Assessment of genetic relationships among *Anthurium* cultivars using nucleotide sequence of chitinase and β-1, 3-glucanase genes

Nattapong Srisamoot[1], Teamjun Srisamoot[2] and Chalita Chookanhom[1]*

Abstract

Chitinase and β-1, 3-glucanase are members of pathogenesis-related proteins (PR proteins) that are strongly induced when host plant cells are faced by pathogen stress. Thus, chitinase and β-1, 3-glucanase constitute an important protein of plants against pathogens. In this study, the genetic relationships of ten *Anthurium* cultivars were analyzed using nucleotide sequences of chitinase and β-1,3-glucanase gene. DNA was extracted and used as a template in the polymerase chain reaction (PCR) using chitinase and β-1, 3-glucanase specific primers. The length of chitinase gene sequences of ten *Anthurium* cultivars ranged from 655 to 722 bp and the average length was 690.70 bp. The length of β-1, 3-glucanase gene sequences ranged from 442 to 490 bp and the average length was 473.20 bp. the genetic relationship analysis using Kimura 2-parameter indicated that the genetic similarity index based on chitinase gene sequence has an average of 47.20%. The lowest value (30.17%) was between Red Hot and Sangtien and the highest value (100%) was between Cherry Pink and Red Hot and between Chompoo Noppon and Red Strong. The genetic similarity index based on β-1, 3-glucanase gene sequence has an average of 53.50%. The lowest value (32.17%) was between Merengue and Plew Tien Phuket and the highest value (100%) was between Red Bar and Sun Red. The genetic similarity index based on a combination of chitinase and β-1, 3-glucanase gene sequence has an average of 49.00%. The lowest value (33.73%) was between Chompoo Noppon and Plew Tien Phuket and the highest value (90.83%) was between Plew Tien Lampang and Sangtien. All dendrograms demonstrated that 10 *Anthurium* cultivar were divided into two major groups but the clustering was not correlated with morphology. The close relationship between high disease resistance cultivar was found.

Keywords: Genetic relationship, *Anthurium andreanum*, chitinase, β-1, 3-glucanase

Introduction

Anthurium andraeanum Hort. (family Araceae) has a common name as Flamingo flower or Tail flower but popularly called *Anthurium* according to the genus name. *Anthurium* is commonly used for cut flowers, gardening and potted plants because of their long-lasting, colorful and quaint (Srisamoot & Padsri, 2018). The unique characteristics and shape of the leaves and spathes are distinctive, so they are used in decoration with other flowers. The *Anthurium* cultivation in Thailand tends to expand more planting areas. Production capacity in the country is 5.5 million flowers a year. The domestic selling price is 12-15 baht per flower. The export price is 20-30 baht per flower. Export markets have been expanded to Japan, Dubai, Myanmar, China, Taiwan, and the United States. The business value is worth over 100 million baht per year (Inspire Anthurium, n.d.). From the market demand for uniform plants with high-quality flowers and high-yield, so the novel *Anthurium* cultivars were breeding to get the varieties that look great and strange.

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In order to increase the efficiency of *Anthurium* production, more knowledge about the genetic diversity, genome structure and phylogenetic relationships of the hybrids cultivars and wild types was required. Moreover, the breeding program for improvements of the domestic cultivars and importing new cultivars from abroad resulted in a great genetic diversity of the *Anthurium*. However, the genetic information of *Anthurium* is rare.

Molecular marker, especially polymerase chain reaction based (PCR-based) types, is the essential tool to estimate genetic diversity and establish genetic information. A large number of molecular techniques are now available for assessing the genetic diversity of plants including random amplified polymorphic DNA (RAPD), variable number tandem repeats (VNTR), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP). This marker can be directly used to evaluate at the DNA level and eliminating the influence of environmental variations (Souza Neto et al., 2014). Recent advances in DNA sequencing technology have resulted in generating sequence data providing the opportunity to analyze the genetic relationships of the plant. This method is called DNA barcoding, which has been developed to a rapid and practical method for evaluating plant genetics at a species level using specific DNA sequences (Gao et al., 2008). Many genes from nuclear and chloroplast genomes such as internal transcribed spacers (ITS), Ribulose bisphosphate carboxylase large chain (*rbc*L), intergenic spacer between the Photosystem II protein D1 (*psb*A) gene and the histidine transfer RNA (*trn*H), DNA-directed RNA polymerase subunit beta (*rpo*B) and the ribosomal RNA maturase (*mat*K) genes have been useful for deducing the phylogenetic relationships of plant species. These sequences can be compared with sequences produced from previously specified samples that are easily accessible for taxonomic confirmation. However, the DNA barcoding technique is intended to distinguish plant species using genetic differences (Chanaeng et al., 2017). The use of highly differentiated nucleotide sequences may not be related to the proximity of evolution. In addition, sequences of chloroplast DNA, including noncoding regions, usually diverge too slowly to resolve close relationships at the interspecific level (Sang, 2002). ITS sequence variation is not always sufficient to resolve closely related species. Thus, low-copy nuclear genes with rapidly evolving introns, are needed for a full resolution of interspecific phylogenies (Toluei & Toluei, 2018). It has recently become clear that low-copy nuclear genes are particularly helpful in resolving close interspecific relationships in plants (Ali et al. 2015). These shows that nucleotide sequence analysis of low-copy nuclear genes which encoding proteins that perform the same function to determine genetic relationships may provide more consistent results. The advantages of low-copy nuclear genes include the availability of many genes, their overall faster rate of evolution, and their biparental inheritance (Small et al., 2004). Examples of low-copy nuclear genes that are used to study the phylogenetic relationships of plant species such as arginine decarboxylase (Galloway et al., 1998), malate synthase (Lewis & Doyle, 2002), phosphoribulokinase (Lewis & Doyle, 2002), alcohol dehydrogenase (Sang et al., 1997) and phytochrome C (Silva et al., 2016). Pathogenesis-related proteins (PR proteins) gene are another group of low-copy nuclear gene that can be used for phylogenetic reconstruction. However, the lack of the universal markers or universal PCR primers of nuclear genes has obstructed their phylogenetic utility (Ali et al. 2015).

PR proteins are plant proteins that are created to prevent the invasion of pathogens. The important PR proteins are chitinase (EC 3.2.1.14) and β-1, 3-glucanase (EC 3.2.1.39), which have the ability to digest cell walls of fungi and bacteria, respectively (Wu et al., 2001). Chitinase and β-1, 3-glucanase are found in both monocotyledon and dicotyledon plants. These enzymes are synthesized when the occurrence of pathogens, wounds and pressure from chemicals and the environment. The chitinase and β-1, 3-glucanase activities are increased after artificial inoculation with pathogen (Srisamoot et al., 2017). Chitinases are

specific enzymes which can degrade the chitin by hydrolyzing β-glycosidic bonds. As chitin is the primary component of most of the fungal cell wall (Jain et al., 2016). β-1, 3-glucanase hydrolyses β-glucans, one of another major components of fungal cell wall (Jain et al., 2016). Furthermore, oligomeric products of digested chitin and β-glucan can act as signal molecules to stimulate further defense responses (Li et al. 2001). The genes of these enzymes have received great interest and have become a very important resource in plant genetic engineering of crop plants for disease resistance (Li et al., 2001). The expression patterns of chitinase and β-1, 3-glucanase are extensively studied in various plants, but the analysis of genetic relations from the nucleotide sequence of those genes is still very little. Thus, we have analyzed the genetic relationship of 10 *Anthurium* cultivars on the basis of sequence variations of the chitinase and β-1, 3-glucanase genes. The information obtained can be used for the application of *Anthurium* breeding programs, estimating the genetic similarity among cultivars and for the identification of cultivars.

Materials and methods

Sample collection and DNA extraction

Twenty-six Anthurium cultivars were collected from ornamental plant market in the Central and the Northeast region of Thailand and planting to preserve germplasm at Kalasin University, Kalasin. (listed in Table 1). The cultivar names were classified by the seller. Fresh young leaves were collected from the field, packed in plastic bags and kept in iceboxes.

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	Cherry Pink	20 cm tall, less tillering and small petioles.	Pink with green edge	Pink	Nakhon Ratchasima
2	Chompoo Noppon	30 cm tall, tillering and heart shape with spine tail leaf.	Pink	Yellow to green	Nonthaburi
3	Merengue	30 cm tall, less tillering and small petioles.	White	Pink	Pathumthani

Table 1 (Cont.)

No.	Taxon	Morphological characters			Source (Province in Thailand)
		Over view	Spathe Color	Spadix Color	
4	Plew Tien Lampang	40 cm tall, less tillering and big leaf with thick petioles.	Light pink	pink	Nakhon Ratchasima
5	Plew Tien Phuket	40 cm tall, less tillering and big leaf with thick petioles.	Pink	Red	Nakhon Ratchasima
6	Red Bar	25 cm tall, less tillering and heart shape with spine tail leaf.	Red	Green	Khon Kaen
7	Red Hot	25 cm tall, less tillering and small petioles.	Pink	Pink	Pathumthani
8	Red Strong	25 cm tall, tillering and heart shape with spine tail leaf.	Red	Pink to green	Khon Kaen

Table 1 (Cont.)

No.	Taxon	Morphological characters			Source (Province in Thailand)
		Over view	Spathe Color	Spadix Color	
9	Sangtien	30 cm tall, less tillering and spear shape with spine tail leaf.	White	Pink	Pathumthani
10	Sun Red	25 cm tall, tillering and heart shape with spine tail leaf.	Red	Yellow to green	Khon Kaen

DNA was extracted using the modified cetylmethylammonium bromide (CTAB) method (Doyle & Doyle, 1990). The fresh plant tissues sample (300 mg) were grounded in liquid nitrogen and transferred to a 1.5 mL sterile reaction tube and addition of 1000 μL of CTAB extraction buffer (20 g/L CTAB; 1.4 M NaCl; 0.1 M Tris-HCl; 20 mM Na_2EDTA) and 20 μL of Proteinase K (20 mg/mL). The mixture was vortexed and incubated at 65 °C for 60 min. Further, 20 μL of RNase A (10 mg/mL) were added and the sample was homogenized, incubated at 37 °C 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 quantity was measured using a spectrophotometer estimation at 260 and 280 nm and the DNA samples were diluted to 20 ng/μL for polymerase chain reaction (PCR) amplification.

Primer design and PCR amplification

The design of the primers to be used in polymer chain reactions was carried out by analyzing the conserved region of the chitinase and β-1, 3-glucanase genes of various plants from the GenBank database by multiple alignment using the ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The nucleotide sequence of the conserved region is being used as a model for synthesis of forward primers and reverse primers, with approximately 23-25 nucleotide is shown in Table 2.

Table 2 Nucleotide sequences of primers used for amplified the chitinase and β-1, 3-glucanase gene.

Gene	Primer name	Nucleotide sequences	Annealing temperature (°C)	Expected size (bp)
chitinase	CHI - F1	5′-GCTACTGCTTCAAGGAGGAGAAACA-3′	56	750
	CHI - R1	5′-CTGGTTGTAGCAATCCAGGTTATCG-3′		
β-1, 3-glucanases	BGL - F1	5′-ATGGCGAAACCAAGTTCATCAGT-3′	51	500
	BGL - R1	5′-CGCTTAGTTGAAATTGATTGGGTAT-3′		

The chitinase and β-1, 3-glucanase genes were amplified from the extracted DNA by PCR, using a thermocycler (TGRADIENT, Biometra). The total volume of 25 μL contains 1X PCR buffer, 0.4 mM dNTP, 2.0 mM $MgCl_2$, 0.25 μM forward primer, 0.25 μM reverse primer, 0.5 unit *Taq* polymerase (Vivantis) and 20 ng DNA template. PCR conditions were as follows: initial denaturation at 94 °C for 3 min, 35 cycles consisting of denaturation at 94 °C for 30 sec, annealing at 51 or 56 °C (depends on the type of primer) for 30 sec and extension at 72 °C for 50 sec, and a final extension step at 72 °C for 5 min.

DNA fragment purification and sequencing

PCR products were electrophoresed in 1.5% agarose gels in 1 × TAE buffer. The gel was stained with ethidium bromide for 30 minutes and then visualized under UV light. For sequencing, PCR products were excised from a 1.5% agarose gel and purified using a PureLink Quick Gel Extraction Kit (Invitrogen) following the manufacturer's instructions. The purified DNA fragments were sequenced on an ABI Prism 3730 automatic sequencer (Gibthai Co., Ltd) using both forward and reverse primers.

Sequence analysis

Genetic relationships of *Anthurium* cultivar were estimated from chitinase and β-1, 3-glucanase genes sequences by using the MEGA7 software (Kumar et al., 2016). The dendrogram was drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. The dendrogram was constructed using unweighted pair group method with arithmetic means (UPGMA) method.

Results and Discussion

The DNA extracted using the modified CTAB method has a large clear genomic DNA band and the same position as the Lambda/*Hin*DIII marker on 0.8% agarose gel. The quality of extracted DNA was good and can be used as a template for gene amplification by polymerase chain reaction. The average concentration of DNA extracted from 10 *Anthurium* cultivars was 88.50 μg/mL. The lowest concentration is Cherry Pink (30.0 μg/mL) while the highest is Red Strong (220.0 μg/mL) (data not shown). Amplification of the chitinase gene placed at approximately size 750 bp on 1.5% agarose gel (Figure 1A) in all the *Anthurium* accessions used in this study. The presence of a single band of PCR products demonstrated that the designed primer has high accuracy for amplification of chitinase gene. In contrast, the amplification product of β-1, 3-glucanase gene has more than one band in several samples. The expected size should be approximately 500 bp on 1.5% agarose gel (Figure 1B). The appearance of a non-specific band or multiple bands may be due to the following two reasons. First, the target sequence of the primer is more than one copy on the template DNA. In this case, it is necessary to design a new primer. second, the annealing temperature is not well adjusted (lower than optimum) causing the primer to bind to the DNA template without specificity. This problem may be solved by using Hot-Start PCR or using high primer annealing temperatures. However, templates with GC-rich changes in cycling conditions, including high primer annealing temperatures to decrease unspecific primer-binding were not helpful (Hommelsheim et al., 2014). Howsoever, the occurrence of multiple bands does not hinder the nucleotide sequence analysis. Because only band that similar in size to the desired size were excised in the DNA fragments purification step.

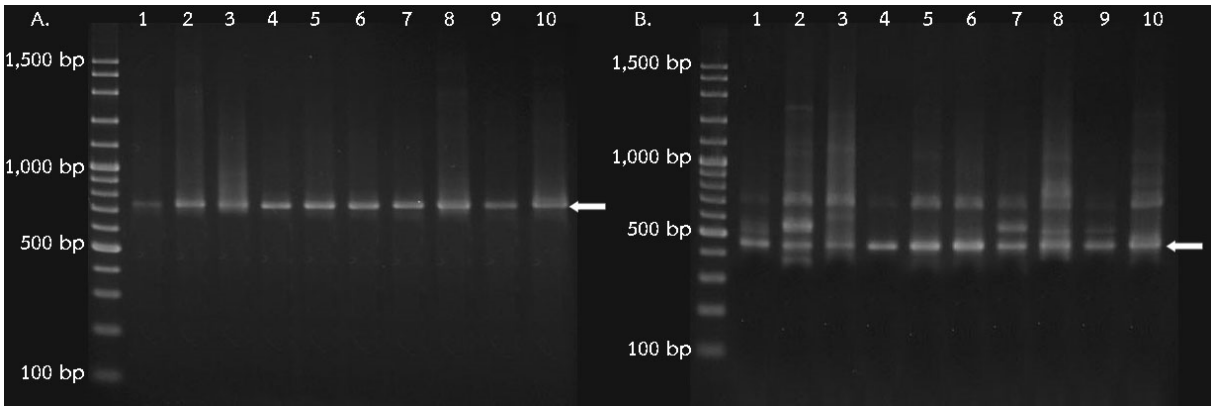

Figure 1. The amplification products of chitinase gene (A) and β-1, 3-glucanases (B). 1-10 number of *Anthurium* cultivars were shown in Table 1. M was 1kb DNA ladder marker. An expected band was indicated by white arrow.

The sequence characteristics of the chitinase gene and β-1, 3-glucanase gene include length variation and base composition are presented in Table 3 and 4, respectively. The alignment result indicated that each sample has a different length of the chitinase gene and β-1, 3-glucanase gene sequence. For chitinase gene, the length was from 655 to 722 bp and the average length was 690.70 bp. The average CG content was 49.01%. The highest was Plew Tien Lampang and the lowest was Sun Red with CG content of 54.40% and 38.50%, respectively. The length of β-1, 3-glucanase was from 442 to 490 bp and the average length was 473.20 bp. The average CG content was 48.78%. The highest was Cherry Pink and Sangtien and the lowest was Sun Red with CG content of 52.70% and 43.80%, respectively.

Table 3 Analysis of nucleotide sequence of the chitinase gene of 10 anthurium cultivars.

No.	Cultivar	Length (bp)	Base Content				
			(A)	(T)	(G)	(C)	%CG
1	Cherry Pink	655	143	159	158	195	53.9
2	Chompoo Noppon	667	161	174	163	169	49.8
3	Merengue	722	146	201	202	173	51.9
4	Plew Tien Lampang	717	160	167	199	191	54.4
5	Plew Tien Phuket	683	212	153	180	138	46
6	Red Bar	684	214	188	127	155	41.2
7	Red Hot	715	157	172	175	211	54
8	Red Strong	690	170	193	161	166	47.7
9	Sangtien	675	163	156	175	181	52.7
10	Sun Red	699	222	208	122	147	38.5
	Average	**690.7**	**174.8**	**177.1**	**166.2**	**172.6**	**49.01**

Table 4 Analysis of nucleotide sequence of the β-1, 3-glucanase of 10 anthurium cultivars.

No.	Cultivar	Length (bp)	Base Content				
			(A)	(T)	(G)	(C)	%CG
1	Cherry Pink	490	110	122	135	123	52.7
2	Chompoo Noppon	489	100	141	141	107	50.7
3	Merengue	478	124	126	147	81	47.7
4	Plew Tien Lampang	442	122	108	108	104	48.0
5	Plew Tien Phuket	472	105	140	91	136	48.1
6	Red Bar	470	101	162	81	126	44.0
7	Red Hot	460	113	118	134	95	49.8
8	Red Strong	473	104	131	132	106	50.3
9	Sangtien	476	117	108	113	138	52.7
10	Sun Red	482	106	165	85	126	43.8
	Average	**473.2**	**110.2**	**132.1**	**116.7**	**114.2**	**48.78**

Genetic similarity analysis was conducted using the Kimura 2-parameter model (Kimura, 1980). Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. The chitinase gene sequences of the 10 *Anthurium* cultivars were aligned and resulted in 978 characters with 805 variable sites (82.31%). A genetic similarity index was showed in Table 5. The average genetic similarity index was 47.20%. The lowest value was 30.17% between Red Hot and Sangtien and the highest value was 100% between Cherry Pink and Red Hot and between Chompoo Noppon and Red Strong. For β-1, 3-glucanase gene, the sequences of the 10 *Anthurium* cultivars were aligned and resulted in 562 characters with 445 variable sites (79.18%). A genetic similarity index was showed in Table 6. The average genetic similarity index was 53.50%. The lowest value was 32.17% between Merengue and Plew Tien Phuket and the highest value was 100% between Red Bar and Sun Red. The combination of both sequences of chitinase gene and β-1, 3-glucanase gene demonstrated alignment result in 1,359 characters with 1,179 variable sites (86.75%). The average genetic similarity index was 49.00%. The lowest (33.73%) and highest (90.83%) genetic similarity index was found between Chompoo Noppon and Plew Tien Phuket and between Plew Tien Lampang and Sangtien, respectively.

Table 5. Estimates of genetic similarity index between chitinase gene of 10 *Anthurium* cultivars.

No.	Taxon	Similarity coefficient (%)									
		1	2	3	4	5	6	7	8	9	10
1	Cherry Pink	100.00									
2	Chompoo Noppon	41.38	100.00								
3	Merengue	55.46	40.23	100.00							
4	Plew Tien Lampang	32.76	39.08	33.05	100.00						
5	Plew Tien Phuket	32.47	39.66	32.76	98.85	100.00					
6	Red Bar	33.91	46.84	34.77	66.95	68.10	100.00				
7	Red Hot	100.00	41.38	55.46	32.76	32.47	33.91	100.00			
8	Red Strong	41.38	100.00	40.23	39.08	39.66	46.84	41.38	100.00		

Table 5 (Cont.)

No.	Taxon	Similarity coefficient (%)									
		1	2	3	4	5	6	7	8	9	10
9	Sangtien	30.17	38.51	30.46	87.93	87.36	66.95	30.17	38.51	100.00	
10	Sun Red	32.76	45.98	33.05	37.07	38.22	68.97	32.76	45.98	37.07	100.00
Average		**47.20**									

Table 6. Estimates of genetic similarity index between β-1, 3-glucanase gene of 10 *Anthurium* cultivars.

No.	Taxon	Similarity coefficient (%)									
		1	2	3	4	5	6	7	8	9	10
1	Cherry Pink	100.00									
2	Chompoo Noppon	78.55	100.00								
3	Merengue	49.06	50.13	100.00							
4	Plew Tien Lampang	39.41	38.61	32.98	100.00						
5	Plew Tien Phuket	39.95	38.34	32.17	71.05	100.00					
6	Red Bar	56.84	58.18	35.12	51.21	80.16	100.00				
7	Red Hot	61.39	62.73	68.10	34.85	35.12	43.70	100.00			
8	Red Strong	79.62	95.98	49.33	40.75	40.48	60.32	61.93	100.00		
9	Sangtien	38.34	39.41	34.32	81.50	67.56	47.72	36.46	42.09	100.00	
10	Sun Red	56.84	58.18	35.12	51.21	80.16	100.00	43.70	60.32	47.72	100.00
Average		**53.50**									

Table 7. Estimates of genetic similarity index from combination sequences of chitinase and β-1, 3-glucanase gene of 10 *Anthurium* cultivars.

No.	Taxon	Similarity coefficient (%)									
		1	2	3	4	5	6	7	8	9	10
1	Cherry Pink	100.00									
2	Chompoo Noppon	64.98	100.00								
3	Merengue	53.82	48.06	100.00							
4	Plew Tien Lampang	35.25	34.43	35.84	100.00						
5	Plew Tien Phuket	34.55	33.73	36.31	74.62	100.00					
6	Red Bar	46.30	48.18	42.07	44.07	49.59	100.00				
7	Red Hot	80.73	65.92	56.76	36.08	35.14	42.19	100.00			
8	Red Strong	57.46	60.87	48.65	38.78	38.90	64.04	53.00	100.00		
9	Sangtien	36.19	35.25	35.02	90.83	69.68	43.01	36.90	38.54	100.00	
10	Sun Red	48.18	48.88	42.19	39.60	45.24	89.19	44.42	64.04	38.54	100.00
Average		**49.00**									

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The dendrogram for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (Kumar et al. 2016). The dendrogram analysis based on chitinase gene sequence, β-1, 3-glucanase gene sequence and a combination of both gene sequence of 10 *Anthurium* cultivars is shown in Figure 2, 3 and 4, respectively. The clustering analysis of all dendrogram indicated that the ten *Anthurium* cultivars were divided into two major groups. The first major group consists of five cultivars namely Cherry Pink, Chompoo Noppon, Merengue, Red Hot and Red Strong while the second major group consists of rest five cultivars, Plew Tien Lampang, Plew Tien Phuket, Red Bar, Sangtien and Sun Red. The high genetic relationship between Chompoo Noppon and Red Strong was found in a dendrogram of chitinase and β-1, 3-glucanase gene sequence. This is consistent with the UPGMA clustering analysis using the Pearson coefficient of ISSR marker (Srisamoot & Padsri, 2018). The closely related genetic of high disease resistance cultivar, Plew Tien Lampang and Plew Tien Phuket, was found. Plew Tien Lampang and Plew Tien Phuket were reported to be resistant to leaf blight and anthracnose, respectively (Srisamoot et al., 2017). Such genetic closeness may be a direct result of the similarity of PR protein. Although, the clustering of Plew Tien Lampang and Plew Tien Phuket is in agreement with the similarity of their morphological characteristics such as large size, pink spathe and big leaf with thick petioles. Or another one couple, Red Bar and Sun Red, shared some common characters showed the high similarity index. Most of the samples in this study, which different even in size, spathe color and spadix color were clustered together. This is because the use of only two gene sequences are not enough to represent the entire genome. Therefore, there is no correlation between gene sequences and morphological data. However, showing genetic closeness from gene sequences related to disease resistance may be useful in planning the anthurium breeding. By choosing the desired characteristics cultivar and the closely related to high resistance cultivar first.

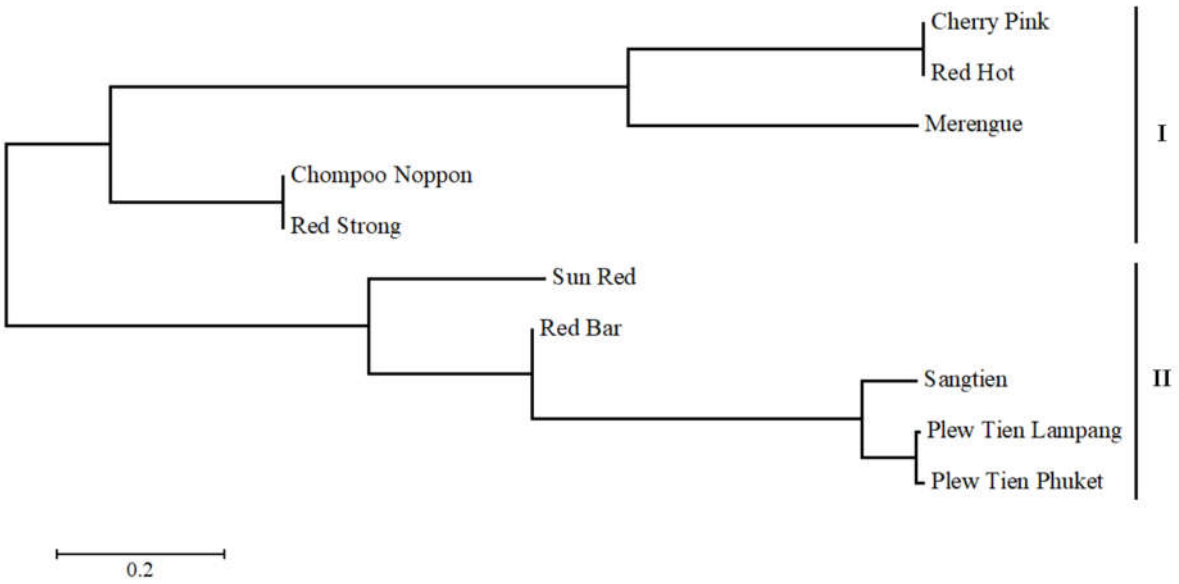

Figure 2. Molecular Phylogenetic analysis by Maximum Likelihood method based on chitinase gene sequence of 10 *Anthurium* cultivars.

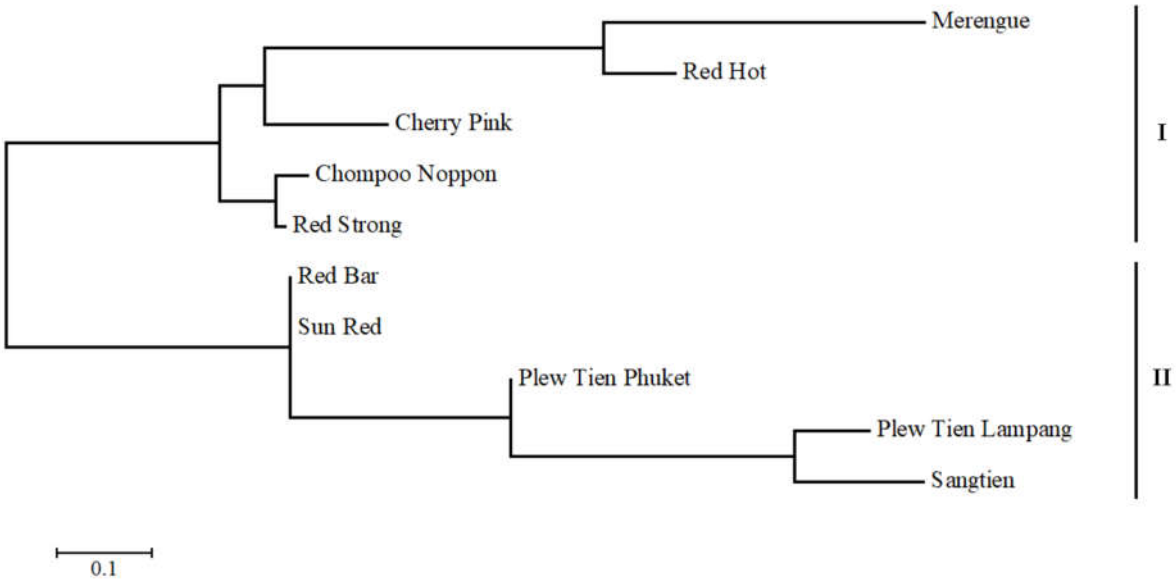

Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method based on β-1, 3-glucanase gene sequence of 10 *Anthurium* cultivars.

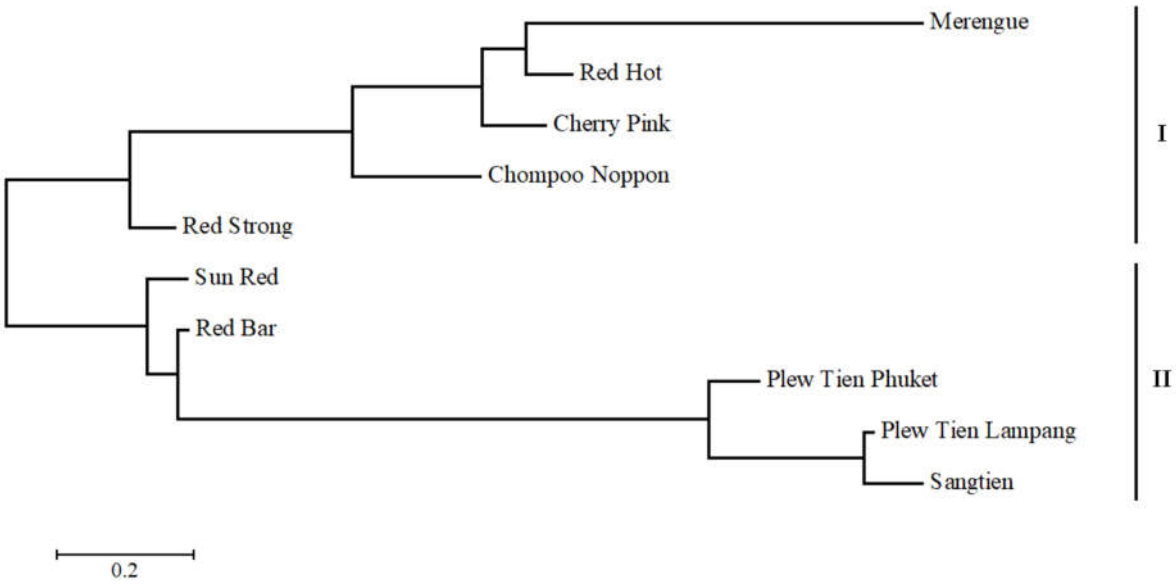

Figure 4. Molecular Phylogenetic analysis by Maximum Likelihood method based on a combination of chitinase and β-1, 3-glucanase gene sequence of 10 *Anthurium* cultivars.

Conclusion

The length of the chitinase gene was 655 to 722 bp with the average was 690.70 bp. The CG content was from 54.40% to 38.50% with the average was 49.01%. The length of β-1, 3-glucanase gene was 442 to 490 bp with the average was 473.20 bp. The CG content was from 52.70% to 43.80% with the average was 48.78%. An average genetic similarity index from chitinase gene sequence, β-1, 3-glucanase gene sequence and a combination of both gene sequences were 47.20, 53.50 and 49.00, respectively. The genetic similarity index form chitinase gene sequence alignment was lowest (30.17%) between Red Hot and Sangtien and highest (100%) between Cherry Pink and Red Hot and between Chompoo Noppon and Red Strong. For β-1, 3-glucanase, the genetic similarity index was lowest (32.17%) between Merengue and Plew Tien Phuket and highest (100%) between Red Bar and Sun Red. The genetic similarity index based on a combination of chitinase and β-1, 3-glucanase gene sequence was lowest (33.73%) between Chompoo Noppon and Plew Tien Phuket and highest (90.83%) between Plew Tien Lampang and Sangtien. All dendrograms in this study divide 10 *Anthurium* cultivars into two major groups. The first major group included Cherry Pink, Chompoo Noppon, Merengue, Red Hot and Red Strong while the second major group included Plew Tien Lampang, Plew Tien Phuket, Red Bar, Sangtien and Sun Red. Although the clustering was not consistent with the morphological character, it is useful for the *Anthurium* breeding plan.

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