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Genetic divergence of *Anthurium affine* germplasm using morphoagronomic and molecular descriptors[1]

Divergência genética do germoplasma de *Anthurium affine* utilizando descritores morfoagronômicos e moleculares

Clara Yohana Maia[2], Nazaré Suziane Soares[2], Ana Cecília Ribeiro Castro[3], João Ravelly Alves de Queirós[2], Fernando Antonio Souza de Aragão[3] and Patricia do Nascimento Bordallo[4]*

ABSTRACT - *Anthurium affine* is an ornamental Brazilian native plant very appreciated due to its long-lasting durability. To explore the genetic diversity available, 21 *A. affine* half-sibling progeny were evaluated using morphoagronomic and molecular descriptors. The morphoagronomic descriptors used were: plant height, plant diameter, growth habit, leaf number, leaf blade length, leaf blade width, petiole length, petiole diameter, leaf margin undulation, general appearance; soil cover rate and Random Amplified Polymorphic DNA (RAPD) markers the molecular one. All the descriptors within the progeny showed variability and general appearance, leaf margin undulation and soil cover rate are the ones that most contribute to the plants divergence. From 45 RAPD primers tested, 19 presented clear polymorphic bands (42.2%). Those generated 200 molecular markers, 81 (40.5%) of them being polymorphic. The number of polymorphic bands per RAPD primer ranged from one (OPB10) to eight (OPW11) and polymorphism ranged from 16.6% (OPB10) to 70% (OPW05). Grouping analysis of morphoagronomic and molecular descriptors formed five and four groups respectively. The dendrograms showed differentiation between accessions although there was no association between morphoagronomic and molecular groupings. There is genetic divergence within the half-sibling progeny of *A. affine* plants evaluated, which opens up possibilities for increase variability.

Key words: Clustering. Characterization. Genetic variation. Ornamental. RAPD.

RESUMO - *Anthurium affine* é uma planta ornamental nativa do Brasil sendo muito apreciada pela sua longa duração. Para investigar a divergência genética disponível, 21 progênies de meio-irmãos *A. affine* foram avaliados utilizando descritores morfoagronômicos e moleculares. Os descritores morfoagronômicos utilizados foram: altura da planta, diâmetro da planta, hábito de crescimento, número de folhas, comprimento da folha, largura da folha, comprimento do pecíolo, diâmetro do pecíolo, ondulação da margem foliar, aparência geral, taxa de cobertura do solo, e marcadores do tipo Polimorfismo de DNA Amplificado ao Acaso (RAPD). Todos os descritores dentro da progênie apresentaram variabilidade e os descritores aparência geral, ondulação da margem foliar e taxa de cobertura do solo foram os que mais contribuíram para a divergência entre as plantas. Dos 45 primers testados, 19 apresentaram inequívocas bandas polimórficas (42.2%). Estas geraram 200 marcas moleculares, sendo 81 (40.5%) polimórficas. O número de bandas polimórficas por primers variou de um (OPB10) a oito (OPW11) e o polimorfismo variou de 16.6% (OPB10) a 70% (OPW05). Análises de agrupamento dos descritores morfoagronômicos e moleculares formaram cinco e quarto grupos, respectivamente. Os dendrogramas apresentaram diferenciação entre os acessos embora não haja associação entre os agrupamentos dos descritores morfoagronômicos e moleculares. Existe divergência genética dentro da progênie de meios-irmãos de *A. affine*, o que abre possibilidades para aumento da variabilidade.

Palavras-chave: Agrupamento. Caracterização. Variação genética. Ornamental. RAPD.

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*Author for correspondence
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[1]Trabalho parte do projeto Embrapa código 11.15.02.002.09.00
[2] Departamento de Fitotecnia, Universidade Federal do Ceará/UFC, Fortaleza-CE, Brasil, cmaia1892@gmail.com (ORCID ID 0000-0002-3786-8407), suzianesoares05@gmail.com (ORCID ID 0000-0002-4349-5141), ravellyalves@hotmail.com (ORCID ID 0000-0003-4987-8589)
[3]Embrapa Agroindústria Tropical, Fortaleza-CE, Brasil, cecilia.castro@embrapa.br (ORCID ID 0000-0001-6441-9888), fernando.aragao@embrapa.br (ORCID ID 0000-0002-4041-7375)
[4]Laboratório de Biologia Molecular, Embrapa Agroindústria Tropical, Fortaleza-CE, Brasil, patricia.bordallo@embrapa.br (ORCID ID 0000-0003-4032-3106)

INTRODUCTION

The commercial floriculture sector in the Brazilian agribusiness is both dynamic - it demands constant changes and new products - and promising - this market traded R$ 6.9 billion in 2017 (US$ 1.77 billion) (JUNQUEIRA; PEETZ, 2018).

A component of the pot market is the tropical foliage, characterized by a diversity of shapes, colors and uses that come to meet both consumer market and producers' demand for novelties (CASTRO *et al.*, 2010).

Among the tropical foliage, the genus *Anthurium* excels in the ornamental market due to its rich diversity in shapes, beautiful leaves and durability. The Anthurium inflorescence is a set of bisexual flowers arranged in a spike named spadix, protected by a modified bract called spathe, and the spadices and spathes can vary in shape, size, and color between species (DIAZ *et al*., 2019).

There are many species of this genus in the Brazilian flora (COELHO; VALADARES, 2019). The presence of species with ornamental potential in native flora comes as an opportunity for Brazilian floriculture to follow a trend of the ornamental market: the search for innovation (JUNQUEIRA; PEETZ, 2018; TANIGUCHI *et al.*, 2018). However, only a few species of *Anthurium* are commercially exploited (PESSOA; CASTRO; GALLÃO, 2013). In this context, one native species of Brazil that is promising to the ornamental market of potted plants is *Anthurium affine*.

A. affine can be considered ideal for pot plant consumer due its compact architecture and very undulate leaf blades. Its leaves are flexible, wavy and shiny, showing high ornamental performance, a high post-harvest durability that makes this species suitable for commercial exploitation, especially due to the high demand for green ornamental plants with exuberant appearance and high durability and easy care (MORAIS *et al.*, 2017). The species has a wide distribution in Brazil and is considered adapted to different growing conditions (MORAIS *et al.*, 2017).

Given the potential of *A. affine* to be marketed, it is recommended that research be conducted just so this potential plant genetic resource be sustainably assessed, avoiding having extractivism as the only source of specimens. And one kind of research that can be considered fundamental to this process is the study of genetic diversity.

Molecular and phenotypic evaluations are essential towards the effective use of plant genetic resources in plant breeding programs and prebreeding activities (BYRNE *et al.*, 2018; PESSOA *et al.*, 2018). This characterization and evaluation of germplasm allows an evaluation of genetic variability within accessions and could be used to identify promising accessions. As in other crops, the evaluation of a progeny of half-siblings from parental that meet the standards for ornamental use is made to more efficiently select superior genotypes for commercial use (OLIVEIRA *et al.*, 2013). With sufficient information acquired in the characterization process, it is possible to determine the genetic distances between the individuals that compose a progeny by genetic-statistical analyzes. Several procedures can be used for this purpose; however, those based on multivariate techniques could be an advantageous in analyzing genetic diversity, because classify and order large numbers of breeding material and trait combinations (ZANKLAN *et al.*, 2018).

It is in this context that the present study had as objective to evaluate genetic divergence of 21 half-sibling of *A. affine* through joint analysis of morphoagronomic and molecular characters and to select the most informative morphoagronomical descriptors for ornamental use.

MATERIAL AND METHODS

This study evaluated 21 half-sibling of *A. affine* plants from Brazil, selected from the Araceae germplasm collection, which conserves 70 accessions at Embrapa Agroindústria Tropical, Fortaleza, Ceará, Brazil (3°45'05" S - 38°34'36" W), passport data available at alelobag.cenargen.embrapa.br/AleloConsultas/Passaporte/. The female parent (BRA 59245-1) was chosen because of its erect and compact growth habit, with highly undulate leaf margins, standing out from the other accessions. The half-siblings came from the same inflorescence and due to the species open-pollination feature; the second parental plant was not identified. The number of flowers formed in *A. affine* can vary greatly, the spadix of cultivated accessions ranges between 6 - 9 cm long, 1.0 - 2.0 cm diameter containing more than 100 flowers. The flowers matured from base to apex, and the mature pistil takes about one week after opening of the inflorescence and the androecium at about 5 weeks. The berries formed are red to dark maroon at apex, vary in number, depending on pollination, from 30 to 100 fruits or more with one or two seeds. (data not published in full text articles). The seeds were grown in an 80% sun blocker shade house and, by the time of the characterization the plants were 2 years old, when they were already in the reproductive phase and had final size with the leaves showing their maximum dimension. This species, as a seedling or potted plant, is sold in various sizes, but the larger the size, the higher the market price.

Morphological characterization was performed by evaluating plant height (PLH), plant diameter (PLD), growth habit (GRH), leaf number (LEN), leaf blade length

(LBL), leaf blade width (LBW), petiole length (PEL), petiole diameter (PED) and leaf margin undulation (LMU). The general appearance (GEA) and the soil cover rate (SCR) were also evaluated through score inference. The morphological characteristics were evaluated according to aspects relevant to the ornamental uses and terminologies of the CATE (Creating a Taxonomic E-science) Araceae database, described by Morais *et al*. (2017).

The characterization of GRH took into account the distribution of the leaves with compact erect (1) or erect expanded (0). All leaves were considered fully expanded in the leaves count stage. Leaf descriptors were evaluated on all fully expanded leaves of each plant and mean values of each descriptor were obtained for the whole plant. Thus, LBW was obtained by measuring the widest portion of the leaf blade. The LBL was measured from the base of the leaf blade to the apex. PEL was obtained from the measurement from the base of the petiole to the base of the leaf blade. The measurements were taken using tape measure (cm) and digital caliper (mm).

The variables LMU, GEA and SCR were scored by five evaluators on a rating scale of scores (Table 1). LMU leaves were characterized as sinuous or wavy; sinuous margins had softer or absent ripples and wavy leaves had margins with more pronounced ripples. GEA was characterized by vigor, sanity and uniformity. SCR was determined by the percentage of substrate area covered by the plant (photo taken with a camera 1.5 m distant and perpendicular to the plant).

Preliminary protocols for genomic DNA extraction of *A. affine* were tested using different plant tissues. After testing, genomic DNA from root tissue was extracted as proposed by Inglis *et al.* (2018), with adjustments. Subsequently, all DNA was quantified using a Nanodrop® 2000 spectrophotometer and diluted to a concentration of 10 ng mL^{-1}.

The amplification reactions for each sample containing 50 ng of DNA had a total volume of 16 µl that also included 2.5 µM of each primer, 1 X PCR Buffer, 2.5 mM $MgCl_2$, 200 µM dNTP and 1 U Taq polymerase (Promega). Forty five RAPD primers (OPA02; OPA05; OPA08; OPB10; OPB20; OPC20; OPD08; OPD09; OPD20; OPF03; OPF06; OPF12; OPF13; OPF15; OPG02; OPG08; OPG10; OPL02; OPN05; OPN07; OPN14; OPN17; OPO08; OPO10; OPP06; OPR01; OPR03; OPR05; OPR06; OPR16; OPS11; OPW03; OPW05; OPW08; OPW11 – Operon Technologies Inc.; and UBC 185; UBC 30; UBC 303; UBC 304; UBC 317; UBC 319; UBC 324; UBC 338; UBC 339 and UBC 341 – University of British Columbia) were tested for polymorphic bands following Oliveira *et al.* (2013) and Souza Neto *et al.* (2014).

The amplification program consisted of initial DNA denaturation at 95 ºC for 5 min, followed by 40 cycles for 1 min at 94 °C of DNA denaturation, annealing for 1 min at 35 °C and DNA amplification for 1 min at 72 °C; and a final extension step for 5 min at 72 °C. The PCR was performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, USA). The PCR products were subjected to horizontal electrophoresis using a 1% agarose gel containing in TBE buffer, pH 8.0. Electrophoresis was performed at 130 W of constant power, and gels were stained with ethidium bromide (10 µL mL^{-1}) and a molecular marker ladder of 1 kb (Plus DNA Ladder Invitrogen®). Molecular data were obtained from the visualization of bands, considering the polymorphic markers and their respective alleles, which were determined by the presence or absence of bands and by the sizes of DNA fragments at each locus.

About the statistical analysis of morphological data, Euclidean distances were obtained for evaluation of the variability within the access and the estimation of the genetic divergence. With the genetic similarity estimated, the dendrogram was constructed by the UPGMA method. This is an unweighted method of pairing by the arithmetic mean that avoids using extreme values of the data to characterize the dissimilarity between the genotypes (CRUZ; CARNEIRO; REGAZZI, 2014).

In addition to the cluster analysis, the principal component analysis and the correlation between the

Table 1 - Score system to evaluate general appearance, leaf margin undulation and soil cover rate in *Anthurium affine* Schott plants selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical

Score	General appearance (GEA)	Leaf margin undulation (LMU)	Soil cover rate (SCR)
5	Healthy and uniform appearance	Highly wavy	100% of soil covered
4	Healthy appearance with low variability	Wavy	75% of soil covered
3	Good appearance, but not uniform	Moderate wavy	50% of soil covered
2	Deficient appearance	Slightly wavy	25% of soil covered
1	Uneven and deficient appearance	Not wavy	0% of soil covered

characters were also performed. The analyzes were carried out through the computational application GENES (CRUZ, 2013) and Minitab (MINITAB Inc., 2010).

As for the statistical analysis of the molecular data, the band patterns, also called markers, of each polymorphic RAPD primers were encoded in a binary sheet based on the band presence or absence, designated as 1 and 0, respectively. The polymorphism produced by each accession was used to determine the genetic distance matrix, based on the complementary Jaccard's coefficient. A dendrogram was generated from the matrix being UPGMA the chosen clustering method. The mean of the genetic distance matrix was used as the cut-off point of the dendrogram. The optimal number of markers was calculated according to the correlation coefficient between the original matrix and samples according to Kruskal (1964). Additional parameters such as stress coefficient and distortion were also evaluated in order to improve the reliability of the study. All analyses were conducted using the GENES software (CRUZ, 2013).

RESULT AND DISCUSSION

It was observed variability within the half-sibling progeny for all evaluated morphoagronomic descriptors (Table 2): petiole length (PEL) (2.6 to 9.6 cm); petiole diameter (PED) (5.1 to 11.5 mm); leaf blade length (LBL) (29.8 to 53.8 cm) and leaf blade width (LBW) (9.8 to 21.3 cm); plant diameter (PLD) (66 to 122 cm); plant height (PLH) (36 to 70 cm) and leaf number (LEN) (3 to 11).

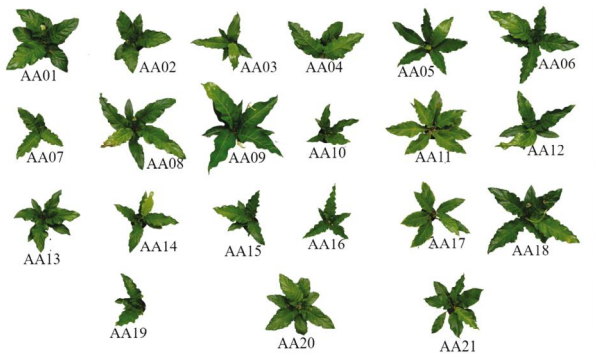
The characters general appearance (GEA) and soil cover rate (SCR) (Figure 1) ranged from 2 to 5 in the criteria. The plants also showed differences in relation to growth habit (GRH) [i.e., nine plants have their growth

Table 2 - Averages and values referring to the morphological descriptors evaluated for 21 half-siblings of *Anthurium affine* Schott plants selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical

Plant	PEL	PED	LBL	LBW	PLD	PLH	LEN	GEA	LMU	SCR	GRH
AA01	4.3	9.8	46.3	21.3	104	62	8	5	3	5	0
AA02	5.1	9.7	43.6	20.1	79	44	5	3	3	3	0
AA03	4.5	5.8	34.3	11.3	80	40	8	3	2	4	0
AA04	4.6	10.5	46.3	18.7	93	53	5	2	4	3	0
AA05	9.6	8.4	48.8	14.3	90	59	7	5	2	2	1
AA06	5.4	9.9	51.4	18.9	116	60	7	4	4	4	1
AA07	3.9	10.5	43.8	17.5	70	46	4	3	5	4	1
AA08	6.8	7.9	53.8	14.6	122	69	8	3	3	4	1
AA09	4.9	7.1	46.2	15	95	70	11	3	2	4	1
AA10	6.1	8.4	41.8	11.9	69	65	5	2	4	2	1
AA11	4.8	10	44.8	17.6	106	57	10	5	3	2	0
AA12	5.7	8.5	53.7	18.8	105	58	5	3	2	5	1
AA13	5.1	9.4	37.3	13.8	84	51	9	4	5	4	0
AA14	5.7	6.8	37.4	11	82	55	8	4	3	2	1
AA15	3.1	6.6	32.5	12.1	80	44	8	4	3	3	0
AA16	2.7	5.1	29.8	9.8	72	60	10	4	4	5	1
AA17	8.1	10.3	46.6	16	90	53	7	5	1	2	1
AA18	5.6	10	50.3	18	114	70	8	5	4	4	1
AA19	6.3	11.5	45	19	66	63	3	2	4	3	1
AA20	2.6	9.7	42.2	17.1	95	46	9	5	3	5	0
AA21	5.6	8.5	41.4	16.1	85	36	8	5	2	3	0
Mean	5.3	8.8	43.7	15.9	90.3	55.3	7.3	3.8	3.1	3.5	0.6

PEL - petiole length (cm); PED - petiole diameter (mm); LBL - leaf blade length (cm); LBW - leaf blade width (mm); PLD - plant diameter (cm); PLH - plant height (cm); LEN - leaf number; GEA - general appearance (1 to 5); LMU - leaf margin undulation (1 to 5); SCR - soil cover rate (1 to 5); GRH - growth habit (0 or 1)

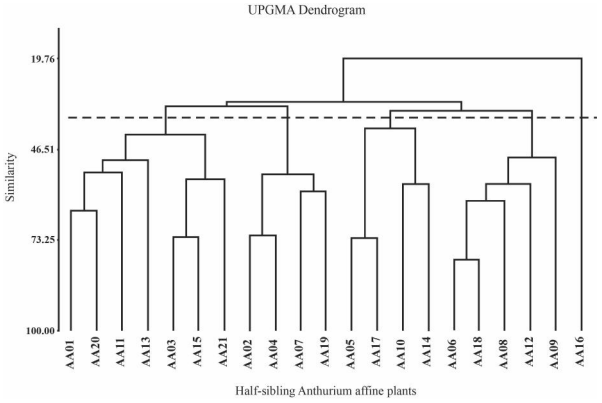
Figure 1 - Half-siblings individuals evaluated in the characterization of *Anthurium affine* Schott plants selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical



classified as erect expanded (0); 12 plants had their habit classified as compact erect (1)].

The UPGMA analysis resulted in five groups (Figure 2). The largest one grouped one third of the individuals (AA01, AA03, AA11, AA13, AA15, AA20 and AA21). The plants included in this group have GRH erect expanded and similar LEN (ranging from 8 to 10). Thus, this first group is composed of plants of more open architecture and with many leaves.

Figure 2 - Dendrogram obtained by the UPGMA method from the Euclidean distance considering 11 morphoagronomic descriptors, evaluated in 21 half-sibling individuals of *Anthurium affine* Schott based on similarity measures



Another group consisted of four individuals (AA02, AA04, AA07 and AA19). The plants in this group have similar PED (from 9.7 to 11.5 mm) and LBL (from 43.6 to 46.3 cm). In relation to the other individuals, the plants in this group present average PED and LBL values.

Four individuals (AA05, AA10, AA14 and AA17) formed another group that had similar PLH values (from 59 to 65 cm), equal SCR scores (2), and compact erect GRH. Therefore, this group was constituted by tall plants, with architecture more closed and compact, which explains the low value of SCR.

One fourth group consisted of five individuals (AA06, AA08, AA09, AA12 and AA18), which had the highest values for LBL (from 46.2 to 53.8 cm). These are also the plants with the highest PLD values (from 95 to 122 cm). Thus, in addition to the broad plants in this cluster, they all have GRH erect compact.

The 16th individual formed the latter group alone. Phenotypically, it was different from the others by having smaller leaves, with lower values of PED, LBL and LBW. However, it is a tall plant with a considerably large number of leaves.

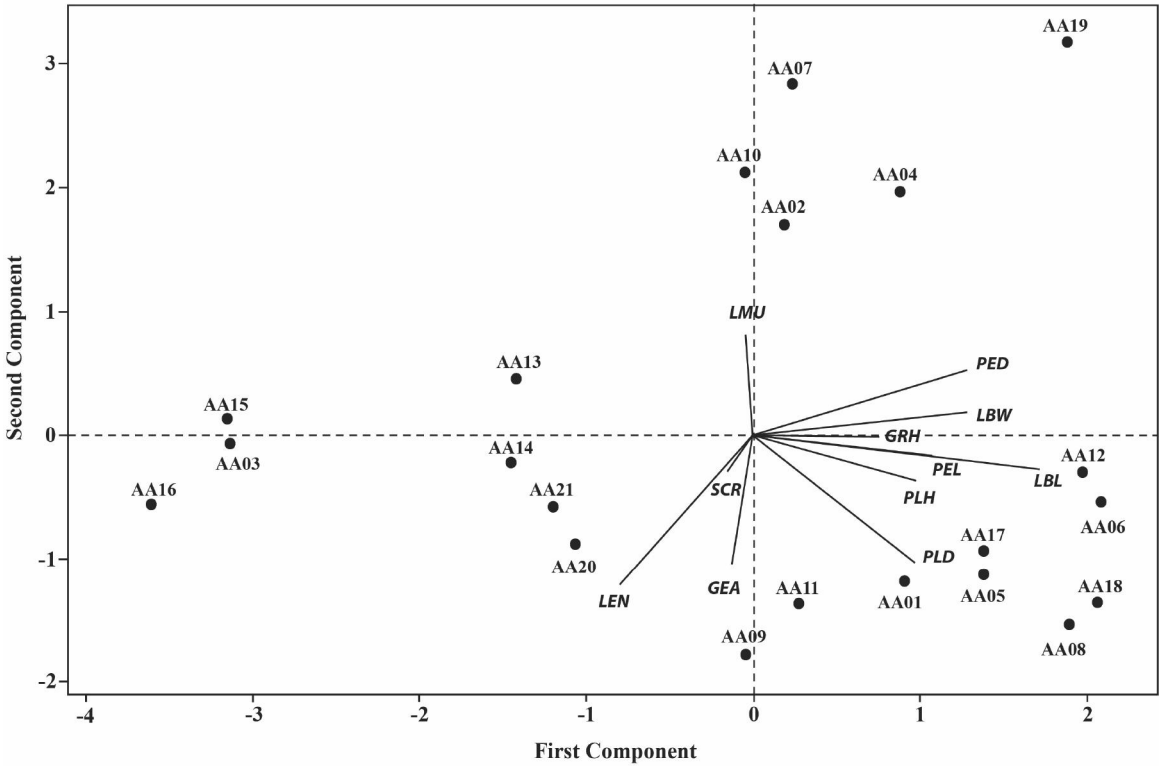
Principal components analysis (PCA) revealed that the first and second principal components accounted for 45.8% and 27.9% of the data variation, respectively. These two components together explained 73.9% of the total variation observed in the data (Figure 3).

The wide distribution of individuals in the PCA shows considerable divergence between them. Because *Anthurium* is an allogama species and presents protoginy, there is greater variability within populations (POLI; TEMPONI; COAN, 2017), which may explain the great dissimilarity between plants. Genetic divergence within half-sib progeny may also suggest the genetic distance of the parents. This is because the male parent is not known and any plant from the collection can be the parent (the compatibility between *A. affine* and other *Anthurium* species is unknown). The genetic divergence observed in this progeny may be useful for future studies on germplasm banks or collections and studies focused on the development of cultivars.

The differences between the distribution of the individuals in the PCA and the cluster analysis can be explained by the fact that an analysis of principal components presents only part of the total variation (CRUZ; CARNEIRO; REGAZZI, 2014). This may explain why PCA and UPGMA analysis differed in the clustering of individuals. Since PCA components explained approximately 74% of the total variation, the contribution of some characters in the remaining 26% of the total data variation is shown to be decisive for the clustering pattern observed.

The position of the vectors shows the degree of influence of one over the other. Vectors in opposite directions show negative correlation, vectors that overlap or with acute angles between them are highly correlated

Figure 3 - Principal components analysis (PCA) for 11 morphological descriptors evaluated in 21 half-sibling individuals *Anthurium affine* Schott plants selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical



PEL - petiole length; PED - petiole diameter; LBL - leaf blade length; LBW - leaf blade width; PLD - plant diameter -; PLH - plant height; LEN - leaf number; GEA - general appearance (1 to 5); LMU - leaf margin undulation (1 to 5); SCR - soil cover rate (1 to 5); GRH - growth habit (0 or 1)

and perpendicular vectors do not correlate (CRUZ; CARNEIRO; REGAZZI, 2014). These authors also argue that, in addition, the length of the vectors shows the divergence of the individuals in a given character. In this sense, the LEN and LBL characters had the highest and SCR had the lowest variation. Thus, there is a greater difference between the extreme phenotypic expressions of these characters. The PCA analysis revealed that: (1) characters PLD, PLH, PEL and LBL were the most influential in the distribution of subjects AA01, AA05, AA06, AA08, AA11, AA12, AA17 and AA18; (2) characters GEA, SCR and LEN were those that contributed to the distribution of individuals AA03, AA09, AA14, AA16, AA20 and AA21; (3) character LMU contributed to the distribution of individuals, AA13, AA15, and especially AA10 because it is positioned exactly in the region of greatest positive influence of the vector; (4) characters PED and LBW contributed to the distribution of individuals AA02, AA04, AA07 and AA19; (5) character GRH remained on the ordinate axis (it positively interfered with both the first and fourth quadrants).

Besides the direct and positive influence of the characters in the distribution, there are secondary influences that must also be emphasized. For example, the second group formed by the individuals AA02, AA04, AA07 and AA19 (Figure 2) was positioned entirely in the fourth quadrant. Thus, in addition to the similar phenotypes for LBL and PED, these individuals were also strongly influenced by LMU, as can be seen from their position in the principal components graph (Figure 3).

The correlation analysis between some of the descriptors in the PCA was confirmed and quantified (Table 3). The LMU character was the only one that showed no significant correlation with any other, even though the PCA has indicated negative correlation of this character with other characters. This means that it is not possible to infer about this character using another character in the present study, which justifies its importance in the characterization process of *A. affine*.

There was a negative correlation of PEL with SCR and positive correlation of SCR with GRH and LBL. This means that longer petioles tend to open more space between the stem and leaf blade, so the soil will not be fully covered. In relation to the positive correlations, it has been observed that the longer the petioles, the easier the leaves are erectly arranged, and that long petioles mean longer leaf blades. Studies that make use of these

Table 3 - Correlation coefficients (R) among the 11 morphoagronomic descriptors[1] evaluated in 21 half-sibling individuals of *Anthurium affine* schott plants selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical

R	PEL[1]	PED	LBL	LBW	PLD	PLH	LEN	GEA	LMU	GRH
SCR	**-0.57	-0.13	0.04	0.2	0.27	0.08	0.2	0.02	0.2	-0.07
GRH	*0.46	-0.06	0.37	-0.17	0.04	**0.65	-0.21	-0.19	0.03	
LMU	-0.39	0.28	-0.16	0.06	-0.2	0.14	-0.24	-0.31		
GEA	0.06	0.01	-0.02	0.04	0.37	-0.14	**0.58			
LEN	-0.27	*-0.52	-0.29	-0.39	0.34	0.1				
PLH	0.31	0.07	*0.50	0.08	0.42					
PLD	0.17	0.16	**0.69	0.4						
LBW	0.06	**0.81	**0.67							
LBL	*0.54	**0.57								
PED	0.23									

[1]PEL - petiole length; PED - petiole diameter; LBL - leaf blade length; LBW - leaf blade width; PLD - plant diameter; PLH - plant height; LEN - leaf number; GEA - general appearance (1 to 5); LMU - leaf margin undulation (1 to 5); SCR - soil cover rate (1 to 5); GRH - growth habit (0 or 1). *,**/5% and 1% significantly different trough t test, respectively

characters are rare and do not analyze the correlations between them, reassuring the importance of the analysis of these characters presented here, highlighting that SCR is a characteristic related to the visual quality of the potted plant and, from a practical point of view, it is important for plant selection (CAMPOS *et al.*, 2019).

In *A. affine*, the taller the plants, the wider and longer the leaves. This is shown by the positive correlations between PED - LBL and PED - LBW along with the positive correlations between LBL - PLH, PLD - LBW. Vectors associated to characters related to both leaves and plant size occupied the same position in PCA and in such angle that reinforces the correlation presented here (Table 3).

For the first component, the characters PED, LBL and LBW stood out among the descriptors that most influenced the distribution of the 21 half-sibling individuals evaluated. As shown by the correlation analysis, these characters are related to the size of the leaf, confirming once again the correlation between them. For the second component, the most influential descriptors were the PLD, LEN and GEA characters, which are related to the overall phenotype of the plant.

It was concluded that the individuals positioned to the right of the abscissa axis and above the axis of ordinates presented higher phenotypic expressions for the PED, LBL, LBW, PLD, LEN and GEA characters. These are descriptors considered important for selection of individuals with larger leaves and better appearance for the landscape environment.

Based on the characters of greatest contribution in explaining the global variation of the data, it was possible to estimate the genetic divergence of individuals from the progeny. Dissimilarity works can be performed without verification and characterization of all organs of the plant, since only a few characters already express most of the genetic divergence. These results suggest that future work on breeding of these genotypes is needed. The characters shown to explain the total phenotypic variation may be useful for future characterization of banks and collections and the elaboration of descriptors for the genus.

There is a great variability within the *A. affine* accessions regarding the leaf morphological characteristics, useful for characterization. Furthermore, using descriptors that are linked to aspects that are of interest of producers and consumers, like leaf morphology, for example, may offer ease and feasibility for selection of material in the future.

The genetic diversity found is the main prerequisite for starting a genetic improvement program for a species. The pre-breeding stages include, among others, the evaluation and characterization of the germplasm. Therefore, it is important to have a broad knowledge of the genetic variability available for breeding and according to market requirements, offering new product options in a market as eager for novelties as ornamental plants. Also, the available data of the genetic variability in the collection are useful in the conservation of the species, as the accessions present in it must be representative of the genetic variation of the original population. Its characterization allows to increase the genetic base, to provide a source of alleles for genetic improvement, as well as to be used in the reconstitution of habitats.

Regarding the molecular analysis, the chosen extraction protocol was appropriate to extract the DNA

from the 21 half-sibs of *A. affine*. Preliminary tests using both root and leaf tissues indicated a better quality of DNA when extracted from roots due to its lowest rates of oxidation and the high amount of mucilage found in the leaf tissue.

From the total of 45 RAPD primers tested, 19 (OPA02; OPA05; OPA08; OPB10; OPC20; OPD09; OPF03; OPF06; OPF12; OPF15; OPG10; OPN07; OPN14; OPR03; OPR05; OPS11; OPW05; OPW11 and UBC 185) revealed clear polymorphic bands (42.2%). Those generated 200 molecular markers; 81 of them polymorphic (40.5%). Among the RAPD primers, the number of polymorphic bands ranged from one (OPB10) to eight (OPW11) and polymorphism ranged from 16.6% (OPB10) to 70% (OPW05). The analysis of the optimal number of markers indicated that with 175 markers, the correlation with the genetic distance matrix of all bands was 0.95, distortion was 1.3, and the stress value was 11.6, which represents a sufficient number of fragments to obtain stable associations among the individuals sampled according to Kruskal (1964). Therefore, the number of markers was considered appropriate to determine genetic diversity. The data was analyzed using the software GENES (CRUZ, 2013) (Figure 4).

The data obtained from the selected markers was also used to generate a genetic distance matrix and a dissimilarity dendrogram based on the unweighted pair-group method using arithmetic averages (UPGMA). The genetic distances among the half-sibs ranged from 5.4% to 15%. This matrix of genetic distance was used for construction of a genetic dissimilarity dendrogram that had 0.827 of cophenetic correlation. This value can be considered high, considering 0.60 as a minimum value [as suggested by Manly and Alberto (2016)]. The average genetic dissimilarity between pairs of accessions was 0.109887, which was also the dendrogram cut point, clustering the accessions in four main groups (Figure 5).

The four main groups depicted in the dendrogram were as follows: (1) the largest one was composed of 61.9% of the assessed individuals (AA01, AA02, AA03, AA04, AA06, AA07, AA08, AA10, AA13, AA14, AA15, AA17 and AA18); (2) a second group was composed of 23.8% of the individuals (AA05, AA09, AA11, AA19 and AA21); a third group was composed of two individuals (AA12 e AA16) and one last individual did not clustered (AA20).

Due to *A. affine's* reproductive nature as an open pollinator and the process to obtain the half-siblings,

Figure 4 - Graphical analysis of the optimal number of markers obtained using the correlation coefficient (0.95) between 200 molecular markers obtained from 21 half-sibling individuals selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical

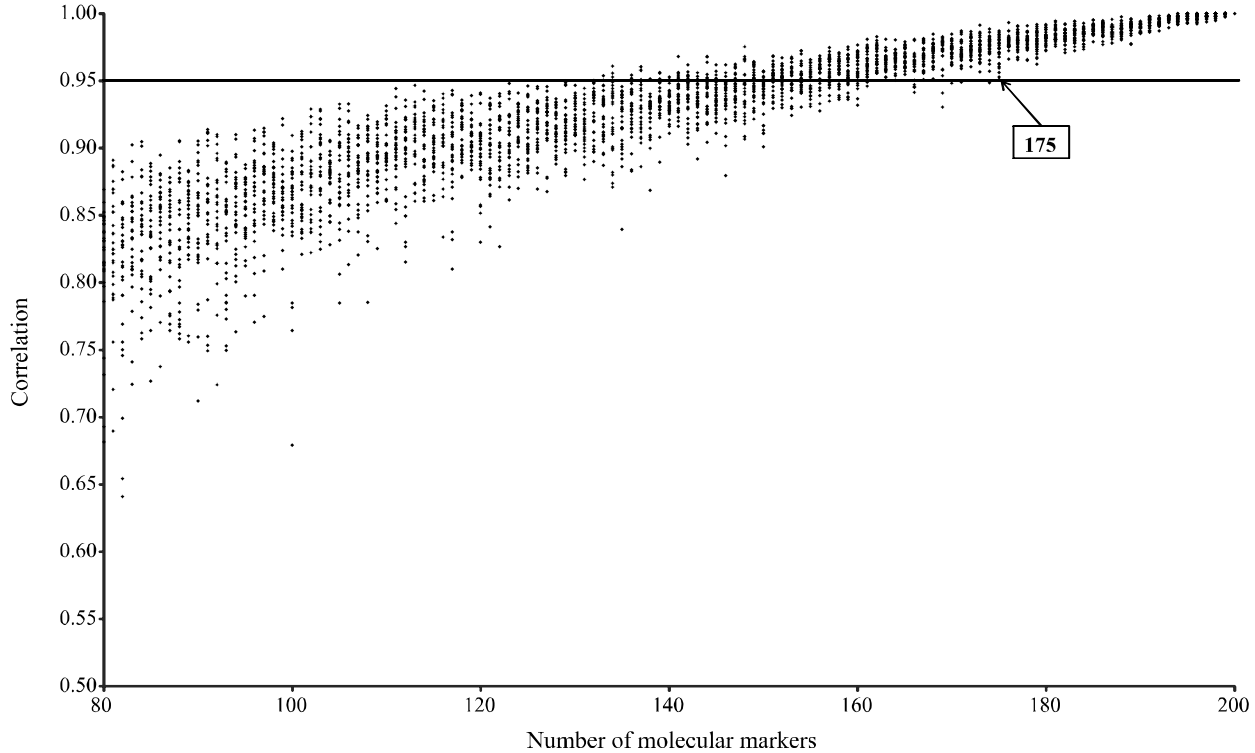
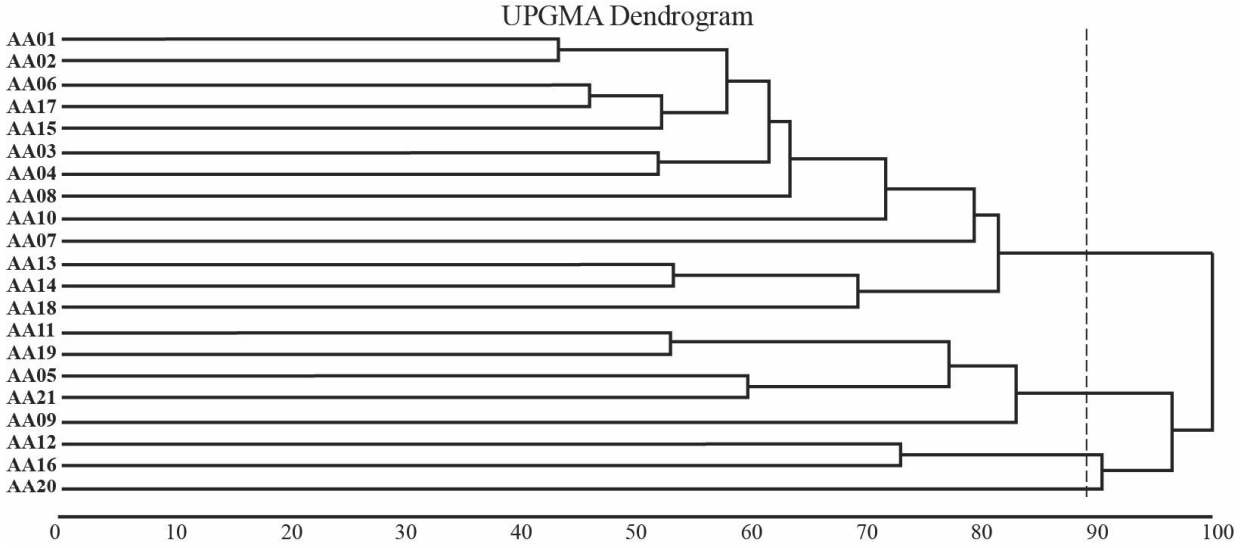


Figure 5 - Dendrogram obtained by the UPGMA method considering the genetic data obtained from 21 half-sibling individuals of *Anthurium affine* Schott selected from the Araceae germplasm collection of Embrapa Agroindústria Tropical



the expected absence of duplicates in the germplasm collection is now confirmed by the genetic data collected. The obtained information, therefore, could be useful for the improvement of the collection and guidance for future breeding programs. The molecular markers can assist in the characterization of the collection's germplasm, in its management by identifying redundant entries, in the selection of genotypes and in the planning of crosses with the use of genetically contrasting genotypes, which opens up possibilities for crosses to increase variability of the collection.

CONCLUSIONS

There is genetic divergence within the half-sibling progeny of *Anthurium affine* plants at the Araceae germplasm collection of Embrapa Agroindústria Tropical. Among the morphoagronomic descriptors, general appearance (GEA), leaf margin undulation (LMU) and soil cover rate (SCR) are the ones that most contribute to the divergence of the plants. The molecular markers that most contribute to the analysis were OPW05, OPW11 and OPD09, due to the highest amount of polymorphism observed. The morphoagronomic characters and the molecular markers are not correlated.

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