



RESEARCH COMMUNICATION

Infraspecific variability in *Asystasia dalzelliana* Santapau from southern Western Ghats of Kerala - An overview

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Abstract

During field expeditions, *Asystasia dalzelliana* specimens were collected from different geographical locations from the state of Kerala. The collected populations varied with respect to the color and size of the flowers. Subsequently, critical evaluation has been done using qualitative and quantitative traits. The DL3 and DL4 types were found to have restricted distribution patterns. The affinities among the collected specimens have been interpreted through two-dimensional scatter plot and PCA which proves that the variations are solely restricted in the flower color and banding of anthers. The study resolves the ambiguity among the color variants of *A. dalzelliana*.

Keywords

Asystasia; phenetic variance; Principal Component Analysis; scatterplot; UPGMA

Introduction

The family Acanthaceae consists of 220 genera and nearly 4000 species, mostly tropical in distribution. *Asystasia* Blume, a common taxon of the family consists about 50 species distributed in the tropics of the Old World (1, 2), chiefly in India and Africa (3). In India, thirteen species, one subspecies and two varieties were reported for the genus *Asystasia* (4-6), of which 6 taxa and two varieties are from Kerala (7). Recently, the taxonomic status of one variety reported from Kerala (*A. gangetica* var. *krishnae*) has been revised and the number of varieties reduced to one (8). The diagnostic features of the genus include tubular flower with regular slightly curved corolla (9). *Asystasia gangetica* is a variable species with several flower colors (10). *Asystasia dalzelliana* var. *alba* V.S.A. Kumar and Deepa Lekshmi, is endemic to Kerala and has recently been reported from southern western Ghats of Kerala (5).

Species of the genus *Asystasia* have been reported to have many therapeutic potentials (11-13). Certain taxa are applied in traditional medicine also, owing to their therapeutic potentials. The Indian folk medicine exploits the species as medicine which could be one of the reasons why the species is not having bulk distribution (8, 14). The status of the species in floras is mentioned as rare. Cumulative habitat destruction, anthropogenic activities and climate change will serve as factors for the extinction of the species in future, demanding serious conservation approaches.

In *Asystasia gangetica*, flower color variation is a common phenomenon which has been well documented recently (10). However, *A. dalzelliana* is not reported to have variability in flower color and the most common distribution is seen for the violet color variant. During field expeditions, the authors came across four morpho forms of this species from various geographical locations of the state. Such infraspecific variants, hereby mentioned as morphoforms can be the intermediate forms resulted as part of evolutionary process. As part of previous studies, the white

flower color variant has been established as a variety (5). The comparative analysis of the four collected morphoforms of the species formed the stem of the present study, as no previous reports are available regarding this.

Materials and Methods

Collections have been carried out in different districts of Kerala during June 2021- January 2023. Moderate climatic conditions such as humid climate and bright indirect sunlight are found to be essential for the growth of the taxon. The species resumes growth on monsoon and they flourish in summer. The study observes that environmental factors are not the cause of such morphoforms as the same morphoforms have been collected from more than one location. Four different morpho forms of *A. dalzelliana* have been collected from various geographical regions of Kerala (Fig. 1, Table 1). The morpho forms were represented as DL-1, DL-2, DL-3 and DL-4 (Fig. 2) and the collected specimens were subjected to detailed taxonomic analysis. Population analysis of the four morpho- forms have been performed for number of plants per population selected. A total of 22 quantitative and qualitative morphological characters were analyzed and recorded for all the collected specimens of the four morpho forms. A total of 17 populations of DL-1, 8 populations of DL- 2, 3 populations of DL -3 and 2 populations of DL -4 were evaluated for comparison.

Statistical Analysis

Data were recorded for 19 quantitative and 12 qualitative characters for the collected color variants (Table 2). To determine the significant differences among the quantitative morphometric variables, one way analysis of variance (ANOVA) was performed (IBM SPSS statistics 22). P<0.05 were considered as significant and the results were represented in mean ± standard error. To analyze the quantitative and

Table 1. Collection locations of flower color variants of *Asystasia dalzelliana* Santapau

Accession	Location	Coordinates
DL 1	Kulathupuzha	8° 53' 46.3"N 77° 3' 39.4"E
	Vayalkal	8° 55' 1.4" N 76° 51' 16.2"E
	Neyyar	8°32'04.4" N 77°08'31.4"E
	Kulappada	8° 35' 57.8" N 77° 3' 52.5"E
	Arakulam	9° 48' 59.8" N 76° 49' 53.6"E
	Puliyamala	9° 47' 11.9" N 77° 10' 36.2"E
	Painav	9° 51' 15.9" N 76° 56' 50.2"E
	Mattakkara	9°38'42.3" N 76° 37' 55.6"E
	Meenachil	9° 42' 20.8" N 76° 42' 0.7"E
	Vellarikund	12° 21' 58.9" N 75°17' 23.7"E
	Seethangoli	12°35'15.8" N 75°00'03.8"E
	Mallam	12°31'09.0" N 75°05'57.3"E
	Ottapalam	10°46'42.9" N 76°22'39.3"E
	Neriyamangalam	10°03'02.2" N 76°46'38.2"E
Cherupuzha	12°16'19.1" N 75°21'56.0"E	
DL 2	Karur	10°21'52.6" N 76°17'15.6"E
	Madathara	8°49'03.6" N 77°00'53.6" E
	Neyyar	8°32'03.0" N 77°08'46.5" E
	Ponmudi	8°45'34.2" N 77°06'56.3" E
	Vayalkal	8°55'33.8" N 76°51'16.3" E
	Bonacaud	8°40'47.3" N 77°10'09.3" E
	Vithura	8°40'31.9" N 77°04'19.8" E
DL 3	Kallar	8°42'34.6" N 77°07'43.4" E
	Nedumangad	8°36'44.4" N 77°00'48.5" E
	Iriyanni	12°29'55.1" N 75°07'47.6" E
DL 4	Bovikanam	12°29'36.6" N 75°08'00.5" E
	Mulleria	12°32'44.5" N 75°09'54.6" E
DL 4	Kaipally	8° 55' 45.356" N 76° 55' 05"E
	Kaipally	8° 55' 45.358" N 76° 55' 07" E

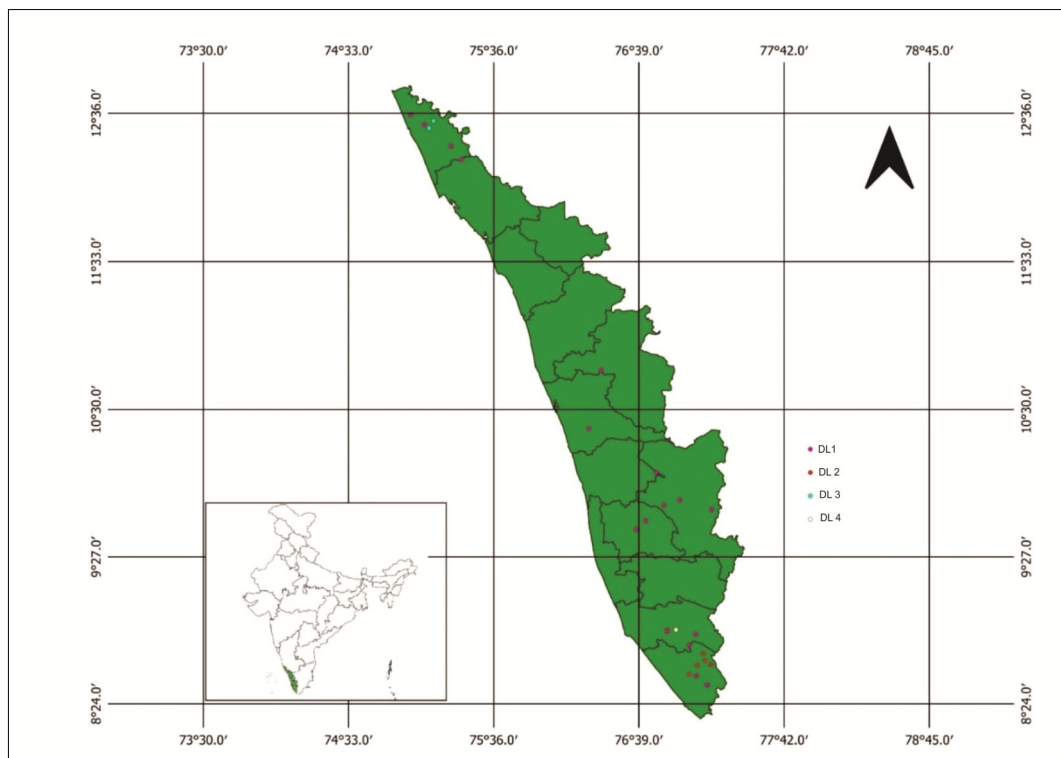


Fig. 1. Distribution map of color variants of *Asystasia dalzelliana* Santapau.

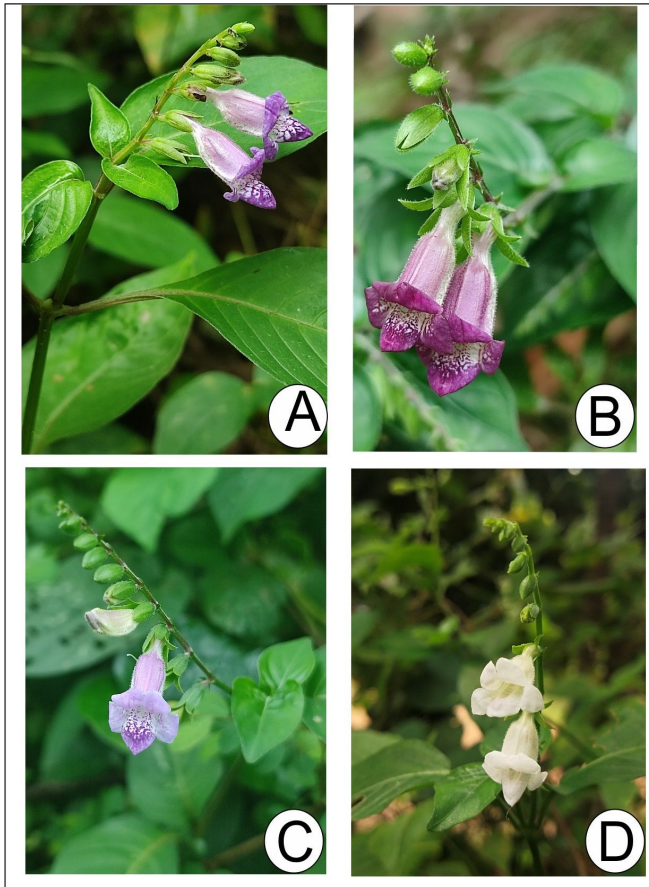


Fig. 2. Flower color variants of *Asystasia dalzelliana* Santapau. A- DL1, B- DL2, C- DL3, D - DL4.

qualitative characters that best define the group, a correlation based Principal Component Analysis (PCA) was performed using R software by considering 19 quantitative characters and 3 significant qualitative traits (Table 3, 4). The percentage variation for the first two PCA axes was used to construct the two-dimensional scatter plot, based on normalized variance - covariance matrix. UPGMA clustering method was used to represent distance matrix as a phenogram. Two-dimensional scatterplot and dendrogram were constructed and the data interpreted.

Results and Discussion

The most widespread flower color form of the species is DL-1 with purple coloration for corolla (Fig. 3), collected from several locations have been analyzed. The accession DL-2 (Fig. 4), though apparently resembles DL-1, has a deep purple corolla color with slight variation in quantitative measurements of floral traits viz. length of flower (2.5 cm in DL-1 vs 2.1 cm in DL-2), length of calyx (0.8 cm-1.0 cm vs 0.6 cm - 0.9 cm), length of lateral bracteole (0.15 cm vs 0.1 cm), length of longer filament of anther (0.7 cm vs 1.2 cm), length of gynoeceium (1.4 cm vs 1,2 cm) and length of style (1.1 cm vs 0.9 cm). DL-3 (Fig. 5), collected from Iriyanni of Kasaragod district, has a pale-purple corolla shows floral traits viz. flowers (1.8 cm long), calyx (0.5 - 0.8 cm long), lateral bracteole (0.25 cm long), longer filament of anthers (0.56 cm long), ovary (1.4 cm long) and style measuring 1 cm length with comparatively smaller seeds. The DL-4 (Fig. 6) has

Table 2. Comparison between accessions of *A. dalzelliana*

Features	<i>A. dalzelliana</i> (DL-1)	DL- 2	DL-3	DL 4
Height & habit	60-90cm Erect herb	50-80 Erect herb	30-50cm Erect herb	40-50cm,scandent herb
Stem	Quadrangular stem with prominent groove, internodal length 5.8-9.5cm	Quadrangular stem with prominent groove. Internodal length 5- 6.2cm	Quadrangular stem with groove. Not prominent as others.4.5- 5.2cm.	Quadrangular stem with prominent groove, small hairs at nodal region, Internodal length 6-12cm
Leaves	opposite, decussate; petiolate; Ovate to rounded 3.5-8cm long; densely pubescent on both side of lamina;8-10 pairs of lateral veins, leaf base rounded, leaf tip broadly acuminate, petiole 1.5-3.4cm long	opposite, decussate; petiolate; ovate to lanceolate 4.8-9 cm; pubescent on both side of lamina; 6-8 pairs of lateral veins, leaf base round, leaf tip acute to acuminate, petiole 1.7- 2.3cm long	opposite, decussate; petiolate; Ovate to rounded 3.3-4.1cm long; sparsely pubescent on both side of lamina; 6 pairs of lateral veins. Leaf base acute, leaf tip acute to acuminate. Petiole 1.3- 1.9cm long	opposite, decussate; petiolate; Ovate to elliptical 3.5-6.2cm long; pubescent on adaxial side of lamina;9-12 pairs of lateral veins, leaf base cuneate, leaf tip broadly acuminate, petiole 2 -2.5cm long
Inflorescence	10-12 flowers 10-20 cm long;	10-15 flowers; 14-23 cm long	9-12 flowers; 3.5-4 cm; peduncle green color and brown at nodes of pedicel	9-12 flowers 5-6.4cm long peduncle, green
Flower	Violet; 2.5cm long. pedicel is brownish green color	Dark violet purple blotch on the middle lower lip. 2.1cm	white flower with pale violet blotch on the middle lower lip. 1.8cm	White. Flower 1.5- 1.9cm.pedicel green 0.2cm
Calyx	5 valvate free, lanceolate 0.8- 1cm,pubescent	5 valvate free, lanceolate 0.6-9cm,pubescent	5 valvate free, lanceolate 0.5- 8cm,pubescent	5 valvate free, lanceolate 0.7 -0.9cm,pubescent
Bract	3,lanceolate,middle bracteole 0.3cm and lateral bracteole with 0.15cm	3,lanceolate,middle bracteole 0.3cm and lateral bracteole with 0.1cm	3,lanceolate,middle bracteole 0.3cm and lateral bracteole with 0.25cm	3,lanceolate,middle bracteole 0.2cm and lateral bracteole with 0.1cm
Stamens	4; epipetalous. Minute hairs can be seen on filament.0.7 and 0.5cm, white with a vertical violet-black bands on the sides	4; epipetalous; filament of shorter stamen 1cm,filament of longer stamen 1.2cm; Anthers oblong white with a vertical violet-black bands on the sides	4; epipetalous; filament of shorter stamen 0.34cm,filament of longer stamen 0.56cm; Anthers oblong white with a vertical violet-black bands on the sides.	4; epipetalous. Minute hairs can be seen on filament, white , filament of shorter stamen 0.6cm,filament of longer stamen 0.8cm.Anthers oblong white
Gynoeceium	White, lower portion of style have pubescence,1.4cm long, style 1.1cm	White, lower portion of style have pubescence,1.2cm long, style 0.9cm	White, lower portion of style have pubescence,1.3cm long, style 1cm	White, lower portion of style have pubescence,1.3cm long, style 1cm
Fruit	capsule, clavate, acute tip, 3.2 cm long, 0.5cm width	capsule, clavate, elongated tip, 2.8cm long, 0.4cm width	capsule, clavate, elongated tip, 2.3 cm long, 0.3cm width	capsule, clavate, elongated tip, 2.6 cm long, 0.4cm width

Table 3. Quantitative traits considered for PCA analysis

Plant height	Length of flower
Lamina breadth	Breadth of flower
Lamina length	Length of sepal
Petiole length	Length of inflorescence
Leaf length	Length of peduncle
Leaf area	Length of stamen
Petiole index	Length of gynoecium
Internodal length	
Number of lateral veins	Length of fruit
Number of flower	Breadth of fruit

Table 4. Qualitative traits considered for PCA analysis

1	Flower Colour
2	Anther colour
3	Bands on anther

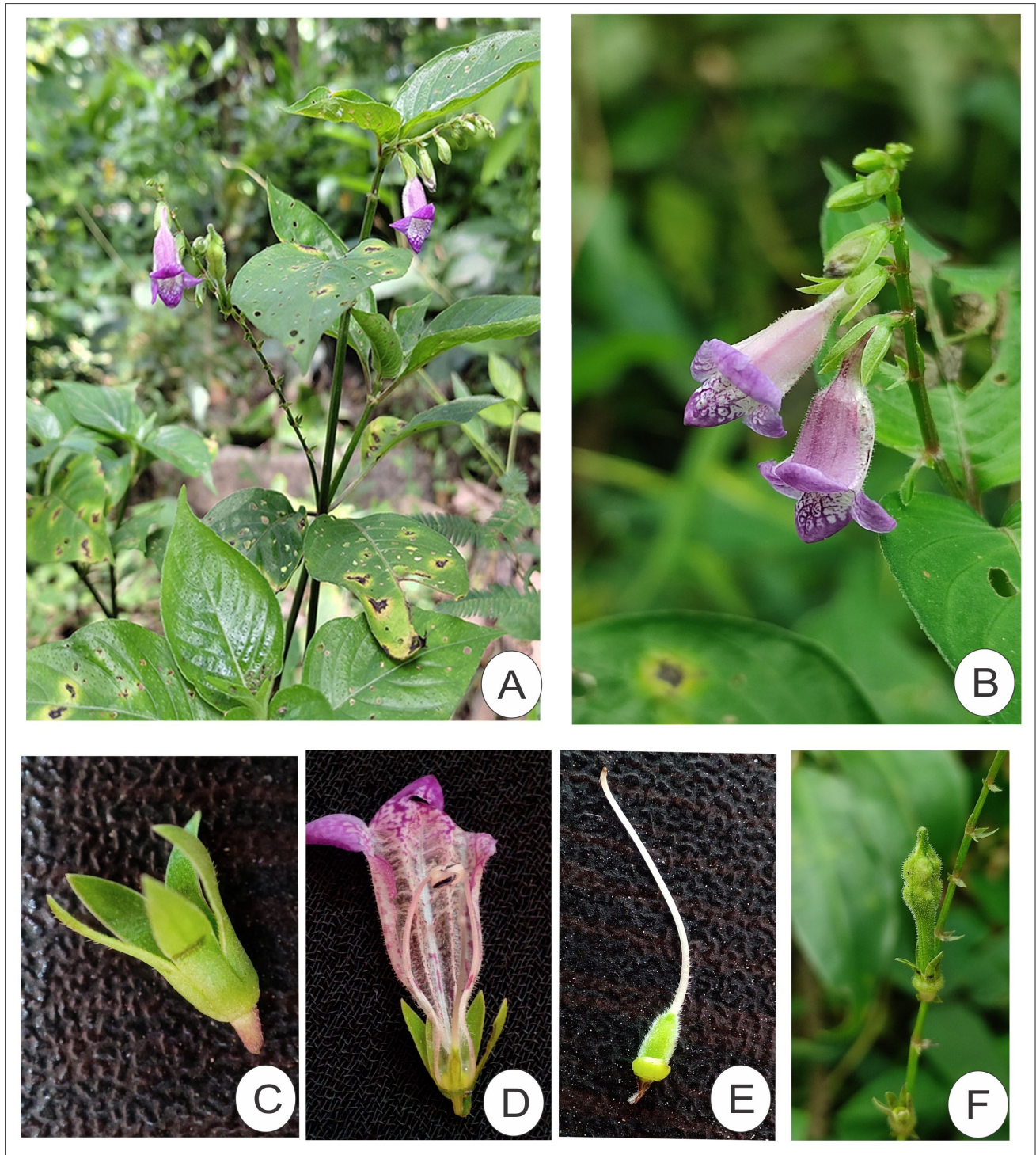


Fig. 3. *Asystasia dalzelliana* Santapau - color variant DL- 1. **A-**Habit, **B-** Inflorescence, **C-** Corolla front view, **D-** Stamen, **E-** Gynoecium, **F-** Opened corolla, **G-** Sectional view of flower, **H-** Sepal, **I-** Fruit.

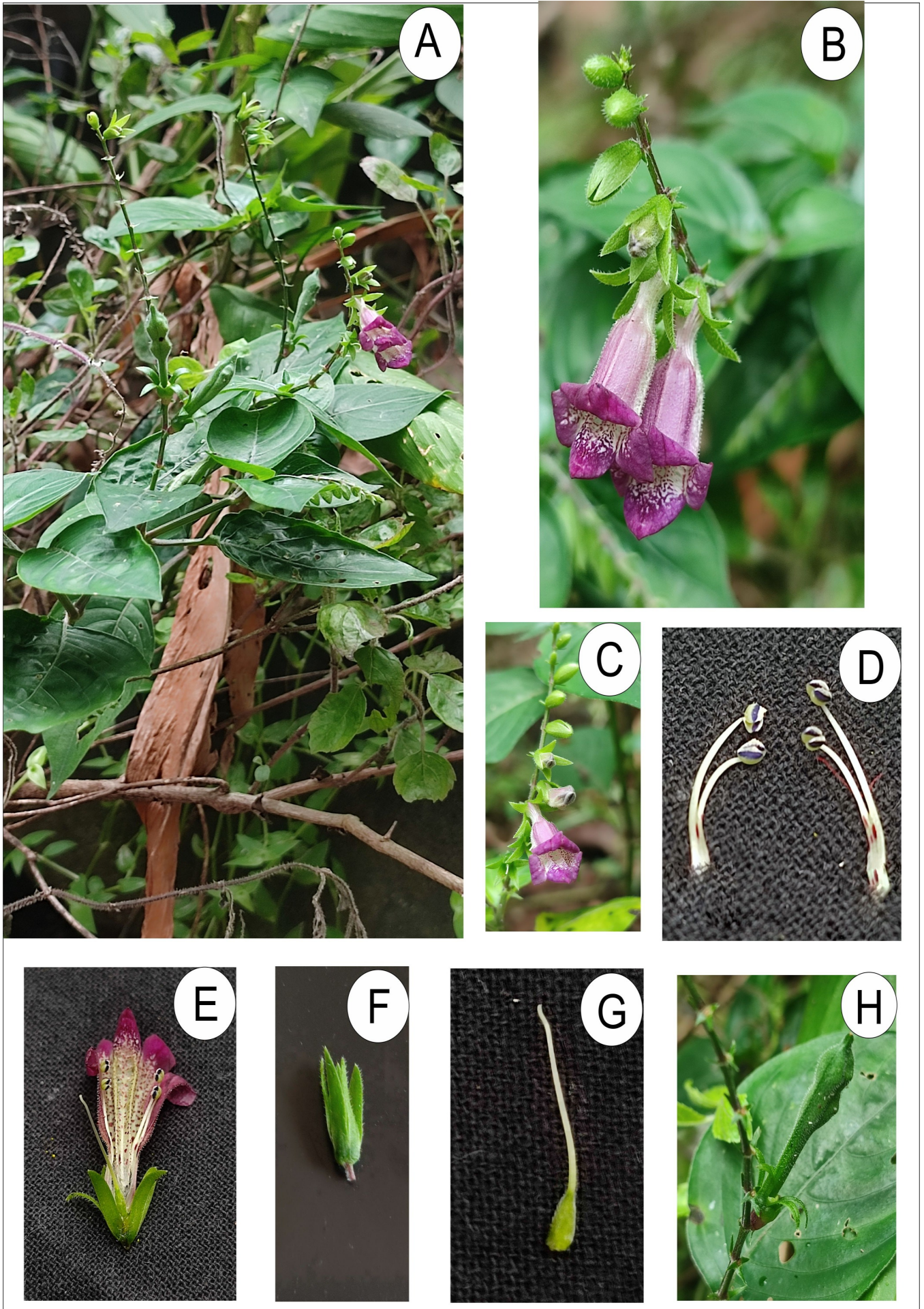


Fig. 4. *Asystasia dalzelliana* Santapau - color variant DL- 2. **A-** Habit, **B-** Inflorescence, **C-** Corolla front view, **D-** Stamen, **E-** Opened corolla, **F-** Sepal, **G-** Gynoecium, **H-** Fruit.

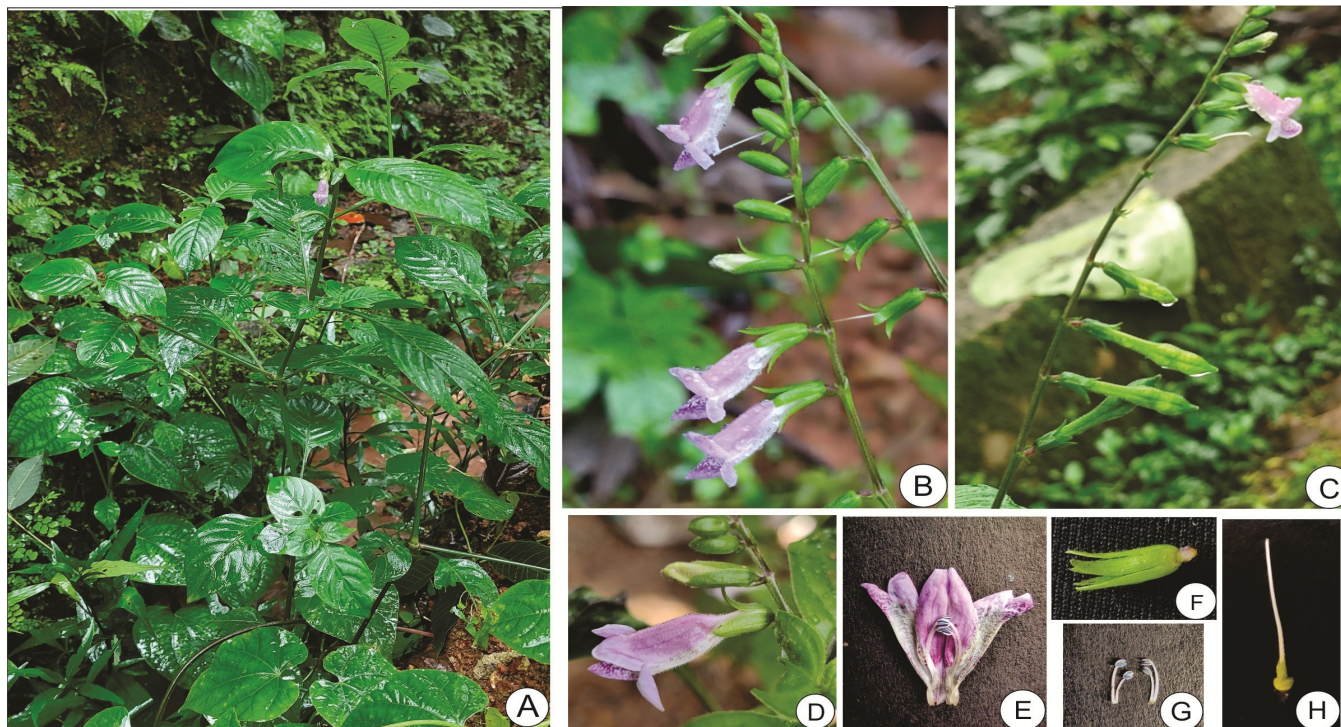


Fig. 5. *Asystasia dalzelliana* Santapau - color variant DL- 3. **A-**Habit, **B-** Inflorescence, **C-** Fruit, **D-** Flower side view, **E-** Opened corolla, **F-** Sepal, **G-** Stamen, **H -** Gynoecium.

distinctly different floral features (length of flower 1.5-1.9 cm; length of calyx 0.7 - 0.9 cm; length of lateral bracteole 0.1 cm; longer filament of anthers 0.8 cm; length of ovary 1.4 cm and style measuring 1 cm) when compared to the other three (Table 2).

As the Table 2 indicates, the variant accessions of *A.dalzelliana* show dissimilarities in the flower color as well as presence or absence of bands on anther. However, analysis of populations of individual variants do show the range of variability in their quantitative traits with respect to leaf length and breadth, plant height, length of petiole, pedicel, calyx, corolla and filament of anthers. The accessions DL-3 and DL-4 displayed highly restricted geographical distribution.

Population analysis of the four-flower color morpho - forms have been done for number of plants per population. A total of 25 populations were observed for DL 1, with a range of 10-15 plants per population; 15 populations observed for DL 2, with 7 - 12 plants per population. DL 3 and DL 4 shows limited number of populations with a range of 5 - 10 plants per population. DL 4 has least density of population and confined to the type locality at Kollam district of Kerala state (5). The population density was highest for DL-1 followed by DL-2, DL-3 and DL -4 (Fig.7).

There might be some kind of genetic modifications which would have contributed to the observed morphological variabilities which needs in depth investigations. However, the ecological factors have not been found to influence the observed morphological variations as the distribution of the morphoforms were not strictly confined to a particular geographical region except for *A.dalzelliana* var. *alba*. Furthermore, the easily observable gross morphological traits were seldom affected by the prevailing environmental conditions of the collection sites.

In the PCA, first three axes were considered for analysis. The first and second principal component of the accessions of *A. dalzelliana* color variants generated phenetic variance of about 41.56 % with eigen value 7.89 and 12.98 % with 2.46 eigen value. PC3 contributed 8.02 % phenetic variance with 1.52 eigen value (Table 5). The qualitative and quantitative characters selected for PCA are found to be influential in the PCA axis. The quantitative morphological characters like petiole length, internodal length, number of lateral veins, number of flowers, length of flower, length of sepal, length of inflorescence and peduncle as well as length of fruit have significant contribution to the first and second variable axis.

In the PCA of qualitative data of the accessions of *A.dalzelliana* color variants, the first and second principal component were analyzed as 76.18 % phenetic variance with eigen value 2.28 and 21.55 % with eigen value 0.64. The variability percent of PC3 is 2.26 % with eigen value 0.06. The anther color and presence or absence of black blue bands on anther are influential characters which largely control PCA axis (Table 6). The quantitative traits except number of flower, breadth of flower, length of sepal and length of gynoecium exhibit P value >0.05, which indicate its less significance in the study (Table 7).

The two-dimensional scatterplot for PC1 and PC2 axis were constructed, based on normalized variance - covariance matrix. The analysis resulted in the clustering of the accessions of *A. dalzelliana* morpho forms into three. Cluster1 includes accessions of DL1 and DL 2, cluster 2 and 3 include only the accessions of DL 3 and DL 4 respectively. The cluster analysis divides the four variants into three clusters, indicating the infraspecific phenotypic variability among the accessions (Fig. 7).

The UPGMA phenogram displays clustering pattern with DL-1 and DL-2 in a similar clade, DL-3 and DL-4 in

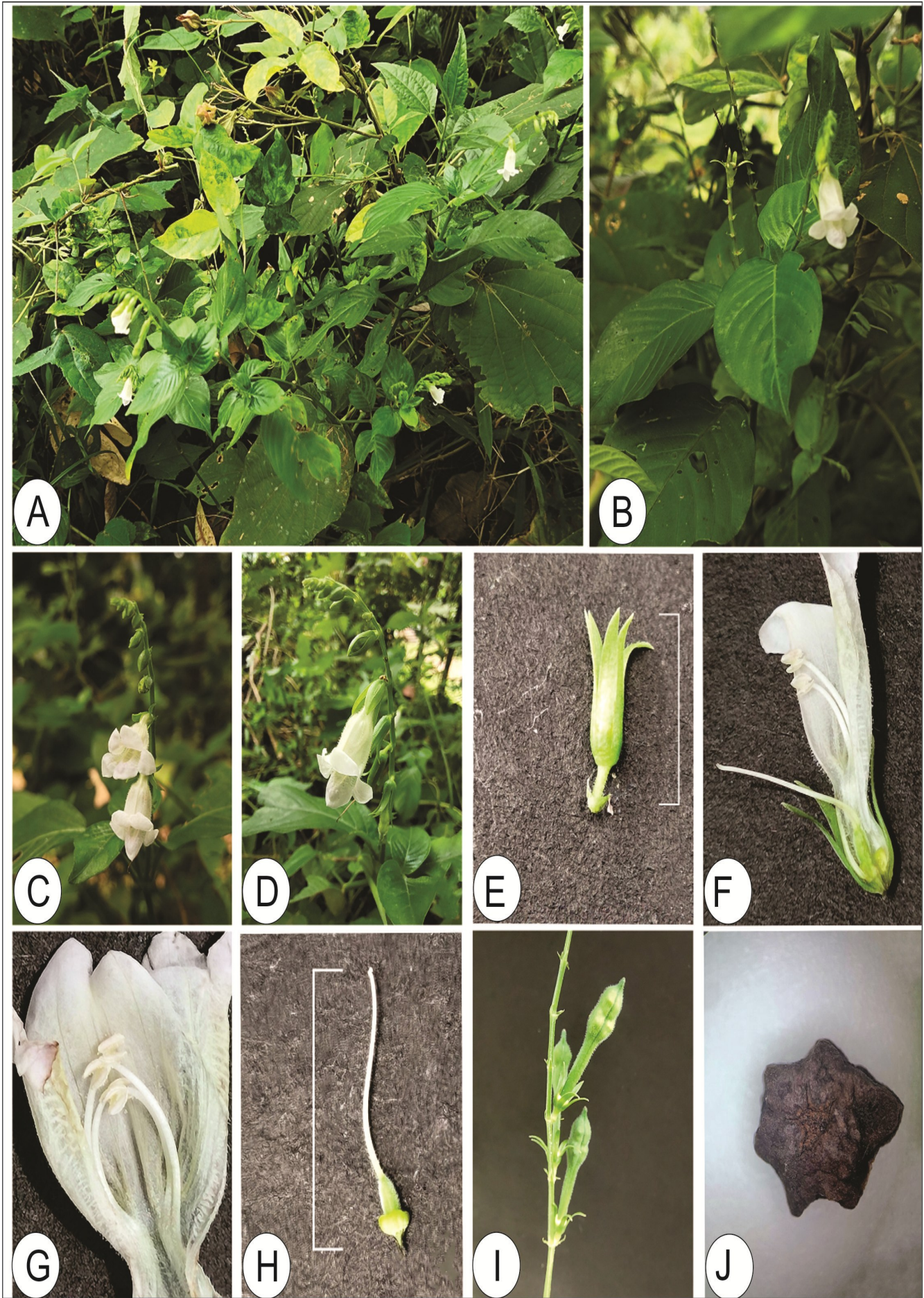


Fig. 6. *Asystasia dalzelliana* Santapau var. *alba* - color variant DL-4. **A-**Habitat, **B-** Habit, **C-** Corolla front view, **D-** Inflorescence, **E-** Sepal, **F-** Sectional view of flower **G-** Opened corolla, **H-** Gynoecium, **I-** Fruit, **J-** Seed.

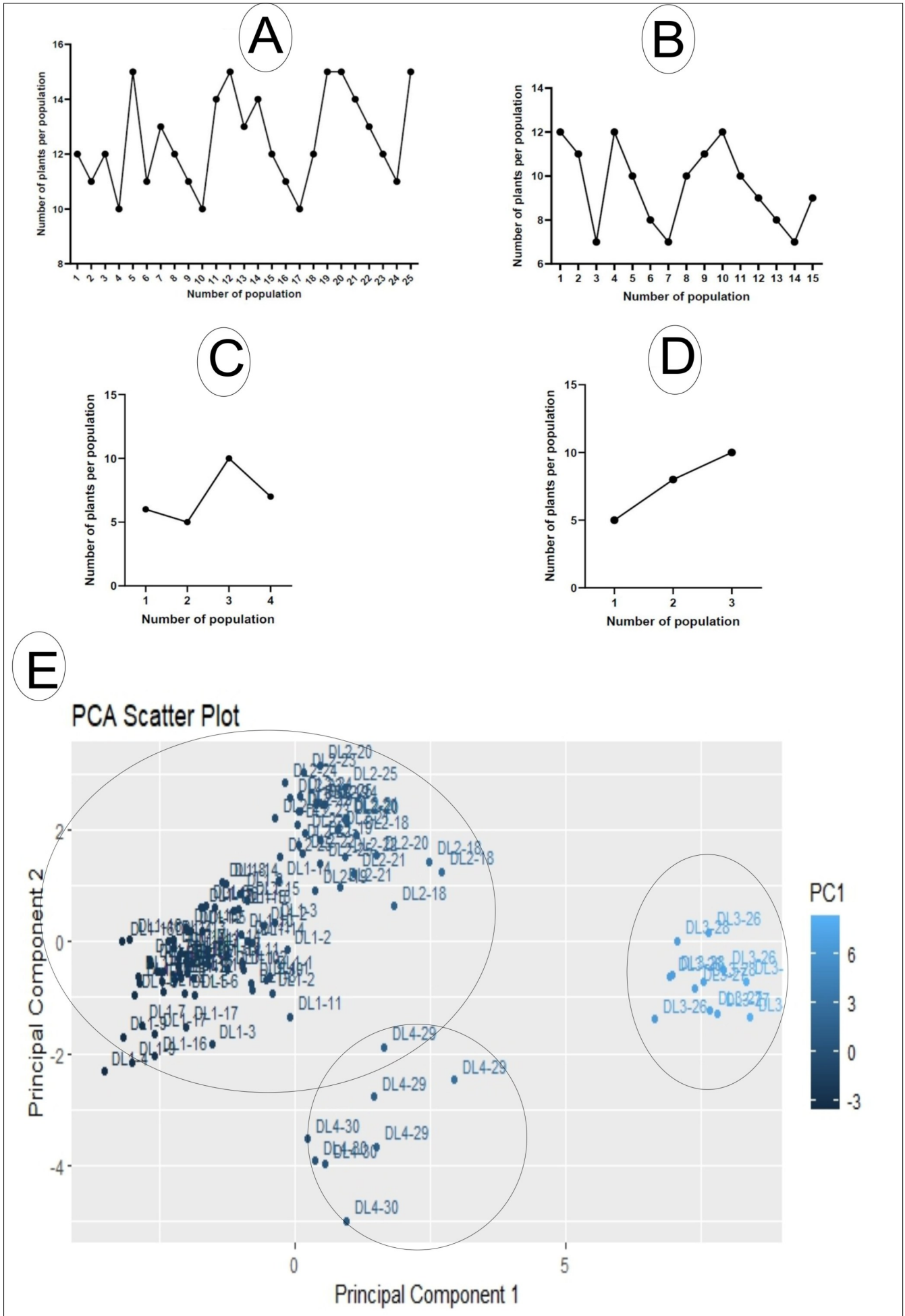


Fig. 7. Population density analysis of four color variants of *Asytasia dalzelliana* Santapau **A**- DL1, **B**- DL2, **C**- DL3, **D**- DL4. **E**- Two dimensional scatterplot.

Table 5. Principal component analysis for quantitative traits

Principal component analysis	Axis 1	Axis 2	Axis 3
Eigen value	7.89	2.46	1.52
Variability	41.565	12.983	8.025
Cum. Percentage	41.565	54.548	62.573
PCA variable loadings	F1	F2	F3
plant height	0.768	0.284	-0.219
lamina breadth	0.742	-0.493	-0.180
lamina length	0.891	-0.282	0.102
petiole length	0.912	-0.107	0.032
leaf length	0.934	-0.245	0.087
leaf area	0.836	-0.466	-0.100
petiole index	0.655	0.171	0.071
internodal length	0.840	-0.077	-0.168
no. 20 lateral veins	0.273	-0.413	0.650
number of flower	-0.033	0.381	0.250
length of flower	0.626	0.086	-0.571
breadth of flower	0.337	0.029	-0.023
length of sepal	-0.248	-0.561	0.349
length of inflorescence	0.531	0.719	0.239
length of peduncle	0.549	0.702	0.191
length of stamen	0.529	0.183	0.485
length of gynoecium	0.078	-0.093	-0.172
length of fruit	0.847	0.204	-0.050
breadth of fruit	0.443	-0.091	0.293

Table 6. Principal component analysis of qualitative traits

Principal component analysis	Axis 1	Axis 2
Eigen value	2.286	0.647
Variability	76.185	21.551
Cum. Percentage	76.185	97.737

Table 7. Variance for 19 quantitative characters

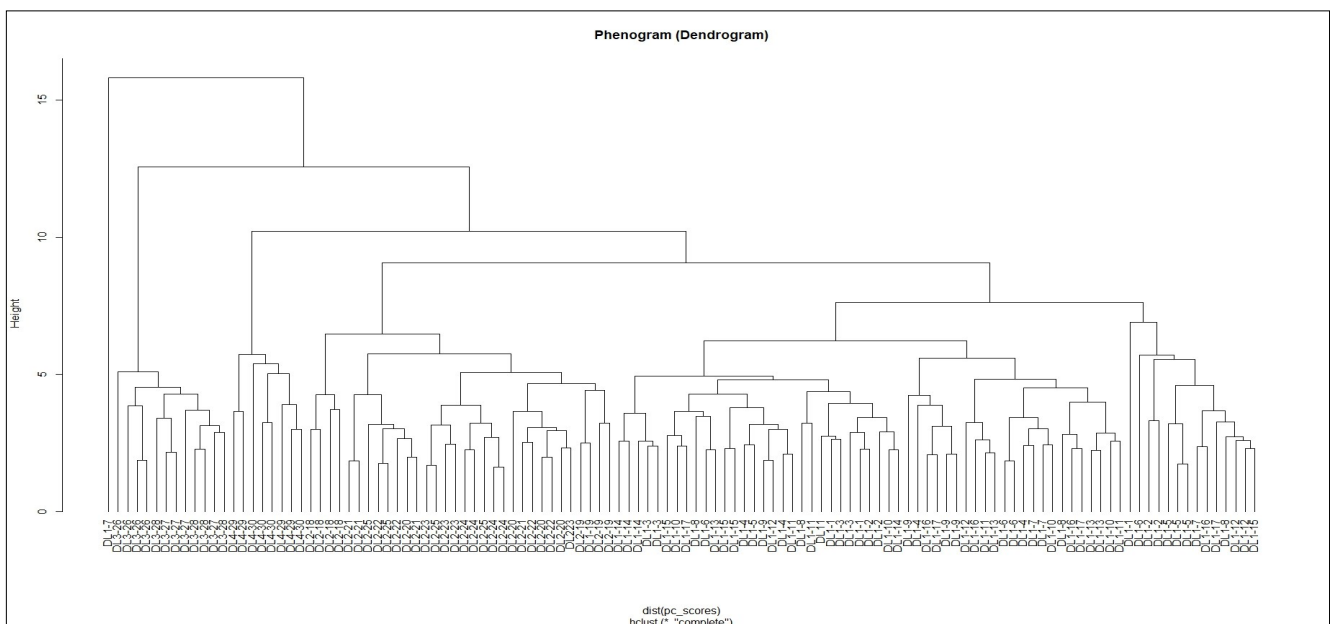
Character	F value
Plant height	32.506***
Lamina length	29.431***
Lamina breadth	40.306***
Petiole length	25.403***
Leaf length	52.686***
Leaf area	43.709***
Petiole index	7.609***
Intermodal length	16.451***
No.of secondary lateral vein	4.615***
No.of flower	1.750
Length of flower	16.302***
Breadth of flower	2.021
Length of sepal	2.220
Length of inflorescence	59.371***
Length of peduncle	40.344***
Length of stamen	4.759***
Length of gynoecium	0.997
Length of fruit	14.099***
Breadth of fruit	2.917***

***Significance at P<0.05

separate distinct groups (Fig. 8). The two-dimensional scatter plot substantiates the grouping of the collected accessions as displayed in the scatterplot.

The most justifiable interpretation for the obtained results is that the observed morpho forms of *A.dalzelliana*, spread across the geographical regimes of the state, may cause confusion to field botanist at first glance. Distinct variability could be observed with the variants DL4 owing to

its white flower color and lack of pattern in the anthers. This has already been hierarchically categorized. It also seems that the habitat or the environmental conditions of these morpho-forms seldom affect the color variability as the authors could collect the same variant from more than one location which were quite distant. It is reasonable to assume that the variability in flower color can be the

**Fig. 8.** Cluster analysis based on morphological characters.

manifestation of some gene expression, unbiased by environmental conditions and the distribution of the same morphoform in different locations could be the result of migration. Further, it can even be considered as some sort of evolutionary transitional forms, which needs much more time for accurate prediction. The conservation strategies of such morpho forms are equally important in the case of medicinal plants like *Asystasia* as the overexploitation of the taxa can lead to extinction in near future.

Conclusion

The study evaluates the diversity among *A. dalzelliana* morphoforms and their distribution pattern. The traits selected for the analysis demarcates the morphoforms which has been clearly reflected in the grouping patterns of two-dimensional scatterplot as well as dendrogram. The variations shown, though seems to be apparent, banding pattern on anthers as well as nature and color of corolla can serve as reliable features for hierarchical ranking. The observed population frequency reveals the importance of conservation to ensure the survival of the biologically interesting morphoforms as there exists high degree of possibility for the extinction of these taxa, coupled with depletion of biodiversity. Conservation of various morphoforms documented from different regions through *ex situ* or *in situ* means is highly warranted.

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Authors' contributions

The authors contributed equally to the present work.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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