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Evaluation of *Adenium* genotypes for physio-chemical and flowering characters

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Abstract
Thirty three genotypes were evaluated for physio-chemical and flowering characters. Plant compactness was recorded maximum in Cross 6 (Arrogant × Vithoon's White). Maximum chlorophyll a content of leaves (3.08 mg/100 g) was recorded in Cross 14 (Mor Lok Dok × Double Sweet Heart) and Chlorophyll b content of leaves (1.61 mg/100 g) was found in Cross 12 (Harry Potter × Vithoon's White). Maximum anthocyanin content in petals (31.92 mg/100 g) was recorded in Cross 18 (Taiwan Dwarf × Deang Udam Sap). Maximum carotenoid content in leaves (5.04 mg/100g) was recorded in Picottee. Maximum superoxide dismutase activity (6.46 unit/g protein) was recorded in Cross 13 (Mor Lok Dok × Deang Udam Sap) and guaicol peroxidase activity (5.01 µ mol/min/g protein) was recorded in Cross 1 (Sudarshan × Deang Udam Sap). *In-situ* longevity (18.63 days) was observed maximum in Cross 15 (Picottee × Deang Udam Sap). Thus, Cross 6, Taiwan Dwarf, Cross 20, Cross 14, Cross 21, Cross 4 and Cross 5 has been found for plant compactness and Cross 15 and Cross 13 has been found for enhanced flower longevity.

Keywords: *Adenium*, enzymes, pigments and plant compactness

Introduction
Adenium obesum (Forssk.) Roem and Schult. Known as desert rose is becoming very popular as a pot plant. It belongs to the family Apocynaceae. It is a genus of spectacular succulents and having very heterozygous genetic nature. *Adenium obesum* is a highly variable taxon in growth and flowering habit and is found across all Africa, South Sahara, Kenya and from Senegal to Sudan. Most of the species of *Adenium* are diploid (2n=24). *Adeniums* are best suited for container culture as they are slow growing, can tolerate salinity and most important they responds well to pruning Chavan *et al.* (2017) [2], Singh *et al.* (2017) [11] and Singh *et al.* (2019) [12]. The present experiment has been investigated to evaluate the genotypes of *Adenium* for phyio-chemical parameters and flowering character. In reference to flowering parameters, flower colour and flower longevity are important characteristics that influence plant value. Pigments influence flower colour and antioxidative enzymes *viz.*, Guaiacol Peroxidase activity (µ mol/min/g protein) (GPOX) and Superoxide dismutase enzyme activity (unit/g protein) (SOD) have been known to affect flower longevity Prajapati, 2013) [9]. Hence, the present study was conducted to evaluate physio-chemical and flowering characters.

Materials and methods
The present study was carried out at the Advance Technology Centre for production of various crops in soilless systems, at the Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari-396450, Gujarat during 2017-18 to 2018-19. The experiment was laid out in randomized block design with three replications, consisted thirty three genotypes of *Adenium*. Grafting was done on two years old plants. The experimental observations were taken on different morphological traits and recorded on five randomly selected plants from each genotype in each replication were used for data analysis. The data was recorded for two years *i.e* 2017-18 and 2018-19 and pooled. The whole data thus obtained was analyzed by using M statistical software Assex at 5% level of significance. Observations were recorded on five competitive plants randomly selected. *In-situ* longevity by counting number of days from flower bud opening till the day of

flower senescence, plant compactness was recorded by number of nodes divided by shoot length and average was recorded. Plant pigments *viz*., chlorophyll and carotenoid content in leaves, anthocyanin content in leaves and enzymatic activity were estimated as given below:

Chlorophyll content in leaves (mg/100g)

Chlorophyll content was estimated by following the procedure of Sadasivam and Manickam (1996) [10]. One gram of fresh leaves was ground to fitness in 20 ml of 80 per cent acetone. Centrifuged (5000 rpm for 5 min.) and transferred the supernatant to a 100 ml volumetric flask. Then ground the residue with 20 ml of 80 per cent acetone, centrifuged and transferred the supernatant to the same volumetric flask. This procedure was repeated until the residue was colourless. The mortar and pestle was washed thoroughly with 80 per cent acetone and the clear washings were collected in the volumetric flask. The volume was made upto 100 ml with 80 per cent acetone. Read absorbance of the solution at 645 and 663 nm wavelength against the solvent (80% acetone) blank. The amount of chlorophyll present in the extract (mg g^{-1} tissue) was calculated using the following equations:

$$\text{mg chlorophyll 'a'}/100\text{ g} = 12.7\,(A663) - 2.69\,(A645) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll 'a'}/100\text{g} = 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll }/100\text{ g} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W}$$

Where,
A = absorbance at specific wavelengths
V = final volume of chlorophyll extract in 80 per cent acetone
W= fresh weight of tissue extracted

Carotenoids content in leaves (mg/100 g)

The fresh plant material was cut and ground. A known amount of the ground plant material (3g) was taken in a mortar with 10-15 ml of acetone and few crystals of anhydrous sodium sulphate to the plant material and then filtered on a Buchner funnel through Whatman's No. 42 filter paper. The same procedure was repeated until the tissue was free from pigments. The filtrate was partitioned thrice with equal volume of petroleum ether using a separatory funnel, added 10-15 ml petroleum ether and mix thoroughly. Two layers separated out on standing. The lower layer was discarded and upper layer was collected in a 100 ml volumetric flask. Make up to 100 ml with petroleum ether and O.D. of the solution was measured at 450 nm using petroleum ether as blank Sadasivam and Manickam (1996) [10].

The total carotenoid content was calculated using the following formula

$$C = \frac{D \times V \times f \times 10}{2500}$$

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

Anthocyanin content in petals (mg/100g)

Anthocyanin pigment was estimated by following the procedure of Swain and Hillis (1959) [12]. One gram of petals from outer whorl of the corolla was ground to fitness in 20 ml acidified ethanol (1% solution of HCl in 80% ethanol). This mixture was transformed into another beaker, covered with para film and stored overnight at 4 ºC. The mixture was filtered next day through No.1 Whatman's filter paper in a funnel and the filtrate was collected in a flask. After the filtration was over, macerate (left in the filter paper) was again mixed with 10 ml of extracting solvent and filtered through another No.1 Whatman's filter paper into the flask containing earlier filtrate. The final volume was made up to 30 ml by the addition of extraction solvent. From that solution, 10 ml was taken into another beaker and made up to 20 ml by the addition of extracting solvent. Then this solution was stored in dark for two hours at room temperature and the spectrophotometer reading was recorded at 535 nm wavelength against the blank and the anthocyanin pigment was estimated according to the formula given 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

Enzymatic activities (SOD and GPOX)

Extract for determination of superoxide dismutase (SOD) and guaiacol peroxidise (GPOX) activities was prepared from 0.3 g of leaves homogenized with a pre-chilled mortar and pestle under ice cold condition in 3 ml of extraction buffer, containing 50 mM sodium phosphate buffer (pH 7.4) with the addition of 1 mM EDTA and 1 per cent (w/v) polyvinylpyrolidone (PVP). The homogenates were centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for the assay (Costa *et al*., 2002).

Superoxide dismutase enzyme activity (SOD)

Total SOD (EC 1.15.1.1) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (NBT). The 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8) 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0.1 ml enzyme extract, riboflavin was added at last (Van Rossum *et al*., 1997) [15].

Test tubes were shaken and positioned 30 cm below from a light blank consisting of four 15-W fluorescent lamps. The reaction was allowed to run for 10 minutes and stopped by switching the light off. The photo reduction in NBT was considered as increase in absorbance at 560 nm. Blanks and controls were run the same way but without illumination and enzyme, correspondingly. One unit of SOD was defined as the amount of enzyme essential to slow up the reduction of NBT by 50 per cent in a reaction mixture. Enzyme unit of SOD was calculated according to the formula given by Constantine and Stanley (1977) [3].

$$\text{SOD unit} = \frac{\text{O.D.control (without enzyme)}}{\text{O.D.sample}} - 1 \times \frac{1}{\text{enzyme conc.(g)}}$$

$$\text{SOD U/g protein} = \frac{\text{SODunit}}{\text{mg/g protein}}$$

Guaiacol Peroxidase enzyme activity (µ mol/min/g protein)

GPOX (EC 1.11.1.7) activity was determined in the homogenates by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol (ε = 26.6 mM cm^{-1}) in a reaction mixture containing 50 mM sodium phosphate buffer pH 7.0, 0.1 mM EDTA, 0.05 ml enzyme extract, 10 mM guaiacol and 10 mM H_2O_2 Costa *et al*. (2002) [4].

Result and Discussion

With regard to physiological parameter, plant compactness was recorded maximum in Cross 6 (Arrogant × Vithoon's White) which was statistically at par with Taiwan dwarf, Cross 20 (Harry Potter × Double Sweet Heart), Cross 21 (Mor Lok Dok × Double Sweet Heart), Cross 4 (Arrogant × Deang Udam Sap) and Cross 5 (Arrogant × Double Sweet Heart). Minimum plant compactness was recorded in Picottee. Plant compactness indicates more number of internodes and less internodal space. This is good parameter for pot plants. Thus variation in genotypes indicates difference owing to their genetic makeup (Table 1).

With regard to plant pigments maximum chlorophyll a content of leaves (3.08 mg/100 g) was recorded in Cross 14 (Mor Lok Dok × Double Sweet Heart) followed by Cross 2 (Sudarshan × Double Sweet Heart) and Chlorophyll b content of leaves (1.61 mg/100 g) was observed in Cross 12 (Harry Potter × Vithoon's White) followed by Cross 5 (Arrogant × Double Sweet Heart). Further, maximum carotenoid content in leaves (5.04 mg/100g) was recorded in Picottee followed by Cross 2 (Sudarshan × Double Sweet Heart) (Table 2). It may be attributed to good vegetative growth in these genotypes. Flower colour is mainly based on the presence of two major groups of pigments: carotenoids and flavonoids. Red, blue and lilac flower colours are mainly provided by anthocyanins, an important flavonoid class, whereas yellow flower coloration is in most cases formed by carotenoids. With regard to petal pigments maximum anthocyanin content in petals (31.92 mg/100 g) was found in Cross 18 (Taiwan Dwarf × Deang Udam Sap) which was statistically at par with Cross 5 (Arrogant × Double Sweet Heart) and Cross 22 (Sudarshan × Deang Udam Sap). The difference in anthocyanin content in different genotypes may be due to their genetic makeup. In general, flower coloration in the range of orange-red to red is determined by a combination of anthocyanins and/or carotenoids. Flowers acquire their characteristic due to these pigments in combination with other chemical and physical localization of pigments and the optical properties of petal epidermal cells by Kay *et al*., (1981) [6] and Mol *et al*. (1998) [8] and by Kishimoto *et al*. (2007) [7] in Chrysanthemum and by Tatsuzawa *et al*. (2005) [14] in orchid by Hatamzadeh *et al*. (2012) [5] and Prajapati (2013) [9] in gerbera. In reference to enzymatic activity *viz*., SOD and GPOX, maximum superoxide dismutase activity (6.46 unit/g protein) was recorded in Cross 13 (Mor Lok Dok × Deang Udam Sap) followed by Cross 1 (Sudarshan × Deang Udam Sap) and guaicol peroxidase activity (5.01 µ mol/min/g protein) was recorded maximum in Cross 1 (Sudarshan × Deang Udam Sap) followed by Cross 13 (Mor Lok Dok × Deang Udam Sap). *In-situ* flower longevity (18.63 days) was observed maximum in Cross 15 (Picottee × Deang Udam Sap) which was at par with genotype Cross 13 (Mor Lok Dok × Deang Udam Sap), as shown in Table 3. SOD and GPOX have indicated as anti-oxidative enzymes. Higher anti-oxidative enzymatic activities, in these genotypes reflects direct influence on higher longevity. Thus, genetic makeup is responsible for such difference in enzymatic activity. Similar results of variation in enzymatic activities and flower longevity have been recorded in different ornamental plants by Chakraborty *et al*. (2009) [1] in *Hemerocallis* and by Prajapati, (2013) [9] in gerbera.

In conclusion, genotypes Cross 6, Taiwan Dwarf, Cross 20, Cross 14, Cross 21, Cross 4 and Cross 5 have been found suitable for plant compactness. Further, Cross 15 and Cross 13 have been found suitable for enhanced flower longevity.

Table 1: Variation in plant compactness in different genotypes of *Adenium*

Genotypes	plant compactness
Sudarshan	0.58
Arrogant	0.71
Mung Siam	0.60
Harry Potter	0.60
Mor Lok Dok	0.66
Picottee	0.51
Taiwan Dwarf	0.89
Deang Udam Sap	0.70
Double Sweet Heart	0.71
Vithoon's White	0.55
C-1 (S × DUS)	0.72
C-2 (S × DSH)	0.65
C-3 (S × VW)	0.69
C-4 (A × DUS)	0.83
C-5 (A × DSH)	0.81
C-6 (A × VW)	0.90
C-7 (MS × DUS)	0.76
C-8 (MS × DSH)	0.72
C-9 (MS × VW)	0.74
C-10 (HP × DUS)	0.68
C-11(HP × DSH)	0.67
C-12 (HP × VW)	0.68
C-13 (MLD × DUS)	0.68
C-14 (MLD × DSH)	0.79
C-15 (P × DUS)	0.78
C-16 (P × DSH)	0.73
C-17 (P × VW)	0.75
C-18 (TD × DUS)	0.72
C-19 (TD × DSH)	0.79
C-20 (HP × DSH)	0.88
C-21 (MLD × DSH)	0.86
C-22 (S × DUS)	0.61
C-23 (HP × DSH)	0.79
S.Em.+	0.03
C.D. at 5%	0.09
C.V. %	11.28

Table 2: Variation in different pigments of *Adenium* genotypes

Genotypes	chlorophyll a (mg/100 g)	Chlorophyll b (mg/100 g)	Carotenoid content in leaves (mg/100 g)	Anthocyanin content in petals (mg/100 g)
Sudarshan	2.12	1.46	3.36	8.79
Arrogant	2.44	1.17	4.45	19.41
Mung Siam	2.29	1.34	3.74	9.86
Harry Potter	2.27	1.37	4.06	14.90
Mor Lok Dok	2.39	1.30	3.74	0.46
Picottee	2.15	1.08	5.04	5.15
Taiwan Dwarf	2.22	1.01	2.70	14.45
Deang Udam Sap	2.29	1.35	1.22	19.68
Double Sweet Heart	2.05	0.96	3.23	12.08
Vithoon's White	2.41	1.35	3.81	0.57
C-1 (S × DUS)	2.42	1.36	2.65	30.23
C-2 (S × DSH)	2.94	1.15	4.50	26.88
C-3 (S × VW)	2.32	1.36	2.79	13.49
C-4 (A × DUS)	1.00	0.46	3.07	23.06
C-5 (A × DSH)	2.33	1.55	3.42	31.90
C-6 (A × VW)	2.32	1.36	4.29	27.03
C-7 (MS × DUS)	2.72	1.42	4.40	21.33
C-8 (MS × DSH)	2.36	1.35	3.39	23.70
C-9 (MS × VW)	2.40	1.56	4.19	5.07
C-10 (HP × DUS)	2.30	1.36	2.48	19.61
C-11(HP × DSH)	2.29	1.36	4.11	18.73
C-12 (HP × VW)	2.49	1.61	2.27	13.41
C-13 (MLD × DUS)	2.78	1.41	3.47	16.24
C-14 (MLD × DSH)	3.08	1.31	3.01	0.85
C-15 (P × DUS)	2.41	0.46	4.18	18.84
C-16 (P × DSH)	2.82	1.15	3.48	16.78
C-17 (P × VW)	2.32	1.30	2.09	15.20
C-18 (TD × DUS)	2.44	1.30	3.07	31.92
C-19 (TD × DSH)	2.36	1.33	3.04	12.58
C-20 (HP × DSH)	2.31	1.32	2.80	8.08
C-21 (MLD × DSH)	2.36	1.35	3.29	0.48
C-22 (S × DUS)	2.43	1.33	2.36	31.57
C-23 (HP × DSH)	2.93	1.48	4.34	22.31
S.Em.+	0.03	0.02	0.02	0.30
C.D. at 5%	0.08	0.06	0.05	0.86
C.V. %	3.00	3.94	1.40	4.59

Table 3: Variation in enzyme activity and longevity of different genotypes of *Adenium*

Genotype	SOD enzyme activity (unit/g protein)	GPOX enzyme activity (µ mol/min/g protein)	*in-situ* longevity (days)
Sudarshan	1.17	1.27	7.57
Arrogant	1.30	2.06	9.57
Mung Siam	0.37	1.16	7.50
Harry Potter	0.98	1.55	8.60
Mor Lok Dok	0.27	1.40	8.23
Picottee	0.30	1.67	8.73
Taiwan Dwarf	0.80	0.90	6.90
Deang Udam Sap	1.32	1.89	9.97
Double Sweet Heart	1.39	1.77	8.93
Vithoon's White	1.42	1.43	8.97
C-1 (S × DUS)	5.75	4.96	17.03
C-2 (S × DSH)	4.00	3.28	14.97
C-3 (S × VW)	1.45	2.43	11.93
C-4 (A × DUS)	1.75	2.31	10.90
C-5 (A × DSH)	3.90	2.57	11.97
C-6 (A × VW)	1.97	2.67	13.20
C-7 (MS × DUS)	1.51	2.46	12.13
C-8 (MS × DSH)	3.69	2.32	11.60
C-9 (MS × VW)	1.90	2.29	11.47
C-10 (HP × DUS)	1.80	2.20	9.83
C-11(HP × DSH)	1.98	2.67	13.47
C-12 (HP × VW)	4.81	3.36	16.27
C-13 (MLD × DUS)	6.46	4.09	18.33
C-14 (MLD × DSH)	2.35	2.63	12.47
C-15 (P × DUS)	4.88	3.83	18.63
C-16 (P × DSH)	2.48	3.03	13.50

C-17 (P × VW)	4.29	3.09	14.37
C-18 (TD × DUS)	4.54	3.22	15.30
C-19 (TD × DSH)	2.83	2.31	11.23
C-20 (HP × DSH)	4.13	3.14	14.73
C-21 (MLD × DSH)	3.07	2.09	10.17
C-22 (S × DUS)	4.73	4.27	16.80
C-23 (HP × DSH)	3.02	2.76	13.50
S.Em.+	0.06	0.05	0.18
C.D. at 5%	0.16	0.15	0.51
C.V. %	5.18	5.12	3.69

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