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Development of novel microsatellite markers for effective applications in Anthurium cultivar identification

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Abstract *Anthurium andraeanum* is one of the most economically important floral crops and potted flowers marketed worldwide. Microsatellite markers are currently the preferred molecular marker owing to the many desirable attributes, including hypervariability, codominance, and amenability to high-throughput genotyping; however, there are few polymorphic molecular markers available for Anthurium. The object of this study was to develop and characterize novel microsatellite markers using the Araceae sequences in GenBank of the National Center for Biotechnology Information (NCBI) to contribute to molecular identification for cultivar protection. Using 1,579 Araceae expressed sequence tags (ESTs) and the related nucleotide sequences, 100 candidates contained simple sequence repeat (SSR) motifs that were suitable for primer design. Furthermore, 100 pairs of SSR primers were screened against a set of 28 diverse genotypes representing 24 cultivars that included four registration cultivars which were bred from the Taiwan Agricultural Research Institute (TARI) and 20 commercial cultivars, appended with three hybrid progeny and a mutant line. From the selected six polymorphic SSR loci, 52 alleles were amplified and 27 distinct genotypes were found, except for 'Tropical' and its mutant, with a mean number of eight alleles per locus. The polymorphism information content (PIC) ranged from 0.86 to 0.93. Based on these results, we proposed a key identification set using four microsatellite markers that is sufficient to discriminate among 24 cultivars. Because the Anthurium microsatellite markers developed in this study are primarily from expressed sequence tags or related genomic sequences, they can be used for cultivar identification and, accordingly, contribute to genetic evaluations in breeding programs.

Keywords Anthurium · Expressed sequence tags · Microsatellite marker · Cultivar identification

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Introduction

Anthurium andraeanum is a perennial flower of the Araceae family plants that are suitable for growth in subtropical regions. Among Anthurium, approximately 20 species can be used as ornamental plants, with *Anthurium andraeanum* being the most popular for potted plants and cut flowers (Henny et al. 1988; Kamemoto and Kuehnle 1996). According to the statistics of 2006 from the Dutch Flowers Auctions Association (VBN), Anthurium was ranked fourth as

an economic potted plant, after phalaenopsis, chrysanthemum, and kalanchoe. Since 1997, the Taiwan Agricultural Research Institute (TARI) has enhanced breeding programs in response to market demands; aiming to promote the diversification of flower types and colors, the stability of the year-round quality, and cultivation adaptability. Six new cultivars have been registered from 2006 to date. However, effective molecular markers need to be established to assist the genetic background analysis and hybrid selection in this breeding program. In addition, specific molecular markers can establish the identity of the registration material and cultivar protection.

In recent years, biotechnology has developed rapidly, and molecular markers have become important tools in genetic research and assist in breeding efficiency. Among them, simple sequence repeat (SSR) markers are relatively abundant in plant genomes (Condit and Hubbell 1991; Wang et al. 1994); these markers show hypervariability, are multiallelic in nature, representing codominant genetic traits, and are suitable for high-throughput analysis (Rajwant et al. 2011). SSR markers have been widely applied to construct linkage maps of the rice (McCouch et al. 2002) and tomato (Frary et al. 2005) genomes. Such markers can also be used to assist in selection in breeding and backcrossing for a specific trait or quantitative traits, in genotyping species (He et al. 2003; Gong and Geng 2010; Zorrilla-Fontanesi et al. 2011), and in cultivars identification (Tantasawat et al. 2010; Zhang et al. 2010) and serve as effective tools in plant germplasm diversity analysis (Guilford et al. 1997; Gomez et al. 2008; Mujaju et al. 2010).

There have been relatively few reports regarding the use of molecular markers to characterize genetic variation within Anthurium cultivars. Isozymes were the first molecular marker used for genotypic characterization in Anthurium (Kobayashi et al. 1987), yet those markers are very limited and display low polymorphism. Since then, a number of genomic markers based on DNA polymorphism, such as random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) molecular markers, have been developed and used for genetic diversity analyses (Wang et al. 2001; Nowbuth et al. 2005). However, RAPD and ISSR markers are dominant, limiting their use for population genetic studies. Additionally, the RAPD markers are also sensitive to the reaction conditions, interfering with the reproducibility of banding patterns between experiments. The present study aims at mining the genomic information of Anthurium and other Araceae genera for developing novel and specific SSR markers and to select critical markers for fingerprint analyses in the future. For this purpose, we screened the NCBI GenBank of publicly available nucleotide sequences and expressed sequence tags (ESTs) for the SSR motifs, designed flanking primer pairs to amplify the SSR-containing markers, evaluated a key subset of polymorphic primers for the identification of cultivars, and tested the marker transferability across the Araceae family.

Materials and methods

Plant materials and DNA extraction

Twenty-eight genotypes of *Anthurium* spp., including four new cultivars ('TN01 Pink Panther', 'TN02 Orange Storm', 'TN03 Green Elf', and 'TN04 Pink Girl') which had been bred by the TARI, four comparison cultivars ('Marian Seefurth', 'Nette', 'Midori', and 'Lunette'), 16 commercial cultivars, three hybrid progeny, and a mutation of 'Tropical' (Tropical-P), were selected as the test materials (Table 1).

The genomic DNA of the 28 genotypes had been isolated from young leaves following the CTAB method described by Iqbal et al. (1995) with some modifications. For each sample, approximately 100 mg of fresh tissue was homogenized with 1 ml of CTAB extraction buffer (2 % CTAB, 100 mM Tris–HCl [pH 8.0], 20 mM EDTA [pH 8.0], 1.4 M NaCl, and 0.1 % β-mercaptoethanol), placed into a 1.5 ml Eppendorf tube and incubated at 65 °C for 30 min. To each tube, 500 µl of 24:1 chloroform/isoamyl alcohol was added, and the solution was vortexed and centrifuged at 13,000 rpm for 10 min. The DNA was then precipitated by mixing 500 µl supernatant with an equal volume of isopropanol, incubating at 4 °C for 1–2 h and then centrifuging at 13,000 rpm for 5 min. The DNA pellet was washed with 75 % ethanol, dried to remove the alcohol, and dissolved in 100 µl water at 4 °C overnight. Lastly, the supernatant containing the DNA was collected after microcentrifugation at 12,000 rpm for 5 min and diluted to a concentration approximately 50 ng/µl for the PCR reaction.

Table 1 The plant materials of *Anthurium* spp. and their inflorescent characteristic used in the identification of simple sequence repeats (SSRs)

Cultivar/ genotype	Spathe Color	Spadix Color	Peduncle Color	Origin
Acropolis	White	Yellow	Green	Anthura (Netherlands)
Alexis	Red	Green	Green	AVO (Netherlands)
Anneke	Salmon	Yellow	Brown	AVO (Netherlands)
Atlantis	Red	Yellow	Brown	Unknown
Carnaval	White and red boder	Yellow	Brown	Anthura (Netherlands)
Champion	White and red vein	Red	Green	Anthura (Netherlands)
Cuba White	White	Green	Green	AVO (Netherlands)
Fantasia	Cream and red vein	Red	Green	Anthura (Netherlands)
Fla-Exotic	White and red boder	Yellow	Brown	Flamingo (Netherlands)
Lady Jane	Pink	Pink	Green	Florida University
Lunette	Pink	Pink	Brown	AVO (Netherlands)
Marian Seefurth	Pink	Green	Green	Hawaii University
Merengue	White	Pink	Green	Anthura (Netherlands)
Midori	Green	Green	Green	Anthura (Netherlands)
Sonate	Pink	Pink	Green	Anthura (Netherlands)
Southern Blush	Purple	Pink	Brown	Florida University
TN01	Obake Pink	Green	Green	TARI[a]
TN02	Obake Orange	Yellow	Brown	TARI
TN03	Green	Green	Green	TARI
TN04	Obake Pink	Pink	Green	TARI
Tropical	Red	Green	Green	Anthura (Netherlands)
Tropical-P[b]	Pink	Green	Green	Tropical mutation
Tulip	Pink	Pink	Green	Unknown
Champagne	Cream	Green	Green	Anthura (Netherlands)
123(Nette)	Orange	Yellow	Brown	AVO (Netherlands)
943954-1	White	Yellow	Green	TARI
943954-2	White	Yellow	Green	TARI
943954-4	White	Yellow	Green	TARI

[a] TARI: Taiwan Agricultural Research Institute

[b] Tropical-P is a mutant from the Tropical

SSR motif search and primer design

A set of 55 nucleotide sequences of *Anthurium* from NCBI (GenBank) were screened for the presence of perfect SSRs using a combination of a simple sequence repeat identification tool (SSRIT, Temnykh et al. 2001) and primer 3 (Rozen and Skaletsky 2000). In the present study, a microsatellite was defined as a DNA sequence containing at least 15 repeated units for mononucleotide repeats, seven units for dinucleotide repeats, and four units for tri-, tetra-, penta-, and hexa-nucleotide repeats. All of the primers were designed using the following parameters: (1) a minimum of seven dinucleotide or five trinucleotide repeats in the ESTs; (2) a primer length of 18–27 nucleotides (nt), with 20 nt considered optimal; (3) expected PCR products between 80 and 400 bp; (4) an optimal annealing temperature 60 °C, and (5) a GC contents from 20 to 80 %, with 50 % as the optimum.

A total of 32 *Colocasia esculenta* microsatellite sequences were searched in GenBank to verify for a number of repeats and motifs. SSR primers flanking the repeat sequences were designed using the primer3 software program. These primers were used to assess the transferability to reveal genetic polymorphisms in Anthurium.

We screened 1,492 *Zantedeschia aethiopica* ESTs available at NCBI (GenBank) for di-, tri-, tetra-, and penta-nucleotide motifs in microsatellite containing ≥15 base pairs (bp) and hexa-nucleotide motifs

≥18 bp, using a combination of msatcommander (Faircloth 2008) and primer3 (Rozen and Skaletsky 2000). Sufficient flanking sequences of appropriate quality for primer design were screened, and the polymorphisms among 28 genotypes of Anthurium were characterized.

Polymerase chain reaction (PCR) amplification and SSR markers selection

The PCR reactions were performed in 96 well plates using the Perkin Elmer Cetus Thermal Cycler 9700 (Applied Biosystems) amplification program. Each 25 µl reaction mixture contained 50 ng Anthurium genomic DNA, 0.4 µM forward and reverse primers, 200 µM each dNTP, 2.5 µl 10X PCR buffer containing 50 mM Tris–HCl [pH 8.3], 50 mM KCl, 50 mM $(NH_4)SO_4$, and 1 unit *Taq* DNA polymerase (Roche-FastStart). The PCR amplification conditions were programmed as one cycle of denaturation at 95 °C for 5 min, followed by 35 cycles with a 45 s denaturation at 94 °C, a 25 s annealing at the *Tm* (*Tm* 55–60 °C, adjusted to the individual primers) and a 45 s extension at 72 °C. The final extension was at 72 °C for 8 min.

The PCR products were separated by electrophoresis on 6 % polyacrylamide gels in 0.5X TBE buffer (40 mM Tris acetate [pH 8.0] and 1 mM EDTA) at a constant power of 90 V for 60 min using a MGV-202-33 (C. B. S. SCIENTIFIC) apparatus. The gels were stained with ethidium bromide (1 µg/ml) and visualized under UV light using an IS 2000 Digital Imaging System (Alpha Innotech.). A 25 bp DNA Step Ladder (Promega) and a Low Molecular Weight DNA Ladder (NEB) were used as the molecular size standard.

Data analysis

All 28 Anthurium genotypes were used to screen for SSR primers, PCR amplification, and product-length polymorphisms. For the primers that produced the expected PCR product size, the number of alleles was recorded, and the polymorphism information content (PIC) of each SSR locus was calculated as described by Saal and Wricke (1999). The genetic similarity coefficient estimation was based on the method described by Nei and Li (1979). Furthermore, 28 Anthurium genotypes were clustered based on the estimated Dice's similarity coefficient. The phylogenetic tree (or dendrogram) was inferred using the BioNumerics Software (Applied Math), and the clustering method of the Un-weighted Pair Group Method Using Arithmetic Average (UPGMA).

Results

Development of novel Anthurium SSR markers

A total of eight (14.5 %) SSR-containing primers were designed from 55 nucleotide sequences of *Anthurium*. Of these, six primer pairs (Am1–Am3 and Am6–Am8) contained dinucleotide repeat motifs (75 %), and two primers (Am4 and Am5) contained trinucleotides motifs (Table 2). When the eight working SSR primer pairs were further tested using the 28 genotypes, four loci (50 %) were able to produce the expected PCR products, whereas the other four failed to amplify the expected fragments. All of the 4 amplified loci revealed polymorphisms and detected 40 alleles, and the amplicon size ranged from 92 to 200 bp (Table 2). The number of alleles at each polymorphic SSR locus ranged from 9 to 11, with an average of 10 alleles (Table 2). The PIC value of SSR markers was from 0.87 to 0.93 (Table 2). SSR-PCR profiles obtained from the 4 loci of Am2, Am7, and Am8 were clear and easy to interpret (Fig. 1).

All of the target sequences of thirty-two primer pairs designed from the 32 *Colocasia esculenta* microsatellite sequences contained dinucleotide repeat motifs. Among them, 25 of the SSR-primers contained dinucleotides of perfect/compound repeats, and seven primers contained interrupted compound dinucleotide repeats. Testing the 28 genotypes of *Anthurium* using the 32 SSR-primers of *Colocasia esculenta* revealed that only locus Cm16 was able to produce the expected PCR products with polymorphisms, with 9 polymorphic alleles being detected and fragment sizes of 206–299 bp. Moreover, the locus of Cm16 was highly polymorphic, with a PIC value of 0.9, and the profile was easy to interpret (Table 2; Fig. 1).

Transferability of Zantedeschia SSR markers for Anthurium

Of the 1,492 *Zantedeschia aethiopica* EST sequences, sufficient flanking sequences of appropriate quality for SSR primers design were present in 350 unigenes

Table 2 Microsatellite primer sequences and characteristics of the SSR markers developed from the GenBank sequences of Anthurium, *Colocasia esculenta* and *Zantedeschia aethiopica*

Locus and GenBank accession no.	Repeat motif	Primer sequences 5′ → 3′	SSR location	Expected size (bp)	Product size (bp)	Allele no.	PIC
Am1 (AY054729)	$(AT)_{17}$	F: TCCTGAGCCAAATCCTTGTT R: TCGGGTCGTGATTAATCGTT	Intron	221	–	0	–
Am2 (AY232494)	$(CA)_{20}(AT)_6(AG)_{10}$	F: TGCTCCATCGATCTCTCCTT R: GTGCATCATCCTCGCAGATT	5′UTR	165	96–191	9	0.91
Am3 (AY236866)	$(CA)_{19}(AT)_5(AG)_{10}$	F: GACACAGTTGCCTCCGATTT R: AGCTGTTGTTTATAGAGGCAGAA	CDS	154	112–189	11	0.87
Am4 (AY251054)	$(CGC)_5$	F: GTGAAGAACTCGTGGGGAAC R: TGAGATGGTGAAGGGCTAGG	CDS	209	–	0	–
Am5 (AY251055)	$(TCC)_5$	F: CTCACCTTTGAGCCTTCGAC R: TACAGCAGCCTCACCTCCTC	CDS	153	–	0	–
Am6 (AY555155)	$(AT)_9$	F: TCCTGAGCCAAATCCTTGTT R: TCGGGTCGTGATTAATCGTT	CDS	205	–	0	–
Am7 (DQ421809)	$(AG)_{31}$	F: GAAAAGGTAGGGTGTTTTCTCG R: CGGAACAAGTACCTCGGTTG	CDS	162	92–200	9	0.90
Am8 (EF079870)	$(CA)_{11}(AT)_9(AG)_{13}$	F: GCGTAGGGTAGACACAGTTGC R: CAGCTGTTGTTTATAGAGGCAGA	CDS	154	121–198	11	0.93
Cm16 (AM183320)	$(AG)_{19}$	F: ACTGGGCCACCAAATAAACA R: ACGACCTGGACTTCATGACC	–	207	206–299	9	0.90
Zm32 (AJ700607)	(T)^15. (GTTTT)^4	F: GCCGATGTGTCCTCAGTGTA R: AGCAAGGGCACAGAGAAGAA	–	162	163–171	3	0.86

(Fig. 2). Among these SSRs, trinucleotide repeats were the most abundant (38.3 % of all SSRs), followed by interrupted compound repeats (35.1 %), and dinucleotide repeats (22.5 %). Of these, 60 working SSR primer pairs were further tested using the 28 genotypes. However, only the Zm32 locus was able to produce the expected amplicon size, with three polymorphic alleles detected at this locus; the amplicon size ranged from 163 to 171 bp, with a PIC value of 0.86, and the profile was easy to interpret (Table 2; Fig. 1).

Establishing the optimal combination of SSR markers to discriminate Anthurium cultivars

Table 3 lists the allelic profiles of 28 genotypes for the four selected polymorphic SSR loci; a number of genotypes sharing the same profile vary at different loci. For example, the cultivar 'Cuba White' and 'Fantasia' had the same profiles within the loci of Am2, Am8 and Cm16, but revealed different allele markers at the Am7 locus. In particular, the 'Southern Blush' cultivar had three specific profiles, including amplicons of 96 and 157 bp at the Am2 locus, 111 bp at Am7, 121 and 156 bp at Am8, and 214, 227 and 255 bp at Cm16 (Table 3).

Because the use of an individual SSR locus may not differentiate many cultivars, the combination of several loci could increase the efficiency when used for identification (Table 3; Table 4). Regarding the Am8 locus, only nine cultivars (or 11 genotypes) could be distinguished, whereas a combination of all the alleles within four selected loci in Table 4 can discriminate among 24 cultivars. Furthermore, 27 genotypes were discriminated by the combination of 65 SSR markers which amplified from 4 selected loci, with the exception of 'Tropical' and its mutant 'Tropical-P' with the same profile at each of the 4 loci (Tables 3, 4).

Genetic diversity of Anthurium cultivars

The assessment of the genetic diversity of the 28 genotypes was based on the amplification of four selected SSR loci (Am2, Am7, Am8, and Cm16)

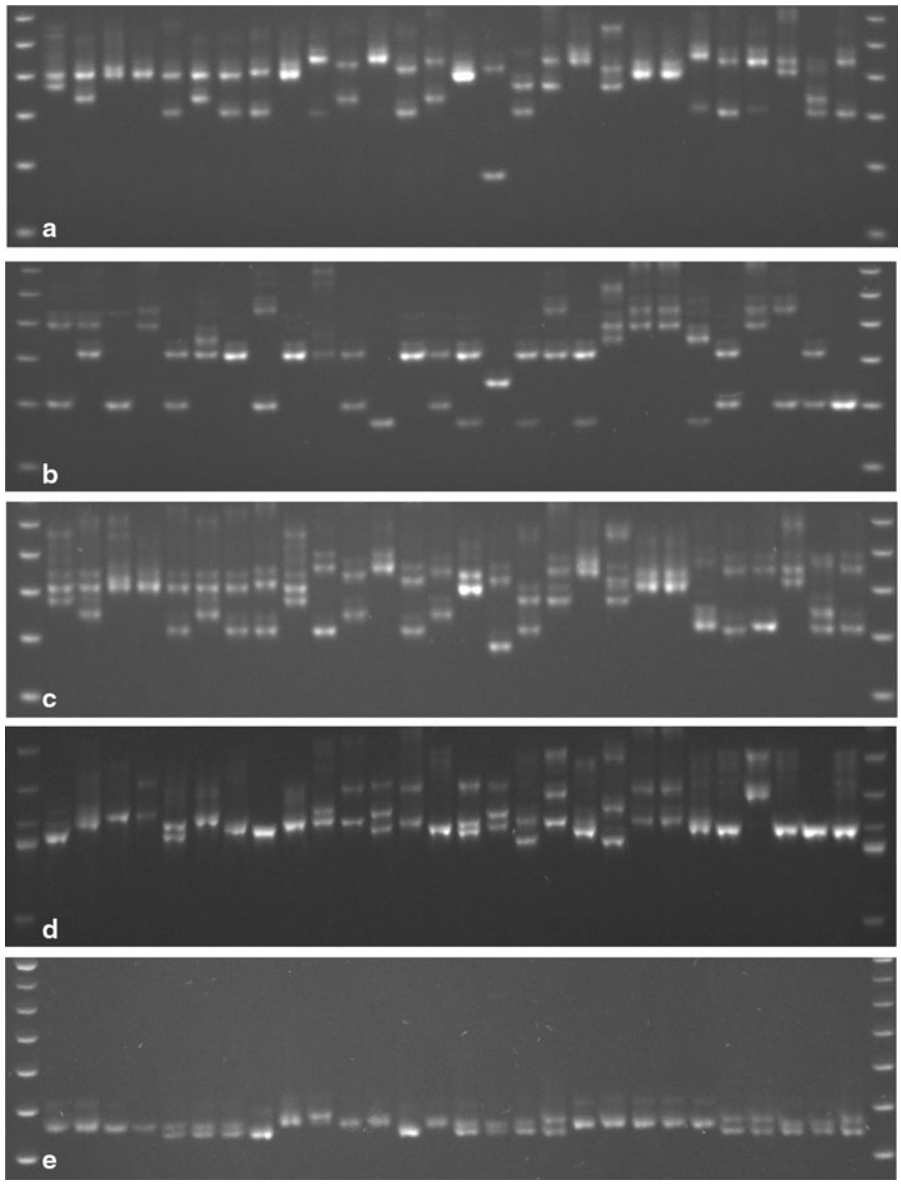

Fig. 1 PCR-amplified SSR profiles of the Am2 (**a**), Am7 (**b**), Am8 (**c**), Cm16 (**d**), and Zm32 (**e**) loci among 28 genotypes of Anthurium after separation on 6 % polyacrylamide gels (The order of the lanes from left to right follows the order from top to bottom in Table 1. Marker: 25 bp DNA step ladder (Promega) and low molecular weight DNA ladder (NEB))

giving a total of 65 polymorphic SSR markers. By the cluster analysis the 28 genotypes were separated into three main clusters, likely matching with their origin (i.e., breeders) (Fig. 3; Table 1), as the secondary main cluster (containing 12 genotypes) reveals that 9 of the commercial cultivars bred in the Netherlands grouped together with 3 progeny from TARI (Fig. 3; Table 1). However, 'Tropical', 'Tropical-P', 'Sonate' and 'Anneke' which were bred in the Netherlands are separated in the third cluster (Fig. 3; Table 1). Moreover, 'TN01' and 'TN04' bred at TARI, in addition to 'Acropolis' and 'Tulip' appear to be differentiated from the other cultivars (Fig. 3). In particular, 'Southern Blush', bred at the University of Florida showed the most difference from the other 27 genotypes (Fig. 3).

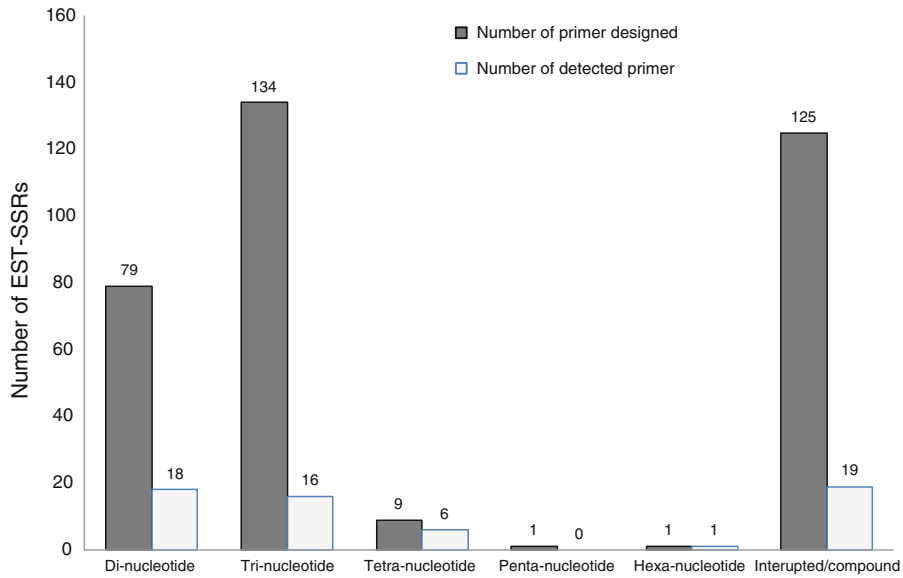

Fig. 2 Distribution of the primers designed and detected using *Zantedeschia aethiopica* ESTs, as based on the motif length

Discussion

The main goal of this study was to develop novel SSR markers in Anthurium. Primer pairs were constructed for 55 nucleotide sequences of Anthurium in GenBank, with four loci being successfully amplified. In addition, we selected two inter-genus SSR markers from *Colocasia* and *Zantedeschia* and assessed their transferability to Anthurium. Further PCR amplification indicates that the 28 genotypes of Anthurium consist of the characters for six SSR markers with significant polymorphism (Table 2). Most of the polymorphic loci are dinucleotide $(AG)_n$ motif repeats; three of the SSRs were located in coding regions, one was found in a 5′UTR and the other one was a genomic SSR (Table 2). It has been reported that a general property of plant genomes is a large abundance of AG/CT repeats. The functional significance of SSRs in genic regions in plants is unclear, but such homopurine–homopyrimidine stretches as $(AG)_n/(CT)_n$ in 5′UTRs have been reported to be related to the gene regulation genes involved in transcription, nucleic acid metabolism and the regulation of gene expression (Scaglione et al. 2009; Varshney et al. 2005). High proportions of AG/CT repeats have also been found in ESTs: e.g., pineapple (Wohrmann and Weising 2011), and kiwifruit (Fraser et al. 2004) and in almost all ESTs of various dicotyledonous plants (Kumpatla and Mukhopadhyay 2005). For example, the length of the polymorphism in a $(CT)_n$ microsatellite in the 5′UTR of the waxy gene in rice proved to be correlated to the amylose content (Ayers et al. 1997). However, a microsatellite located in a coding region can affect the activation of a gene and, therefore, the expression of a protein. It has been reported that the markers derived from transcribed sequences may be less polymorphic but more robust compared with genomic SSRs (Varshney et al. 2005).

Based on the number of alleles identified, the genic-SSR markers developed in this study appear to be as polymorphic as genomic SSRs (Table 2) and, thus represent a valuable tool for the genetic characterization of Anthurium. However, the 60 SSR primer pairs designed using the *Zantedeschia aethiopica* EST sequence and the 32 SSR primer pairs designed using the *Colocasia eculenta* microsatellite sequence resulted in a difference only one pair of primers (<4 %) and produced expected fragment. The results of this study indicate that the cross-genus transferability of the SSR primers for Anthurium designed using the ESTs of *Zantedeschia aethiopica* and the microsatellite sequences of *Colocasia eculenta* are extremely low. Because the report of Wohrmann and Weising (2011) indicated that the cross-species SSR marker transferability of Bromeliaceae is approximately 17.9 %, we speculate that the cross-species transferability may be higher than the cross-genus transferability in Araceae.

To use microsatellite markers for cultivar differentiation, we present four representative polymorphic SSR loci showing easily scorable alleles and the

Table 3 Allelic sizes and profiles of four selected SSR loci for the 28 genotypes of Anthurium

Cultivar/genotype	Specific alleles (bp) in the selected SSR locus			
	Am2 (AY232494)	Am7 (DQ421809)	Am8 (EF079870)	Cm16 (AM183320)
Acropolis	144;152	100;149	144;152;162;190	206
Alexis	135;152	128;149	137;152;162	214
Anneke	152	100	152	221
Atlantis	152	149;161	152	221;255
Carnaval	127;152	100;128	129;152;162	206;214
Champion	135;152	128;138	137;152;162	214
Cuba White	127;152	128	129;152;162	214
Fantasia	127;152	100;161	129;152;162	214
Fla-Exotic	152;157	128	144;152;162;190	214
Lady Jane	127;163	128;200	129;166;174	221;227
Lunette	135;163	100;128	137;162	221;255
Marian Seefurth	163	92	166;174	214;227;255
Merengue	127;157	128	129;156;166	221;255
Midori	135;163	100;128	137;162	214
Sonate	152	92;128	152;162	214;221;255
Southern Blush	96;157	111	121;156	214;227;255
TN01	127;144	92;128	129;144;152	206;221
TN02	144;163	128;161	144;152;162;174	221;247;299
TN03	163	92;128	162;166	214;286
TN04	144;157;191	138;149;181	144;156;166;190	206;234;299
Tropical	152	149;161	152;162	221;255
Tropical-P	152	149;161	152;162	221;255
Tulip	127;167	92;138	129	214
Champagne	127;163	100;128	129;162	214
123(Nette)	127;163	149;161	129;162	247;299
943954-1	152;163	100;161	156;162;198	214
943954-2	127;135;157	100;128	129;137;166	214
943954-4	127;163	100	129;162	214
Number of cultivars discriminated	8	9	9	10
Number of SSR allele markers within 28 genotypes	16	15	18	16

Table 4 Efficiency of the optimal combination of the four selected informative SSR loci to discriminate 27 Anthurium genotypes

Numbers of combination	Locus and combination	Number of cultivars distinguished	Total number of genotypes distinguished
1	Am8	9	11
2	Am8 + Am7	12	14
3	Am8 + Am7 + Am2	13	14
4	Am8 + Am7 + Am2 + Cm16	24	27

profiles of 28 Anthurium genotypes (Table 3; Fig. 1). A diagnostic banding pattern could be found for all 27 of the genotypes except for a mutant of 'Tropical'. It is suggested that SSR markers are suitable cultivar-specific markers of Anthurium; in addition, the SSR allele profiles can provide effective tools for

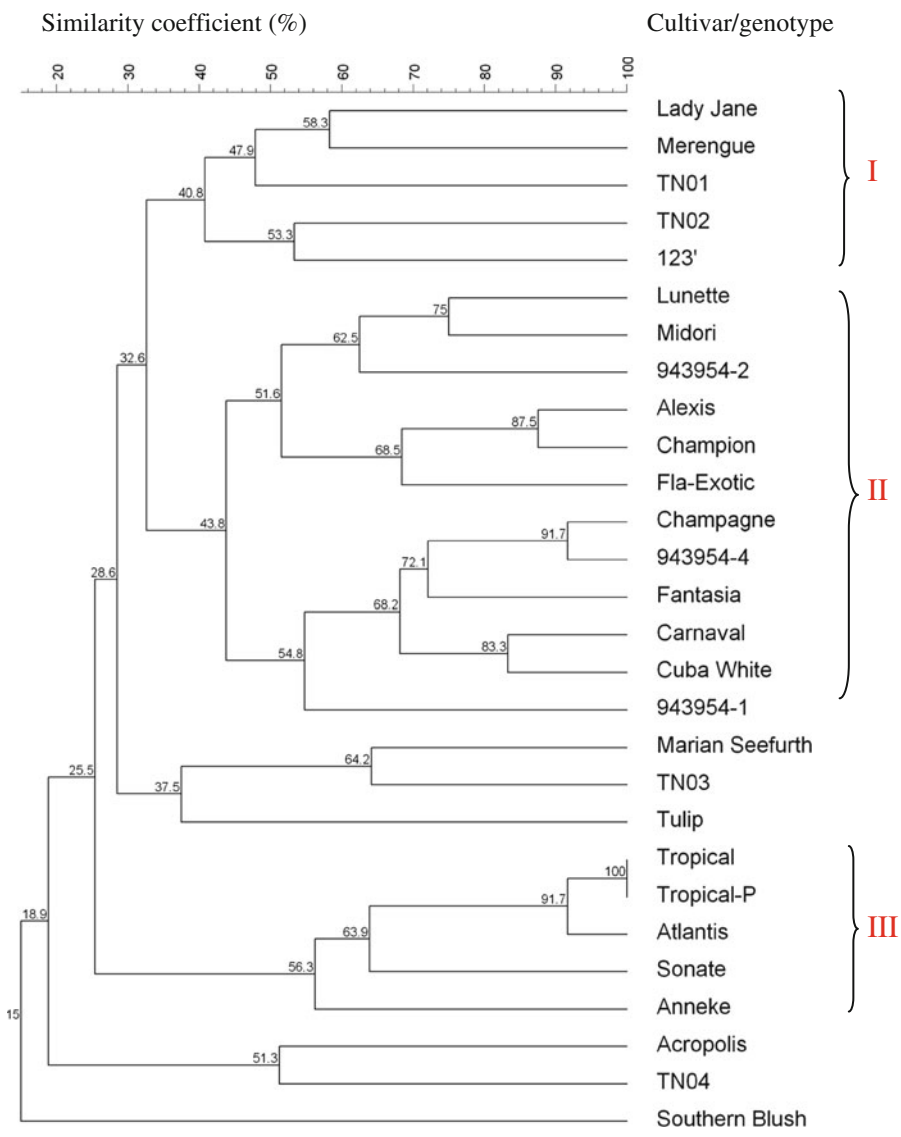

Fig. 3 Dendrogram presenting the association among 28 Anthurium genotypes based on the UPGMA cluster analysis of 65 amplified SSR markers (an estimated similarity coefficient indicated on the position of the cluster node)

differentiating or fingerprinting Anthurium. Furthermore, the higher is the average number of polymorphic markers, the higher is the characterization among genotypes. Microsatellite markers have also been shown to be highly polymorphic and efficient for phylogenetic studies (Ashkenazi et al. 2001). In this study, the genetic diversity analysis revealed that 'Southern Blush', which originated from the University of Florida was different from the other 27 genotypes (Fig. 3). According to a related report, 'Southern Blush' is a hybrid of *Anthurium andraenum* and *Anthurium amnicola* (Henny et al. 1988); therefore, it differs significantly from the other genotypes that belong to *Anthurium andraenum*. Performing cluster analysis of genetic similarity using SSR markers could help to distinguish genomic backgrounds and resources.

In conclusion, we developed and characterized the first six microsatellite markers for use in Anthurium fingerprinting and genetic diversity studies. Furthermore, we have shown a high level of polymorphism of the Anthurium SSR loci. Through further development in the future, these SSR markers should provide an efficient tool for cultivar discrimination and genetic diversity assessment and will be beneficial for marker-assisted selection in Anthurium.

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