Assessing genetic diversity of some *Anthurium andraeanum* Hort. cut-flower cultivars using ISSR markers

Nattapong Srisamoot*, Intorawatch Padsri

Division of Biotechnology, Faculty of Agro-Industrial Technology, Kalasin University, Kalasin 46000, Thailand

**Corresponding author: nattapong2.sr@ksu.ac.th*

ABSTRACT

Anthurium andraeanum Hort. cultivars are an important ornamental plant with a very high genetic diversity. Most cultivars are characterized based only on the phenotype of inflorescence which are difficult to distinguish between very closely related cultivars. This research aimed to assess the genetic diversity of 26 *Anthurium* cultivars using inter simple sequence repeat (ISSR) markers. The ten ISSR primers generated 122 total bands and 113 polymorphic bands with an average percentage of polymorphism of 91.64%. The average polymorphic information content (PIC) was 0.26, the average effective multiplex ratio (EMR) was 10.54, the average marker index (MI) was 2.84 and the average resolving power (Rp) was 11.32. Genetic similarity matrix from Pearson coefficient between cultivars ranged from 0.01 between Joa Sua and Cherry Pink to 0.87 between Red Bar and Sun Red, with a mean of 0.58. Cluster analysis using the unweighted pair group method with arithmetic mean analysis (UPGMA) divided 26 cultivars into two major groups. The first group comprised only Thai native foliage anthurium while the second group included all cut-flower cultivars. Close relationship between cultivars with different appearance was found. These clustering was acceptable with Cophenetic Correlation Coefficient (CCC) was 0.92. The results obtained from this study can be useful for cultivar identification and genetic diversity assessment of *Anthurium*.

Keywords: *Anthurium*; genetic diversity; DNA markers; DNA fingerprint, ISSR

INTRODUCTION

Anthurium (family Araceae) is a popular long lasting, colorful and quaint ornamental plant. The shape of the leaves and spathes are unique, therefore they are used for decoration along with other flowers. Many *Anthurium* species are produced and distributed internationally as cut-flowers, flowering potted plants and landscape plants. *Anthurium*, which includes more than 600 species (Souza Neto *et al*., 2014), is possibly one of the most complex genera in the Araceae family (Nowbuth *et al*., 2005). It is believed that most of the cut-flower anthuriums are hybrids of *Anthurium andraeanum* Linden ex André with several closely related species and have been referred to as *Anthurium andraeanum* Hort (Nowbuth *et al*., 2005). A demanding market requires uniform plants with high-quality flowers and a high yield (Stancato and Tucci, 2010). The novel anthurium cultivars are breed to get the varieties that look great and strange. To fulfill these purposes, the knowledge regarding genetic diversity and genetic information of the anthurium cultivars are important for plant improvement programs. However, the characterization of anthurium cultivar has been based only on the phenotypic traits of inflorescence and the genetic information of *Anthurium* is scarce.

Molecular marker is an essential tool to estimate the genetic diversity and to establish genetic information. This marker can be directly used to evaluate diversity at the DNA level and eliminating the influence of environmental variations (Souza Neto *et al*., 2014). In past years, biotechnology has been developed rapidly. Molecular markers became the important tools in genetic research and assisted in breeding efficiency. A large number of molecular techniques are now available for assessing the genetic diversity of plants. Among them, inter-simple sequence repeats (ISSRs) have been shown to provide a powerful, rapid, simple, reproducible and inexpensive means to assess genetic diversity and identify the closely related cultivars in many species (Srisamoot and Sootsuwan, 2016). ISSR technique involves the use of a microsatellite core unit bearing oligonucleotide primers, usually 16–25 bp long, non-anchored or anchored at the 5’ or 3’ end with 1–4 degenerate nucleotides. ISSR can be a rapid and easy technique for identifying gene-specific trait because it overcomes many technical limitations of RAPD and AFLP due to its high reproducibility and simplicity (Bornet and Branchard, 2001). The ISSR technique has been extensively used for several applications especially to detect the genetic similarities or dissimilarities in

various plants, such as pineapple (*Ananas comosus* (L.) Merr.) (Souza *et al*., 2017), *Rheum* (Tabin *et al*., 2016), sorghum (*Sorghum bicolor* (L.) Moench) (Basahi, 2015), *Lilium* (Zhao *et al*., 2014), wheat (*Triticum aestivum* L.) (Bararkat *et al*., 2013; Najaphy *et al*. 2011), alfalfa (*Medicago sativa* L.) (Rashidi *et al*. 2013) and mulberry (Kalpana *et al*., 2012). For *A. andraeanum*, ISSR markers have been used in current breeding programs, estimating the genetic similarity among genotypes, for the identification of cultivars and population structure by various workers (Tabin *et al*., 2016). Thus, ISSR markers are used to analyze the genetic diversity of *Anthurium* cultivars that are very diverse.

MATERIALS AND METHODS

Sample collection and DNA extraction

Twenty-six *Anthurium* cultivars were collected from ornamental plant markets in the Central and the Northeastern regions of Thailand (listed in Table 1) and subsequently planted to preserve germplasms at Kalasin University, Kalasin.. The cultivar names were classified by the seller.

Table 1 List of *Anthurium* cultivars used in the present study and its morphological characters.

No.	Taxon	Morphological characters			Source (Province in Thailand)
		Over view	Spathe Color	Spadix Color	
1	Arizona	30-cm tall, large leaf with small petioles.	Red	Yellow	Bangkok
2	Cherry Pink	20-cm tall, less tillering and small petioles.	Pink with green edge	Pink	Nakhon Ratchasima
3	Choke Koa Chan	25-cm tall, less tillering and spear shape leaf.	Green	Brown	Nakhon Ratchasima
4	Chompoo Noppon	30-cm tall, tillering and heart shape with spine tail leaf.	Pink	Yellow to green	Nonthaburi
5	Dakota	40-cm tall, tillering and thick petioles.	Red	Yellow to green	Bangkok
6	Dakotae	20-cm tall, tillering and heart shape with spine tail leaf.	Red	Yellow	Nonthaburi
7	Gemini	20-cm tall, tillering and thick petioles.	Pink	White to yellow	Nonthaburi
8	Jenny Red	30-cm tall, less tillering and heart shape with spine tail leaf.	Red	Yellow	Nakhon Ratchasima
9	Joa Sua	30-cm tall, less tillering and big invert spear shape leaf with short petioles.	Red with green edge	Violet	Nakhon Ratchasima
10	Hoo Chang	40-cm tall, tillering and big leaf with thick petioles.	Green	brown	Nakhon Ratchasima
11	La Quinta	45-cm tall, less tillering and heart shape with spine tail leaf.	Pink	Yellow to green	Nonthaburi
12	Marshall	40-cm tall, less tillering and big leaf with thick petioles.	Pink with green edge	Yellow to pink or red	Pathumthani
13	Mayonce	25-cm tall, less tillering and triangle shape with spine tail leaf.	Pink	Yellow	Nakhon Ratchasima
14	Merengue	30-cm tall, less tillering and small petioles.	White	Pink	Pathumthani
15	Nakato	30-cm tall, less tillering and small petioles with spine tail leaf.	Red	Red	Nonthaburi
16	Pink Champion	25-cm tall, less tillering and heart shape with spine tail leaf.	Pink	Pink to green	Nakhon Ratchasima
17	Pistache	40-cm tall, less tillering, small petioles and big spine tail leaf.	Green	Pink and green	Nonthaburi
18	Plew Tien Lampang	40-cm tall, less tillering and big leaf with thick petioles.	Light pink	pink	Nakhon Ratchasima
19	Plew Tien Phuket	40-cm tall, less tillering and big leaf with thick petioles.	Pink	Red	Nakhon Ratchasima
20	Red Bar	25-cm tall, less tillering and heart shape with spine tail leaf.	Red	Green	Khon Kaen
21	Red Strong	25-cm tall, tillering and heart shape with spine tail leaf.	Red	Pink to green	Khon Kaen
22	Sangtien	30-cm tall, less tillering and spear shape with spine tail leaf.	White	Pink	Pathumthani
23	Smithson	25 cm-tall, tillering and spear shape with thick petioles.	Pink	Pink to green	Nakhon Ratchasima
24	Sun Red	25-cm tall, tillering and heart shape with spine tail leaf.	Red	Yellow to green	Khon Kaen
25	Sweetheart Pink	30-cm tall, less tillering and heart shape with spine tail leaf.	Pink	Pink	Nakhon Ratchasima
26	Ta Ole	20-cm tall, less tillering and spear shape with thin petioles.	Pink	Pink	Nonthaburi

Fresh young leaves were collected and kept in iceboxes. DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). Fresh plant tissue samples (300 mg) were grounded in liquid nitrogen and transferred to a 1.5 mL sterile reaction tube. One thousand µL of CTAB extraction buffer (20 g/L CTAB; 1.4 M NaCl; 0.1 M Tris-HCl; 20 mM Na2EDTA) and 20 µL of Proteinase K (20 mg/mL) were added. The mixture was vortexed and incubated at 65 ºC for 60 min. Further, 20 µL of RNase A (10 mg/mL) were added and the sample was homogenized and incubated at 37 ºC for 30 min. Debris was pelleted by centrifugation and the supernatant was extracted twice with phenol:chloroform:isoamylalcohol (25:24:1). DNA was precipitated with the same volume of ice cold isopropanol and centrifugation. The supernatant was discarded and the pellet was washed twice with 70% ethanol, dried and re-suspended in 50 µL of TE buffer (10 mM Tris–HCl; 1 mM EDTA, pH 8.0). DNA quality was evaluated on a 0.8% agarose gel stained with ethidium bromide. DNA concentration was measured using a spectrophotometer and the DNA samples were diluted to 60 ng/µL for polymerase chain reaction (PCR) amplification.

ISSR Fingerprinting

For PCR amplification, 10 ISSR primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18bp) were used (Table 2). These were carried out in a final volume of 25 µL, containing 60 ng of DNA template, 0.5 U *Taq* polymerase, 2.5 µL of 10X reaction buffer, 3.0 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 µM ISSR primer. DNA amplifications were performed in the thermo cycler (TGradient 96, Biometra), under the following conditions: preliminary step of 10 min at 94 ºC, followed by 40 cycles of 40 sec denaturation at 90 ºC, 45 sec for annealing temperature depending on the primers (Table 2) and 90 sec extension at 72 ºC with a final 7 min extension at 72 ºC. PCR products were separated on 1.5% agarose gels electrophoresis at 110 V in TBE 1X buffer. The gels were stained with ethidium bromide (0.05 mg/mL) and the images were visualized under UV and photographed. The obtained bands were compared with the 1kb DNA ladder marker (Invitrogen). In order to evaluate the reproducibility of the DNA profile, PCR reactions were carried out 3 times, and only well-defined and reproducible bands were considered.

Table 2 Nucleotide sequence and annealing temperature (T_a) of ISSR primers used in this study and some summarized results.

Primer	Nucleotide Sequence	Ta (ºC)	No. of total bands	No. of polymorphic bands	Polymorphism (%)	PIC	EMR	MI	Rp
ISSR02	CTCTCTCTCTCTCTCTAC	54.4	9	9	100	0.29	9.00	2.61	7.31
ISSR03	CTCTCTCTCTCTCTCTGC	58.0	15	15	100	0.35	15.00	5.25	14.08
ISSR04	CACACACACACAAC	50.8	8	6	75.00	0.21	4.50	0.95	7.62
ISSR06	CACACACACACAAG	50.4	13	12	92.31	0.33	11.08	3.66	13.85
ISSR07	CACACACACACAGG	52.9	13	13	100	0.27	14.00	3.78	7.23
ISSR10	GAGAGAGAGAGACC	49.0	9	8	88.89	0.24	7.11	1.71	13.69
ISSR13	GAGGAGGAGGC	45.8	15	13	86.67	0.15	11.27	1.69	12.62
ISSR14	GAGGAGGAGGC	45.8	17	17	100	0.30	17.00	5.10	16.85
ISSR15	GTGGTGGTGGC	48.9	9	8	88.89	0.24	7.11	1.71	7.92
ISSR16	ACTGACTGACTGACTG	54.9	13	11	84.62	0.21	9.31	1.95	12.00
Total			122	113	916.38	2.59	105.38	28.41	113.17
Mean			12.2	11.3	91.64	0.26	10.54	2.84	11.32

Data analysis

DNA banding patterns generated by ISSR-PCR amplification, those with a band on the same migration position on agarose gel were scored as present (1) and those without were scored as absent (0). These binary data were used to analyze the genetic similarity index with Pearson coefficient using the unweighted pair group method with arithmetic averages (UPGMA) and this matrix was used to construct a phylogenetic dendrogram and cellulate the Cophenetic Correlation Coefficient (CCC) using the online dendrogram construction utility DendroUPGMA

(http:// genomes.urv.es/UPGMA/) (Garcia-Vallvé *et al*., 1999). The 1000 bootstrap replicates dendrogram was constructed. The obtained dendrogram was imaged with TreeView 3.0 software (Page, 1996). Because ISSR is dominant markers, so Polymorphism Information Content (PIC) was calculated using the formula by De Riek *et al*. (2001) i.e. PICi = 1-[fi ^{2} + (1 - fi)^2], where PICi is the polymorphic information content of marker i, fi is the frequency of the marker bands present and (1-fi) is frequency of the marker bands absent. Effective multiplex ratio (EMR) is calculated as total number of polymorphic loci (per primer) multiplied by the proportion of polymorphic loci per their total number i.e. EMR = np(np/n), where np is the number of polymorphic loci, and n is the total loci number. The higher the value of EMR, the more efficient the primer system is (Chesnokov and Artemyeva, 2015). Marker index (MI) is a statistical parameter used to estimate the total utility of the maker system. Marker index is the product of the PIC value and EMR, MI = PIC x EMR (Chesnokov and Artemyeva, 2015). Resolving power (Rp) is a parameter characterizing ability of the primer combination to detect the differences among the large numbers of genotypes (Gilbert *et al*., 1999; Prevost and Wilkinson, 1999). Rp was calculated using the formula Rp = Σ IB (Prevost and Wilkinson, 1999), where IB (band informativeness) takes the value of: 1 – [2 × (0.5 – p)], p being the proportion of the 26 genotypes (*Anthurium* cultivars analyzed) containing the bands.

RESULTS AND DISCUSSION

DNA detection

Genomic DNA extracted from 26 *Anthurium* cultivars were visualized by agarose gel electrophoresis, the results showed that the loading wells were clean, the DNA bands were clear and no diffusion. The ratio of A260 nm/A280 nm was between 1.75 -1.82 (data not show). These results indicated that the extracted genomic DNA of the young *Anthurium* leaves were high quality enough for further experiment.

Polymorphism exhibited by the ISSR markers

Primers sequences, number of total bands scored, number of polymorphic bands, percent of polymorphism, polymorphic information content, effective multiplex ratio, marker index and resolving power of primers were showed in Table 2.

ISSR amplification from 26 *Anthurium* cultivars using 10 selected ISSR primers produced a total of 122 strong, clear, and reproducible bands, of which 113 were polymorphic (Table 2). The ISSR amplification generated an average of 12.2 DNA bands per primer. The size of the amplified fragments ranged from 300 to 2,000 bp (Figure 1). The primer ISSR14 generated the highest number of scorable bands (17) while the lowest (8) was generated by primer ISSR04. The average percentage of polymorphism was 91.64%. These results coincided with Souza Neto *et al*. (2014) which reported that 17 ISSR primers generated 257 total bands, of which 234 were polymorphic, yielding 91.05% polymorphism. The average polymorphism percentage from 10 primers was high enough to analyze for genetic relationship of these taxon. Similar numbers of primers were previously used to determine genetic diversity of pistachio and wheat cultivars which generated only 63.0% and 80.2% polymorphism, respectively (Tagizad *et al*, 2010; Najaphy *et al*., 2011). While 6 ISSR primers were used to detect polymorphism of 20 *Lilium* species (Zhao *et al.*, 2014). These results indicated that the average polymorphism percentage does not rely on the number of primer used. The primers ISSR02, ISSR03, ISSR07 and ISSR14 produced bands with 100% polymorphism. The lowest percentage of polymorphism was 75% with the primer ISSR04. The results showed that there was significant genetic difference and high genetic diversity among *Anthurium* cultivars. This concurs with the work of Zhao *et al*. (2014) on the genus *Lilium* in China. This may be due to the technique as reported by Salimath *et al*. (1995) which found a larger number of polymorphism using ISSR than RFLP or RAPD analyses. On the other hand, Kalpana *et al*. (2012) demonstrated that polymorphism generated by the RAPD primers was higher than ISSR primers suggested that the percentage of polymorphism depends upon samples used, the polymorphism between different species in this study is high but opposite within cultivars. However, higher percentage of polymorphism using ISSR markers than RAPD markers were reported (Srivastava *et al*., 2004; Souza Neto *et al*., 2014). A comparison of the diagnostic capacity of primers or techniques is, thus, almost invariably based on results from small-scale studies (Prevost and Wilkinson, 1999).

The PIC value ranged from 0.15 (ISSR13) to 0.35 (ISSR03) with a mean of 0.26. For dominant markers, the highest value of PIC should be 0.5 (Tabin *et al*. 2016). In general, each ISSR primer is used as both the forward and reverse primers. PIC can be determined based on both the number and frequency of the amplified fragments. The average PIC (0.26) in this study confirmed that ISSR provided a good marker system for distinguishing between different cultivars as a maximum PIC values of 0.5 for

dominant markers (Guo *et al*., 2014). Some related values, EMR and MI, were also analyzed in this study. The value of EMR indicated the efficiency of the marker system. This feature varied from 4.50 to 17.00 with an average value of 10.54. In this study, the primers that showed that the higher polymorphism had the higher EMR values which is consistent with results from Najaphy *et al*. (2011). MI is a feature of a marker which calculated for all the primers. The MI was the lowest with the primer ISSR04 (0.95) and highest with the primer ISSR03 (5.25) with an average of 2.84. Because MI is the product of PIC and EMR, therefore the higher polymorphism provides higher EMR (Najaphy *et al*., 2011) and also higher MI.

The Rp of ISSR primers ranged between 7.23 (ISSR07) and 16.85 (ISSR14) with an average of 11.32. RP was positively correlated with the number of total amplified bands. Rp value provides a moderately accurate estimate of the number of genotypes identified by a primer (Prevost and Wilkinson, 1999). Six of the ISSR primers (ISSR03, ISSR06, ISSR10, ISSR13, ISSR14, and ISSR16) occupied the high Rp values (14.08, 13.85, 13.69, 12.62, 16.85 and 12.00, respectively) and therefore seem to be the most informative primers for distinguishing the *Anthurium* cultivars.

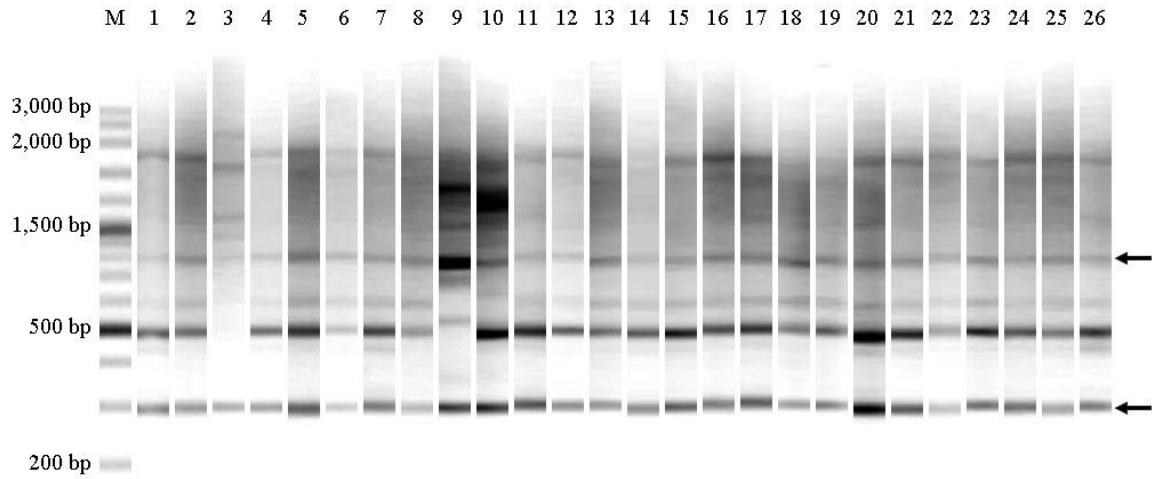

Figure 1 ISSR amplification pattern of 26 *Anthurium* cultivars obtained with primer ISSR13. M is 1kb-DNA ladder marker (Invitrogen), lanes 1-26 refer to number of *Anthurium* cultivars listed in Table 1. Arrows indicate the monomorphic band.

Genetic relationships among *Anthurium* cultivars

Similarity matrix based on ISSR binary data across the 26 *Anthurium* cultivars were computed with Pearson coefficient are varied from 0.01 (Joa Sua vs. Cherry Pink) to 0.87 (Red Bar vs. Sun Red) (Table 3). The results confirmed that Red Bar and Sun Red cultivars are genetically very close. Both cultivars have common morphological characters such as small size with heart shape with spine tail leaf and red spathe. Similar to data from Souza Neto *et al*. (2014), the most closely related cultivars shared common characteristics such as their origin, spathe color, spathe shape, young leaf color, lobe type, and spadix color. The average similarity matrix values of *Anthurium* cultivars were 0.58. These results further showed a high level of genetic diversity across the tested cultivars. The exploitation of genetic variability within *Anthurium* cultivars might be based on the improvement programs for breeding and develop of new cultivars.

Clustering analysis of 26 *Anthurium* cultivars

A dendrogram was generated using the UPGMA method. The clustering analysis implied that the 26 *Anthurium* cultivars can be divided into two major groups. The first group consists only foliage anthurium (Joa Sua, Choke Koa Chan and Hoo Chang) which are Thai native plants and had 0.48 genetic similarity index. The second group was divided into three subgroups (Figure 2): Group IIA included five cultivars, Plew Tien Lampang, Plew Tien Phuket, Smithson, Nakato and, Ta Ole with 0.58 genetic similarity index; Group IIB included five cultivars, Marshall, Dakotae, Mayonce, Red Strong and, Chompoo Noppon with 0.56 genetic similarity index; and Group IIC comprised of the remaining thirteen cultivars with 0.66 genetic similarity index. This clustering corresponds to results from a RAPD study (Nowbuth *et al.* 2005), which is inconsistent with the external appearance. However, in each subgroup, a

Table 3 Similarity matrix computed with Pearson coefficient among the 26 *Anthurium* cultivars

No.	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	Arizona	1.00																									
2	Cherry Pink	0.66	1.00																								
3	Choke Koa Chan	0.18	0.14	1.00																							
4	Chompoo Noppon	0.57	0.47	0.23	1.00																						
5	Dakota	0.60	0.60	0.23	0.48	1.00																					
6	Dakotae	0.51	0.47	0.29	0.49	0.51	1.00																				
7	Gemini	0.61	0.75	0.15	0.46	0.59	0.49	1.00																			
8	Jenny Red	0.62	0.64	0.38	0.53	0.54	0.40	0.52	1.00																		
9	Joa Sua	0.06	0.01	0.29	0.21	0.04	0.02	0.02	0.13	1.00																	
10	Hoo Chang	0.20	0.16	0.70	0.29	0.29	0.24	0.21	0.30	0.44	1.00																
11	La Quinta	0.67	0.75	0.23	0.62	0.59	0.48	0.64	0.68	0.11	0.25	1.00															
12	Marshall	0.32	0.25	0.37	0.54	0.39	0.44	0.34	0.38	0.14	0.32	0.40	1.00														
13	Mayonce	0.51	0.47	0.25	0.60	0.44	0.60	0.49	0.43	0.16	0.30	0.55	0.58	1.00													
14	Merengue	0.70	0.74	0.26	0.49	0.64	0.51	0.82	0.67	0.11	0.29	0.72	0.36	0.51	1.00												
15	Nakato	0.49	0.52	0.24	0.51	0.39	0.43	0.41	0.53	0.16	0.17	0.48	0.38	0.60	0.52	1.00											
16	Pink Champion	0.64	0.84	0.26	0.49	0.60	0.38	0.72	0.67	0.08	0.22	0.66	0.33	0.44	0.70	0.52	1.00										
17	Pistache	0.58	0.68	0.28	0.40	0.67	0.42	0.66	0.51	0.05	0.28	0.63	0.34	0.45	0.67	0.39	0.64	1.00									
18	Plew Tien Lampang	0.46	0.47	0.30	0.44	0.44	0.44	0.45	0.50	0.11	0.19	0.46	0.46	0.54	0.47	0.52	0.54	0.41	1.00								
19	Plew Tien Phuket	0.44	0.51	0.25	0.43	0.44	0.38	0.52	0.51	0.05	0.12	0.46	0.47	0.45	0.54	0.55	0.54	0.44	0.80	1.00							
20	Red Bar	0.71	0.62	0.22	0.46	0.55	0.49	0.64	0.59	0.02	0.15	0.67	0.27	0.45	0.75	0.44	0.62	0.59	0.45	0.39	1.00						
21	Red Strong	0.57	0.54	0.20	0.76	0.51	0.52	0.59	0.53	0.18	0.22	0.62	0.51	0.56	0.59	0.44	0.55	0.54	0.44	0.43	0.56	1.00					
22	Sangtien	0.57	0.64	0.27	0.58	0.61	0.49	0.63	0.67	0.11	0.29	0.68	0.54	0.56	0.72	0.54	0.62	0.57	0.55	0.56	0.56	0.62	1.00				
23	Smithson	0.44	0.42	0.25	0.53	0.45	0.35	0.37	0.49	0.19	0.15	0.50	0.44	0.45	0.49	0.61	0.42	0.36	0.52	0.56	0.40	0.53	0.53	1.00			
24	Sun Red	0.73	0.72	0.30	0.51	0.69	0.55	0.67	0.68	0.01	0.22	0.77	0.36	0.41	0.79	0.44	0.72	0.70	0.49	0.43	0.87	0.58	0.65	0.44	1.00		
25	Sweetheart Pink	0.60	0.62	0.26	0.51	0.59	0.41	0.67	0.72	0.08	0.25	0.67	0.40	0.51	0.72	0.48	0.72	0.57	0.55	0.50	0.70	0.58	0.65	0.40	0.73	1.00	
26	Ta Ole	0.48	0.46	0.18	0.46	0.39	0.43	0.31	0.46	0.09	0.11	0.51	0.44	0.46	0.49	0.67	0.42	0.32	0.45	0.52	0.44	0.40	0.46	0.57	0.44	0.37	1.00
Average		0.58																									
CCC		0.92																									

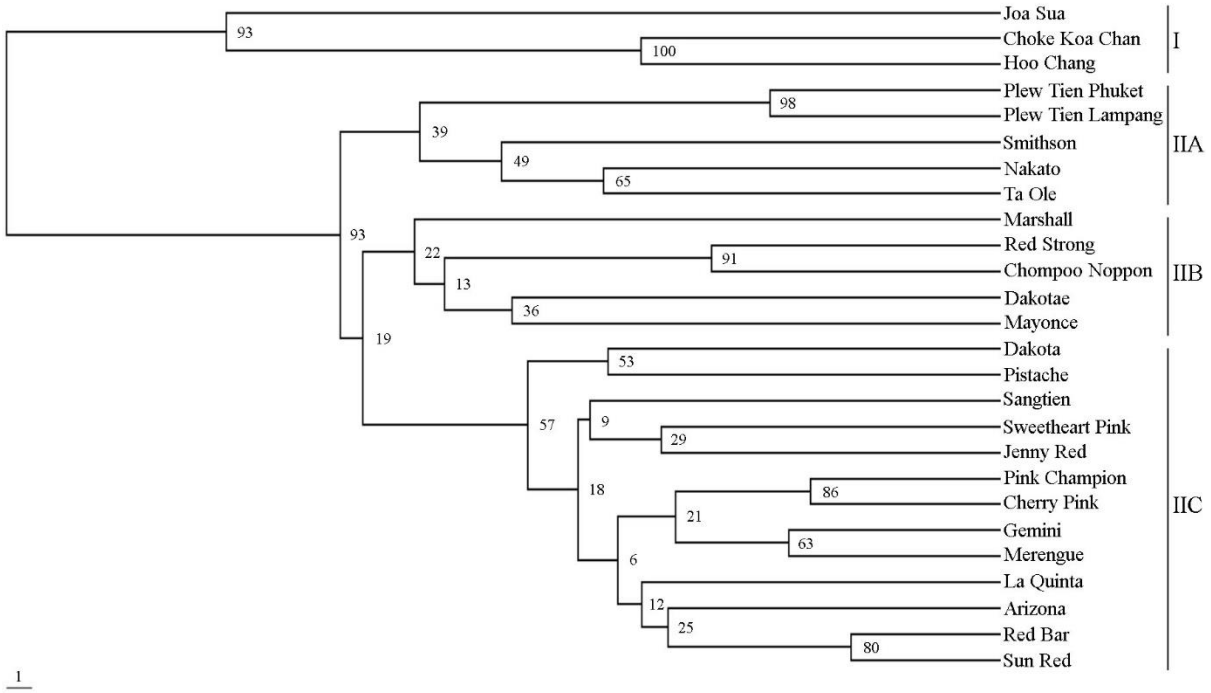

Figure 2 Dendrogram with bootstrap values derived from UPGMA clustering analysis using Pearson coefficient of ISSR marker.

couple of cultivars with high genetic similarity sharing at least one features. Subgroup IIA, Plew Tien Lampang and Plew Tien Phuket shared some common characters such as large size, pink spathe and big leaf with thick petioles. Subgroup IIB, Dakotae and Mayonce shared common characters that are small size, yellow spadix and spine tail leaf. Subgroup IIC, Cherry Pink and Pink Champion shared common characters that are small size, pink spathe and pink spadix. Another couple, Red Bar and Sun Red which had the highest similarity matrix values showed a common character as describe above. The clustering analysis based on ISSR fingerprint also distinguished foliage anthurium from cut-flower cultivars. Surprisingly, Nakato and Ta Ole were clustered together but they are very different in size, spathe color and spadix color. It seems that there is no correlation between the primer and the morphological data. The reasons might be that the ISSR polymorphic band were not corresponding to the morphological characteristics, thus, the clustering was not related with the morphological traits (Zhao *et al.*, 2014). In addition, *Anthurium* morphology is quantitative traits that are multifactorial, meaning that they are influenced by several polymorphic genes and environmental conditions. Moreover, these clusters are based on low bootstrap value (65%). A bootstrap value of 50-70% indicates the low confidence of the genetic relationship. This result clarifies their greater genetic diversity.

The calculated Cophenetic Correlation Coefficient (CCC) was 0.92 (data not show). The CCC is the Pearson's linear correlation coefficient between the matrix of distances among cultivars as a result of the original data and the similarity matrix resulting from the dendrogram. According to the magnitude of a CCC value approaches 1, the consistency of the clustering pattern is greater (Souza Neto *et al*., 2014). The calculated CCC from this study implied the good fit between the original data matrix and the matrix resulting from the dendrogram. Therefore, the clustering was acceptably consistent. Consequently, ISSR marker is a very useful and effective method for assessing genetic diversity of some *Anthurium* cultivars. The present ISSR data generated by ten primers indicated a high genetic diversity in the examined cultivars and could be used for cultivars identification and assessment of genetic diversity among *Anthurium*. However, the generated ISSR fingerprint in this study does not cover the various cultivars of *Anthurium* which available in the ornamental plant market. With further development in the future, these ISSR markers may be an effective tool for cultivar identification and genetic diversity assessment of all *Anthurium*.

ACKNOWLEDGEMENTS

The authors would like to thank Division of Biotechnology, Faculty of Agro-Industrial Technology, Kalasin University, Kalasin, Thailand for the equipment and laboratories to conduct research.

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