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Molecular studies of coralberry (*Ardisia crenata* Sims; Primulaceae) from Thailand based on SCoT markers

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Abstract. *Suparman S, Pornpongrungrueng P, Chantaranothai P. 2023. Molecular studies of coralberry (*Ardisia crenata *Sims; Primulaceae) from Thailand based on SCoT markers. Biodiversitas 24: 3183-3189.* A coralberry (*Ardisia crenata* Sims) is the most common species of *Ardisia* Sw. distributed widely in Thailand. The grouping of the species for intraspecific based on morphometric characters was unstable. Some molecular primers were used by researchers to separate species. In this molecular study, six selected SCoT of 36 primers, i.e., primers no. 13, 14, 15, 19, 20, and 21, displayed high polymorphic and resolving power in *A. crenata* from 14 different natural populations in Thailand. This marker separated all taxa into two groups from different floristic regions. In contrast, this result did not support the classification of the intraspecific level of *A. crenata* based on continued morphological characteristics. For the conclusion that this study was recorded as the first one using the SCoT marker on the *Ardisia* and the primer worked well in *A. crenata* and was recommended for other species in *Ardisia.*

Keywords: *Ardisia*, *mata ayam*, PCR primers, Thailand floristic region

INTRODUCTION

Ardisia crenata Sims (1818) is a common species of *Ardisia* Sw. with a very wide distribution from India, Myanmar, China, Japan, Indochina, the Philippines, the Malay Peninsula, and Indonesia, mainly in the tropics (POWO 2023). The species are known as coralberry (English), *tapet takai* (Thailand), or *mata ayam* (Indonesia), respectively; it has 29 synonyms (The Plant List 2022). Generally, the synonym was created because of misinterpreting the specimen and then recorded as a new species by different botanists. Therefore, molecular markers were widely used to clarify the relationships between intraspecific taxa of flowering plants.

The following techniques are the most used in genetic diversity studies: Restriction fragment length polymorphism (RFLP) (Sarin et al. 2013; Yang et al. 2013), Random amplified polymorphism DNA (RAPD), single sequences repeated (SSR), Amplified fragment length polymorphism (AFLP), and Start codon targeted (SCoT) markers. The results from RAPD, AFLP, or microsatellite markers, provide more suitable data to classify individual organisms into nominal genotype categories. These markers are important in intraspecific genotype variation studies. Moreover, in the past decades, microsatellite has been the most frequently used molecular marker (Lopez 2015). Some researchers have begun to compare Inter Simple Sequence Repeat (ISSR) and ScoT results. Etminan et al. (2016) cited that polymorphism level and molecular variance obtained from SCoT are superior to ISSR markers. For example, in citrus plants (Rutaceae), the marker is very useful in characterizing, identifying, and structuring the species' genetic diversity (Mahjbi et al. 2015). That research shows the possibility and the advantage for the next research of SCoT markers for plant conservation and developing the program to improve the quality, such as on important economic fruits. Although SCoTs target potential coding genomic regions generally producing a dominant marker system, several co-dominant markers are also generated (Zhang et al. 2015). A co-dominant molecular marker is important to track the heterozygosity between populations. This co-dominant marker is SSR and SNP molecular marker for dominant markers such as AFLP, RAPD, and multilocus SSRs (Abuzayed et al. 2017). This dominant and co-dominant type marker affected the percentage index value. In dominant markers with equal population distribution, the value of IC is higher, but the normal range is between 0-0.5 (Chesnokov and Artemyeva 2015). On the other hand, SCoT markers exhibit high polymorphism and resolving power (Satya et al. 2015). Overall SCoT marker is easy because the product was resolved by performing agarose gel electrophoresis. In addition, it uses a longer primer, so the marker is highly reproducible. Several SCoT primers have been designed, and 36 of them have been constructed by Collard and Mackill (2009).

In the case of *A. crenata*, some research was based on nuclear microsatellite marker analysis. The result from China found that the members of this species can be grouped by distribution regions (Mu et al. 2010). On the higher level, nuclear ITS and two chloroplast intergenic spaces were used to reconstruct the phylogeny of Asian

Ardisia and relatives in Myrsinoideae (Yang and Hu 2022). Another molecular marker, RAPD, proved helpful in identifying *Ardisia's* species and indicating the geographical region. However, the markers cannot separate morphological traits based on horticulture, like berry color. Furthermore, no molecular analysis report was based on SCoT markers in *A. crenata*. Thus this marker was selected to investigate the intraspecific relationship for *A. crenata* in Thailand.

MATERIALS AND METHODS

Plant materials

Fourteen samples from different individual plants of *A. crenata* were collected from different places around Thailand (Figure 1). All samples were collected and deposited in the KKU herbarium, Thailand. The samples were divided into three groups based on the leaf shape and length. These groups: Group 1 consisted of plants with oblanceolate leaves, 11.80-14.64 cm long, and leaf apex acute to acuminate; Group 2 consisted of plants with oblong-elliptic leaves, 17.35-20.65 cm long, and leaf apex acute to acuminate; and Group 3 consisted of plants with elliptic to oblanceolate leaf, 6.50-8.70 cm long, with the leaf apex varying between obtuse and round. The detail of the sample showed in Table 1. The young and healthy leaves were cut and cleaned with 70% alcohol. The leaf was then dried in silica gel for DNA extraction.

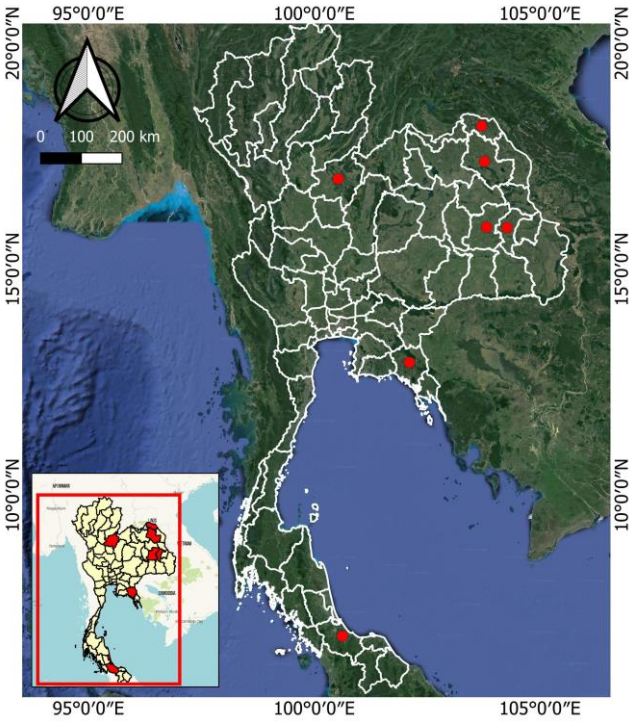

Figure 1. Map of Thailand, written provinces are locations for taking specimens. Map modified from Shorthouse (2023)

Procedures

DNA extraction

The DNA extraction was conducted based on the CTAB method from Doyle and Doyle (1987) and modified by Sookcharoen (2020). The method was non-liquid nitrogen preparation. One to two grams of free midrib leaf tissue was mashed into powder on a sterilized pestle and crucible. The powder was moved to the Eppendorf tube (1.5 mL), and add by 1.2 mL of 2x CTAB extraction buffer (50 mL of 0.1 M tris-HCL, pH 8, 140 mL of 5 M NaCl, 50 mL of 0.25 M EDTA pH 8, 10 g of CTAB, distilled water to 500 mL). Then 3 µL of 2-mercaptoethanol was added and incubated in a water bath at 65°C, ensuring it was all mixed by inversion every 10 minutes. After 90 minutes, 200 µL of Chloroform: isoamyl alcohol (SEVAG) (24:1) was added and mixed thoroughly by inversion for 5 minutes. Next, the tube was centrifuged at 14,000 rpm for 15 minutes, and 700 µL of supernatant was transferred to a new Eppendorf tube. Then, 500 µL SEVAG (24:1) was added and centrifuged at 14,000 rpm for 15 minutes. Next, 500 µL of the supernatant phase was transferred to a new Eppendorf tube and mixed with 700 µL of ice-cold isopropanol. Then, it was stored at -20°C for 2 hours to precipitate the DNA. The DNA was pelleted by centrifugation at 14,000 rpm for 15 minutes; the supernatant was discarded and air-dried at room temperature for 15 minutes. The DNA pellet was resuspended in 100 µL of Tris-EDTA (TE) buffer and 2 µL of RNAse (10 mg/mL) at 37°C for 30 min. Then, 60 µL of 2.5 M sodium oxaloacetate (NaOAc) was added, directly adding 1,000 µL of ice-cold 95% ethanol, then stored in the tube at -20°C overnight. The next day, the supernatant tube was centrifuged for 15 minutes at 14,000 rpm and the supernatant was discarded from the tube, 1,000 µL ice-cold ethanol was added. For last washing, spin at 14,000 rpm in 15 minutes is needed. The pellet contain DNA add by 80 µL of TE and it can be stored at -20°C.

Polymerase Chain Reaction (PCR) amplification and data analysis

The primer screening procedure was done to search for the most suitable and reproducible SCoT primers to generate genetic variation data in *A. crenata*. A total of 36 SCoT primers following Collard and Mackill (2009) were screened by amplification based on the PCR procedure using a selected individual sample of *A. crenata* from the three groups. All primers are 18 bp, and their GC content ranges from 50% to 72% with the various positions of ACT. The final volume for PCR is 10 µL for each mixing tube of primer-DNA genome, consisting of 5 µL of 2XTaq pol master-mix, 3 µL of DDH2O (nuclease-free water), 1 µL primer (10 ng/µL), and 1 µL genomic DNA (30-60 ng/µL). The PCR procedure, according to Sookcharoen (2020) as follows, step 1: initial denaturation, 1 cycle, at 94°C for 4 minutes; Step 2: 35 cycles from denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and extension 72°C for 2 minutes; Step 3: final extension at 72°C for 10 minutes in 1cycled. PCR amplicons were displayed on 1.5% agarose gel electrophoresis and stained with Visafe Red Gel Stain (Vivantis). The PCR product

was run concurrently with a 100 bp DNA ladder. In addition, a stained band of DNA in the gel was visualized under UV light and photographed through a gel documentation system (Biorad, Hercules, USA). The primer that produced a clear band for all groups was selected as the candidate primer. Then, all candidate primers were used to amplify all the samples and observed for the total and polymorphic bands. Next, the primer that provided 100% polymorphic and Polymorphism information content (PIC) value of more than 0.3 were selected and tested for reproducibility by repeating the PCR amplification step three times. Finally, the reproducible primer was selected as the most suitable primer for studying the genetic diversity of *A. crenata*.

The amplified bands resulting from every PCR SCoT primer were scored as present (1) or absent (0). Then, the score was counted to generate the binary data matrix. The number of the polymorphic band (NPB), the PIC, Number of Polymorphic Loci (NPL), Percentage of Polymorphic Loci (PPL), Observed Number Alleles (Na), Effective Number of Alleles (Ne), Nei's gene diversity (H), Shannon's information index (I), the total genetic diversity (Ht), the mean within-population genetic diversity (Hs), the genetic differentiation coefficients among the different population (Gst), and the Estimate of gene flow (Nm) were calculated. The PIC value was calculated online using https://gene-calc.pl/pic (Binkowski and Miks 2018), while other genetic parameters were calculated using POPGENE 1.32 (Yeh et al. 1999). The category of PIC is 0-0.2 (uninformative), 0.2-0.3 (moderate informative), and 0.31-0.5 (high informative polymorphic). Furthermore, the PIC value is calculated for dominant markers as described: PIC = 1 - [f2 + (1 -f)2]. Where f is the marker frequency in the data set; for the dominant markers, the maximum PIC value is 0.5. Note that the PIC values are higher for the markers with equal distribution in the population. Even though the values are much higher for the markers with multiple alleles, the PIC value also depends on the distribution frequency of the alleles (Chesnokov and Artemyeva 2015).

Another necessary value is the Resolving power (RP). RP is based on the distribution of alleles within the sampled genotypes and strongly correlates with the ability to distinguish among analyzed samples. The division of samples into two groups is based on the presence or absence of a band, ideally present in one part of the samples while absent from the other. Bands can be weighed according to their similarity to the optimal condition (50% of genotypes containing the band), where Ib or Band informativeness is represented on a scale of 0-1. This Ib band is defined as $Ib = 1 – (2 \times |0.5 – p|)$, where p is the portion of the samples containing the observed band. Next, using this value, the resolving power or the ability of a primer (technique) to distinguish between genotypes could be represented by the sum of these adjusted values for all generated bands (Amiryousefi et al. 2018).

The binary data was then analyzed in multivariate analyses with two different methods; cluster analyses with UPGMA to generate groups among samples and principal coordinate analysis (PCoA) to confirm alternative grouping among the individual samples. Both methods were performed with Paleontological Statistics Software Package for Education and Data Analysis (PAST) software version 4.03 (Hammer et al. 2001).

RESULTS AND DISCUSSION

SCoT primer screening

Thirty-six SCoT primers were tested for the first screening, leaving 17 selected SCoT primers (Table 2). The selected primers produced 12-25 bands. The percentage of the polymorphic band was 100% polymorphic from 16 primers, and only SCoT 6 had 95% PPB. PIC value ranges are various, with the lowest being 0.2268 and the highest being 0.4016. The next screening, from 17 SCoT primers, leaves only six SCoT primers with a 100% polymorphic band, and PIC is more than 0.3. Thus, these six primers were selected (detailed information in Table 3). The average PIC value is 0.353. The bands produced by the selected primers were then coded to binary data (0 and 1) and input into an Excel worksheet. The PAST software generated a dendrogram from the clustering method, especially the unweighted pair group method with arithmetic mean (PGMA) method and scatterplot visualization from the Principal Coordinate Analysis (PCoA) method. Finally, the genetic diversity was analyzed from the data of six selected SCoT primers (Table 4).

As many as 129 total polymorphic bands from six selected SCoT primers are 100% polymorphic. The PIC primer was from 0.328 to 0.4026 with an average of 0.352, and the band length is 150 to 2,500 base pairs. SCoT primer 14 (0.337) produced the lowest PIC value and the highest from SCoT primer 19 (0.491). The value of resolving power generated from all the primers is 55.14, with the lowest from SCoT primer 13 (7.86) and the highest produced by SCoT primer 21 (12.43). The DNA profile is shown in Figure 2.

Table 1. The samples of *A. crenata* were divided into three groups based on leaf size and shape

Groups	Sample code	Location: voucher specimens
I	PP 71	Phu Phan National Park, Sakon Nakhon: *Suparman 76*
I	TNC 88	Ton Nga Chang, Songkhla: *Suparman 94*
I	TSL 80	Thung Salaeng Luang National Park, Phitsanulok: *Suparman 84*
II	RE 10.1	Roi Et: *Suparman 32*
II	RE 10.2	Roi Et: *Suparman 33*
II	RE 10.3	Roi Et: *Suparman 34*
II	RE 10.4	Roi Et: *Suparman 35*
II	RE 10.5	Roi Et: *Suparman 36*
II	RE 10.8	Roi Et: *Suparman 38*
II	PSU 89	Songkhla: *Suparman 95*
II	CT 1	Chanthaburi: *Suparman 111*
III	YAS 1	Yasothon: *Suparman 45*
III	TSL 78	Phitsanulok: *Suparman 82*
III	PW 2B	Buengkan: *Suparman 09*

Population structure

Genetic diversity values were generated, including: PIC, Percentage of Polymorphic Loci; Number observed alleles (Na); Effective Number of alleles (Ne); Nei's gene diversity (H); Shannon's information index (I); The total genetic diversity (Ht); The mean within-population genetic diversity (Hs); The genetic differentiation coefficients among the different population (Gst); and Estimate of gene flow (Nm). The average observed number of alleles (Na) and an effective number of alleles (Ne) for all the groups ranged between 1.50-1.76 and 1.25-1.31, respectively. For the species level, the number of alleles is 2, with the effective number being 1.30. Similarly, Nei's gene diversity (H) and Shannon's Information index (I) ranged between 0.16-0.198, with an overall total genetic diversity of 0.21. The percentage of polymorphic loci (PPL) was estimated in the range of 37.61-75.79% for the total genetic group, and the species level is 100%. The genetic differentiation among the different populations and the gene flow value is 0.13 and 3.37, respectively.

The gene flow among the group was very high (Nm = 3.37), automatically revealing the low genetic differentiation between-groups (Gst = 0.13). All values indicated only around 13% of the total genetic variation occurred in inter-groups, and the rest (87%) was within each group of *A. crenata*. In this study, the estimate of gene flow was very high, with very little differentiation in genetics among the population of *A. crenata*. But indeed, the species has very high variation in morphology, especially for continuous variation.

Cluster analyses and PCoA

The dendrogram generated from UPGMA (Figure 3) separated 14 individual samples into two main groups (Co-phenetic correlation 0.69). Group I consists of six individual samples; the rest are in Group II. Samples in group I are from different subgroups assigned based on morphology and different location. The group is divided into three small subgroups, i.e., subgroups A, B, and C. Sample CT 1, from Chanthaburi and YAS 1, from Yasothon joined in subgroup A. Subgroup B consist of sample TNC 88 and PSU 89, both are from Songkhla Province. Two samples from Thung Salaeng Luang (TSL 80 & TSL 78) merge in subgroup C. Group II consists of six samples from Roi Et (RE 10.1-10.5 & RE 10.8); the rest was from Sakon Nakhon (PP 71) and Buengkan (PW 2B).

Table 2. The result from 17 SCoT primers was selected from the first primer screening step

SCoT Primers	TNB	NPB	PPB	PIC
4	19	19	100	0.2719
6	19	18	95	0.3615
8	18	18	100	0.2984
12	19	19	100	0.2972
13*	19	19	100	0.3322
14*	20	20	100	0.3243
15*	15	15	100	0.3448
17	17	17	100	0.2796
19*	19	19	100	0.4016
20*	20	20	100	0.3448
21*	12	12	100	0.3299
23	19	19	100	0.2659
28	17	17	100	0.2852
31	19	19	100	0.2761
34	23	23	100	0.2268
35	17	17	100	0.274
36	25	25	100	0.249

Note: TNB: Total Number of bands, NPB: Number of polymorphic bands, PPB: Percentage of Polymorphic band, PIC: Polymorphism information content. *Primer chose only SCoT Primer with PPB 100% and PIC over 0.3 (PIC range is 0-0.5). SCoT Primer 13, 14, 15, 19, 20, and 21 were calculated for genetic diversity

Table 3. Statistical characteristics of the six SCoT markers used in the present study

SCoT primers	TNB	NPB	PPB	PIC	RP*	Range of band size
13	18	18	100	0.342	7.86	150-1250
14	20	20	100	0.337	8.57	200-1100
15	20	20	100	0.333	8.57	150-200
19	19	19	100	0.401	8.57	200-1500
20	20	20	100	0.352	9.14	300-2000
21	31	31	100	0.328	12.43	200-2500
Average:	-			0.353	-	
Total:	129			-	55.14	

Note: RP*: resolving power, calculated with a calculator in https://irscope.shinyapps.io/iMEC/ (Amiryousefi et al. 2018)

Table 4. Genetic information from six selected SCoT primers based on group and species level

	N	NPL	PPL	Na	Ne	H	I	Ht	Hs	Gst	Nm
Group 1	3	49	44.95	1.65± 0.48	1.31± 0.33	0.19± 0.18	0.30± 0.27	0.21± 0.014	0.18 ± 0.01	0.14	3.06
Group 2	8	87	79.82	1.76± 0.43	1.29± 0.27	0.19± 0.15	0.31± 0.22				
Group 3	3	41	37.61	1.50± 0.50	1.25± 0.23	0.145±0.19	0.25 ±0.26				
Species	14	109	100	2	1.30±0.21	0.21±0.16	0.35±0.15				

Note: N: Sample Size, NPL: Number of Polymorphic Loci, PPL: Percentage of Polymorphic Loci, Na: number observed alleles, Ne: Effective Number of alleles, H: Nei's gene diversity, I: Shannon's information index, Ht: The total genetic diversity, Hs: The mean within-population genetic diversity, Gst: The genetic differentiation coefficients among the different population, Nm: Estimate of gene flow, Nm: 0.5(1-Gst)/Gst

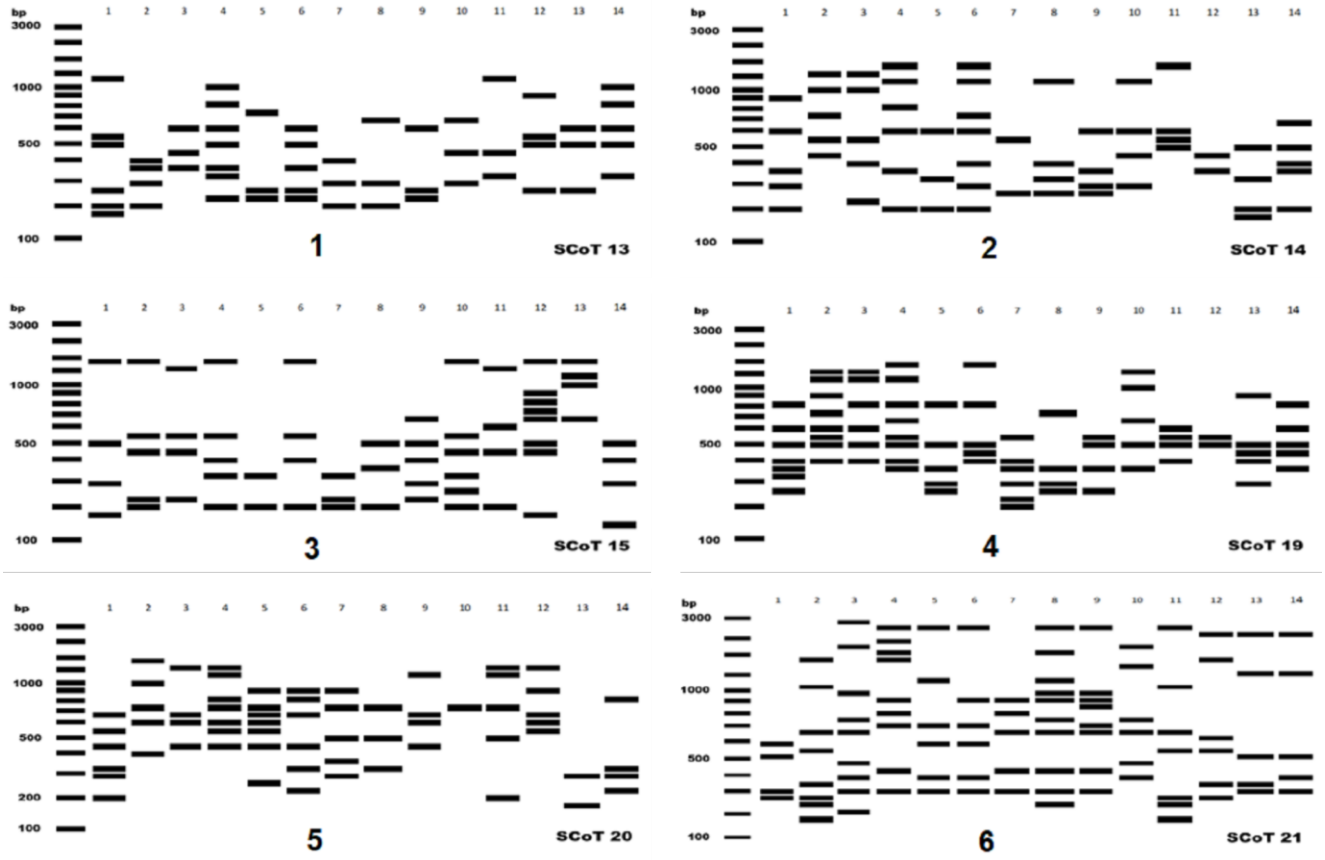

Figure 2. A diagram representing the DNA profiles of *A. crenata* in Thailand that were produced by six selected SCoT markers

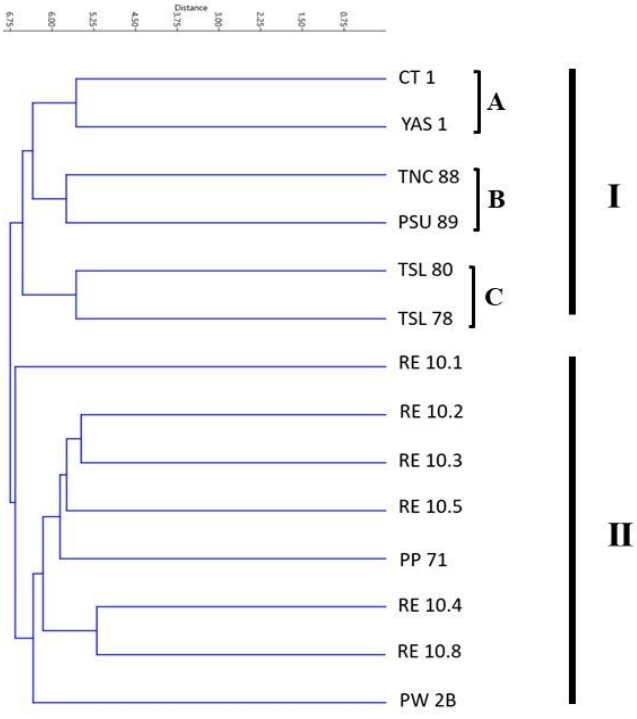

Figure 3. Dendrogram of 14 samples of *A. crenata* based on six selected SCoT primers. The sample code refers to Table 1

The SCoT data were subjected to a principal coordinate analysis (PCoA) to obtain an alternative view of the grouping among the individual samples. This analysis resulted in a PCoA plot (Figures 3-4) and revealed two major groups of *A. crenata*, as same as the group resulting from cluster analysis. Likewise, the group members are absolutely the same as the group produced by UPGMA. Therefore, both analyses show consistent results between cluster analysis and PCoA. Furthermore, the statistical summary from PCoA is shown in Table 6. The total percentage variation of coordinates 1, 2, and 3 is 44.95%. The value indicates the complex multidimensional nature of SCoT variation.

Discussion

The recent study recorded first using the SCoT marker in *A. crenata* for indicating genetic variation among some different natural populations. The result indicated that the SCoT marker could be used to examine genetic information at an intraspecific level for *A. crenata*. The Polymorphic information content (PIC) generated from six SCoT primers in 14 individual samples of *A. crenata* show high informative polymorphic (PIC=0.35 on average). The lowest and the highest are 0.328 to 0.402, respectively.

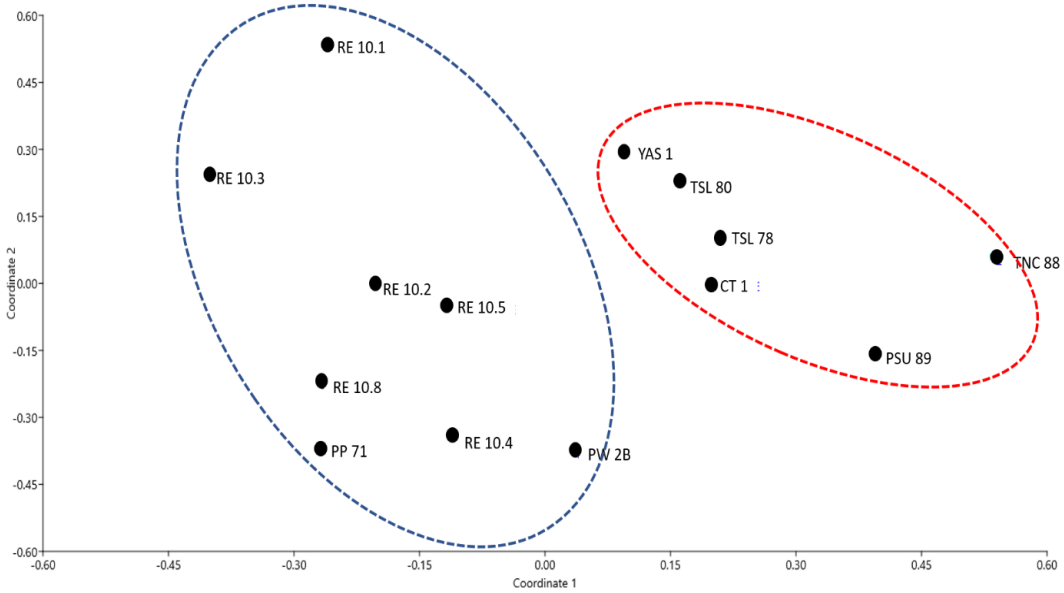

Figure 4. Scatterplot based on coordinate 1 and coordinate 2 of principal coordinate analysis of 14 samples of *A. Crenata*

This PIC value is higher than other studies using SCoT in different plants. For example, Sookcharoen (2020) reported the PIC value for the genus *Laggera* (Asteraceae) as 0.325 on average, with the highest value being 3.99 and the lowest being 0.2763. On the other hand, the studies of *Elymus sibiricus* (Poaceae) in China recorded higher PIC, i.e., PIC is 0.50 (Zhang et al. 2015). The resolving power (RP) value for SCoT primer in *A. crenata* is comparable to another plant. The value ranges from 7.89 to 12.43, while the RP value in Siberian wheatgrass was between 1.40 and 6.49. In potatoes, the highest SCoT primer RP is 9.75. These PIC and RP are two important parameters as benchmarks in selecting effective fingerprint primers and the method's accuracy (Bhattacharyya and van Staden 2018). Generally, the resolving power of the SCoT marker in coralberry is still the highest among plants in previous research.

The genetic structure of *A. crenata* based on SCoT primer was variable. The value of the Estimate of gene flow (Nm) is 3.37. The value is much higher than Nm in *Laggera* spp. reported by Sookcharoen (2020), that only 0.22 and 0.25 for *L. alata* and *L crispata*, respectively. However, the value of the genetic differentiation coefficient among the population (Gst) is reversed. Gst in *A. crenata* is 0.13, and the values for *L. alata* and *L. crispata* are 0.68 and 0.67, respectively.

Cluster analyses and association among 14 samples of *A. crenata* in Thailand were consistent in two main groups (Figures 3-4). That result could be discussed based on the floristic region. Thailand, according to the Flora of Thailand, has seven floristic regions, i.e., Northern, North-Eastern, Eastern, Central, South-Eastern, South-Western, and Peninsular (Chayamarit and Balslev 2022). The grouping was generated from the SCoT dataset, with samples grouped into non-Eastern and Eastern groups. Group I (Figure 4), the non-Eastern group, was compiled from three subgroups of four provinces and four floristic regions. Subgroup A was from two regions that are South-eastern and Eastern. Subgroup B consisted of two samples from Songkhla, Peninsular region, and the third consisted of two samples from Phitsanulok, North region. Group II consisted of samples from the Eastern and North-eastern regions. This group could be predicted because both regions are close and intersect. Based on its geography, both Eastern and North-eastern are under the North-eastern region. There was only one sample from Yasothon, also from the Eastern region, which separated into Group I. This phenomenon may be due to a lack of samples for cluster analysis. A grouping based on the SCoT marker also revealed the same in Thai *Manilkara zapota* (Vanijajiva 2020). Some *Manilkara* from the Central region were separated into two groups, and each was grouped with other regions, but generally, the dominant clustered was based on the floristic region.

SCoT markers used in this study are more likely to separate member of *A. crenata* due to regional differences rather than morphology. SCoT marker ability in separated individuals from different regions was revealed in many studies. For example, Zhang et al. (2015) used 16 SCoT primers to cluster among *Elymus sibiricus* accessions. The result indicated that the marker could classify the accession from the different origins in China. In coneflower (*Echinacea*), SCoT primer is also useful for examining genetic diversity studies (Jedrzejczyk 2022). The latest publication provides updated information about the usefulness of SCoT primers in many applications for plants with economic value (Rai 2023). Another finding of the recent study regarding the separation based on the region was supported by earlier research by Mu et al. (2010). Interestingly, genetic variation in *A. crenata* was also revealed by nuclear microsatellites. For example, in south China, *A. crenata* can roughly be separated into eastern and

western groups, consistent with the floristic region of the Sino-Himalayan Forest.

The classification resulting from SCoT in *A. crenata* also revealed some morphological characteristics that cannot be used to separate samples based on those characters. In this case, leaf size and apex are used to define three intraspecific groups of *A. crenata*. Two samples with a similar range of leaf length are separated into different groups. Samples RE 10.9 and PSU 89, both were grouped in group II based on leaf size and shape but separated by SCoT marker in different group. Conversely, two different ranges of leaf size are merged in one group, i.e. sample TSL 78 was in group II and TSL 80 was in group I based on leaf size. However, SCoT marker merged both samples into one group (Figure 3). Thus, the continued morphological characteristic cannot be used to classify *A. crenata* into the intraspecific level. On the other hand, SCoT marker worked in separating sample based on floristic region for intraspecific level.

In conclusion, this study was recorded for the first use of SCoT markers as a specific marker which worked well for intraspecific levels on genus *Ardisia*. The very small amount of genomic DNA for PCR in the SCoT marker was one of the important advantages. Six selected SCoT primers showed high polymorphic data and resolving power in *A. crenata.* It separated the species from 14 different populations in Thailand. Two consistent groups were produced from UPGMA and PCoA analyses, and the result did not support the classification of the intraspecific level of *A. crenata* based on continued morphological characteristics. Briefly, the SCoT marker supported the classification in *A. crenata* based on floristic region.

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