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GENETIC DIVERSITY OF *ADENIUM OBESUM*: MORPHOLOGICAL AND MOLECULAR APPROACHES

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ABSTRACT: Thirty-five genotypes of *A. obesum* belonging to three full-sib progenies were characterized using morphological and molecular markers. With respect to corolla pigmentation, a predominance of red and magenta colors was observed, and 46% of the hybrids presented double petal arrangement. In the first filial generations (F_1), no genotypes with absence of corolla pigmentation (white) and purple corolla pigmentation were observed. Of the 35 genotypes evaluated, three main color groups were observed and 33.33% showed petal arrangement with five petals (single), 53.33% showed double petal arrangement (10 petals), and 13.34% showed 15 petals (triple petal arrangement). The simultaneous analyses for the qualitative and quantitative morphological characteristics indicated the existence of high genetic variability. ISSR molecular markers detected a high level of polymorphism (97%) and the highest proportion of variation was found within progenies (82.14%). The genotypes from the cross between ICA-bd x ICA-rs presented the highest mean values for the quantitative, vegetative, and floral traits evaluated. The morphological traits with great ornamental interest were the total number of flowers, number of annual blooms, number of days from anthesis to senescence of flowers and caudex diameter. Nine genotypes were selected for the next stage of the breeding program: ICA-br 2, 6, 8, 9 and 15; ICA-vr 4 and 9, and ICA-vb 8 and 2. This study demonstrated the importance of the combined use of morphological and molecular markers to obtain estimates of genetic diversity in *A. obesum*.

Keywords: Ornamental breeding, *Adenium*, desert rose, ISSR markers, multivariate analysis

INTRODUCTION

Adenium obesum (Forssk.) Roem. & Schult is an ornamental plant, known as desert rose, desert azalea, lily-impala, or adenium. It is cultivated as an ornamental plant globally and belongs to the Apocynaceae family, which also includes several genus as *Alamanda*, *Plumeria*, and *Spirea*. It is a succulent shrub with a sculptural appearance and is non-lactescent and branched with a short and thick stem that functions as a water reserve organ. Hence, it exhibits high resistance to drought (Plaizier, 1980; Lorenzi, 2008).

The improvement of several crops is mainly based on the induction or generation of genetic variations via hybridization between distinct genotypes with the objective of obtaining segregating populations. In this process, superior individuals or lines are selected. Hence, studies on genetic divergence can be crucial because they provide estimates for the identification of genitors and progenies, which, when crossed, will increase the chance of selection of superior genotypes in advanced generations (Swarup *et al*., 2021; Salgrota and Chauhan, 2023).

Estimates of genetic diversity obtained via morphological and molecular characterizations are relatively simple and efficient for selecting individuals (Sharma *et al*., 2022). In addition to characterization performed using different markers, the choice of the appropriate method for analysis and obtaining estimates is another fundamental component. In desert rose, studies are scarce, and data are insufficient for an adequate characterization of genetic diversity in the species. Chavan *et al*. (2018) demonstrated a wide genetic variability for numerous vegetative and floral traits among the 22 genotypes of *A. obesum* evaluated, indicating a heterozygotic nature of high complexity in this species. Recently, Ramos *et al*. (2022) also reported an expressive genetic variability in commercial accessions of *A. obesum*.

Based on the information available in the literatures, despite its high ornamental potential, the species is still in the process of domestication. Hence, the scarcity of information regarding the botanical, physiological, genetic, and germplasm facets of the species presents both a constraint and a significant prospect for researchers.

Considering the information presented, the objective of this study was to evaluate the genetic variability in 35 hybrids of *A. obesum* obtained through controlled hybridization using morphological and molecular markers via multivariate analysis and to select superior genotypes.

MATERIALS AND METHODS

The genitors of *A. obesum* used in the present study were selected based on corolla pigmentation and petal arrangement. Three genitors with the following characteristics were selected: ICA-rs (G2): purple pigmented corolla and simple petal arrangement (five petals); ICA-bd (G1): white pigmented corolla and double petal arrangement (10 petals), and ICA-vt (G3): red pigmented corolla and triple petal arrangement (15 petals) (Ramos *et al*., 2022). Thirty-five lines of three hybrids were obtained via manual and controlled greenhouse crosses between the three genitors: ICA-rs, ICA-bd, and ICA-vt.

To ensure a higher germination rate, the seeds were germinated in biological oxygen demand chambers (BOD) in germination boxes at 30 °C and a 12 h photoperiod. The germinated seedlings were kept in a greenhouse in 2-L pots containing commercial Bioplant® substrate. The seedlings were maintained under protected cultivation throughout the experimental period of 24 months. Fertilization, irrigation management, and pest and disease control were carried out according to the needs of the crop (Mendes *et al*., 2021).

The following qualitative and quantitative morphological traits were evaluated: plant height (H), number of branches (NB), caudex diameter (CD), number of flowers per plant year 1 and year 2 (NFlo1 and NFlo2), corolla pigmentation (CP), flower length (FL), flower width (FW), corolla diameter (CDi), number of petals per flower (NP), number of days from anthesis to senescence year 1 and year 2 (ND1 and ND2), and number of annual blooms year 1 and year 2 (NAF1 and NAF2). All evaluations of vegetative quantitative characters (H, CD, NB) were performed on the all 35 genotypes, and data were collected at the final occasion of the experiment, 24 months after seedling germination. The quantitative floral characters (NFlo, FL, FW, CDi, NP, ND, NAF) were averages of all productive plants that varied according to the individual from two to four plants and five flowers per plant were evaluated. The floral qualitative trait of CP was evaluated in all blooms during two years, with the evaluation of three flowers per plant. The characterization of corolla pigmentation was performed using the color catalogue of the Royal Horticultural Society (RHS, 1995). The 35 genotypes were distributed in a greenhouse in a completely randomized design, with three treatments and one plant per experimental plot.

DNA was extracted from young leaves of the 35 genotypes of *A. obesum* following the method described by Doyle and Doyle

(1987). Thirteen ISSR (Inter Simple Sequences Repeats) primers from the UBC collection (*Primers* developed by the University of British Columbia Biotechnology Laboratory, collection no. 9) were used, namely, UBC 800 to UBC 900 to evaluate all 35 hybrids.

The distances between genotypes were calculated based on Jaccard's similarity coefficient. A cluster analysis based on genetic distances was performed using the unweighted pair group method with arithmetic mean (UPGMA) to generate a dendrogram (Sneath and Sokal, 1973) using the R program (R Core Team, 2022). The determination of the number of groups was performed according to the protocol outlined by Mojena (1997).

For the estimation of dissimilarity among the 35 genotypes, based on morphological data, the average standardized Euclidean distance was used for quantitative data and the frequency of coincidence for qualitative data. For simultaneous evaluation of qualitative and quantitative data, the matrix of average distances between the average standardized Euclidean distance and the frequency of coincidence were used. Based on the distance matrices, dendrograms were generated by the average divergence grouping method (UPGMA). The determination of the number of groups for the dendrograms was performed according to the cut-off of Mojena (1997). The analyses were performed in the R software (R Core Team, 2022) using the Multivariate Analysis Package (Azevedo, 2021).

In the present study, the term genotypes were applied to individuals from the first filial generation considering that the genitors (G_1, G_2 and G_3) presented at least one different character among them. Hence, regarding the volume of data generated, we will present the evaluation of all individuals together for the morphological markers. For the ISSR molecular markers, collectible data and those for each of the progenies, namely, BR (ICA-bd x ICA-rs), VR (ICA-vt x ICA-rs), and VB (ICA-vt x ICA-bd) obtained will be presented.

RESULTS

Of the 35 lines evaluated, three main color groups and 16 distinct codes were observed according to the RHS color catalog classification (Table, 1 and Figs., 1, 2 and 3). Out of the genotypes obtained from the cross between ICA-bd x ICA-rs (BR), 33.33% showed petal arrangement with five petals (single), 53.33% showed double petal arrangement (10 petals), and 13.34% showed 15 petals (triple petal arrangement). The progeny ICA-vt x ICA-bd showed 40% triple petal arrangements, 30% simple arrangements, and 10% plants with double petal arrangement. Finally, out of the hybrids resulting from the cross between ICA-vt x ICA-rs, 30%, 60%, and 10% showed of simple, double, and triple arrangements, respectively.

The 35 genotypes were sorted into five groups with respect to quantitative traits only. Group- I: included two genotypes, ICA-vb 2 and ICA-vb 7. Group- II: included the following genotypes: ICA-vb 1 ICA-vr 8, ICA-vb 9, ICA-vb 4, and ICA-vr 1. Group- III: included a single genotype, ICA-vb 3. The fourth group included the hybrids ICA-vr 2 and ICA-vr 6, and the fifth group comprised the largest number of genotypes, 25 of the 35 evaluated. All genotypes from the cross between ICA-bd x ICA-rs were sorted in group- V (Fig., 4A).

Regarding the mean values, the genotypes from the cross between ICA-bd x ICA-rs presented the highest mean values for the quantitative, vegetative, and floral traits evaluated (Fig., 4B).

The simultaneous analysis of quantitative and qualitative characters also indicated the formation of five groups. Group- I: consisted of a single genotype, ICA-vr 3. Group- II: involved genotypes of ICA-vb 4, ICA-vr 1, ICA-vb 2, ICA-vb 7, ICA-vb 1, ICA-vb 9, and ICA-vr 8. Group- III: included genotypes of ICA-vr 2 and ICA-vr 6, and group- IV: involved genotypes of ICA-br 2, 4, and 5.

Table 1. Identification (ID), description of codes and colors according to the Royal Horticultural Society (RHS) catalog in three genitors (G_1, G_2 and G_3) and 35 genotypes of *A. obesum,* Montes Claros, Brazil.

N°	ID	RHS code	RHS Color	N°	ID	RHS code	RHS Color
G_1	ICA-bd	NN155C	White-I	**17**	ICA-vr 2	46C	Red-III
G_2	ICA-rs	N89A	Violet-X	**18**	ICA-vr 3	53C	Red-III
G_3	ICA-vt	53B	Red-III	**19**	ICA-vr 4	N45C	Red-III
1	ICA-br 1	71B	Magenta	**20**	ICA-vr 5	46B	Red-III
2	ICA-br 2	53B	Red-III	**21**	ICA-vr 6	46C	Red-III
3	ICA-br 3	51A	Red-III	**22**	ICA-vr 7	53C	Red-III
4	ICA-br 4	51A	Red-III	**23**	ICA-vr 8	N45C	Red-III
5	ICA-br 5	51A	Red-II	**24**	ICA-vr 9	53C	Red-III
6	ICA-br 6	46C	Red-III	**25**	ICA-vr 10	73C	Magenta-XI
7	ICA-br 7	61C	Magenta-XI	**26**	ICA-vb1	53B	Red-III
8	ICA-br 8	61D internal 61C external	Magenta-XI	**27**	ICA-vb 2	67B	Magenta-XI
9	ICA-br 9	61B	Red-III	**28**	ICA-vb 3	N45B	Red-III
10	ICA-br 10	61C	Red-III	**29**	ICA-vb 4	53D	Red-III
11	ICA-br 11	71C external 69C internal	Magenta-XI	**30**	ICA-vb 5	53C	Red-III
12	ICA-br 12	71C external 69C internal	Magenta-XI	**31**	ICA-vb 6	45B	Red-III
13	ICA-br 13	45C	Red-III	**32**	ICA-vb 7	63A	Magenta-XI
14	ICA-br 14	60D	Magenta-XI	**33**	ICA-vb 8	64B external 63D internal	Magenta-XI
15	ICA-br 15	61C	Magenta-XI	**34**	ICA-vb 9	67B external 69D internal	Magenta-XI
16	ICA-vr 1	45C	Red-III	**35**	ICA-vb 10	63B	Magenta-XI

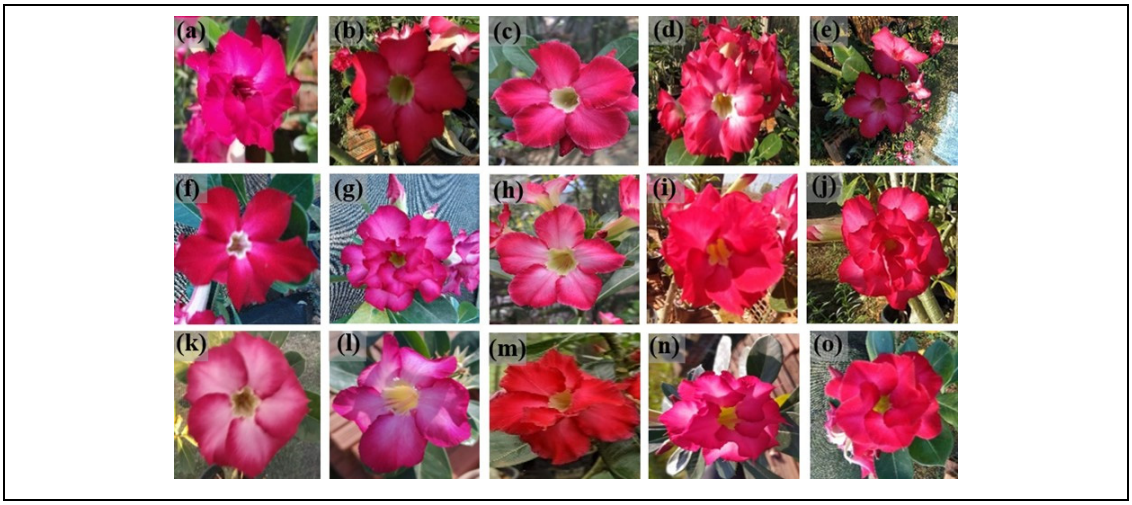

Fig. 1. Variations in corolla pigmentation and petal arrangement observed in genotypes from the cross BR of *A. obesum*, Montes Claros, Brazil. a.ICA-br 1, b. ICA-br 2, c. ICA-br 3, d. ICA-br 4, e. ICA-br 5, f. ICA-br 6, g. ICA-br 7, h. ICA-br 8, i. ICA-br 9, j. ICA-br10, k. ICA-br 11, l. ICA-br 12, m. ICA-br 13, n. ICA-br 14, o. ICA-br 15.

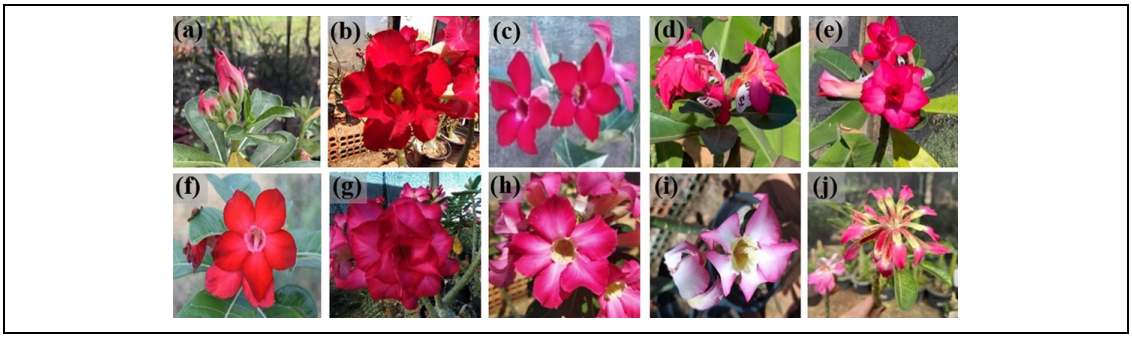

Fig. 2. Variations in corolla pigmentation and petal arrangement observed in genotypes from the VB cross of *A. obesum*, Montes Claros, Brazil. a.ICA-vb1, b. ICA-vb 2, c. ICA-vb 3, d. ICA-vb 4, e. ICA-vb 5, f. ICA-vb 6, g. ICA- vb 7, h. ICA-vb 8, i. ICA-vb 9, j. ICA-vb10.

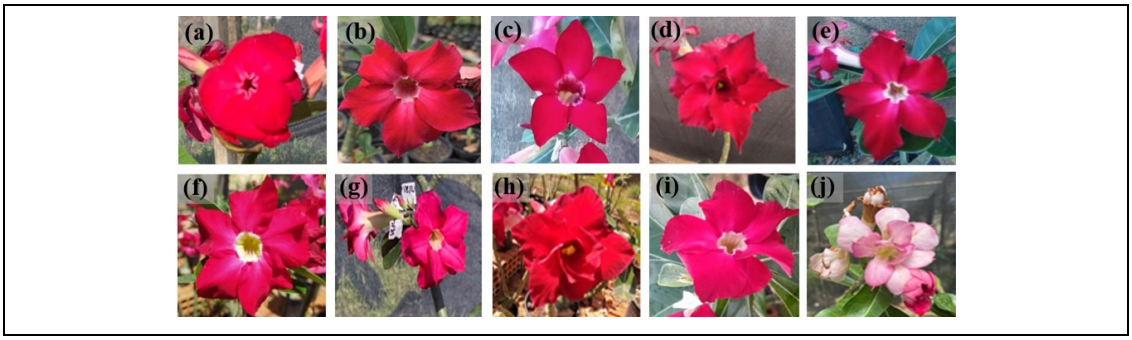

Fig. 3. Variations in corolla pigmentation and petal arrangement observed in genotypes from the VR cross of *A. obesum*, Montes Claros, Brazil. a.ICA-vr1, b. ICA-vr 2, c. ICA-vr 3, d. ICA-vr 4, e. ICA-vr 5, f. ICA-vr 6, g. ICA-vr 7, h. ICA-vr 8, i. ICA-vr 9, j. ICA-vr 10.

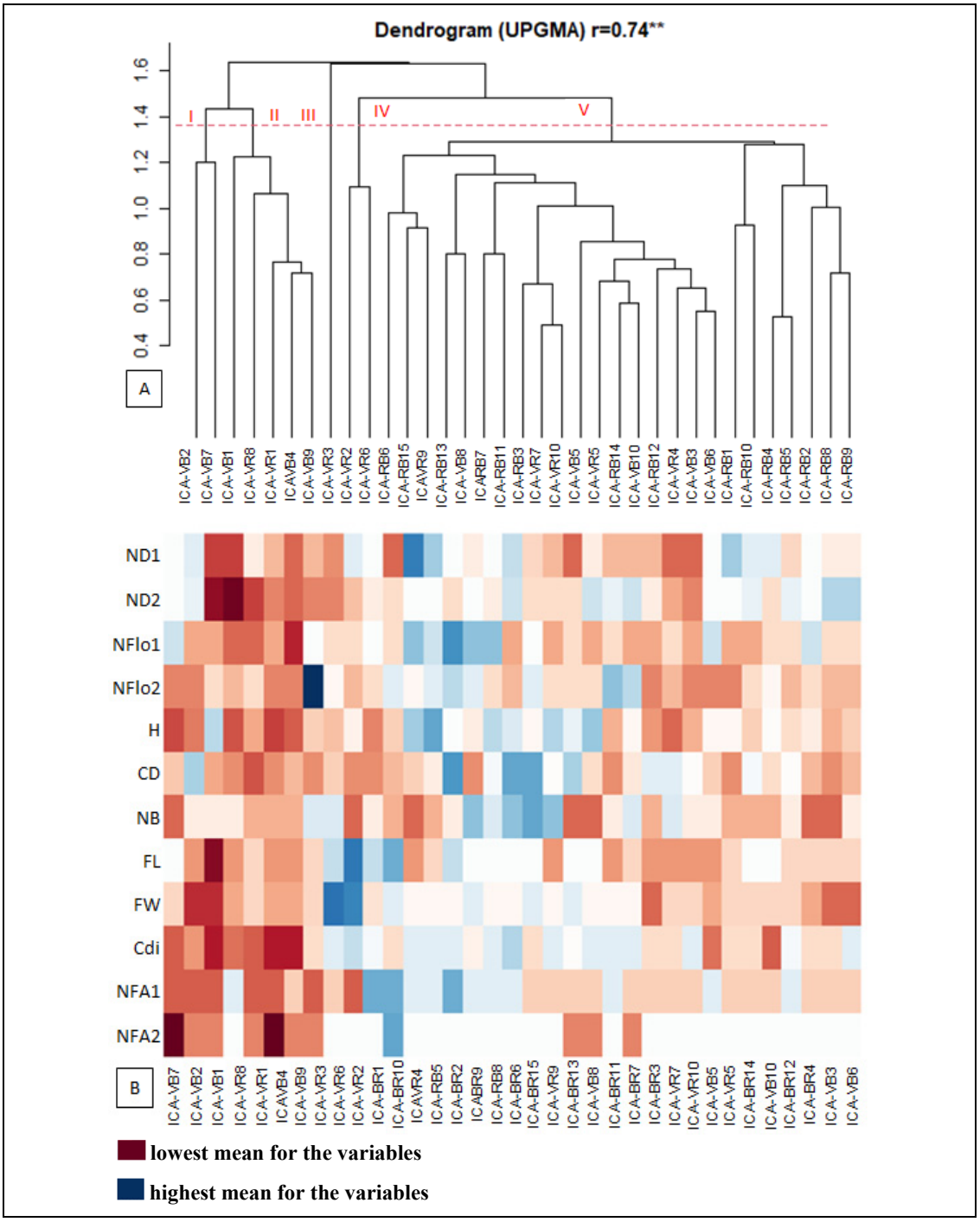

Fig. 4. Dendrogram obtained via the average divergence method (UPGMA) (A) and representation of the average values (B) for 35 genotypes of *A. obesum* for the following characters: number of days from anthesis to senescence year 1 and year 2 (ND1 and ND 2), total number of flowers per plant, year 1 and year 2 (NFlo1 and NFlo 2), plant height (H), caudex diameter (CD), number of branches (NB), flower length (FL), flower width (FW), corolla diameter (CDi), and number of annual blooms year 1 and year 2 (NFA1 and NFA 2). r = cophenetic correlation, ** = significant at 1% by Mantel test.

Group-V: included the largest number of genotypes, 24 of the 35 genotypes evaluated (Fig., 5). The dissimilarity data indicated that the genotypes closest to each other were ICA-br 3 and ICA-vr 7, whereas the most distant one was the genotype ICA-vr 3 (Fig., 5).

A total of 46 loci were visualized after the amplification with 13 ISSR primers, ranging from a minimum of three to a maximum of six loci per primer, with a polymorphism percentage of 97% (Table, 2).

Based on the molecular analysis, the dissimilarities among the 35 genotypes were determined with the formation of six groups (Fig., 6). Only ICA-vr1 was included in group- I. Group- II: included genotypes ICA-vr 6, ICA-vr7, ICA-vr8, ICA-vr 9, and ICA-vr10. Group- III: involved ICA-rb11. Group-IV: included BR (6, 3, 9, 10, 2, 7). Group- V: included the genotypes of progenies BR (1,15,12, 13, 14, 8, 4 and 6) and VB (6, 8, 4, 5, 7, 9 and 10). Group-VI: included the genotypes VB (1, 2, and 3) and VR (2, 3, 4, and 5) (Fig., 6).

Dissimilarity analyses for each of the progenies were also performed, and the hybrids from the ICA-rs x ICA-bd cross were sorted into three groups. The group-I: it was formed exclusively by the genotype ICA-br 11. The other genotypes were included in groups II and III. The genotypes with the lowest dissimilarity were ICA-br 13 and ICA-br 14 (Fig., 7A).

The hybrids of progeny VB (ICA-vt x ICA-bd) were separated into two groups. Group- I: included the genotypes ICA-vb 1, ICA-vb 2 and ICA-vb-3, whereas other hybrids were included in the other group. The highest similarity was observed between genotypes ICA-vb 9 and ICA-vb 10 (Fig., 7B). The genotypes of the progeny from the ICA-vt x ICA-rs cross were also grouped into two groups. Group I included ICA-vr genotypes 2, 3, 4, and 5, whereas group II included the remaining genotypes (Fig., 7C).

The results obtained from AMOVA analysis showed that most of the genetic variations were concentrated within the progenies. It was observed that 82.14 % of the

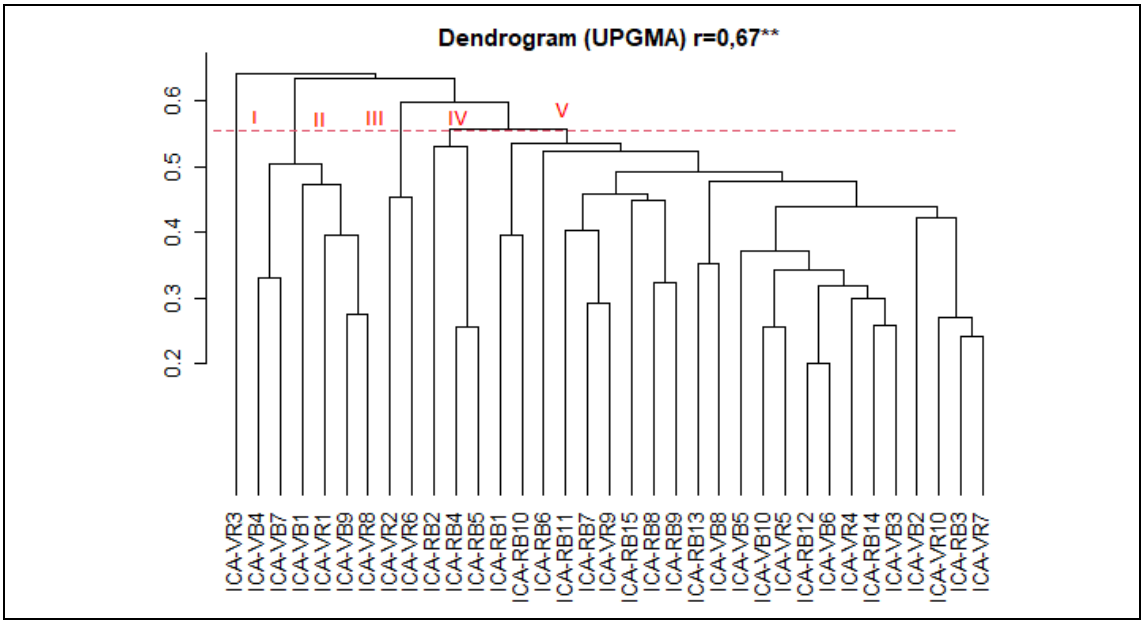

Fig. 5. Representation of dissimilarity among 35 genotypes of *A. obesum.* for quantitative and qualitative characters, the discrimination between the groups with the distance matrix was analyzed using the average standardized Euclidean distance for quantitative data and coincidence index for qualitative data. r = cophenetic correlation, ** = significant at 1% by Mantel test.

Table 2. List of ISSR primers, sequence motifs, annealing temperatures and total number of loci amplified in the 35 genotypes of *A. obesum*, Montes Claros, Brazil.

Initiator	Sequence Motifs (5'-3')	Annealing temperature (ºC)	Total number of loci
UBC 807	(AG)8 T	50	03
UBC 810	(GA)8 T	50	03
UBC 811	(GA)8 C	53	03
UBC 813	(CT)8 T	50	03
UBC 814	(CT)8 A	50	03
UBC 817	(CA)8 A	50	04
UBC 820	(CA)9 RC	60	06
UBC 822	(TC)8 A	50	04
UBC 827	(AC)8 G	50	04
UBC 836	(AG)8 YA	51	03
UBC 842	(GA)8 YG	50	04
UBC 846	(CA)8 RC	50	03
UBC 849	(GT)8 YA	50	03
	Polymorphism: 97%		

R= Purine (A or G); Y= Pyrimidine (C or T); D= G, A or T; B= G, T or C; H= A, C or T; V= G, C or A

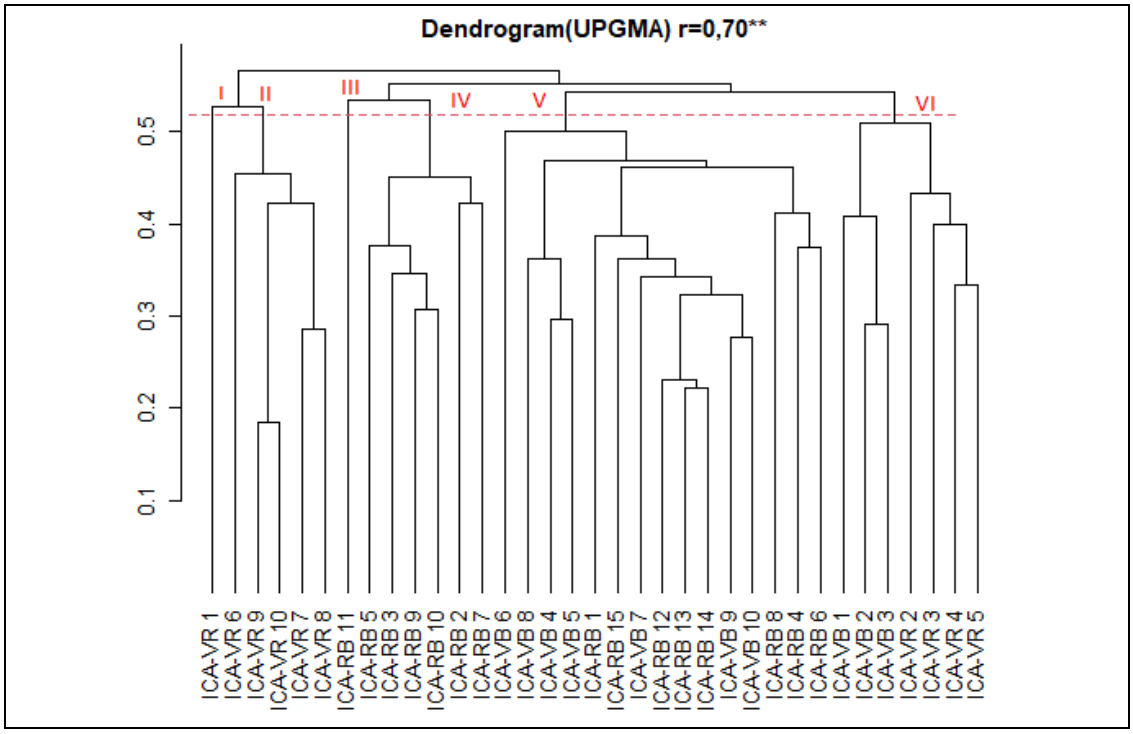

Fig. 6. Dendrogram obtained using the average divergence (UPGMA) clustering method for ISSR primers in 35 genotypes of *A. obesum*, Minas Gerais, Brazil. r = cophenetic correlation, ** = significant at 1% by Mantel test.

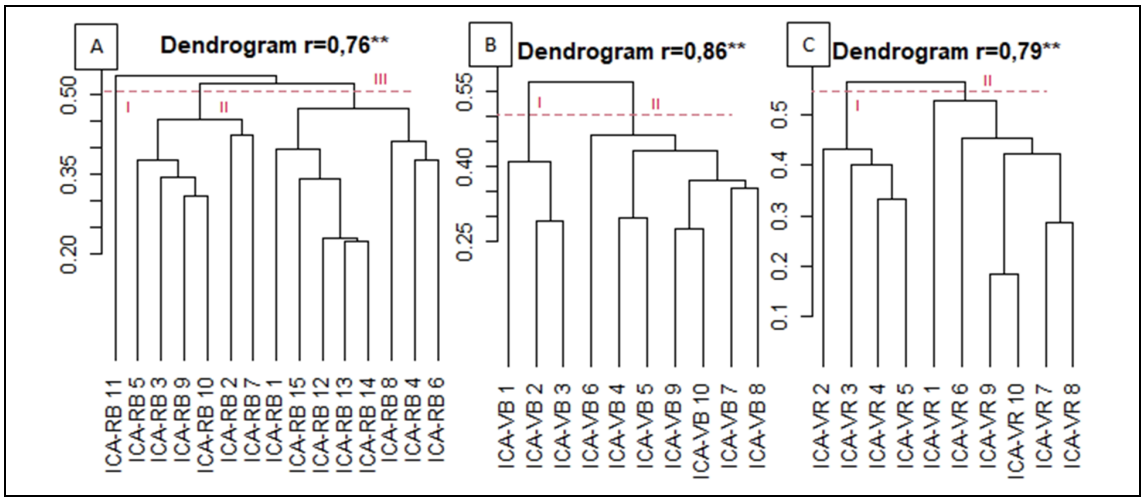

Fig. 7. Dendrogram obtained based on the average divergence clustering method (UPGMA) for ISSR primers in 15 genotypes **from ICA-bd x ICA-rs cross (A), 10 hybrids obtained from the ICA-vt x ICA-bd cross (B) and 10 hybrids resulting from the ICA-vt x ICA-rs cross (C) of *A. obesum*, Minas Gerais, Brazil. r = cophenetic correlation, ** = significant at 1% by Mantel test.**

total variation corresponded to intra-population variation and 17.86 % corresponded to variation among progenies (Table, 3).

DISCUSSION

The progenies were evaluated to examine morphological (vegetative and floral) and molecular characteristics. The analyses were performed considering important aspects from the ornamental and commercial perspective, encompassing quantitative and qualitative descriptors. The results indicated significant genetic variabilities among the hybrids, as the 35 genotypes showed variabilities for corolla pigmentation, that predominated ranged from red to magenta. In the first filial generations (F_1), no genotypes with corolla pigmentation with absence of pigmentation (white) and purple pigmentation were observed. Despite the variation observed, we verified that the corolla pigmentation was not the most important factor for discrimination between hybrids. However, it should be considered in a breeding program since the corolla color is one of the attributes with a remarkable ornamental value.

Few studies on the inheritance of flower color in *Adenium* have been conducted. Tangwisit *et al*. (2015) performed hybridization between plants with corolla with absence of pigmentation (white corolla) and a pink corolla plant and concluded that the inheritance of the absence of pigmentation (white pigmented corolla) seems to be monogenic recessive. The pink pigmented corolla presented with complete dominance type allelic interaction. Qualitative descriptors, such as corolla pigmentation, are generally easy to visualize and distinguish among individuals. Genes whose effects are easy to identify and relatively stable control some commercially important traits in ornamental plants. These include flower color and resistance to certain diseases (Narbona *et al*., 2021).

Although no other studies have been conducted examining the inheritance of corolla pigmentation in the genus *Adenium,* it is important to note that the variability of corolla pigmentation is significant. More than 500 distinct corolla color patterns have been catalogued in desert rose. In addition to the solid colors, the species also presents variegations, with whitish, yellowish, or reddish and purplish zones with different intensities (Nietsche *et al*., 2021). We believe that this remarkable variability may be associated with a large number of alleles

Table 3. Analysis of molecular variance (AMOVA) between and within three progenies of *A. obesum* based on ISSR markers, Montes Claros, Brazil.

Source of variation	DF	SQ	QM	TV (%)	ΦST
Between progenies	2	0.859	0.430	17.853	0.179
Within progenies	32	3.947	0.123	82.147	
Total	34	4.807	0.141		

DF: degree of freedom; SQ: sum of mean squares; QM: mean square of the residue; TV (%): total percentage of variation

and/or genes due to possible allelic and gene interactions governing this character.

Regarding the types of petal arrangements, we examined three genitors with distinct patterns in the hybridizations: single (corolla with five petals), double (corolla with 10 petals), and triple (corolla with 15 petals). The results of the first filial generation in the three progenies evaluated (BR, VB, and VR) indicated substantial variation in the types of petal arrangements, with predominance for the double petal arrangement in the crosses of ICA bd x rs and ICA vt x rs, as well the triple petal arrangement in the hybridization ICA vt x bd. The data indicated a dominance of the double and triple petal arrangements over the single one. However, further studies are required to elucidate the inheritance, and therefore, assist breeders in hybridization and genotype selection processes.

No scientific studies have been published so far on the inheritance of the characteristic number of petals in the corolla in desert rose. Only one important report has been made available by Ramos (2024). According to author, the petal arrangement of *A. obesum* flowers is digenic in nature, with trait inheritance controlled by dominant–recessive epistasis, where the simple petal arrangement is dominant over the compound petal arrangement.

The other quantitative, vegetative, and floral characters evaluated required the use of measuring gauges, several observations, and more accurate methods of analysis. Among the quantitative floral characteristics evaluated, we can emphasize certain descriptors that are regarded as highly pertinent within the flower production sector, namely the number of flowers per plant and per branch, dimensions of the floral structure, and number of days from anthesis to flower senescence. With respect to these quantitative descriptors and the other vegetative characters evaluated, significant variations were also observed among the 35 hybrids.

The cluster analyses successfully discriminated the 35 desert rose hybrids. The analyses of quantitative and quantitative traits simultaneously discriminated the 35 hybrids into five groups, considering the UPGMA method. Additionally, it was also possible to clearly identify the most divergent hybrids as well as the most similar ones. The UPGMA method also allowed to discriminate the hybrids in two of the three progenies evaluated. As the progenies were presented in distinct dissimilarity groups, a strong indication of genetic diversity was noted, and the possibility of combinations between these progenies can be helpful for the genetic improvement of *A. obesum*.

For the quantitative descriptors of the vegetative part, particularly, plant height, caudex diameter, and number of branches per plant, important variations were also observed among the genotypes evaluated. For these characteristics, three hybrids belonging to progeny BR, ICA-br 5, 2, and 15, respectively, need to be highlighted. The genotypes ICA-br 4, ICA-vr 3, ICA-vr 2, and ICA-br 10 demonstrated prominence in relation to floral attributes of significance, such as the duration from anthesis to senescence, total flower count per plant, flower length, and frequency of annual flowering, respectively. The traits described above exhibit a polygenic and quantitative (controlled by many genes) inheritance

patterns and show strong environmental influence. These traits are crucial commercially for selective plant breeding (Mekapogu *et al*., 2022). Langton (1991) considered some quantitative characters important for ornamental plants, such as the number and dimensions of floral structures, the length of the floral scape, and the length and width of the leaf. The author also highlighted the relevance of visual appearance (qualitative characteristic) that should be considered at all stages of the selection process of flowers and ornamental plants.

In the analysis with ISSR molecular markers, it was evident that the species presented considerable genetic variability. The results indicated a high average level of polymorphism (97%), reinforcing those published by Chavan *et al*. (2018). In this study, when applying the molecular marker of the RAPD type, a high level of polymorphisms among the accessions of *A. obesum* were also observed. Of the 17 RAPD primers used, 12 showed 100% polymorphic bands, and the lowest level of polymorphism was 66%.

One of the advantages of using ISSR markers is their informative capacity, presenting a greater number of polymorphic loci, which allows the distinction between accessions even with similar morphology and regardless of environmental conditions (Zhang *et al*., 2012). We also emphasize that ISSR markers are efficient both in distinguishing populations that are vegetatively propagated and naturally tend to present greater genetic uniformity and in accessing the genetic diversity of populations propagated by seeds (Luz *et al*., 2020).

The analysis has been effective for the quantification of the genetic diversity of individuals, serving as a subsidy for strategies aimed at the conservation and maintenance of the species (Costa *et al*., 2015).

The cluster analysis of the three progenies evaluated jointly and individually efficiently discriminated the progenies and accessions within and between progenies. In the joint analysis, it was possible to observe the formation of six groups. Accessions belonging to all the progenies were not observed in any of the six groups. The grouping of individuals from one progeny with another progeny, as in the case of groups V and VI, which exclusively grouped accessions from progenies VB and BR, may be associated with the degree of inbreeding in the population or with the low number of ISSR markers used. Individuals from groups I, II and III showed the highest genetic divergence among the other accessions from groups IV, V and VI. These individuals can be selected and used as parents in future crosses aiming at the formation of new populations for the improvement of the species.

Although the 35 genotypes were obtained from controlled hybridizations and had at least one common parent, the estimates of genetic parameters showed moderate to high genetic diversity. The analysis of molecular variance allowed the division of the total variance into its components between and within progenies. The highest proportion of variation was found within progenies (82.14%), whereas a significantly lower variation was observed (17.9%) among progenies.

Different factors can affect the levels of genetic diversity in a population, among which the breeding system, gene flow, population size and selection processes (Charlesworth and Wright, 2001). In the present study, the observed diversity can be explained by considering the reproduction mode of the species. *A. obesum* is allogamous, with evidence of self-incompatibility. It requires cross-pollination, directly impacting the level of genetic diversity (Possobom *et al*., 2021). Thus, the low value of distinction between progenies ($\Phi ST = 0.179$) are typical of species whose reproduction system requires obligatory cross-fertilization to obtain offsprings (Hamrick and Godt, 1996).

Finally, for the next cycles, we suggest new recombinations between individuals,

with emphasis on the following hybridizations aiming at the formation of new types of cultivars: ICA-vr4 (red color and triple arrangement) x ICA-br2 (red color and single arrangement), ICA-vr9 (single red) x ICA-br9 (triple red), ICA-vb2 (triple magenta) x ICA-br6 (single red) and ICA-vb8 (single magenta) x ICA-br15 (double magenta). Genotype selection was carried out considering corolla color and petal arrangement. Both characteristics, color and number of petals, directly affect ornamental merit as they are indicative of quality and, therefore, of commercial value, in addition to their function of attracting pollinators or protecting organs (Zhao *et al*., 2024).

Our study demonstrated the importance of the combined use of morphological and molecular markers to obtain estimates of genetic diversity, as well as to access the individuals with the greatest ornamental potential and dissimilarities. Integrating molecular markers into long-term breeding pipelines can significantly accelerate genetic gains and improve breeding efficiency. Recommendations include: 1) Utilizing molecular markers for genomic selection,which uses marker data to predict the breeding value of plants, allowing for earlier selection and reduced generation intervals and 2) Employing markers for marker-assisted selection (MAS), targeting specific genes or quantitative trait loci (QTLs) for desirable traits.

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