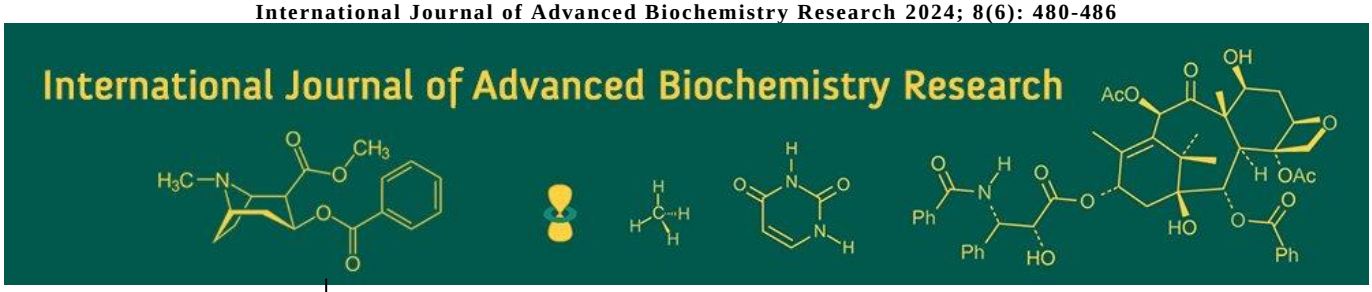

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Diversity in pigment evaluation of different genotypes of *Adenium obesum*

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Abstract
This study examines pigment diversity indices in eleven genotypes of *Adenium obesum*, focusing on anthocyanin, carotenoids, and chlorophyll. Chlorophyll shown the highest diversity with a dominance index of 0.09272, Simpson's index of 0.9073, and Shannon's index of 2.388. It also demonstrates high evenness (0.9898) and equitability (0.9957), indicating a balanced distribution. Carotenoids, though fewer in number, exhibit significant diversity with a Menhinick index of 1.78 and Fisher's alpha index of 5.174. In contrast, anthocyanin, despite of its major changes in content (1.47 to 64.14mg/100g), It showed the lowest diversity and evenness. These findings also suggested that the genetic variability in pigment biosynthesis in adenium of eleven genotypes and that can provides opportunities for a breeder to enhance the ornamental and aesthetic chacter choosen in *Adenium obesum spp.*

Keywords: Anthocyanin, aarotenoids, chlorophyll, *Adenium* and diversity

Introduction
Adenium commonly known as the desert rose, is a prominent ornamental plant renowned for its strikingly colourful flowers and distinctive caudex. Originating from the arid regions of the Sahel, East Africa, and the Arabian Peninsula, this species has become a favourite among horticulturists and plant enthusiasts due to its adaptability and the aesthetic appeal of its blossoms (Gilman & Watson, 1993) [9]. The plant's popularity has driven significant interest in understanding the genetic and environmental factors that influence its phenotypic diversity, particularly flower pigmentation. Other notable species include *Adenium arabicum*, *Adenium multiflorum*, *Adenium somalense*, *Adenium swazicum*, and *Adenium socotranum*, showcasing natural diversity.

Flower colouration in Adenium obesum is primarily determined by anthocyanins, carotenoids, and betalains, with anthocyanins being the most prevalent pigments responsible for the red, pink, and purple hues (Tanaka *et al*., 2008) [30]. These pigments not only contribute to the plant's visual appeal but also play critical roles in attracting pollinators and providing protection against environmental stresses (Schaefer *et al*., 2004; Grotewold, 2006) [21, 11].

Variations in the genetic makeup of different genotypes can result in significant differences in flower colour, which is crucial for breeding programs aimed at developing new varieties with enhanced ornamental traits (Chandler & Sanchez, 2012) [4]. The manipulation of genes involved in pigment biosynthesis can lead to the creation of flowers with novel colours and patterns (Davies *et al*., 2012; Tatsuzawa *et al*., 2021) [6, 31].

Materials and Methods
The study was conducted at Hi-Tech Horticulture Park, College of Horticulture, Junagadh Agricultural University in Gujarat, India, in 2022 within the South Saurashtra Agro-Climatic Zone. Eleven Adenium genotypes were used: ADWB-78, Sunny Bunny, ADWB-1, Dancing Santa, Beauty Queen, ADWB-22, Dragon Blood, ADWB-4B, Butterfly Dream, ADWB-50, and Local. Plants were grown in pots with a soil, vermicompost, cocopeat, and sand mixture (2:1:1:2) and placed outdoors in full sun, with manual irrigation twice a week. Cultural treatments followed the recommended guidelines. Monthly pigment trait analysis was conducted from June to September 2021. Statistical analysis involved eleven treatments and three replications using OP Stat, Past 3.0, and Clust Vis software.

Pigments analyzed included anthocyanin, carotenoids, and total chlorophyll content in the leaves.

Anthocyanin content in petals (mg/100g)

Estimated the amount of anthocyanin pigment through Swain and Hillis's, 1959 methodology. Twenty ml of acidified ethanol (a 1% HCl solution in 80% ethanol) was used to grind one gramme of the outer whorl of the corolla's petals to fit. This mixture was transferred into a second beaker, wrapped in parafilm, and kept at 4 ºC overnight. The following day, the mixture was filtered using a funnel and No. 1 Whatman's filter paper. The filtrate was then gathered in a flask. Following filtering, the macerate (which was still in the filter paper) was combined with 10 ml of extracting solvent and passed through a second No. 1 Whatman's filter paper before being transferred into the flask that had previously held the filtrate. The extraction solvent was added to get the total amount up to 30 ml. 10 ml of that solution was transferred to a second beaker, where 20 ml was obtained by adding the solvent. After that, this solution was left at room temperature for two hours in the dark. Then, the spectrophotometer reading at 535 nm was recorded in comparison to the blank, and the anthocyanin pigment was approximated using the formula below.

Anthocyanin content: (mg/100 g)
= D535 × Dilution factor x $10/Avg^{E1\%}535$
= (D535 × Dilution factor)/98.2

Where,
D535= O.D. at 535 nm wavelength
Dilution factor = (original extract × dilution amount)/ extract taken for dilution

Carotenoid content in leaves (mg/100 g)

Fresh plant material was crushed and chopped. Whatman's No. 42 filter paper was used to filter a known quantity of pulverized plant material (3g) through a Buchner funnel after it had been combined with 10–15 ml of acetone and a few crystals of anhydrous sodium sulphate. Until the tissue was pigment-free, the same process was repeated. The filtrate was divided into three equal portions using a separatory funnel, to which 10-15 ml of petroleum ether was added and thoroughly mixed. On standing, two layers are separated. The top layer was gathered in a 100 ml volumetric flask, while the lower layer was disposed of. To measure the O.D. of a solution, add up to 100 ml of petroleum ether and use it as a blank at 450 nm (Sadasivam and Manickam, 1996) [19].

The total carotenoid content was calculated using the following formula

C = D×V ×f ×10/2500

Where,
C = Total amount of carotenoids (mg)
D = Absorbance at 450 nm
V = Volume of the original extract in ml f = Dilution factor and
2500 = Average extinction coefficient of the pigments

Total Chlorophyll content (mg/100g)

Chlorophyll was extracted in 80% acetone and the absorption was taken at 663 nm and 645 nm wavelength in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll was calculated (Goodwin, 1976). One gram of fresh leaves was mashed in 20 ml of 80% acetone. Centrifuged at 5000 rpm for 5 minutes, then transferred the supernatant to a 100 ml volumetric flask. The residue was then ground with 20 mL of 80% acetone, centrifuged, and the supernatant transferred to the same volumetric flask. The operation was repeated until the residue was colourless. The mortar and pestle were completely cleaned with 80% acetone, and the clear washings were collected in the volumetric flask. The volume was increased to 100 ml with 80 percent acetone. Read the solution's absorbance at 663 and 645 nm wavelengths against the solvent blank (80% acetone). The extract's total chlorophyll content (mg g^{-1} issue) was estimated using the following equations:

$$\text{Chlorophyll} - \text{a}\left(\frac{\text{mg}}{\text{g}}\right) = 13.36\,(\text{A663}) - 5.19\,(\text{A645}) \times \frac{\text{V}}{1000 \times W}$$

$$\text{Chlorophyll} - \text{b}\left(\frac{\text{mg}}{\text{g}}\right) = 27.43\,(\text{A645}) - 8.12(\text{A663}) \times \frac{\text{V}}{1000 \times W}$$

$$\text{Total Chlorophyll}\left(\frac{\text{mg}}{\text{g}}\right) = 5.29(\text{A663}) + 22.24(\text{A645}) \times \frac{\text{V}}{1000 \times W}$$

Where, A= Absorbance at a specific wave length
V= Final volume of chlorophyll extract in 80% alcohol
W= Fresh weight of tissue extracted

Results and Discussion

Experimental results are depicted in figure 1 and Table. 1. This clearly indicated that significant variation was observed in pigmentation also confirming the potential genotypes for diverse flower colors in 11 genotypes (Figure 3). However, flower color was not the main factor in distinguishing genotypes. Rare and unique genotypes have higher ornamental value, despite the lack of consumer preference studies on flower colors in *A. obesum* (Singh *et al.*, 2017) [26].

Anthocyanin content in petal

The variation in anthocyanin content among different genotypes of Adenium obesum can be attributed to genetic factors influencing pigment biosynthesis pathways (Hughes &, 2007) [14]. Genetic studies suggest that differences in gene expression and regulation lead to varying levels of anthocyanin accumulation in plant tissues (Tanaka *et al*., 2008) [30]. The data showed the wider variability in all the genotypes. the Dragon Blood genotype exhibits a high anthocyanin content (64.14 mg/100g), possibly due to upregulation of genes involved in anthocyanin synthesis, while Sunny Bunny shows a lower content (1.74 mg/100g), indicative of reduced level in petals and also may have gene expression in these pathways (Britton, 1995) [3].

Carotenoid content in leaves

Carotenoids in adenium leaves play crucial roles in photo protection, antioxidant defense, vibrant coloration, resilience to stress, and potential nutritional benefits, underscoring their physiological and ornamental significance. The data from leaves shown that ADWB-22 exhibited the highest carotenoid content (5.45 mg/100g), followed by ADWB-4B, while lowest (2.32 mg/100g) was observed in ADWB-78 Variability in carotenoid levels among adenium genotypes

reflects genetic influences on biosynthesis pathways (Hughes & Smith, 2007; Sindhuja *et al*., 2020) [14, 25]. Studies indicate that genetic factors also found the carotenoid accumulation through level of expression and regulatory mechanisms in pigments (Tanaka *et al*., 2008; Kishimoto *et al*., 2007) [30, 16].

Total chlorophyll content in leaves

Total chlorophyll content in adenium leaves plays a critical role in photosynthesis, nutrient absorption, stress response, and overall plant health (Hughes & Smith, 2007; Britton, 1995) [14, 3]. Optimal chlorophyll levels are essential for enhancing both growth and ornamental appeal in landscaping and gardening. ADWB-1 recorded the highest total chlorophyll content at 6.06 mg/100g, indicating efficient photosynthetic capability and robust plant health (Hughes & Smith, 2007; Britton, 1995) [14, 3]. This genotype likely possesses genetic factors that support chlorophyll biosynthesis pathways, leading to higher chlorophyll accumulation. Conversely, ADWB-50 and ADWB-22 showed lower total chlorophyll content (3.64 mg/100g), possibly due to genetic variations affecting chlorophyll biosynthesis or enhancing breakdown processes (Tanaka *et al*., 2008) [30].

Pigment diversity indices

Pigment diversity indices were calculated using Past 3.0 software (Hammer *et al*., 2001). (Table 2). Taxa_S (Species Richness): All three pigment categories had an equal number of taxa, with 11 species each (Ellstrand & Elam, 1993; Vega & Orellana, 2017). Individuals: The number of individuals varied significantly across pigments, with Anthocyanin having the highest number at 237, followed by Chlorophyll with 49, and Carotenoids with 32 (Hughes *et al*., 2017) [15]. Dominance_D: Dominance was lowest for Chlorophyll (0.09272) and highest for Anthocyanin (0.1512), indicating a more even distribution of species in Chlorophyll (Smith & Rausher, 2011) [27]. Simpson_1-D: Simpson's diversity index (Simpson, 1949) [24] was highest for Chlorophyll (0.9073) and lowest for Anthocyanin (0.8488), suggesting greater diversity in the Chlorophyll group (Streisfeld & Rausher, 2009) [28]. Shannon_H: The Shannon index indicated the highest diversity in Chlorophyll (2.388) and the lowest in Anthocyanin (2.078) (Shannon *et al*., 1948. Yoshida *et al*., 2017) [22, 33]. Evenness_e^H/S: Evenness was highest in Chlorophyll (0.9898), indicating a more even distribution of individuals among species, and lowest in Anthocyanin (0.7264) (Schaefer & Ruxton, 2011) [20]. Brillouin Index: The highest Brillouin index was observed for Anthocyanin (1.876), suggesting a higher degree of diversity (Davies *et al*., 2018) [2]. Menhinick Index: The Menhinick index was highest for Carotenoids (1.78), indicating a high diversity relative to the number of individuals (Albert *et al*., 2018) [2]. Margalef Index: Carotenoids showed the highest value (2.885), suggesting greater species richness relative to the number of individuals (Tanaka *et al*., 2008) [30]. Equitability_J: Equitability was highest for Chlorophyll (0.9957), indicating a more even distribution of species (Grotewold, 2006) [11]. Fisher_alpha: The highest value was observed for Carotenoids (5.174), indicating a higher level of species diversity (Zhao & Tao, 2015). Berger-Parker: The index was lowest for Chlorophyll (0.1107), suggesting a lower dominance by the most abundant species (Harborne & Williams, 2000) [13]. Chao-1: The Chao-1 estimator was consistent across all three pigments, indicating equal species richness with an estimate of 11 species (Chao *et al*., 2005; Mworia *et al*., 2011) [5, 18].

Adenium highlights that all three pigment categories contain an equal number of 11 taxa each (Ellstrand & Elam, 1993; Vega & Orellana, 2017) [8, 32]. Nevertheless, significant variations in individual counts and diversity indices among Anthocyanin, Chlorophyll, and Carotenoids underscore distinct distribution patterns and ecological roles within the species (Hughes *et al*., 2017; Smith & Rausher, 2011; Streisfeld & Rausher, 2009) [15, 27, 28]. Chlorophyll demonstrates higher overall diversity and even distribution of species and individuals (Yoshida *et al*., 2017; Grotewold, 2006) [33, 11]. Conversely, Anthocyanin exhibits higher diversity indices, suggesting a more diverse composition within its species despite a less even distribution (Davies *et al*., 2018; Schaefer & Ruxton, 2011) [2, 20]. Carotenoids show the highest species richness relative to the number of individuals (Albert *et al*., 2018; Tanaka *et al*., 2008) [2, 30]. These findings underscore the intricate dynamics of pigment diversity in Adenium obesum, providing insights into their ecological importance and genetic variability.

Grouping cluster

Based (Figure 2.) on hierarchical clusters of the pigments *viz*., anthocyanin, carotenoid content in leaves and total chlorophyll content compounds, the 11 genotypes were grouped into two clusters. Further dendrogram showed two classes with eleven Results of the principal component analysis were also in conformity with the cluster analysis results.

Group I comprised only one genotype the Dragon Blood. Group II comprised two sub-clusters II-1 and II-2. Sub-group II-I comprised genotypes Beauty Queen, ADWB-22, ADWB-4B, Local, Sunny Bunny and Butterfly Dream while sub-group II-2 was comprised of genotypes ADWB-1, ADWB-78, Dancing Santa, and ADWB-50 (Figure 1). It showed that Dragon Blood was superior in terms of pigments. Selection of parents from these diverse clusters for hybridization programme would help in achieving novel recombinants. Results were also suggested by Abreu *et al.* (2023) [1] in Adenium.

Heatmap analysis for pigmnets

Heatmap (Figure 3) showed dendrograms the tree-like structures on the left and top are dendrograms resulting from hierarchical clustering. Row dendrogram shows the clustering of plant genotypes based on similarity in their pigment content. Column dendrogram indicates the clustering of the types of pigment content based on similarity. The colour scale at the top indicates the relative levels of pigment content. Blue: Lower pigment content, red: Higher pigment content, white: Medium pigment content ClustVis, Metsalu *et al.*, 2015 [17].

Clustering of genotypes of adenium are grouped based on the similarity of their pigment content profiles "ADWB-50" and "Dancing Santa" are closely clustered, suggesting similar profiles in the three pigments measured.

Pigment content Profiling through the heat map revealed which plant varieties had higher or lower content of specific pigments "Dragon Blood" shown a high anthocyanin content (dark red) but lower carotenoids and chlorophyll content (blue) "Local" had aso shown the low to medium levels of all three pigments (light blue to white).

Similarity between pigments the clustering with column indicated that how the pigments were throughly distribution across the genotypes for instance, if carotenoids and chlorophyll contents are closely clustered (figure 2), This illustrated figure indicated that two pigments have similar distribution patterns across the genotypes. "Dragon Blood" and "Local" was shown distinctly different from most other varieties in terms of their pigment profiling, as indicated by their unique colours and positions in the clustering.

Genotypes *viz*., "Sunny Bunny" and "Beauty Queen" indicated the balanced pigment contents, as indicated by the lighter colors and the column dendrogram indicates that "Total chlorophyll content" and "Carotenoids content in leaves" were found to be more similar to each other compared to "Anthocyanin content in petals". This type of analysis can useful for understanding the biochemical pigment diversity among plant varieties and also usefull for breeding programs to select a specific trait variation.

Principal component analysis

"A PCA plot generated using ClustVis (Metsalu *et al*., 2015) [17] is depicted in Figure 4. The x-axis, labeled 'PC1 (56.2%)', represents the first principal component, which explains 56.2% of the total variance. The y-axis, labeled 'PC2 (29.5%)', represents the second principal component, explaining 29.5% of the variance. Each point on the plot represents a sample, positioned according to its scores on PC1 and PC2.

The plot visually displays data variation points closer together indicate similar values, while those farther apart indicate dissimilarity. PC1 captures the most variability, followed by PC2, together explaining 85.7% of the variance. Clusters of points suggest groups of similar observations in the dataset, indicating potential patterns or groupings."

Table 1: The average value of different pigments content in eleven genotypes of adenium (mg/100 g)

Genotypes	Anthocyanin content	Carotenoid content	Total Chlorophyll content
ADWB-78	26.62	2.32	3.84
Sunny Bunny	1.74	3.88	5.01
ADWB-1	24.13	2.59	6.06
Dancing Santa	40.00	3.25	5.35
Beauty Queen	14.49	3.82	5.25
ADWB-22	10.95	5.45	3.64
Dragon Blood	64.14	2.89	4.67
ADWB-4B	11.38	4.15	5.38
Butterfly Dream	3.65	3.40	4.75
ADWB-50	36.03	2.87	5.63
Local	8.90	3.57	4.60
S.Em.±	0.49	0.07	0.10
C.D. at 5%	1.44	0.20	0.30
C.V. %	3.83	3.42	3.54

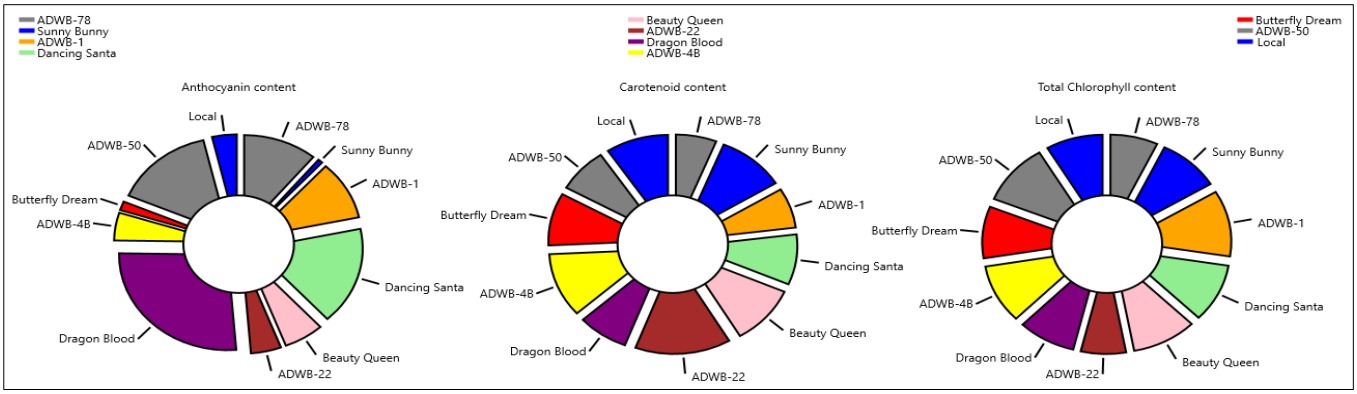

Fig 1: Compositional variation of individual pigments in different genotypes of adenium

Table 2: Diversity indices in pigments found in different genotypes of Adenium

Diversity indices	Pigments		
	Anthocyanin	Carotenoids	Chlorophyll
Taxa_S	11	11	11
Individuals	237	32	49
Dominance_D	0.1512	0.09607	0.09272
Simpson_1-D	0.8488	0.9039	0.9073
Shannon_H	2.078	2.371	2.388
Evenness_e^H/S	0.7264	0.9732	0.9898
Brillouin	1.876	1.416	1.713
Menhinick	0.7071	1.78	1.494
Margalef	1.829	2.885	2.569
Equitability_J	0.8667	0.9887	0.9957
Fisher_alpha	2.374	5.174	4.168
Berger-Parker	0.2644	0.1309	0.1107
Chao-1	11	11	11

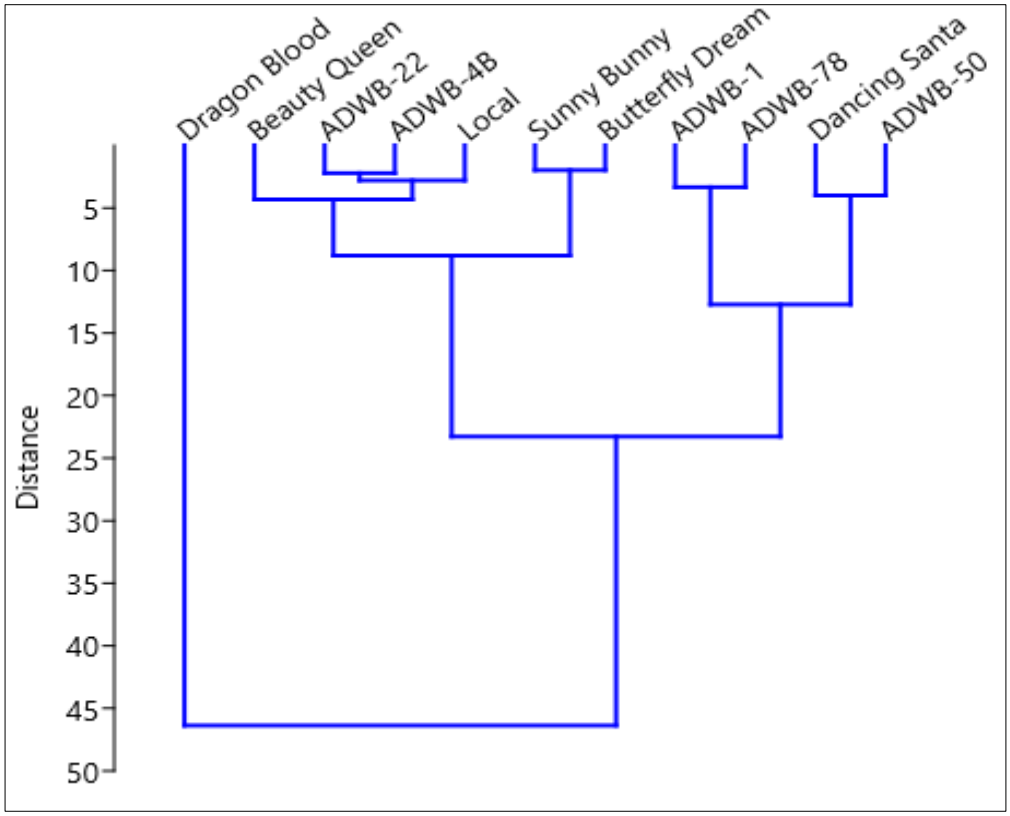

Fig 2: Similarity index (Euclidean) through dendogram in pigments (UPGMA) of 11 genotype of adenium

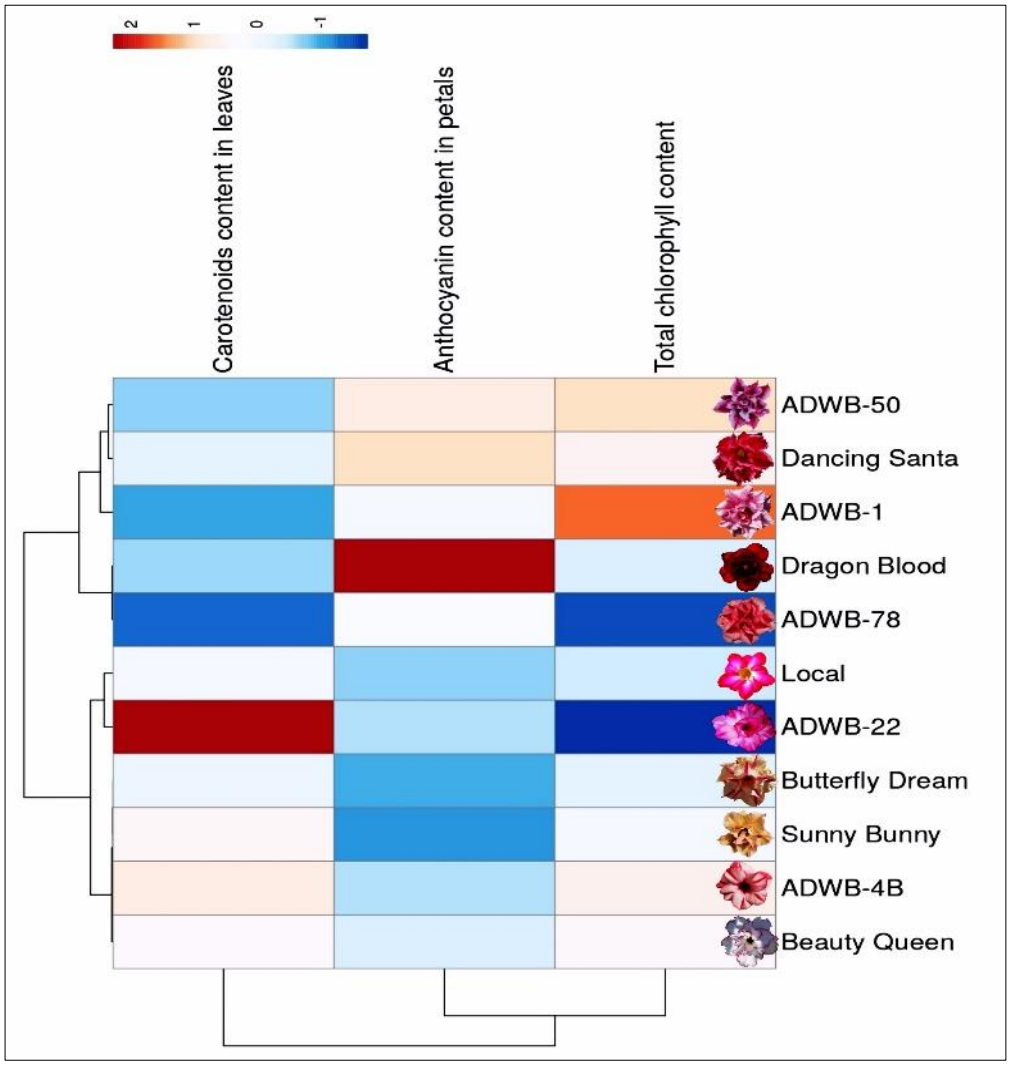

Fig 3: Heatmap for different pigments in different genotypes of adenium

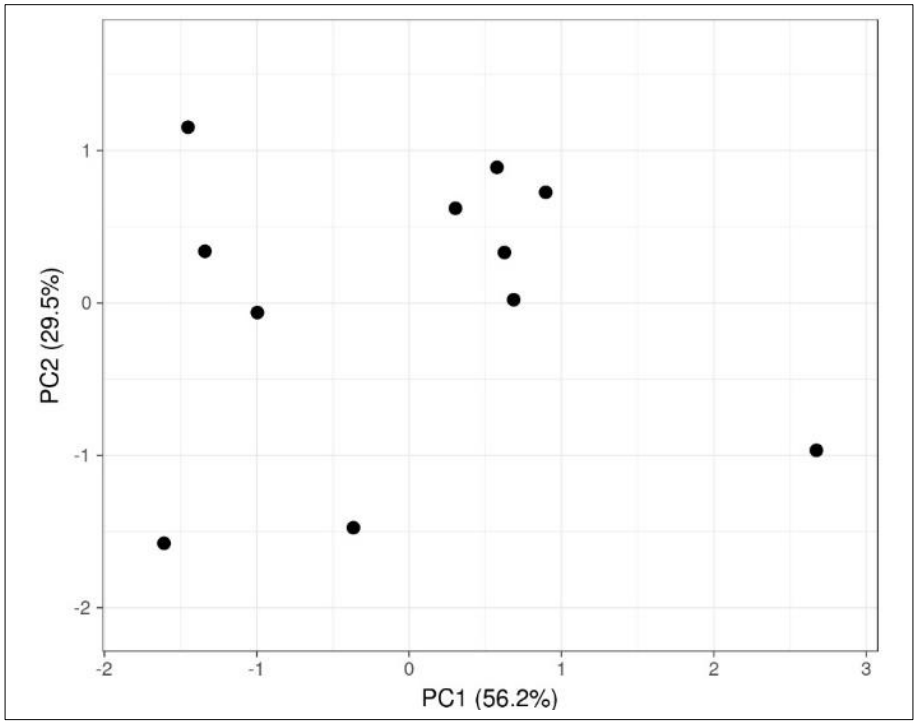

Fig 4: Clust Vis Principal component analysis (PCA) for different pigments for different genotypes of adenium

Conclusion

The study explores pigment diversity in *Adenium obesum*, highlighting significant genetic variation among eleven genotypes. Chlorophyll exhibits the highest diversity and even distribution, followed by carotenoids which show notable species richness. Surprizingly, the diversity tools indicated that anthocyanin displays the lowest diversity and evenness inspite of biochemically varied differences in genotypes. These findings can usefull for selective breeding to enhance the plant's aesthetic appeal by leveraging genetic diversity for developing new flower colors and pattern to maximizing the ornamental potential of *Adenium obesum*.

Future Scope

Diversity in pigment evaluation of *Adenium obesum* genotypes encompasses a broad range of interdisciplinary approaches, combining biochemical, genetic, environmental, and ecological perspectives to comprehensively explore the variability and functional significance of pigment profiles in adenium**.**

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Conflict of Interest

The authors declare that they have no conflict of interest.

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