequences were tri or tetra repeated sequences, compare to the RAPD primers that are random target. So, this made more bands produced by RAPD markers that do not have the specific target compare to ISSR markers. In fact ISSR markers are known to be more sensitive than RAPD; although, this *A. capillaris* study does not agree with this fact.

Cluster analysis performed from combining data of both markers (RAPD and ISSR) generated a dendrogram the samples were grouped into 2 distinct clusters. The genetic distance level ranged from 0.1700 to 0.7400 (Figure 1c). The dendrograms based on RAPD and ISSR combined markers showed partially different genetic distance levels when used individually. But when used together, RAPD-based cluster is similar to the combined cluster than ISSR-based cluster. Our results are in agreement with the studies in *Ficus* species (Hadia et al., 2008). However, in Old World Lupin (*Lupinus sp.*) had found that ISSR-based cluster is more similar to the combined cluster than RAPD-based cluster study by Yorgancilar et al. (2009). *Jatropha curcas* also showed similar result when RAPD and ISSR dendrogram patterns were combined than when ISSR and RAPD based patterns were combined (Gupta et al., 2008).

Hence, both the marker systems RAPD and ISSR either individually or combined can be effectively used in determination of genetic relationships among *A. capillaris* samples. However, various reports suggested ISSR being a better tool than RAPD for phylogenetic studies (Nagaoka and Ogihara, 1997; Ajibade et al., 2000; Galvan et al., 2003).

The similarity index for combine markers RAPD and ISSR ranged from 0.3933 to 0.8468. This range is lower than ISRR based marker, indicating more diversity, but higher than RAPD based marker. In *J. curcas,* combine marker (RAPD and ISSR) more diversity compare to RAPD and ISSR more diversity compare to combine marker (RAPD and ISSR) (Gupta et al., 2008).

Comparative analysis of RAPD and ISSR markers

RAPD markers were more efficient than the ISSR markers with regards to polymorphism detection, as the results indicated that the percentage of RAPD polymorphism bands (95.60%) was higher than ISSR (66.67%). RAPD were also more efficient than ISSR markers for polymorphism detected in *J. curcas* that were reported, RAPD got 84.26% polymorphism compare to 76.54% for ISSR (Gupta et al., 2008). RAPD are more efficient marker compare to ISSR marker for *A. capillaris* study. However, this is contrast to the results that was obtained for barley that produced 98.13% polymorphism for ISSR more than RAPD (77.06%) (Hou et al., 2005).

Also, total number of polymorphic bands and average number of polymorphic bands per primer are more for RAPD (108 and 10.8 respectively) than for ISSR markers (54 and 5.4, respectively) (Table 6). The average number of bands amplified per primer and average number of bands per primer RAPD band (29.1 and 11.3, respectively) was more than ISSR (28.3 and 8.1, respectively).

In this study, it was obvious that the dendrogram based on RAPD markers was not in accordance with the dendrogram based on ISSR markers. Thus, both dendrograms are in agreement with the groups of the geographic origins, but RAPD markers greatly agree with these groups than ISSR markers.

Both RAPD and ISSR markers might detect non coding and therefore more polymorphic DNA by exploiting the different regions of the genome. The differences found among the dendrograms generated by RAPDs and ISSRs could be partially be explained by the different number of PCR products analyzed (291 for RAPDs and

283 for ISSRs) reinforcing again the importance of the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships among *A. capillaris* samples. Another explanation could be the low reproducibility of RAPDs (Karp et al., 1997). Similar results have been observed by Loarce et al. (1996) for barley.

The differences in clustering pattern of genotypes using RAPD and ISSR markers also may be attributed to marker sampling error and the level of polymorphism detected. The putatively similar bands originating for RAPDs in different individuals are not necessarily homologous, although they may share the same size in base pairs. This situation may lead to wrong results when calculating genetic relationships (Fernandez et al., 2002).

The mean of similarity index was 0.4739 for RAPDs, as against 0.7457 for ISSRs, which indicates high genetic variations among those samples that came from different places. The similarities detected with ISSR are greater than the similarities observed with RAPD. But, study from Hou et al. (2005) founded that RAPD are more effectively compare to ISSR in similarities index observed for barley from West China. From the results, RAPD marker shown 1 pair of sample that gave similarity index ≥ 0.8. While, ISSRs shown 4 pairs of samples that indicated similarity index ≥ 0.8. That means RAPD leads to more dissimilarity in samples than ISSRs. The similarity index > 0.8 is reported to be high, indicating narrower genetic variability for a particular crop (Davierwala et al., 2001).

Conclusion

The results of the study can be seen as a starting point for future researches aimed at defining the level of intra- and inter-specific genetic diversity and to detect hybrids among these species. These studies have given important clues in understanding genotype relationship, which may further assist in developing and planning breeding strategies. The two marker systems, RAPD and ISSR used in the present study have also been used as effective tools to evaluate genetic diversity and to throw light on the phylogenetic relationships in different cultivated and uncultivated plants.

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