ARTICLE

Integrating morphological and molecular data to assess genetic diversity in Desert rose

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Abstract: *The integrated analysis of morphological and molecular markers represents an effective approach for estimating genetic variability in ornamental species. In the present study, 37 genotypes of* Adenium obesum *resulting from a single biparental cross were evaluated based on morphofloral traits and ISSR markers. Morphological characterization revealed a predominance of magenta corollas (64.9%), mostly single or double flowers (95%), and variegation in 81% of the genotypes. The mean number of flowers per genotype was 14 and the caudex diameter was classified as "thick" in 20 individuals (> 71 mm). The amplification process generated 97 loci, resulting in 100% polymorphism and an average of 6.47 polymorphic bands per primer. A combined analysis of the morphological and molecular data revealed moderate correspondence between the clustering patterns (entanglement index = 0.582). The results showed that the full-sibling family was genetically diverse and that this diversity could be used to identify elite genotypes.*

Keywords: Adenium obesum*, ISSR marker, genetic variability, ornamental breeding*

INTRODUCTION

Adenium obesum (Forssk.) Roem. & Schult, commonly known as desert rose, belongs to the Apocynaceae family. In its native form, it is a succulent shrub or small tree, native to regions of Africa and the Arabian Peninsula (Plaizier 1980). It is characterized by its exuberant flowers with a wide variety of colors and shapes (single or multiple), in addition to the presence of a dilated structure at the base of the stem, known as the caudex (Colombo et al. 2015, Orozco and Gonzalez 2021).

For species with ornamental potential, genetic improvement aims not only to increase productivity and resistance to biotic and abiotic factors but also to select morphological, qualitative, and quantitative characteristics of interest. However, in Brazil, research on the genetic improvement of desert roses is scarce, despite their variability and genetic resources being essential for the development of new cultivars. In *A. obesum*, the ornamental characteristics most valued by collectors are the color and shape of the flowers, the multipetal floral arrangement, and the caudex (Abreu et al. 2023).

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Ramos et al. (2022), Abreu et al. (2023), and Mendes et al. (2025) evaluated the genetic diversity among desert rose accessions and hybrids. Their results highlighted the rich genetic and morphological diversity among *A. obesum* accessions and hybrids and provides crucial information for breeding programs aimed at developing new varieties with enhanced ornamental characteristics. Identifying promising accessions and understanding their genetic correlations are fundamental to expanding the genetic basis for this species.

Several methodologies exist to assess the genetic variability of species. Among the main resources used in genetic analysis are molecular markers, which allow for the identification of genetic polymorphisms based on the analysis of isoenzymes or DNA sequences. Molecular markers may differ with respect to important features, such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements, and financial investment (Poursakhi et al. 2024). Among the various types, the inter-simple sequence repeat (ISSR) marker stands out and is based on the amplification of regions between microsatellites using PCR with specific primers (Godwin et al. 1997).

Molecular marker systems offer clear advantages over other methods when attempting to genetically characterize organisms and have been successfully applied to a diverse range of plants (Kıraç et al. 2022, Coşkun and Aydın 2024, Erkek et al. 2025, Coşkun et al. 2025). Specifically, in desert rose, Mendes et al. (2025) found that ISSR markers proved to be a valuable tool for characterizing genetic variability in *A. obesum* hybrids. The high genetic diversity detected (97% polymorphism) indicated significant potential for selecting genotypes as parents in breeding programs.

In addition to molecular markers, morphological and ornamental descriptors are valuable tools for assessing the genetic diversity of ornamental plants. By analyzing quantitative and qualitative characteristics, it is possible to estimate the degree of similarity between accessions and cultivars (Dalla et al. 2007). Such descriptors are generally easy to measure and are highly heritable (Ferreira 2008).

Morphological and ISSR analyses have been successfully conducted on many plant species, including ornamental (Tecirli et al. 2018), and crop species (Kırac et al. 2022, Erkek et al. 2025). Mendes et al. (2025) used results from the morphological and molecular analyses of three full-sibling progenies of desert rose to preselect nine superior genotypes that were then used in a breeding program.

The integration of morphological and molecular characterization is a strategic approach for genetic improvement, allowing the identification of promising genotypes, composition of germplasm banks, and selection of parents for crosses (Borém and Miranda 2013, Cruz et al. 2020). This combined approach overcomes the limitations of evaluation based solely on morphological characteristics, providing a more solid basis for breeding decisions.

In this study, we hypothesized that the integration of morpho-ornamental and ISSR molecular analyses would reveal substantial genetic diversity in a full-sib progeny of *A. obesum*, allowing the identification of genetically distinct and ornamentally superior genotypes suitable for use in breeding and cultivar development. Specifically, this study used morphological characteristics and ISSR molecular markers to determine the genetic diversity of a full-sib progeny that contained 37 genotypes. It is important to note that the inferences based on these results are only appropriate for this specific progeny.

MATERIAL AND METHODS

Obtaining progeny

The progenies of full siblings, consisting of 37 genotypes, were obtained by artificial hybridization between two parents, all genotypes belong to the same controlled cross. The female parent selected was the ICA-ro (♀) genotype, characterized by pink petal pigmentation and simple petal arrangement; the male parent used was the ICA-bd (♂) genotype, with white petal pigmentation and double arrangement. Artificial hybridization was performed according to the method described by Ramos et al. (2022). The plants were grown in a greenhouse in 4-L pots that contained a commercial substrate (Bioplant®). The pots were spaced approximately 15 cm apart on a 1.00 m high bench. Nutritional management and pest and disease control followed recommendations for the species (Mendes et al. 2025).

Molecular characterization with ISSR markers

DNA was extracted from leaves according to the methodology described by Doyle and Doyle (1987). The DNA concentration was estimated using a spectrophotometer by measuring the absorbance at 260 nm, with each absorbance unit corresponding to a concentration of 50 ng mL^{-1} of double-stranded DNA (Doyle and Doyle 1987).

Annealing temperature gradient tests were performed using a Robocycler (Eppendorf, Hamburg, Germany). For each test, DNA from two individuals and four ISSR primers were chosen, with temperatures ranging from 45 °C to 65 °C. The temperatures that promoted the highest number of polymorphisms were selected. Thirty-four ISSR primers from the University of British Columbia collection, developed by the Biotechnology Laboratory at the University of British Columbia (collection no. 9), were tested. Of these, 15 were polymorphic and were selected for screening in the progeny of full siblings.

The following conditions were adopted. The total reaction volume was 25 µL and contained DNA (3.0 ng), 1 µL Tris-KCl at pH 8.3 (10 mM/50 mM), $MgCl_2$ (25 mM), dNTPs (0.2 µM), primer *(*0.4 µM), and *Taq* DNA polymerase (0.6 units). Ultrapure water was used to make the volume up to 25 µL. The reactions comprised of an initial denaturation phase at 94 °C for 4 minutes, followed by 35 cycles of denaturation (94 °C/30 seconds), annealing (1 min (gradient from 45 °C to 65 °C, the annealing temperature used for each primer is described in table 1)), and an extension phase at 72 °C for 7 minutes. Immediately afterwards, the temperature was reduced to 4 °C and then the samples were removed. The products resulting from the amplification were separated by electrophoresis in 1.4% agarose gel in 1X SB buffer for approximately 70 minutes, stained in 0.2 mg L^{-1} gel red solution and type IV dye. The gels were subjected to a 120-volt charge and were photodigitized using the L-PIX system (Loccus Biotecnologia, São Paulo, Brazil).

Morphological and ornamental characterization

The evaluation of quantitative vegetative and floral traits was obtained in all 37 genotypes from 18 months of age. The following characteristics were evaluated: total number of flowers per plant (TNF), corolla pigmentation (CP), average length of the floral tube of the flowers (FTL, mm), petal width (PW, mm), corolla diameter (CoD, mm), number of petals per flower (NP), and caudex diameter (CD, mm). The diameter of the caudex was measured at the base of the plant and categorized as thin with a diameter of up to 50.9 mm, medium for caudex with a diameter of 51.0 to 70.0 mm, and thick for caudex greater than 71.0 mm.

Table 1. Relevant ISSR primers for genetic diversity in *A. obesum* genotypes, including primer code, primer sequence, annealing temperature (°C), and total number of loci.

Primer code	**Primer sequence** (5'-3')	**Annealing temperatures** (°C)	**Total number of loci**
810	GAG AGA GAG AGA GAG AT	57	9
811	GAG AGA GAG AGA GAG AC	57	9
818	CAC ACA CAC ACA CAC AG	54	7
823	TCT CTC TCT CTC TCT CC	51	6
824	TCT CTC TCT CTC TCT CG	56	5
825	ACA CAC ACA CAC ACA CT	51	4
826	ACA CAC ACA CAC ACA CC	64	5
830	TGT GTG TGT GTG TGT GG	54	7
836	AGA GAG AGA GAG AGA GYA	49	4
840	GAG AGA GAG AGA GAG AYT	51	6
842	GAG AGA GAG AGA GAG AYG	49	8
850	GTG TGT GTG TGT GTG TYC	56	9
861	ACC ACC ACC ACC ACC ACC	56	6
888	BDB CAC ACA CAC ACA CA	49	3
895	AGA GTT GGT AGC TCT TGA TC	45	9
Loci average: 6.47			
Total polymorphism: 100%			

N = (A, G, C, T); Y = (C, T); D = (A, G, T); B = (C, G, T).

The quantitative floral traits (TNF, FTL, PW, CoD and NP) were evaluated from July 2024 to January 2025, with five flowers per plant in two seasons. The qualitative floral characteristic of corolla pigmentation was evaluated in all blooms (from July 2024 to January 2025), with five flowers per plant using the Royal Horticultural Society (RHS 2015) color catalog. The number of petals per flower was classified as single for flowers with five petals, double for flowers with 10 petals, and triple for flowers with 15 petals. The criteria for quantitative and qualitative characteristics followed the guidelines of the distinguishability, homogeneity and stability form for *A. obesum* (SNPC 2024), as well as data from the literature for other ornamental plants (Kiliç et al. 2024). The experiment used a completely randomized design with 37 treatments and each progeny was a single repetition. Data were collected during the two seasons and different numbers of repetitions were applied to each characteristic.

Analysis of genetic and phenotypic diversity

A data matrix was constructed involving all genotypes in the presence (1) or absence (0) of band fragments to generate a binary matrix. Dissimilarities were obtained using the arithmetic complement of the Jaccard index. Clustering analysis was performed based on genetic distances using the unweighted pair group method with arithmetic mean (UPGMA) to generate a dendrogram using the Multivariate Analysis package/R software (R Core Team 2023) (Azevedo 2021). Each progeny presented a single repetition; quantitative and qualitative morphological data were collected at two stations, and different numbers of repetitions were applied for each characteristic.

Quantitative and qualitative morpho-ornamental data were also subjected to multivariate analyses at a 5% significance level. The mean standardized Euclidean distance was used to obtain a dissimilarity matrix. The dissimilarity between the genotypes of the F_1 progeny of full siblings and their parents was analyzed using a hierarchical clustering heatmap. The dendrogram was obtained using the UPGMA hierarchical clustering method and validated using the cofenetic correlation coefficient and cutoff point defined by Mojena (1977)'s criterion. For these analyses, the "multivariate analysis" package in using R was used (Azevedo 2021).

A tanglegram was developed to visualize the similarities and differences between the clusters generated by the two distinct dendrograms. The UPGMA method was used for hierarchical cluster analysis. The dendrograms, representing morphological and molecular data, were positioned face-to-face, and the correspondence between samples was highlighted by lines connecting the equivalent nodes. Graphs were constructed using the "Dendextend" package in R (version 3.5.2).

RESULTS AND DISCUSSION

The preselection of polymorphic ISSR primers and subsequent amplification in all progenies proved to be a robust and efficient strategy for assessing genetic variability within the *A. obesum* full-sib progeny. The 15 primers used and their respective annealing temperatures are listed in Table 1. Amplification generated 97 loci, with 3–9 loci per primer, all of which were polymorphic, resulting in a 100% polymorphism rate and an average of 6.47 polymorphic bands per primer. The primers 810, 811, 850, and 895 contributed the most to polymorphism in this study. Primers can vary depending on the specific organism and research goal and several University of British Columbia (UBC) primers are highly efficient, show high levels of polymorphism, and are frequently used in a variety of genetic diversity studies, e.g. primer UBC 810.

These results are comparable to the findings of Mendes et al. (2025), who reported 97% polymorphism using 13 ISSR primers in *A. obesum*. The number of detected polymorphisms is an essential component when evaluating genetic diversity because it reflects the evolutionary dynamics and adaptability of a species. However, several researchers have described ISSR markers that show significant differences in polymorphism detection and discrimination capacities in different species (Poursakhi et al. 2025, Sabet et al. 2024).

The Jaccard dissimilarity matrix derived from ISSR data revealed 89 pairs with genetic dissimilarity ≥50%, and four pairs ≥65% [11–10 (65%), 15–13 (70%), 31–30 (65%), and 49–47 (65%)], confirming high intra-population variability. A dendrogram constructed using the UPGMA method (Figure 1) grouped the genotypes into six distinct clusters, supported by a cophenetic correlation coefficient (CCC) of 0.70, indicating good clustering reliability (Cruz et al. 2012). According to Zhou et al. (2023), genetic diversity is strongly influenced by reproductive system, ecological adaptability, and evolutionary history. In predominantly outcrossing ornamental species, such as *A. obesum*, high polymorphism levels are expected to maintain adaptability and facilitate breeding progress.

Morphological characterization specifically from this F_1 population, revealed that the corolla lobe shapes were predominantly oval (68%) or obovate (32%) and the color variation followed the RHS classification (Figure 2, Table S1). Magenta corolla pigmentation was dominant (64.9%), followed by pink/magenta (13.5%), whereas pure pink and red phenotypes were less frequent (10.8% and 2.7%, respectively). Variegation was observed in 81% of the genotypes, demonstrating a wide range of floral aesthetic traits relevant to consumer preferences.

The inheritance pattern of corolla pigmentation is indicative of the dominance of anthocyanin expression in the desert rose. In the present study, a female parent (ICA-ro) exhibiting pigmented petals was used, while ICA-bd, in the absence of anthocyanin, was the pollen grain donor, and all 37 genotypes manifested the presence of anthocyanins in the first filial generation (F_1). Mendes et al. (2025) also used the genitor ICA-bd as a male parent in a hybridization with a red-pigmented corolla as a female genitor, and all 45 genotypes presented a pigmented corolla. This result is consistent with the findings in *Passiflora* hybrids (Souza et al. 2020). Recent studies have shown that anthocyanin biosynthesis genes can be epigenetically regulated, thereby influencing the diversity of floral pigmentation (Mekapogu et al. 2023).

Flower morphology also showed significant variability among the genotypes. Single-flowered plants (five petals) comprised 46% of the flowers, followed by double-flowered (ten petals) (49%) and triple-flowered plants (15 petals) (5%) (Figure 2, Table S1). The mean floral tube length was 64.9 mm, ranging from 52.0 to 79.9 mm, and the mean corolla diameter was 57.2 mm (Table S1). Approximately 73% of the genotypes produced long floral tubes and 13.5% exhibited wide petals. Such diversity in floral dimensions reflects the polygenic control of these traits, as has been observed in other ornamentals (Moyroud and Glover 2017). The 37 genotypes produced 525 flowers, averaging 14 flowers per plant, with genotypes 29, 15, and 19 producing the highest floral count (Table S1). These parameters are directly related to the reproductive vigor and ornamental appeal, which are key selection targets for breeding programs (Singh et al. 2019).

The caudex diameter, a highly valued trait in *A. obesum*, varied significantly among genotypes evaluated, with 20 individuals classified as "thick" (>71 mm). Larger caudices are desirable for visual balance and perceived age, which enhance the market value (Colombo et al. 2015). High heritability of the caudex diameter has been previously reported (Chavan et al. 2018), suggesting a strong potential for selection gains. Moreover, the physiological stability associated with larger caudates could reflect better water storage capacity and stress tolerance (Colombo et al. 2015).

Cluster analysis of the morpho-ornamental traits grouped the genotypes into three main clusters (Figure 3) with a CCC of 0.86, indicating strong internal consistency. Group I included genotypes with larger floral tubes and

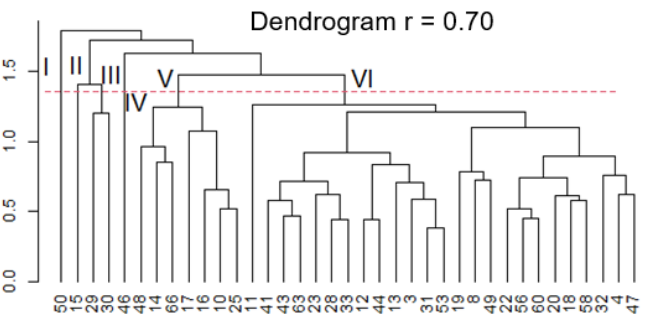

Figure 1. Dendrogram using the average divergence grouping method (UPGMA) for ISSR primers in 37 genotypes of *A. obesum*, Montes Claros, Brazil. r = cophenetic correlation. **Significant at 1% by Mantel test.

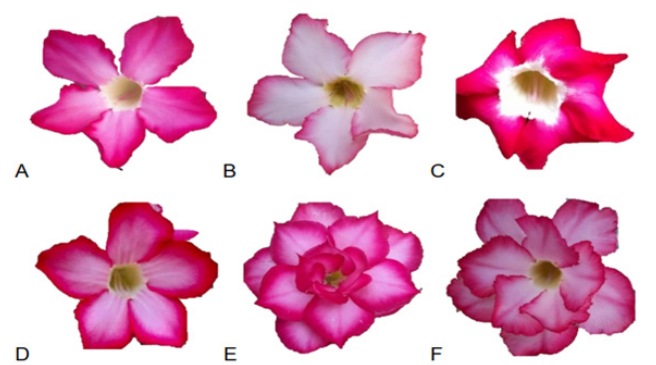

Figure 2. Observed diversity of corolla colors, variegations, and petal arrangement in some genotypes belonging to the progeny of full siblings of *A. obesum*, Montes Claros, Brazil, 2025. (A) Genotype 3: Magenta-colored *A. obesum* flower (N57B) without variegation and a simple petal arrangement. (B) Genotype 11: *A. obesum* flower with a magenta center (61D) and magenta variegation on the margins (N57A) with a simple petal arrangement. (C) Genotype 29: *A. obesum* flower with red coloration (42A) without variegation and simple petal arrangement. (D) Genotype 30: Red *A. obesum* flower (53C) with magenta variegation (N57B) and single-petal arrangement. (E) Genotype 48: Pink *A. obesum* flower (53D) with pink variegation (53A) and triple petal arrangement. (F) Genotype 20: *A. obesum* flower with magenta color in the center (61D) and magenta variegation (N57A) with double petal arrangement.

wider petals, Group II was characterized by genotypes with larger caudices and high flower production (notably red corollas), and Group III consisted of genotypes with high variegation diversity. This differentiation demonstrates the existence of morpho-functional diversity that can be exploited in breeding programs aimed at combining ornamental value with physiological resilience (Sadeghpour et al. 2023, Askari et al. 2024).

The tanglegram revealed differences between morphological and ISSR-based clustering and the datasets based on these two methods (entanglement index = 0.582; Figure 4). The discrepancies between molecular and morphological clustering arose from fundamental differences in how these traits evolve and the extent to which they are affected by external factors. One explanation for the differences is that morphological traits are highly susceptible to **phenotypic plasticity**, which means that a single genotype can produce different phenotypes depending on the environmental conditions. Another explanation for the differences is related to the coverage of the primers by the genome and the lack of correlation between these regions and phenotypic markers. Furthermore, the choice of statistical methods used to construct the phylogenetic trees can also influence the results (Keating et al. 2023, Najafi et al. 2024). Only genotype 33 exhibited a consistent placement across both analyses, confirming the congruence between phenotypic and molecular variability. This genotype presented a magenta corolla in the center and on the margin, a caudex diameter classified as wide (80.5 mm), and a low number of flowers per plant (Table S1).

Integrating molecular and morpho-ornamental analyses is essential for optimizing the selection of superior genotypes, designing efficient crosses, and conserving genetic resources (Borém and Miranda 2013, Cruz et al. 2020). Furthermore, as discussed by Sadeghpour et al. (2023) and Askari et al. (2024), combining genotypic diversity with functional and adaptive traits enhances resilience and long-term cultivar performance. Thus, the identification of promising genotypes in the present study supports the development of new *A. obesum* cultivars that combine high ornamental appeal, genetic distinctiveness, and physiological robustness under various conditions.

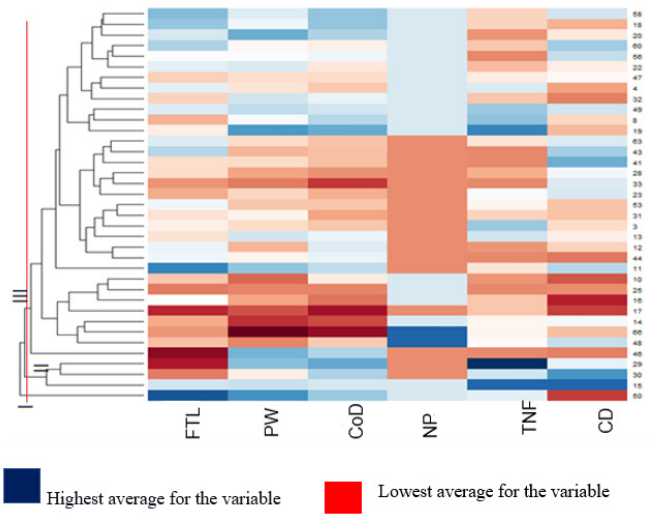

Figure 3. Dendrogram obtained by the UPGMA hierarchical clustering method for morpho-ornamental characteristics. Floral tube length (FTL), petal width (PW), corolla diameter (CoD), number of petals (NP), total number of flowers (TNF), and caudex diameter (CD) of 37 genotypes of *A. obesum*, Montes Claros, Brazil.

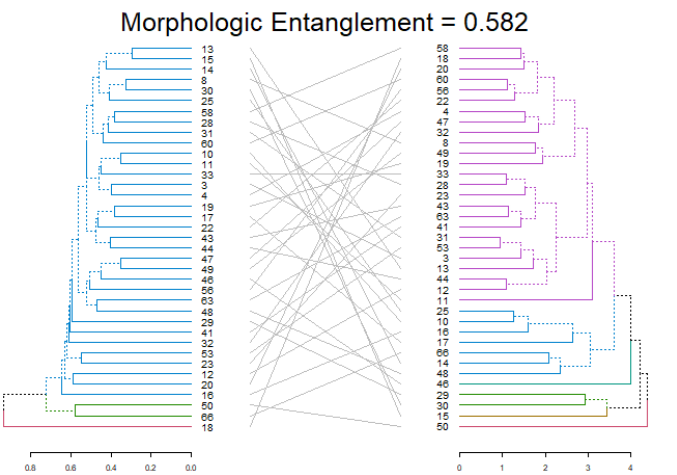

Figure 4. Morphologic entanglement among 37 genotypes of *A. obesum* obtained through genetic diversity considering molecular and morphological characteristics.

The integration of ISSR molecular markers and morpho-ornamental descriptors proved effective in revealing the high genetic and phenotypic variability among *A. obesum* progenies. The 100% polymorphism rate confirmed the efficiency of ISSR primers in detecting genetic diversity, while morphological assessment highlighted broad variations in flower color, shape, and petal number, as well as in the caudex diameter, which are traits of strong ornamental and commercial relevance.

It is important to emphasize that the conclusions from this study are based on data from a single family with full siblings. Furthermore, the genotypes used in future cycles should be recombined. For example: a combination containing genotypes 11 (magenta corolla, five petals, caudex diameter > 84.0 mm and floral tube length > 76.0 mm), 15 (pink/magenta corolla, 10 petals, caudex diameter > 100.0 mm and 35 flowers), 29 (red corolla, five petals, caudex diameter > 79.0 mm and 40 flowers) and 50 (pink corolla, floral tube length > 79.0 mm and 19 flowers) could be used.

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DATA AVAILABILITY

The supplementary file and datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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