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Genetic relationship of *Coccinia grandis* (L.) Voigt accessions, based on RAPD and ISSR markers

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ABSTRACT

Coccinia grandis (L.) Voigt or ivy gourd is widely distributed in nature and has potential for medicinal uses. However, there is little information on the genetic diversity of wild populations in Thailand. Here, 15 ivy gourd accessions were collected from Bangkok and nearby provinces. Angled and lobed leaves were observed. DNA fingerprints were generated from twelve random amplified polymorphic DNA (RAPD) and twelve inter-simple sequence repeat (ISSR) primers. The level of polymorphism in the RAPD profiles was higher than that of the ISSR profiles. Dice's similarity coefficient from RAPD data ranged from 0.73 to 0.82 and was lower than that from the ISSR data (0.88-1.00). The unweighted pair group method with arithmetic mean dendrogram, based on combined RAPD and ISSR data, was similar to the RAPD dendrogram but distinct from the ISSR dendrogram. Two major clusters were found with eleven and four accessions. The genetic relationship between some accessions was relatively consistent with either their locations or leaf shapes. The result indicated that the RAPD and ISSR techniques were suitable for the genetic study of ivy gourd. It also provided the basis for future studies with a higher number of samples for a better understanding of ivy gourd genetic diversity in Thailand.

Keywords: *Coccinia grandis*; ivy gourd; RAPD; ISSR; DNA markers

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1. INTRODUCTION

Coccinia grandis (L.) Voigt, commonly known as ivy gourd, is a dicotyledonous, perennial vine. It belongs to the family Cucurbitaceae (Shaina and Beevy, 2012). In southeast Asia, it is widely distributed in many countries, including the Philippines, Indonesia, Malaysia, and Thailand (Bunkrongcheap et al., 2014). The following details of the botanical features of ivy gourd were described in previous literature (Pekamwar et al., 2013; Wasantwisut and Viriyapanich, 2003). Leaves are simple with palmately reticulate venation. Leaf shapes are angled and resemble a heart or a pentagon. Lobed leaves are also found. The leaf arrangement is alternate. Coil-like tendrils are present and aid the vine in climbing. Ivy gourd is dioecious. Calyx and corolla are found on both male and female flowers. Five subulate, recurved lobes of calyx are present on the hypanthium. The corolla is sympetalous and deeply divided into five lobes. Male flowers contain three stamens, while female flowers have a two-lobed stigma with an inferior ovary. Fruits are of the berry type. Young fruits are green and become red upon ripening. Fruit length is approximately 25-60 mm with a diameter that ranges from 15-35 mm.

There are many types of DNA markers available for the assessment of genetic diversity in plants. Molecular markers were also used in breeding programs for the selection of varieties with desirable traits (Supari et al., 2019). Among these, random amplified polymorphic DNA (RAPD) markers have been extensively used in various living organisms, including microbes (Kaur et al., 2017), plants (Younis et al., 2020), and animals (Hussain et al., 2021). Their major advantage is that DNA fingerprints could be generated without prior knowledge of the genome

sequences (Nasution et al., 2021). Previous studies demonstrated the use of RAPD markers for the assessment of genetic variations in many plant species, e.g., *Rhabdosciadium aucheri* (Kazemeini et al., 2020), *Muntingia calabura* L. (Nasution et al., 2021), and *Calycophyllum spruceanum* Benth. (Saldaña et al., 2021). However, as more genetic information is required for the elucidation of genetic diversity, recent studies have adopted a combination of DNA markers from two or more techniques. Similar to RAPD, PCR-based inter-simple sequence repeat (ISSR) markers were often used in the study of plant genetics (Reddy et al., 2002). Primer sequences contain a repeat sequence with anchor bases on the 3′ end (Godwin et al., 1997). These primers detect genetic variations in DNA regions between two simple sequence repeats (SSR), which are abundant in the genomes (Godwin et al., 1997). Several previous studies employed RAPD and ISSR markers to evaluate the genetic diversity of various medicinal plants, e.g., ginger (*Zingiber officinale*) (Baruah et al., 2019), *Nilgirianthus ciliatus* (Rameshkumar et al., 2019), anise (*Pimpinella anisum*) (Akçali Giachino, 2020), and *Costus pictus* (Naik et al., 2017). These markers were also used for studying the genetic fidelity of micropropagated plantlets (Ahmed et al., 2017; Srinivasan et al., 2021).

The medicinal benefits of ivy gourd were previously described. A study showed that ivy gourd root extract displayed antiadipogenic effects by significantly lowering the intracellular fat level in 3T3-L1 adipocytes (Bunkrongcheap et al., 2014). Expression of the adipocyte differentiation genes was also suppressed by the extract (Bunkrongcheap et al., 2014). Antioxidant, anti-inflammatory, anti-apoptotic, anti-glycation and insulinotrophic activities of ivy gourd extracts have also been reported (Albrahim et al., 2020; Meenatchi et al., 2017; Pekamwar et al., 2013). There have been few studies of genetic variation in ivy gourd. RAPD markers have previously been used to identify genetic elements that may contribute to male and female flowers (Bhowmick et al., 2014; Hossain et al., 2016). ISSR markers were used to elucidate the genetic relationship between ivy gourd and its relatives in the family Cucurbitaceae (Payel et al., 2015). In Thailand, variable leaf shapes of ivy gourd were observed in wild populations, and the consumption of cooked leaves is common (Astiti et al., 2021). However, there have been no reports on the genetic study of ivy gourd in Thailand. Thus, the aim of this study was to examine the suitability of RAPD and ISSR markers for generating DNA fingerprints of ivy gourd accessions collected from Bangkok and nearby provinces. The results also portrayed the genetic relationship between these accessions. It also suggested that RAPD and ISSR markers could be further extended to larger populations for an assessment of genetic diversity of ivy gourd in Thailand.

2. MATERIALS AND METHODS

2.1 Ivy gourd accessions and locations

Fifteen accessions of ivy gourd were collected from four provinces in Thailand. Eight accessions were from four GPS locations in Bangkok. Five accessions were from four GPS locations in Chachoengsao. One accession was collected from Nakhon Nayok and Pathum Thani. The shapes of mature leaves and the plant sexes were recorded. Details of all samples are provided in Table 1.

Table 1. Details of ivy gourd accessions used in this study

Accessions	Leaf shape	Sex	GPS location	District	Province
1	lobed	Female	13°43’38’’N 100°48’09’’E	Ladkrabang	Bangkok
2	angled	Female			
3	lobed	Female	13°43’47’’N 100°49’14’’E	Ladkrabang	Bangkok
4	angled	Male			
5	lobed	Female	13°45′37’’N 100°50’17’’E	Ladkrabang	Bangkok
6	angled	Female			
7	lobed	Female	13°47’41’’N 100°52’11’’E	Nong Chok	Bangkok
8	angled	Female			
9	angled	Male	13°57’42’’N 100°53’44’’E	Lamlukka	Pathum Thani
10	angled	Male	14°08’30’’N 100°58’36’’E	Ongkharak	Nakhon Nayok
11	angled	Female	13°50’08’’N 101°03’20’’E	Bang Nam Priao	Chachoengsao
12	lobed	Female	13°50’05’’N 101°03’29’’E	Bang Nam Priao	Chachoengsao
13	angled	Female	13°45’37’’N 101°04’51’’E	Mueang	Chachoengsao
14	lobed	Female	13°45’46’’N 101°04’56’’E	Mueang	Chachoengsao
15	angled	Female			

2.2 DNA extraction

Fresh tissues of young leaves (100 mg) were used for genomic DNA extraction using the FavorPrep Plant Genomic DNA Extraction Mini Kit (Favorgen, Taiwan), according to the protocol provided by the manufacturer. The integrity of genomic DNA was analyzed using agarose gel electrophoresis with 1% agarose. The DNA concentration was determined based on the absorbance at 260 nm, using Nanodrop (Thermo Fisher Scientific, USA). DNA quality was examined using the ratios between the absorbance at 260 nm and 280 nm as well as 260 nm and 230 nm.

2.3 Amplification of RAPD markers

Equal volumes of genomic DNA (50 ng/µL) from all samples were pooled. This was used for the screening of the RAPD and ISSR primers. A total of 60 RAPD primers were screened. These primers were the OPD (01-20), OPE (01-20), and OPF (01-20) sets. For amplification of the RAPD markers, each PCR reaction (20 µL) consisted of 2

µL of DNA, 1 µL of 10 µM primer, 2 µL of 10X reaction buffer, 2 µL of 2 mM dNTP, 0.6 µL of 25 mM $MgCl_2$, 0.5 U of *Taq* DNA polymerase (NEB, USA), and water. The temperature profile for the reaction was as follows: 94°C for 4 min; 40 cycles of 94°C for 30 s, 35°C for 30 s, and 72°C for 2 min; 72°C for 7 min. Subsequently, twelve RAPD primers were selected for generating RAPD fingerprints from 15 ivy gourd accessions. The RAPD fingerprints were examined using agarose gel electrophoresis with 1% agarose (Table 2).

Table 2. RAPD primers and amplification details for the study of the DNA fingerprints of 15 ivy gourd accessions

Primer	Sequence (5′-3′)	Total markers	% of Polymorphic markers	H	PIC	D	R
OPD-02	GGACCCAACC	12	66.7	0.46	0.35	0.59	4.40
OPD-05	TGAGCGGACA	11	63.6	0.43	0.34	0.52	4.40
OPD-08	GTGTGCCCCA	7	71.4	0.40	0.32	0.48	2.13
OPD-11	AGCGCCATTG	4	75.0	0.46	0.35	0.58	1.20
OPD-13	GGGGTGACGA	6	50.0	0.45	0.35	0.57	1.87
OPD-18	GAGAGCCAAC	10	50.0	0.37	0.30	0.43	2.80
OPE-03	CCAGATGCAC	6	16.7	0.21	0.19	0.23	0.53
OPE-07	AGATGCAGCC	4	50.0	0.38	0.30	0.44	1.60
OPE-15	ACGCACAACC	7	42.9	0.31	0.26	0.35	1.60
OPE-20	AACGGTGACC	9	66.7	0.45	0.35	0.57	3.60
OPF-01	ACGGATCCTG	8	75.0	0.36	0.29	0.41	2.80
OPF-05	CCGAATTCCC	8	37.5	0.31	0.26	0.35	1.60
Average		7.7	55.4	0.38	0.31	0.46	2.38

Note: H = heterozygosity index; PIC = polymorphism information content; D = discriminating power; R = resolving power

2.4 Amplification of ISSR markers

Fifty-nine ISSR primers (University of British Columbia; UBC) were tested. These primers were UBC-807 to -830, UBC-834 to -836, UBC-840 to -862, UBC-866, UBC-870, UBC-873 to -875, UBC-877 to -878, and UBC-880 to -881. The reaction mixture for ISSR marker amplification was similar to the reaction used for the amplification of RAPD markers. The temperature profile for the reaction was as follows: 95°C for 3 min; 40 cycles of 95°C for 30 s, 45°C for 45 s, and 72°C for 90 s; 72°C for 20 min. Twelve ISSR primers (Table 3) were used for generating ISSR profiles of each ivy gourd accession. The PCR products were examined using gel electrophoresis with 1% agarose.

Table 3. ISSR primers and amplification details for the study of the DNA fingerprints of 15 ivy gourd accessions

Primer	Sequence (5′-3′)	Total markers	% of Polymorphic markers	H	PIC	D	R
UBC-813	$(CT)_8T$	6	33.3	0.35	0.29	0.40	1.20
UBC-814	$(CT)_8A$	10	60.0	0.44	0.34	0.55	2.80
UBC-815	$(CT)_8G$	6	33.3	0.38	0.31	0.45	0.93
UBC-834	$(AG)_8YT$	8	37.5	0.37	0.30	0.43	1.47
UBC-835	$(AG)_8YC$	11	45.5	0.34	0.28	0.38	2.53
UBC-840	$(GA)_8YT$	11	45.5	0.22	0.20	0.24	2.13
UBC-842	$(GA)_8YG$	7	42.9	0.26	0.22	0.28	0.40
UBC-844	$(CT)_8RC$	6	33.3	0.28	0.24	0.31	0.80
UBC-857	$(AC)_8YG$	9	11.1	0.11	0.11	0.12	1.07
UBC-866	$(CTC)_6$	10	30.0	0.25	0.22	0.28	0.93
UBC-873	$(GACA)_4$	5	20.0	0.30	0.26	0.34	0.13
UBC-881	$(GGGT)_3G$	9	44.4	0.17	0.16	0.18	1.73
Average		8.2	36.4	0.29	0.24	0.33	1.34

Note: H = heterozygosity index; PIC = polymorphism information content; D = discriminating power; R = resolving power

2.5 Data analysis

All RAPD and ISSR markers of all accessions were converted to binary data, indicating 1 for presence and 0 for absence. The expected heterozygosity (Liu, 1998), polymorphism information content (Botstein et al., 1980), discriminating power (Tessier et al., 1999), and resolving power (Prevost and Wilkinson, 1999) of the RAPD and ISSR primers were calculated, using the software iMEC (Amiryousefi et al., 2018).

The program Dissimilarity Analysis and Representation for Windows (DARwin) (Perrier and Jacquemoud-Collet, 2006) was used for the calculation of Dice's similarity coefficient (Dice, 1945) and the construction of dendrograms. The RAPD and ISSR data were separately used for generating dendrograms, based on the unweighted pair group method with arithmetic mean (UPGMA) method. The combined RAPD-ISSR data were also constructed, using the same method.

3. RESULTS AND DISCUSSION

Fifteen ivy gourd accessions were collected from various locations in Bangkok, Chachoengsao, Nakhon Nayok, and Pathum Thani provinces (Table 1). Some morphological characteristics of these accessions were different. Firstly, variations in leaf shapes were observed among the accessions (Table 1). Nine accessions had angled leaves that resembled a pentagon, while six accessions produced lobed leaves (Figure 1). Twelve accessions were female, while three accessions produced male flowers (Table 1).

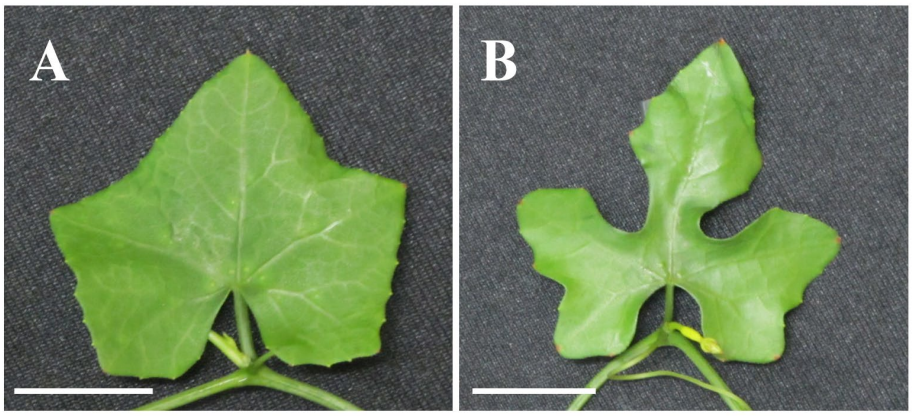

Figure 1. Angled mature leaves of accession 9 (A) and lobed mature leaves of accession 12 (B)
Note: Bar = 2 cm.

Sixty RAPD and 59 ISSR primers were tested for the amplification of DNA markers with pooled genomic DNA. Twelve RAPD (Table 2) and twelve ISSR primers (Table 3) were selected based on the amplification of relatively clear DNA bands (Figure 2). When tested for amplification with 15 ivy gourd accessions, the RAPD primers yielded different numbers of markers ranging from four to twelve. The average number of markers per primer was 7.67. In total, 92 RAPD markers were obtained from the RAPD primers. Among these, 52 markers were polymorphic. The average percentage of polymorphic markers was 55.4% (Table 2). The number of ISSR markers from each primer ranged from five to eleven. The average number of markers per ISSR primer was 8.2. In total, these ISSR primers generated 98 markers. However, only 37 ISSR markers were polymorphic, and the average percentage of polymorphic markers (36.4%) (Table 3) was lower than that of RAPD markers. Expected heterozygosity, polymorphism information content, discriminating power, and resolving power were determined for the RAPD (Table 2) and ISSR primers (Table 3). The average values of these indices of the RAPD primers were relatively higher than those of the ISSR primers. OPD-02 displayed the highest level of expected heterozygosity (0.46), polymorphism information content (0.35), discriminating power (0.59), and resolving power (4.40). In contrast, among the ISSR primers, UBC-814 expressed the highest level of expected heterozygosity (0.44), polymorphism information content (0.34), discriminating power (0.55), and resolving power (2.80).

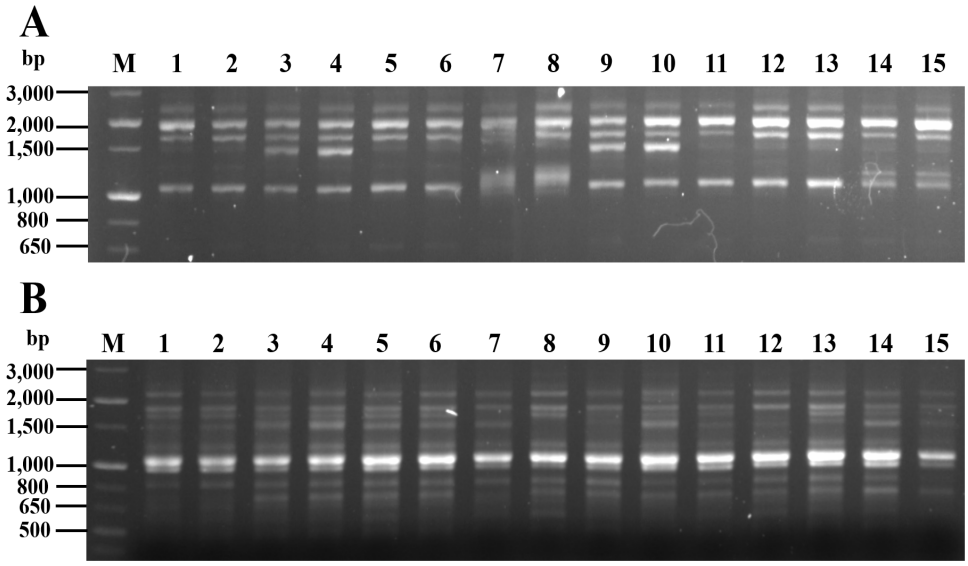

Figure 2. DNA fingerprints of the 15 ivy gourd accessions generated by RAPD primer OPE-03 (A) and ISSR primer UBC-866 (B)
Note: M = 1 Kb DNA ladder (Enzynomics, Korea); 1-15 = ivy gourd accessions 1-15, respectively.

Based on the RAPD data, Dice's similarity coefficient ranged from 0.73 (accessions 3 and 13 and accessions 4 and 13) to 0.98 (accessions 1 and 2), with the average level at 0.85. The lowest coefficient obtained from the ISSR data was 0.88 (accessions 7 and 11), while the highest value was 1.00 (accessions 1 and 2 and accessions 3 and 4). The average value of the coefficient for ISSR markers was 0.92. When the RAPD and ISSR data were combined, accessions 4 and 7, accessions 3 and 13, and accessions 4 and 13 displayed the lowest coefficient (0.84), while accessions 1 and 2 and accessions 3 and 4 showed the greatest coefficient (0.99). The dendrogram generated from RAPD data indicated these 15 ivy gourd accessions formed two major clusters (Figure 3). The first cluster consisted of accessions 1, 2, 5, 6, 7, 8, 9, 12, 13, 14, and 15, while the second cluster comprised the remaining four accessions. Based on this dendrogram, seven pairs of accessions were found to be closely related, including accessions, 1 and 2, 3 and 4, 5 and 8, 7 and 12, 9 and 13, 10 and 11, and 14 and 15. Except for accessions 9 and 13, six of these pairs were also reproduced in the ISSR dendrogram (Figure 4). Accession 9 was distantly related, while accession 13 was more closely related to accessions 1, 2, and 6. Additionally, the clustering pattern of the ISSR dendrogram was somewhat different from that of the RAPD dendrogram. The first cluster contained eight accessions including 1, 2, 3, 4, 6, 13, 14, and 15. Accessions 5, 7, and 8 formed the second cluster, while the third cluster consisted of only accession 12. The fourth cluster contained accessions 10 and 11. Accession 9 was placed as the most distant accession.

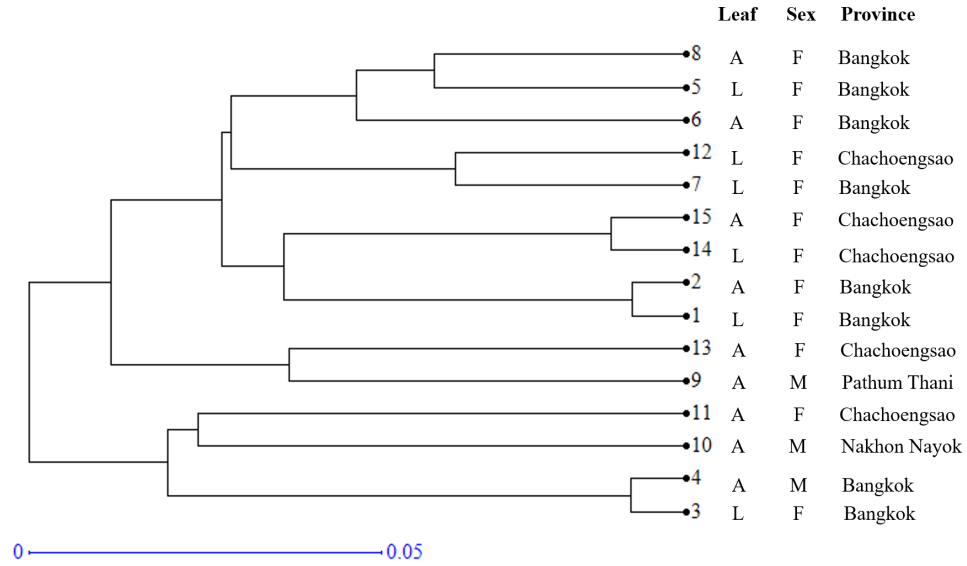

Figure 3. The UPGMA dendrogram based on the RAPD profiles of the 15 ivy gourd accessions
Note: A = angled leaf; L = lobed leaf; F = female; M = male.

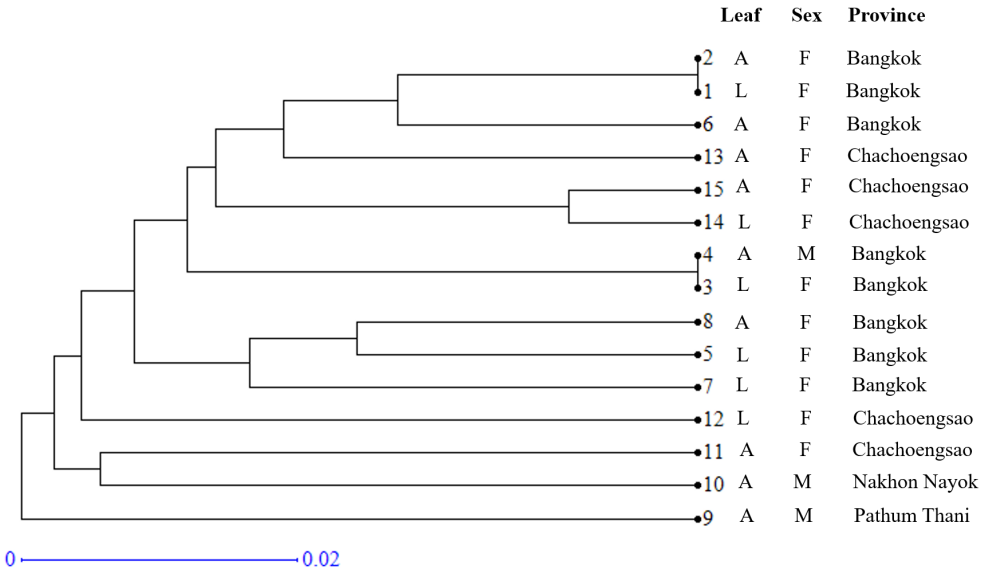

Figure 4. The UPGMA dendrogram based on ISSR profiles of the 15 ivy gourd accessions
Note: A = angled leaf; L = lobed leaf; F = female; M = male.

The dendrogram, based on the combined RAPD and ISSR data, was also constructed (Figure 5). Two major clusters were observed and were similar to those found on the RAPD dendrogram (Figure 3). Except for accession 9, the remaining ten accessions of the first cluster were female. In contrast, the second cluster contained two female plants and two male plants. The close relationships between some of the pairs found in this dendrogram were consistent with their locations. Accessions 1 and 2 were both collected from the same area in Bangkok. A similar result was observed between accessions 3 and 4 (Bangkok) and accessions 14 and 15 (Chachoengsao). Other pairs displayed consistency with their leaf shapes. Accessions 7 (Bangkok) and 12 (Chachoengsao) produced lobed leaves. Leaves of accessions 9 (Pathum Thani) and 13 (Chachoengsao) as well as accessions 10 (Nakhon Nayok) and 11 (Chachoengsao) were angled. In contrast, accessions 5 and 8 produced lobed and angled leaves, respectively, and were collected from different locations in Bangkok. However, both accessions were female plants. It should also be noted that the number of ivy gourd accessions for the two types of leaf shapes, sexes, and sample locations was unequal. Thus, a larger populational study would be needed for the confirmation of these markers as the tools to genetically differentiate ivy gourds of different leaf shapes, sexes, or geographical locations.

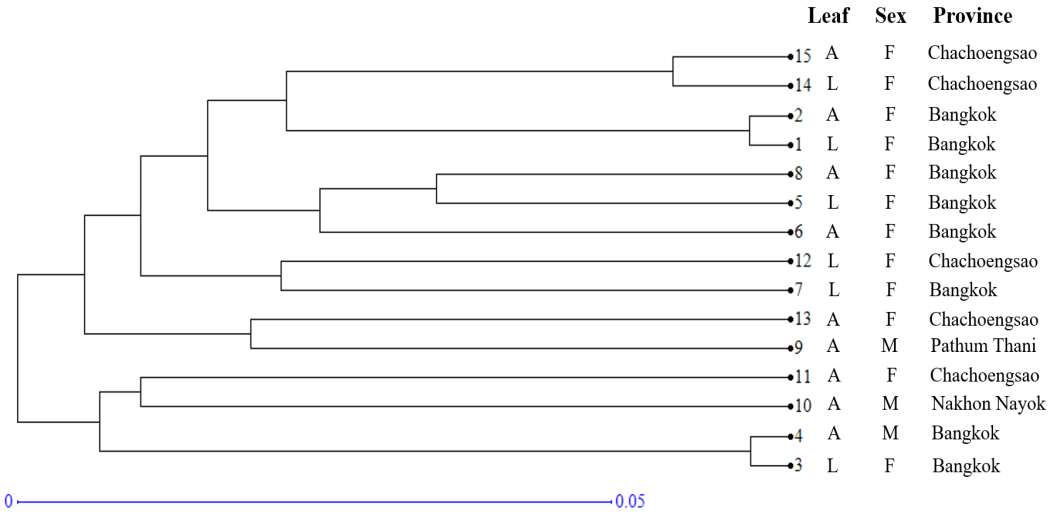

Figure 5. The UPGMA dendrogram based on combined RAPD-ISSR profiles of the 15 ivy gourd accessions
Note: A = angled leaf; L = lobed leaf; F = female; M = male.

Leaf morphology was significant for traditional studies of genetic diversity in plants. Previously, it accounted for 23.8% of the morphological characteristics used for the assessment of evolutionary processes among ivy gourds, collected from various locations in India (Shaina and Beevy, 2012). Variations in leaf shapes were derived from genetic factors. In *Arabidopsis thaliana*, many genes were previously demonstrated for their regulatory roles in leaf shape formation. Dominant mutations of *PHAVOLUTA* and *PHABULOSA* resulted in the formation of rod-shaped mutant leaves, as opposed to flat and expanded leaves in wild-type plants (Ali et al., 2020). Ectopic expression of the *CUC2* and *KNOX* genes caused a change in normal leaves to lobed and crinkly leaves (Ali et al., 2020). Internal interactions between genetic factors and internal cues also regulated leaf formation. Double mutations in the *Nia1* and *Nia2* genes caused impairments in the production of nitrate reductase and consequently the loss of the production of nitric oxide (Pan et al., 2019). The *nia1nia2* double mutant displayed an increase in reactive oxygen species and changes in leaf shapes compared to the wild type (Pan et al., 2019). This suggested that the regulation of reactive oxygen species by nitric oxide played an important role in leaf formation (Pan et al., 2019). In Thailand, ivy gourds are generally found climbing on fences and other trees. It is also sold in local markets. Angled leaves with a tender texture are preferred for cooking in Thai cuisine. However, variations in leaf shapes could still be observed among wild populations. In the present study, two types of leaf shapes were observed. Nine accessions produced angled, pentagon-like leaves, while six accessions had lobed leaves. These leaf-shape variations suggested the existence of genetic distinctions among these accessions. However, in a previous study, UPGMA clustering based on morphological characteristics did not provide a clear distinction between wild and cultivated accessions of ivy gourd (Shaina and Beevy, 2012). Thus, further genetic analysis based on other molecular markers or techniques may be required to elucidate the genetic factors that contribute to leaf formation in ivy gourd.

RAPD and ISSR markers were generated in this study to assess the genetic relationship between 15 ivy gourd accessions, because of their higher levels of genetic information compared to morphological characteristics. The number of generated RAPD markers was relatively lower than ISSR markers. However, the average values of expected heterozygosity, polymorphism information content, discriminating power, and resolving power of the RAPD primers were relatively higher than those of the ISSR primers. The average resolving power of the RAPD primers (2.38) was approximately 1.8-fold that of the ISSR primers (1.34) and displayed the greatest difference among the indices. Because resolving power represented the ability of a primer or a technique to distinguish between genotypes (Prevost and Wilkinson, 1999), the

power of the RAPD primers for distinguishing ivy gourd accessions was higher than that of the ISSR primers. This was consistent with the percentages of polymorphic markers and the ranges of Dice's similarity coefficient obtained from the RAPD and ISSR primers. Similarly, a previous study of *Momordica cochinchinenesis* (Cucurbitaceae) showed that the range of Nei's genetic distance from RAPD fingerprints (0.03 - 0.76) was larger than that from ISSR profiles (0.00 - 0.55) (Wimalasiri et al., 2016). In contrast, a comparative study of *Dalbergia oliveri* (Fabaceae) demonstrated a higher number of ISSR polymorphic markers than RAPD markers (Phong et al., 2011).

Consistent with Dice's coefficient, the dendrogram from the combined RAPD and ISSR data (Figure 5) of the 15 ivy gourd accessions resembled the one from the RAPD markers (Figure 3) rather than that from the ISSR markers (Figure 4). This was in contrast to previous studies of *Vigna mungo* (Fabaceae) and *Citrullus colocynthis* (Cucurbitaceae), where the clustering pattern of combined RAPD and ISSR data was more similar to that of the ISSR data (Souframanien and Gopalakrishna, 2004; Verma et al., 2017). These discrepancies suggested the influence of genomic distinction between plant species on the detection of polymorphisms by different DNA marker techniques. The dendrograms also indicated the genetic relationship between the ivy gourd accessions. However, the tested set of primers, both RAPD and ISSR, was unable to unequivocally demonstrate the distinction between the two morphological types of leaves, sexes, or sample locations.

The levels of polymorphism from the RAPD (55.45%) and ISSR (36.40%) markers observed in the present study were relatively low. This suggested a high level of genetic similarity between the accessions. Alternatively, it might be caused by the short distances between the locations of the ivy gourd accessions, as shown by the pairing of some accessions from the same locations in the RAPD-ISSR dendrogram (Figure 5), in contrast to another RAPD fingerprint study in eight accessions of wild *Muntingia calabura* L. collected from Thailand and Indonesia (Nasution et al., 2021). Twelve RAPD primers displayed levels of polymorphism that ranged from 60% to 100% and were relatively higher than those obtained in the present study. Additionally, another study also demonstrated higher levels of polymorphisms of RAPD (60.98%) and ISSR (74.59%) primers in 54 castor bean varieties obtained from various countries, including India, Jordan, France, Vietnam, Pakistan, Paraguay, Malaysia, Ethiopia, Indonesia, Nigeria, and China (Kim et al., 2021). Thus, a wider range of locations for sample collection should be emphasized in future studies to determine the genetic diversity of ivy gourd.

4. CONCLUSION

The present study demonstrated the genetic relationships of 15 ivy gourd accessions that were collected from ten different locations in Bangkok, Chachoengsao, Nakhon Nayok, and Pathum Thani provinces, Thailand. This was based on the DNA fingerprints obtained from RAPD and ISSR techniques. Comparison of these markers suggested the RAPD markers provided greater power for the detection of the distinction between closely related accessions than the ISSR markers. The relatively low level of polymorphic markers might result from the proximity of the sample locations. However, the construction of the RAPD-ISSR dendrogram somewhat indicated close genetic relationships between ivy gourd accessions that were either from the same locations or had similar leaf shapes. Thus, the result indicated that RAPD and ISSR techniques would be applicable for future populational studies with a higher number of ivy gourd samples from a wider range of locations.

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