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Detection of RAPD Variation in a Forest Tree Species, *Meliantha suavis* Pierre (Opiliaceae) from Thailand

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ABSTRACT *Meliantha suavis* Pierre. is an important wild tree served as a vegetable for local Thai people. Natural populations of this species are currently vulnerable to habitat loss due to deforestation and overexploitation. The population genetic structure is important base line knowledge for conservation of this species. This study used random amplified polymorphic DNA (RAPD) markers to investigate the patterns and distribution of genetic variability in natural populations of *M. suavis* in north and northeast Thailand. Seven 10-mer primers amplified a total of 46 scorable bands, of which 36 (78.3%) were polymorphic. To describe and compare the partitioning of genetic variation, Shannon's information index and Analysis of Molecular Variance (AMOVA) were applied to analyze RAPD data set. Results from Shannon's information measures indicated that twenty-eight percent of genetic variation were found within populations. AMOVA analysis revealed most genetic variation was found within populations comparable to the total genetic variation. AMOVA confirmed the results from Shannon's information index that genetic variability was found to occur within populations of *M. suavis*. Since a high level of genetic variation was found to occur among populations, based on Shannon's diversity, conservation of wild populations would help in the maintenance of the genetic diversity of *M. suavis.*

KEYWORDS: *Meliantha suavis*, conservation genetics, population-genetic structure, RAPD-PCR.

INTRODUCTION

Meliantha suavis Pierre. is a deciduous tree, commonly found in mixed deciduous forest and dry dipterocarp forest in Thailand.[1] Historically, *Meliantha suavis* is an important plant species as a vegetable for people in Thailand and Lao People's Democratic Republic (LPDR). During the early dry season (February to April), young leaves and flowers of *M. suavis* are explored and extensively collected from wild populations by local people. Young leaves, young and/or blooming flowers are used as edible parts. They are always found in local market, even in Bangkok, around Thailand. The market prices sold by the kilo that are relative high (80-100 Baht per kilogram).

Theoretically, the evolutionary potential of a population of plant species depends on the proportions of the amount of genetic variability maintained by a plant species. The long-term persistence of a population requires the production of offspring sufficiently similar to their parental types and these have succeeded in reproducing and are able to sufficiently exploit their environment and cope with populations of biotic factors.[2] Every population has its own genetic system. Through natural selection, the environment continuously removes those genotypes and gene which make their carriers less viable, less fertile, and less successful in reproduction. In evolutionary point of view, populations with low genetic variability have a reduced potential to adapt to environmental change.[3]

Thai people in all regions highly depend on multiuse of local vegetables. Different topography and climate of each part of Thailand result in diverse species of vegetables. Of them, *Meliantha suavis* is an important vegetable for Thai people. The common name of this species for Thai people is "*Phak Waan*".

Recently, natural populations of *M. suavis* are highly vulnerable to habitat loss due to deforestation and overexploiting of flowers, thus this species face decreasing number of plant trees. Besides the reducing of offspring production result from collecting of most flowers from the parental plants in the forest.

In Thailand, little is known about genetics of *M. suavis.* However, the conservation program of *M. suavis* is currently at the stage of exploring its habitat and seed collections from wild populations and grown in a garden (Kasetsart University Research and Development Institute (KURDI), personal communication).

Plant population employs its genetic system as an instrument in order to persist over generations in a changing environment.[2] Thus, in order to plan effectively breeding and gene conservation strategies it is essential to have an understanding of the pattern of genetic variation in the population of interest.

RAPDs are dominant molecular markers developed by Welsh and McClelland[4] and Williams *et al*.[5] They are random pieces of DNA amplified from the genome by a PCR-based technique. RAPD profiling uses single short oligonucleotide primers (10 base) and Taq DNA polymerase to amplify DNA segments between priming sites. Amplified DNA fragments may be visualized on gel, and bands scored as presence / absence character states.

RAPD profiling is being increasingly used in population surveys because of the ease of methodology and the numerous polymorphic distinguishable.[6] Several studies have used RAPDs to assess levels and patterns of variation.[7,8,9,10,11]

This study used random amplified polymorphic DNA (RAPD) markers to investigate the distribution of genetic variability in natural populations of *M. suavis* in north and northeast Thailand. The amounts and patterns of genetic variation are described with a major aim of this study is to provide a basic genetic information relevant to the conservation and restoration of this species.

Materials and methods

Study species

M. suavis Pierre. is a tree species of the Opiliaceae. Young leaves and flowers are similar to those of *Scleropyrum wallichiana*. Thus, it is necessary to consider during specimen collection for *M. suavis*. The author sampled young leaves from ten populations in the forests of North and Northeastern regions of Thailand. The sample size, 2-8 plants per population, was relative small resulting from rarity of the number of individuals in each population. The possible reason for explanation this event is that population sizes have declined because of human interference. This sampling strategy was adopted to ensure that the full geographical range of *M. suavis* in both regions was sampled. However, small number of plants have been used for RAPD analysis such as Fischer and Matthies[19] and Fischer *et al.*[20]. Localities designated in this study are indicated in Table 1. The localities are further grouped into 3 regions as shown in Table 1.

DNA isolation

Samples were stored on wet ice until they arrived in laboratory. DNA was extracted from 1-3 g of leaves tissue using a modified Doyle and Doyle[12] procedure as described by Prathepha and Baimai.[11] DNA concentration was estimated from ethidium bromide stained gels and samples were diluted to 100 ng/ml.

PCR conditions and electrophoresis

Amplification was carried out in a 25 ml volume in 0.5 ml microtube using a Hybaid programmable thermal controller .

The reaction mixture contained 200 mM of each dNTP, 1x Taq polymerase buffer (Promega), 2.0 mM $MgCl_2$, 10 picomoles primer, 100 ng DNA and 0.5 unit of *Taq* Polymerase (Promega). The amplification was performed as follows: initial 1 min 94°C denaturation; 45 cycles of 1 min 94°C, 1 min 36°C, 2 min 72°C; and 5 min 72°C extension. Amplified fragments were separated in 1.4 % agarose gel using 0.5x TBE buffer and were visualized and photographed using a Gel Documentation System, GDS 8000 (UVP Inc., California, USA), after staining with ethidium bromide.

A total of forty primers (Kits B and AM, from Operon Technologies, Alameda, California) were screened for usefulness in an initial survey. Of these, seven primers were found to give clear reproducible results after checking for repeatability of band patterns (Table 2).

The presence or absence of amplified DNA bands was scored for 46 band positions. All bands scored were between 0.25 and 2.0 kb. The final presence-absence matrix contained scores for 45 individuals each at 46 band positions (primer B01: 6 bands, B08: 6, B15: 7, B17: 9 , B18: 6 , AM01: 5, AM04: 7).

Estimating population genetic structure with RAPD data

The estimation of population genetic structure in an analysis of this data was analyzed by assuming that the populations are in Hardy-Weinberg equilibrium, The estimates were calculated using the following methods:

1. The frequency of the 46 RAPD bands detected with the seven primer were calculated and estimates of genetic diversity (H) of each population were obtained using Shannon's information measure;[13] modified for RAPD analysis by Chakraborty and Rao,[14] which is defined as $H = - \Sigma^{k}_{i=1} P_i \log_e P_i$, where P_i is the frequency of the i^{th} RAPD bands in each population.
2. The genetic diversity obtained from Shannon's

Table 1. Origin and description of materials collected for RAPD survey in *Melientha suavis*.

Region	Locality	Province	Code	N
Northeastern region1 (NE1)	Nong Phok district	Roi Et	RE	3
	Phukradueng district	Loei	PKD	4
	Chumphae district	Khon Kaen	KK	4
	Muang district	Surin	SR	4
	Muang district	Mukdahan	MD	4
	Ban DongMan (Muang district)	Mukdahan	MD	4
	Sakon Nakhon National Park	Sakon Nakhon	SKN	8
	Muang district	Chaiyaphum	CYP	4
Northeastern region2(NE2)	Chongmek district	Ubol Ratchathani	UB	2
Northern region (N)	Muang district	Lampang	LP	4
	San Pa Thong district	Chiang Mai	CM	2
	Chom Tong district	Chiang Mai	CM	2

Table 2. Sequences of random primers used in the RAPD analysis of *Melientha suavis* populations.

Code	Sequence (5' to 3')
OPB-01	GTT TCG CTC C
OPB-08	GTC CAC ACG G
OPB-15	GGA GGG TGT T
OPB-17	AGG GAA CGA G
OPB-18	CCA CAG CAG T
OPAM-01	TCA CGT ACG G
OPAM-04	GAG GGA CCT C

index of the 10 populations, detected by the seven primers was used to partition into within (H_{pop}/H_{sp}) and between (H_{sp}-H_{pop}/H_{sp}) population components[7].

3. Nei's original measures of genetic identity and genetic distance[15] were calculated.

4. A dendrogram was constructed from the genetic distance matrix using UPGMA method.

The variation in RAPD patterns was analyzed by analysis of molecular variance (AMOVA).[16] Based on the number of shared amplification products,[17] a matrix of genetic distance between individuals was obtained using a RAPDistance program v. 1.04 calculated from presence/ absence data. Components of variance attributable to differences between regions, between populations within regions, and between individuals within populations were estimated from this matrix using AMOVA. AMOVA variance components were used as estimates of the genetic diversity within and between populations and regions. The number of permutations for significance testing was set at 100 for all analyses. From *phi* statistics (F statistics) gene flow (N_em) can be approximated as $N_em = 1/4\ (1/F_{ST} - 1)$.[18,19]

RESULTS AND DISCUSSION

RAPD variation in *M. suavis* populations

RAPD products electrophoresed in an agarose gel are shown in Fig 1. The number of polymorphic loci and genetic diversity (H) detected with the seven primers for the 10 populations of *M. sauvis* is given in Table 3. The total number of polymorphic loci detected varied between populations. For example, Phukradueng, Ubol Ratchathani, Khon Kaen, Surin, and Chaiyaphum populations were found to be low polymorphisms for all the primers evaluated. While, the Mukdahan (MD) population exhibited relatively high level of number of polymorphic loci. This corresponds with exhibition the highest level of genetic diversity (0.359) for the MD population.

RAPD analysis has been proved to be a high-resolution method for detection of genetic variation among and within populations. Fischer and Matthies[19] pointed out an important suggestion that because of the high resolution of the method, significant genetic variation could be detected within and among populations with relatively small sample sizes. Thus, RAPD is likely to be appropriate method for the analysis of genetic variation in *M. suavis*.

Partitioning of genetic variability between and within *M. suavis* populations

Shannon's information index of genetic diversity was used to partition the diversity into within and between population components (Table 4). H_{pop} provides a measure of the average diversity within populations. Primer OPB-08 and OPB-15 detected the most and the least genetic diversity within populations of *M. suavis*, respectively.

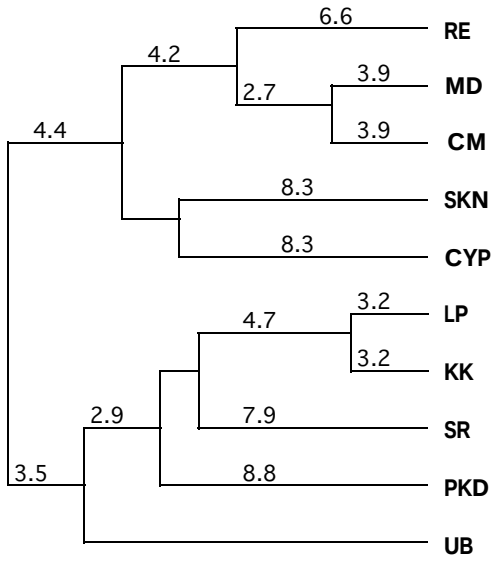

Fig 1. Bands amplified from *Melientha suavis* individuals obtained from primer OPAM-01 (A) and OPB-01 (B); some polymorphic bands are indicated by arrows. Lane 1 = molecular weight marker (1 kb ladder DNA).

Table 3. Number of polymorphic loci and genetic diversity (H) estimated for the 10 populations of *M. suavis* by the Shannon-information index.

Population	No. of polymorphic loci	Percentage of Polymorphic loci	Genetic diversity (H)*
1 (RE)	10	21.74	0.117
2 (LP)	12	26.09	0.147
3 (PKD)	6	13.04	0.064
4 (UB)	3	6.52	0.039
5 (KK)	4	8.70	0.041
6 (SR)	3	6.52	0.039
7 (MD)	27	58.70	0.359
8 (SKN)	15	32.61	0.179
9 (CM)	15	32.61	0.190
10 (CYP)	4	8.70	0.053

* H is the average from over all primers of the Shannon's information index.

$H = - \Sigma^{k}_{i=1} P_i \log_e P_i$, where k is the number of RAPD markers for each primer, and P_i denotes the frequency of the i^{th} RAPD markers in a given population.

Fig 2. Dendrogram (UPGMA method) of 10 populations of *Melientha suavis*. Numbers indicate the genetic distance.

The proportion of genetic diversity present within populations (H_{pop}/H_{sp}) and between populations (H_{sp}-H_{pop}/H_{sp}) indicated that, on average, most of the diversity (71.7 %) occurs between *M. suavis* populations (Table 4). However, the distribution of diversity varying between and within populations detected by the seven primers. For example, primer OPAM-01 and OPB15 detect most diversity between populations (approximately 82%), whereas primer OPB-08 detects most diversity within populations (39.6%).

The results of the AMOVA partitioning of variance are shown in Table 5. RAPD variation among regions, among populations and within populations were not significant differences. It was found that the within-population variance component accounted for 100% of the total variance, while the among-population variance component was not accounted. One pairwise (UB and SKN populations) of the total of 45 pairwise genetic distances (Φ_{ST}) between populations was significant difference (Table 6).

In this study, Shannon's information measures indicated very high genetic differentiation between populations of *M. suavis*. However, because AMOVA resulted in F statistics that showed the genetic variation within and between populations were not different significance (Table 5). Thus, these results have to be interpreted with caution, because power to detect significance with the same F value may not be significant with small sample sizes, but significant with larger sample sizes[6]. For *M. suavis*, thus larger sample size seem necessary for further study because of obvious population differentiation, which was not detected with sufficient power by the AMOVA. However, the strong genetic differentiation (71.7%) found among populations of *M. suavis* (Table 4). This corresponds with low value for the average number of individuals exchanged between populations per generation (N_em = 1.31).

The Nei's estimate of similarity,[15] based on the number of shared RAPD products,[17] was used to generate a similarity and distance (Table 7), The population from Lam Pang and Khon Kaen exhibited the least RAPD variability (0.0641) and the populations from Ubol Ratchathani and Chaiyaphum provinces exhibited the most RAPD variation (0.6411). The proportion of shared RAPD fragments ranged from 0.4783 to 0.9348.

Table 4. Components of genetic diversity (H) in 10 populations of *M. suavis*, partitioned into within (*Hpop/Hsp*) and between populations *(Hsp-Hpop/Hsp*), for seven random primers.

Code	*Hpop*	*Hsp*	*Hpop/Hsp*	*(Hsp-Hpop)/Hsp*
OPB-01	0.1874	0.5226	0.3586	0.6414
OPB-08	0.2372	0.5998	0.3955	0.6045
OPB-15	0.0443	0.2366	0.1872	0.8128
OPB-17	0.0817	0.3389	0.2411	0.7589
OPB-18	0.1060	0.3924	0.2701	0.7299
OPAM-01	0.0645	0.3614	0.1785	0.8215
OPAM-04	0.1443	0.4153	0.3475	0.6525
Mean	0.1236	0.4096	0.2826	0.7174

Table 5. Analysis of Molecular Variance (AMOVA) for *Melientha suavis*.

Source of Variation	d.f.	MSD (x100)	Variance component		
			Variance	%total	*p*[a]
Among regions (AG)	2	11.0	0.00	0.00	0.3465
Among populations Within regions (AP/WG)	7	7.9	0.00	0.00	0.9901
Among individuals Within populations (WP)	35	20.1	0.174	100.0	0.9901
Total	44	39.0	0.174	100	

[a] Levels of significance are based on 100 iteration steps.

Table 6. Pairwise genetic distance (Φ_{ST}) among 10 populations of *Melientha suavis*. Levels of significance : * p < 0.001. P values indicate the probability that a random genetic distance (Φ_{ST}) is larger than the observed distance and are based on100 iterations. Population numerated as in Table 3.

Population	Population								
	1	2	3	4	5	6	7	8	9
2	–0.3113								
3	-0.3113	-0.3333							
4	-0.4759	-0.2515	-0.2515						
5	-0.3113	-0.3333	–0.3333	-0.2515					
6	-0.3113	-0.3333	–0.3333	-0.2515	–0.3333				
7	0.0049	-0.0815	-0.0815	0.0702	-0.0815	-0.0815			
8	0.0049	-0.0815	-0.0815	**0.0702***	-0.0815	-0.0815	–0.1429		
9	-0.3113	-0.3333	–0.3333	-0.2515	–0.3333	–0.3333	-0.0815	-0.0815	
10	-0.3113	-0.3333	–0.3333	-0.2515	–0.3333	–0.3333	-0.0815	-0.0815	-0.3333

Table 7. Estimate values of Nei's unbiased measure of pairwise genetic distance (D) (Nei, 1972) for 10 populations of *M. suavis*.

Population	1	2	3	4	5	6	7	8	9
2	0.3532								
3	0.3841	0.1363							
4	0.4402	0.2015	0.2601						
5	0.3251	**0.0641**	0.1894	0.2566					
6	0.2000	0.1525	0.2027	0.2194	0.1634				
7	0.1054	0.2034	0.2215	0.2651	0.2172	0.1687			
8	0.1772	0.3319	0.2940	0.4443	0.2663	0.1840	0.1763		
9	0.1609	0.1820	0.1566	0.2441	0.2121	0.1349	0.0778	0.2072	
10	0.1604	0.5311	0.4511	**0.6411**	0.4923	0.3080	0.2189	0.1662	0.3664

A dendrogram displaying hierarchical associations is given in Fig 2. The 10 populations were divided into three groups. The UB population appears to form a distinct group. While the major group consists of five populations (Roi Et, Mukdahan, Chiang Mai, Sakon Nakhon and Chaiyaphum) and the other one comprise four populations (Lampang, Khon Kaen, Surin and Phukradueng). The wide separation between populations may result in increased genetic diversity because there may be little cross pollination between adjacent populations. The populations sampled in this study were separated by many kilometers and distance could be a significant factor accounting for the large genetic diversity between populations.

Conclusion

Melientha suavis is an important wild plant species serving as a vegetable for Thai people. Their natural populations are recently highly vulnerable to habitat loss because of deforestation and a few number of offsprings have been produced in each year. The preservation of biodiversity and genetic diversity is a fundamental aim of conservation. The study of population genetics has been recognized as one of the main priorities for conservation.

This study is the first report provides an essential basis on genetic information of *M. suavis* populations. The level of RAPD variation and distribution in populations of *M. suavis* has been investigated. The majority of RAPD variation in this species was found between rather than within populations. When these results are compared to published results, it appears that the distribution patterns obtained from this study agrees with the findings of Chalmers *et al*,[7] based on RAPD variation in a tree species. Thus, it is suggested that RAPD markers could be successfully applied for detecting genetic variability in natural populations of *M. suavis*. Moreover, RAPD markers will have a major impact on the conservation, management and improvement of a tree species, *M. suavis* in Thailand.

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