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RESEARCH ARTICLE

Phenotyping and diversity assessment of *Dendrobium* species by using DUS guidelines

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Abstract

Dendrobiums are sympodial epiphytic orchids belonging to the family Orchidaceae. and have an extraordinary variation in bloom shape, size and colour. They are widely recognized for their enchanting beauty and long-lasting quality. Morphological characterization of *Dendrobium* species was carried out using the DUS guidelines developed by the Protection of Plant Varieties and Farmers Rights Authority (PPV and FRA) in New Delhi. A total of 15 *Dendrobium* species were characterized for 52 vegetative and floral traits. Among the 52 traits, 8 were monomorphic, 12 were dimorphic and 32 were polymorphic, indicating their potential for characterization. Principal component analysis showed 86.14% variation in the first 4 principal components with an eigenvalue >1.0. The first and second principal components explained 41.43% and 19.45% of the total variation respectively. The dendrogram produced by a Ward analysis validated the Principal Component Analysis (PCA) findings. The *Dendrobium* species were grouped into 2 major clusters with three sub-clusters based on the correlation between the morphological traits among the species. Characterization established distinctiveness amongst the *Dendrobium* species. Breeders/farmers can use this study to distinguish between different species and apply for protection through the PPV and FR Authority, New Delhi. The trait-specific data generated while characterizing can be used to develop new inter-specific hybrids, which are common in orchids.

Keywords

characterization; *Dendrobium* orchids; DUS guidelines; ; Farmers Rights Authority; geographical coordinates; Protection of Plant Varieties

Introduction

Orchids contribute around 9% of the total flora and are one of the most prominent families of flowering plants. The total orchid population is estimated to be around 25000 out of which 1350 species have been reported from India. Almost 950 species (nearly 69%) have been documented from North East India (1). Amongst the orchids, *Dendrobium* constitutes the second largest genus of the orchid family, next to *Bulbophyllum*. *Dendrobiums* are epiphytic and native to Southeast Asia and they can be found in their natural habitat from North East India to the islands of New Guinea and Polynesia. *Dendrobium* hybrids are the most popular orchids for

the commercial production of cut flowers and potted plants in the global trade (2).

North East India has been in the spotlight for its rich biodiversity and traditional knowledge, which have greatly interested botanists and plant conservation agencies worldwide. The diversity of *Dendrobiums* in the region offers a large amount of genetic diversity for bio-prospecting. Considering the enormity and diversity of orchids in general and *Dendrobium* species in particular, a networking approach is required, which could share the responsibility of germplasm collection, characterization, conservation evaluation and maintenance of precious genetic resources. This species' study identification and preservation will play a part in meeting crop improvement programmes' present and future needs. Among various tools available for assessing the genetic variability and relatedness among crop genetic resources, morphological characterization is the first step in the description and organization of the germplasm of any crop. Identifying a genetic relationship or genetic divergence in orchids is difficult due to the variable phenotypic expression of different traits. Characterization of genetic diversity and genetic relationships of orchids is essential for conservation and increasing the use of plant genetic resources. For use in crop improvement programmes, it is pertinent to understand the relationship between different *Dendrobium* species and likewise, the morphological relationships can be used to assemble more potential genotypes. A detailed, in-depth study of genetic diversity and its morphological characterization is essential to identify potential genotypes that could be used in hybridization programmes (3).

Despite Dendrobium's enormous genetic diversity and unique characteristics, orchids have not been optimally used in plant breeding programmes. Plant characterization is a method for qualitatively and quantitatively determining plant traits (4). To identify the differences in the characters of each species, morphological characterization is essential to identify the plants for their specific characteristics. It is also a vital step in the conservation process. The morphological parameters vary amongst the species, orders, or families while adapting to distinct environmental conditions. It gives an insight into the closeness of genetic relationships among orchid species in the same genus, which determines the success of plant crossing (5). Characterizing species based on morphological traits provides relevant information about the important agronomic traits, which are expressed due to the genetic makeup of species (6). This enables the classification of similar or dissimilar varieties based on the expression of the traits (7).

Dendrobium orchids are epiphytic and with the rampant destruction of forest areas, the species is facing extinction and needs to be conserved through proper plant breeding programmes. The Protection of Plant Varieties and Farmers Rights Act 2001 (PPV and FR Act) was enacted by the Indian government to encourage public and private investment in plant genotype research and development (8). To be protected under the Act, plant genotypes/species must meet the distinctiveness, uniformity and stability (DUS) criteria. Hence, there is a need to characterize *Dendrobium* species according to DUS testing guidelines (9). Despite the evolutionary significance of the *Dendrobium* orchids, the research reports on the genetic diversity of *Dendrobium* species are scanty which is essential for genetic conservation and plant breeding programmes. Considering the need for conservation and future use in breeding programmes, the present study was conducted to characterize 15 *Dendrobium* species for qualitative and quantitative traits per the DUS guidelines.

Material and methods

Plant material and experimental site

The species were identified using the reference book 'Orchid Flora of Arunachal Pradesh' (10) and by consultation with the scientist from Tippi Orchid Research Centre, West Kameng, Arunachal Pradesh. The experimental materials comprised 15 species collected from different locations in the East Siang district of Arunachal Pradesh: Rengging (5 species), Bodak (3 species), Pasighat (2 species) and Sirki (1 species) (Table 1; Fig. 4) and were established and evaluated at the Department of Floriculture and Landscape Architecture, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, during 2020 to 2022 for two consecutive seasons. The experiment was carried out in three replications, each replication consists of 10 plants. The experiment was laid out in a Completely Randomized Design with three replications.

Distinctiveness, Uniformity and Stability (DUS) Characterization

Aligned with the DUS descriptor (9), this study characterized the plant species for various attributes: plant, leaf and flower. Data were recorded on 10 randomly selected plants from each replication for 52 descriptors.

Plant characters

Plant height (cm), nature of shoot, internode number, internode diameter (cm), inflorescence number per plant, inflorescence orientation, inflorescence length (cm) and peduncle length (cm).

Leaf characters

Number of leaves, leaf length (cm), leaf width (cm), leaf shape and leaf apex.

Flower characters

Flower number per inflorescence, flower length (cm), flower width in front view (cm), flower fragrance, flower longevity on the plant, dorsal sepal length (cm), dorsal sepal width (cm), dorsal sepal shape, dorsal sepal apex, lateral sepal shape, lateral sepal apex, sepal dominant colour, sepal colour pattern inside, petal length (cm), petal width (cm), petal shape, petal curvature, petal apex, petal predominant colour, petal colour number inside, lip length (cm), lip width (cm), lip shape, lip apex, lip curvature, lip lobation, lip predominant colour, lip colour number, lip

Table 1. List of species collected from different locations of East Siang district of Arunachal Pradesh, along with GPS coordinates

Sl. No.	Species (code)	Location	Latitude	Longitude	Elevation
1	*Dendrobium lituiflorum* (D1)	Rengging	28°8'25" N	95°16'39" E	366.35 m
2	*Dendrobium aphyllum* (D2)	Pasighat	28°4'25" N	95°19'48" E	167.25 m
3	*Dendrobium primulinum* (D3)	Bodak	28°14'13" N	95°27'89" E	150.00 m
4	*Dendrobium fimbriatum* (D4)	Rengging	28°5'51" N	95°16'10" E	292.41 m
5	*Dendrobium nobile* (D5)	Rengging	28°8'25" N	95°16'41" E	372.48 m
6	*Dendrobium chrysotoxum* (D6)	Rengging	28°8'26" N	95°16'41" E	335.15 m
7	*Dendrobium densiflorum* (D7)	Rengging	28°8'21" N	95°15'28" E	582.6 m
8	*Dendrobium nobile* var. *alba* (D8)	Rengging	28°9'3" N	95°14'13" E	674.31m
9	*Dendrobium macraei* (D9)	Pasighat	28°06'19" N	95°32'60" E	160 m
10	*Dendrobium jenkinsii* (D10)	Bodak	28°14'13" N	95°27'89" E	150 m
11	*Dendrobium wardianum* (D11)	Sirki	28°8'22" N	95°15'28" E	570 m
12	*Dendrobium thyrsiflorum* (D12)	Bodak	28°90'17" N	95°15'51" E	180 m
13	*Dendrobium devonianum* (D13)	Panging	28°10'9" N	95°13'35" E	320 m
14	*Dendrobium chrysanthum* (D14)	Rengging	28°8'22" N	95°15'29" E	498.05 m
15	*Dendrobium eriiflorum* (D15)	Rengging	28°7'21" N	95°16'25" E	279.82 m

colour pattern, lip margin, lip callus, lip surface texture, column width across stigma (cm), column colour number, column colour pattern, anther cap colour, spur/ mentum length (cm) and flowering season.

Observations of the leaf, shoot and internode were recorded on the flowering shoot. The assessment of inflorescence and the flower were recorded when 50% of the flowers on the inflorescence had opened. The length and width of the flower were recorded on the spread-out positions. All the observations of the sepal, petal, lip and column colour were recorded on the inner side at the apex, mid and base portions using the Royal Horticultural Society (RHS) colour chart (sixth edition).

Statistical analysis

The 15 species of *Dendrobium* were planted in a completely randomized design with three replications**.** The quantitative data were subjected to multivariate cluster analysis based on Ward's method (11) and Principal Component Analysis was carried out using XLSTAT version 2021.2.2 (12).

Results and discussion

Morphological traits have been the most widely used tool by breeders to distinguish between lines or cultivars because of their genetic expression. There were significant variations amongst the 15 Dendrobium species for all the essential characters. The qualitative characters are more consistent and are considered reliable for characterizing species (13). Out of 31 visually assessed DUS descriptors, three were monomorphic, eight were dimorphic and 20 were polymorphic, while out of the 21 measurable traits, 5 were monomorphic, 4 were dimorphic and 12 were polymorphic (Supplementary Table 1). The sepal dominant colour, petal predominant colour, lip predominant and anther cap colours are as per the RHS colour chart (Table 2).

Plant characters

In general, the plant height in 15 species of *Dendrobium* ranged from very small to very large, i.e., very small 3 (D6, D10, D15), small 6 (D3, D8, D9, D11, D13, D14), medium 3 (D5, D7, D12), large 2 (D1, D4) and very large 1 (D2), respectively. Wide variations in plant height were also observed in orchids characterized by using DUS guidelines (14). From the visual observations, four types of nature of shoot were reported amongst the species, *viz.*, cane woody 2 (D2, D13), cane cylindric fleshy 7 (D1, D3, D4, D5, D8, D11, D14), cane clavate fleshy 2 (D7, D12) and bulbous 4 (D6, D9, D10, D15) (Supplementary Fig. 1). Similar variations in nature of shoots in orchids have been reported (15, 16). Depending upon internode number, species were categorized into few in 3 species (D6, D10, D15), medium in 6 species (D7, D8, D9, D11, D12, D13), many in 6 species (D1, D2, D3, D4, D5, D14) and internode diameter was monomorphic, i.e., all 15 species recorded < 2cm diameter (D1 to D15). These traits were also used for conforming distinctiveness amongst the *Dendrobium* species (17) and orchid genera (18). The observations recorded for inflorescence-related traits have revealed that 9 species have few (D4, D6, D7, D9, D10, D11, D13, D14, D15), 3 medium (D5, D8, D12) and 3 had many (D1, D2, D3) inflorescences number per plant. Inflorescence orientation recorded amongst the species was dimorphic, where 2 were arching (D9, D10) and 13 were pendulous (D1, D2, D3, D4, D5, D6, D7, D8, D11, D12, D13, D14, D15). Inflorescence and peduncle lengths were recorded as short for 15 species, i.e., < 20cm and < 10 cm (D1 to D15) respectively. Variations for inflorescence-related traits of a species seem to be genetically controlled, as reported in *Vanda* hybrids (19), *Paphiopedilum* species (20) and *Dendrobium lituiflorum* (21).

Leaf characters

About the number of leaves, the different species of *Dendrobium* under study were noted to have few (<3) leaves for three species (D9, D10), 9 (3-6) as medium (D3,

Table 2. Sepal dominant colour, petal predominant colour, lip predominant and anther cap colour as per RHS colour chart

Sl. No.	Species	Sepal dominant colour	Petal predominant colour	Lip predominant colour	Anther cap colour
1	*Dendrobium lituiflorum* (D1)	White group (NN155D)	White group (NN155D)	Purple group (N78A)	Purple group (N78A)
2	*Dendrobium aphyllum* (D2)	White group (NN155D)	White group (NN155D)	Yellow group (10D)	Yellow group (4D)
3	*Dendrobium primulinum* (D3)	Purple group (N78C)	Purple group (N78C)	White group (NN155A)	Yellow group (4D)
4	*Dendrobium fimbriatum* (D4)	Yellow group (13C)	Yellow group (15A)	Yellow group (5B)	Yellow group (4D)
5	*Dendrobium nobile* (D5)	Purple group (N78A)	White group (NN155C)	Violet group (N79B)	Purple group (N78A)
6	*Dendrobium chrysotoxum* (D6)	Yellow group (3D)	Yellow group (3D)	Yellow group (17A)	Yellow group (4D)
7	*Dendrobium densiflorum* (D7)	Yellow group (8C)	Yellow group (8C)	Yellow group (15A)	Yellow group (4D)
8	*Dendrobium nobile* var. *alba* (D8)	White group (NN155C)	White group (N155D)	Purple group (N77A)	Purple group (N78A)
9	*Dendrobium macraei* (D9)	White group (NN155D)	White group (NN155D)	White group (NN155D)	White group (NN155A)
10	*Dendrobium jenkinsii* (D10)	Yellow group (17A)	Yellow group (17A)	Yellow group (24B)	Yellow group (4D)
11	*Dendrobium wardianum* (D11)	White group (NN155D)	White group (NN155D)	Yellow group (3A)	White group (NN155D)
12	*Dendrobium thyrsiflorum* (D12)	Yellow group (9D)	Yellow group (9D)	Yellow group (14A)	Yellow group (9D)
13	*Dendrobium devonianum* (D13)	White group (NN155D)	White group (NN155D)	Yellow group (5A)	White group (NN155D)
14	*Dendrobium chrysanthum* (D14)	yellow group (16A)	Yellow group (16A)	Yellow group (16A)	Yellow group (11D)
15	*Dendrobium eriiflorum* (D15)	white group (NN155D)	White group (NN155D)	White group (NN157D)	White group (NN157B)

D4, D6, D7, D8, D11, D12, D14, D15) and 4 (>7) as many (D1, D2, D5, D13). Similar findings were also reported in *Dendrobium* species, where they classified the number of leaves into 4 groups (10). The leaf length of 15 species ranged from very short to long, where one species (D10) was very short (<5 cm), 5 (D1, D2, D3, D9, D13) as short (5-10 cm), 7 (D5, D6, D7, D8, D11, D14, D15) as medium (10.1-15 cm) and 2 (D4, D12) as long (>15 cm) were observed. The leaf width for 15 species were grouped into 4 groups, *i.e.,* 4 (D9, D10, D14, D15) were narrow (<2 cm), 9 (D1, D2, D3, D4, D5, D6, D8, D11, D13) medium (2-4 cm), 1 (D12) broad (4.1-6 cm) and 1 (D7) was recorded very broad (>6 cm). All the species were categorized into 3 groups based on their leaf shape, including one species as elliptic (D10), 12 species as lanceolate (D1, D2, D4, D5, D6, D8, D9, D11, D12, 13, D14, D15) and 2 with ovate (D3, D7) leaf shape. About the apex characteristics of the leaves, the observed species were divided into 4 groups, *viz*., acute 7 (D1, D2, D3, D7, D10, D12, D13), obtuse 1 (D9), retuse 5 (D5, D6, D8, D11, D15) and acuminate 2 (D4, D14) species (Supplementary Fig. 2). A similar study on leaf characters using DUS descriptors has also been reported in commercial orchids (14), *Dendrobium* species (17, 22), *Mokara* orchids (23) and in *Coelogyne* species (24).

Flower Characters

Based on the flower number per inflorescence, 11 species (D1, D2, D3, D5, D8, D9, D10, D11, D13, D14, D15) were classified as few (73.33%), 2 species as medium (13.33%) and the other 2 had many (13.33%). It was reported similar results concerning the flower number per inflorescence in *Dendrobium species* (25). In respect to flower length, species have been grouped into 3 categories, *viz.,* small 6 (D2 D9, D10, D12, D14, D15), medium 8 (D1, D3, D4, D5, D6, D7, D8, D11) and long 1 (D13). The flower width in the front view of species was recorded as narrow in 5 (<3 cm) (D7, D9, D10, D12, D15), medium in 6 (3-6 cm) (D1, D2, D3, D4, D6, D14) and broad in 4 (>6 cm) species (D5, D8, D11, D13) (Fig. 1). Similar findings have also been reported in *Dendrobium* species (22) and commercial orchids (15). All 15 species of *Dendrobium* under study were noted to have flower fragrance, which is desirable for the crop improvement programme. Based on the flower longevity, it was grouped into 2 categories: short in 12 (< 15 days) species (D1, D3, D4, D6, D7, D9, D10, D11, D12, D13, D14, D15) and medium in 3 (15- 30 days) species (D2, D5, D8). This was comparable with the study (21) in *Dendrobium lituiflorum*.

Depending on sepal and petal characteristics, dorsal sepal length ranged from short to long; 6 species (D6, D7, D9, D10, D14, D15) were short, 7 medium (D1, D2, D3, D4, D5, D12, D13) and 2 species (D8, D11) were long. Dorsal sepal width was found to be dimorphic, narrow in 7 species (D1, D2, D3, D6, D9, D10, D15) and medium in 8 species (D4, D5, D7, D8, D11, D12, D13, D14). Among sepal shapes, 1 species (D2) was observed to have a linear shape, 6 species (D1, D3, D5, D6, D8, D12) were oblong and 8 species (D4, D7, D9, D10, D11, D13, D14, D15) exhibited elliptic shape. Dorsal sepal apex was grouped as acute 4 (D1, D9, D10, D13) and obtuse 11 (D2, D3, D4, D5, D6, D7, D8, D11, D12, D14, D15).

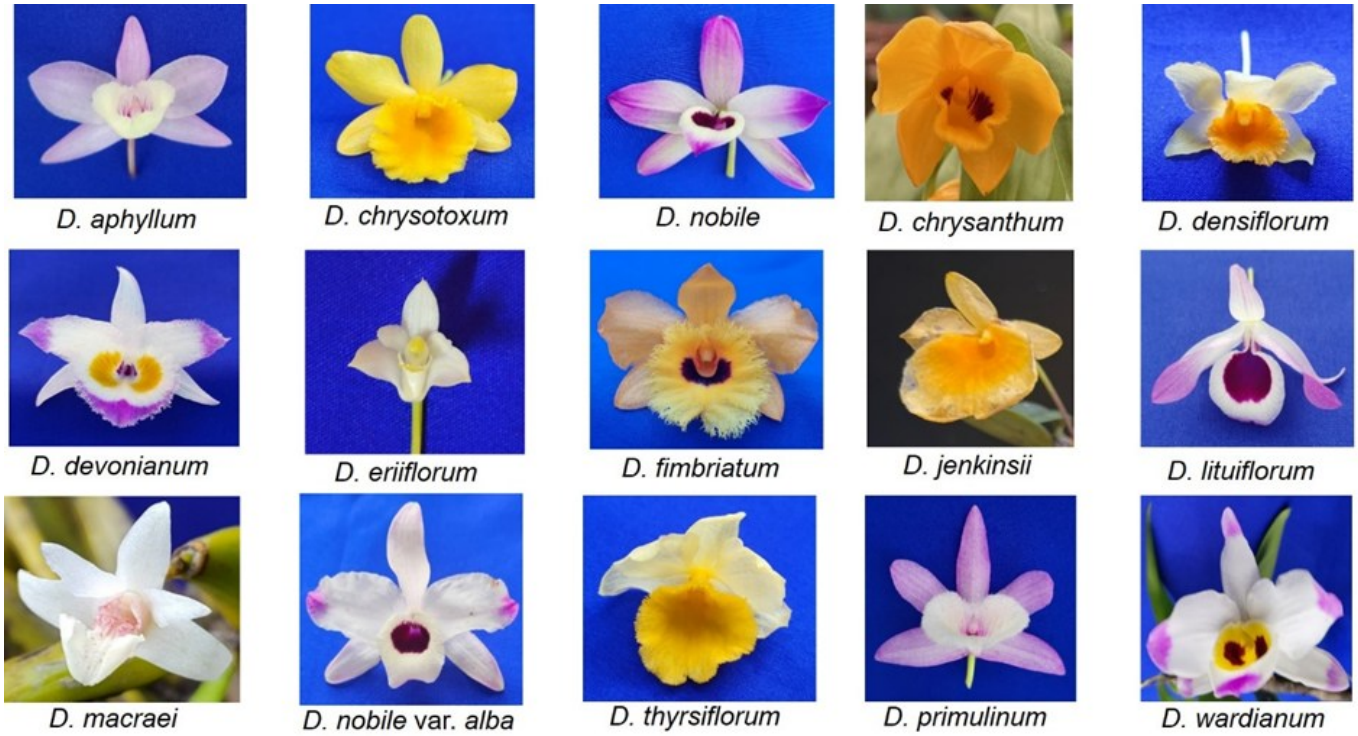

Fig. 1. Variations in flower colour and flower shape among *Dendrobium* species.

Lateral sepal shape was recorded as linear in 1 species (D1), elliptic in 4 (D7, D9, D10, D11), oblong in 4 (D3, D5, D6, D8) and triangular in 6 species (D2, D4, D12, D13, D14, D15). Based on lateral sepal apex, 7 species (D2, D4, D5, D8, D12, D15) were classified as acute, 2 (D1, D13) in acuminate, whereas 6 species (D3, D6, D7, D9, D10, D11, D14) showed obtuse sepal apex. Petal length was reported small in 6 species (D6, D7, D9, D10, D14, D15), 6 medium (D1, D2, D3, D4, D12, D13) and 3 to be long (D5, D8, D11). Petal width was found narrow in 12 (< 2 cm) species (D1, D2, D3, D4, D6, D7, D9, D10, D12, D13, D14, D15) and medium in 3 (2-3 cm) species (D5, D8, D11). *Dendrobium* species were categorized into 5 groups for petal shape, *viz.*, linear 1 (D9), oblong 1 (D3), elliptic 3 (D2, D7, D10), obovate 2 (D4, D12) and ovate 8 species (D1, D5, D6, D8, D11, D13, D14, D15). Based on the petal curvature, species were grouped into 2 categories, *viz.,* straight 6 (D2, D4, D6, D7, D9, D15) and deflexed 9 species (D1, D3, D5, D8, D10, D11, D12, D13, D14). The visual observations on the petal apex showed that 4 species (D5, D8, D10, D13) were classified under acute, 1 (D9) in acuminate, 9 species (D1, D2, D3, D4, D6, D7, D11, D12, D15) under obtuse and 1 species (D3) had a retuse apex. The present findings conform with (20) *Paphiopedilum* species, where they observed different types of sepal and petal characteristics in orchids while characterizing the species per DUS guidelines.

Based on the characteristics of the sepal and petal, 3 classes of sepal dominant colours were reported amongst the species: white, yellow and purple. White colour (NN155D, NN155C) was noticed in 7 species, yellow colour (13C, 3D, 8C, 17A, 9D, 16A) in 6 species and 2 species showed purple colour (N78C, N78A). 5 species were found to have a uniform pattern about the sepal colour pattern, while 10 species exhibited a shaded/striped colour pattern. Results of the petal predominant colour showed that 8 species were white (NN155D), 6 species yellow (15A, 3D, 8C, 17A, 9D,16A) and 1 species had purple (N78C) colour. Species were grouped into 3 categories for the petal colour number inside single 8, double 6 and triple 1. Based on petal colour pattern, out of 15 species of *Dendrobium*, 4 had uniform colour patterns, 10 species striped/shaded and 1 species had netted petal colour pattern (Table 2 and Fig. 1). Similar findings were also reported in *Dendrobium lituiflorum* (21), *Coelogyne* species (24), *Phalaenopsis* species (26) and *Vanda* species (27) where the orchid species were characterized based on sepal and petal characteristics using RHS colour chart.

The characters related to lip, column and spur have shown that the lip length was found to be dimorphic, 3 (<2cm) had short (D6, D9, D15) and 12 (2-4 cm) species (D1, D2, D3, D4, D5, D7, D8, D10, D11, D12, D13, D14) with medium lip. Lip width was categorized as narrow in 5 (< 2cm) species (D6, D9, D10, D14, D15) and medium in 10 (2-4 cm) species (D1, D2, D3, D4, D5, D7, D8, D11, D12, D13). On the basis of lip shape, 3 species (D1, D5, D8) had ovate shape, 2 species (D9, D15) oblanceolate and 10 species (D2, D3, D4, D6, D7, D10, D11, D12, D13, D14) exhibited orbicular lip shape. Lip apex was classified into 4 groups, *viz.,* acute 5 (D5, D8, D11, D13, D15), retuse 4 (D2, D3, D9, D10), obtuse 1 (D1) and praemorse 5 species (D4, D6, D7, D12, D14). Lip curvature was classified into 4 groups, *viz.,* straight 1 (D4), reflexed with straight apex 4 (D2, D7, D11, D14), reflexed with curved apex 7 (D1, D3, D5, D6, D8, D12, D13) and incurved with straight apex 3 (D9, D10, D15). Lip lobation and lip callus were present in all the species of *Dendrobiums* under study. For lip margin, 4 species (D3, D5, D8, D15) had an entire margin, 8 species (D1, D2, D6, D7, D9, D10, D11, D12), undulate, while 3 species (D4, D13, D14) exhibited a fimbriate lip margin. 6 species (D1, D6, D7, D9, D10, D15) were found to be glabrous and 9 species (D2, D3, D4, D5, D8, D11, D12, D13, D14) pubescent for lip surface texture. The column width and spur length were observed to be

narrow (< 0.5 cm) and short (< 1.5 cm), respectively, for all the species under study. These characters are used to know the distinctiveness of a particular trait amongst the species.

Lip predominant colour appeared white (NN155A, NN155D) in 3 species (D3, D9, D15), yellow (10D, 5B, 17A, 15A, 24B, 3A, 14A, 5A, 16A) in 9 (D2, D4, D6, D7, D10, D11, D12, D13, D14), purple (N78B, N77A) in 2 (D1, D8) and violet (N79B) in 1 species (D5) as per the RHS colour chart (Supplementary Fig. 3). For lip colour number, species were grouped into 3 categories, *viz.,* single 5 (D6, D7, D10, D12, D15), double 7 (D1, D2, D3, D4, D8, D9, D14) and triple 3 (D5, D11, D13). In the present study, the lip colour pattern was noted to be uniform in 3 species (D7, D12, D15), mixed in 1 (D11), spotted in 1 species (D9) and striped/shaded in 10 species (D1, D2, D3, D4, D5, D6, D8, D10, D13, D14). Column colour number revealed that it was single in 14 species (D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12, D14, D15) and double in 1 species (D13). Species were grouped into 2 categories, uniform 14 and spotted one, based on column colour pattern (Table 2). Similar variations have been documented earlier for the same characteristics in *Phalaenopsis* (26) and *Dendrobium* orchids (28) using the DUS guidelines.

Visual observations on anther cap colour showed that four species (D9, D11, D13, D15) were white (NN155A, NN155D, NN155B) species, 8 (D2, D3, D4, D6, D7, D10, D12, D14) were yellow (4D, 9D, 11D) and 3 species (D1, D5, D8) were purple (N78A) (Table 2). Based on the flowering season, 14 species (D1 to D14) bloomed during spring and only 1 species (D15) flowered in summer. Qualitative traits such as the shape of the petal, sepal and lip and their colours are more stable across generations; hence, they are reliable for characterization. *Dendrobium lituiflorum* was observed to have the earliest flowering in February, while *Dendrobium wardianum* flowered late during May and all the remaining species flowered in mid-season (March to April). It is evident from the present findings that a more significant number of orchid species bloomed when the temperature remained low and similar findings in *Phalaenopsis aphrodite* have also been noted that it was upregulated under inductively low ambient temperature (29). Although various genes regulating flower growth in orchids have been found, the role of the genes during orchid floral transition remains to be explored through the creation of reliable protocols to cause changes in orchid flowering time (30, 31). In orchids, the initiation of the flower bud varies from species to species and is influenced by the genotype and interactions with the environment for further development (32, 33).

DUS classification and comparison for the morphological characters of *Dendrobium* species revealed that characters like internode diameter, peduncle length, flower fragrance, lip lobation, lip callus, column width across stigma and spur length showed uniformity in expression for all the species. Characters like the nature of the shoot, inflorescence number per plant, flower length, flower width in front view, sepal dominant colour, petal predominant colour, lip predominant colour, lip colour pattern and flowering season were prominent in distinguishing most species.

Cluster analysis of quantitative traits

Cluster analysis was carried out based on the 21 quantitative morphological traits to distinguish possible groups amongst the *Dendrobium* population using Ward's method (Supplementary Table 2). Ward's method is related to changes in the within-cluster variance, which ensures that the linkage method is monotonically increasing in Ward's Agglomerative Hierarchical Clustering (AHC) when applied to Euclidean data. The outcomes of AHC are typically presented as dendrograms. A dendrogram is a binary tree where each node represents a cluster and the leaves of the tree correspond to the original data points being clustered. The Agglomerative Hierarchical Clustering (AHC) divided 15 species into 2 major groups based on the correlation that exists between the morphological traits among the species (Fig. 2). The group 1 cluster comprised 12 populations, which was further divided into 3 subgroups, *viz.,* group 1a containing 5 species, 1b with 4 species and 1c having 3 species. 3 species were classified into group 2, which includes 3 species. In group 1a, all the species had more flowers per inflorescence with small-sized flowers, whereas the species in group 1b had big-sized flowers with few flowers per inflorescence. In group 1c, all the species originated from bulbous-type shoots; in group 2, all the species were short in stature and small-flowered. Similar results were also obtained in Orchid species (34, 35). Therefore, species may be chosen for crossing for further crop improvement based on genetic divergence, as revealed in the dendrogram. Based on the cluster distance, species belonging to *Dendrobium thyrsiflorum, Dendrobium wardianum, Dendrobium primulinum and* species belonging to *Dendrobium densiflorum, Dendrobium nobile* var. *alba, Dendrobium lituiflorum* were most divergent and therefore, making a successful intrageneric crosses amongst the divergent species have a greater chance of hybridization in crop improvement.

Principal component analysis of quantitative traits

To determine the most significant characteristics of the data set and the distance between the genotypes, the same set (15 species × 21 quantitative traits) used for cluster analysis was subjected to PCA. The analysis helped to understand the significant contribution of the morphological characters across the species used for grouping (Supplementary Table 3). The first principal component (F1) explained 41.43% of the total variation and F2 explained 19.45%. The scatterplot of the first 2 principal components, accounting for 62.88% of the cumulative variance, supported the result of the cluster analysis (Fig. 3). Loadings are utilized to identify the most significant impact on each component that distinguishes the *Dendrobium* species from different traits. Loadings typically range from -1 to 1, with values near -1 or 1 indicating that a variable has a strong influence on a particular principal component.

In contrast, loadings close to zero suggest that the variable

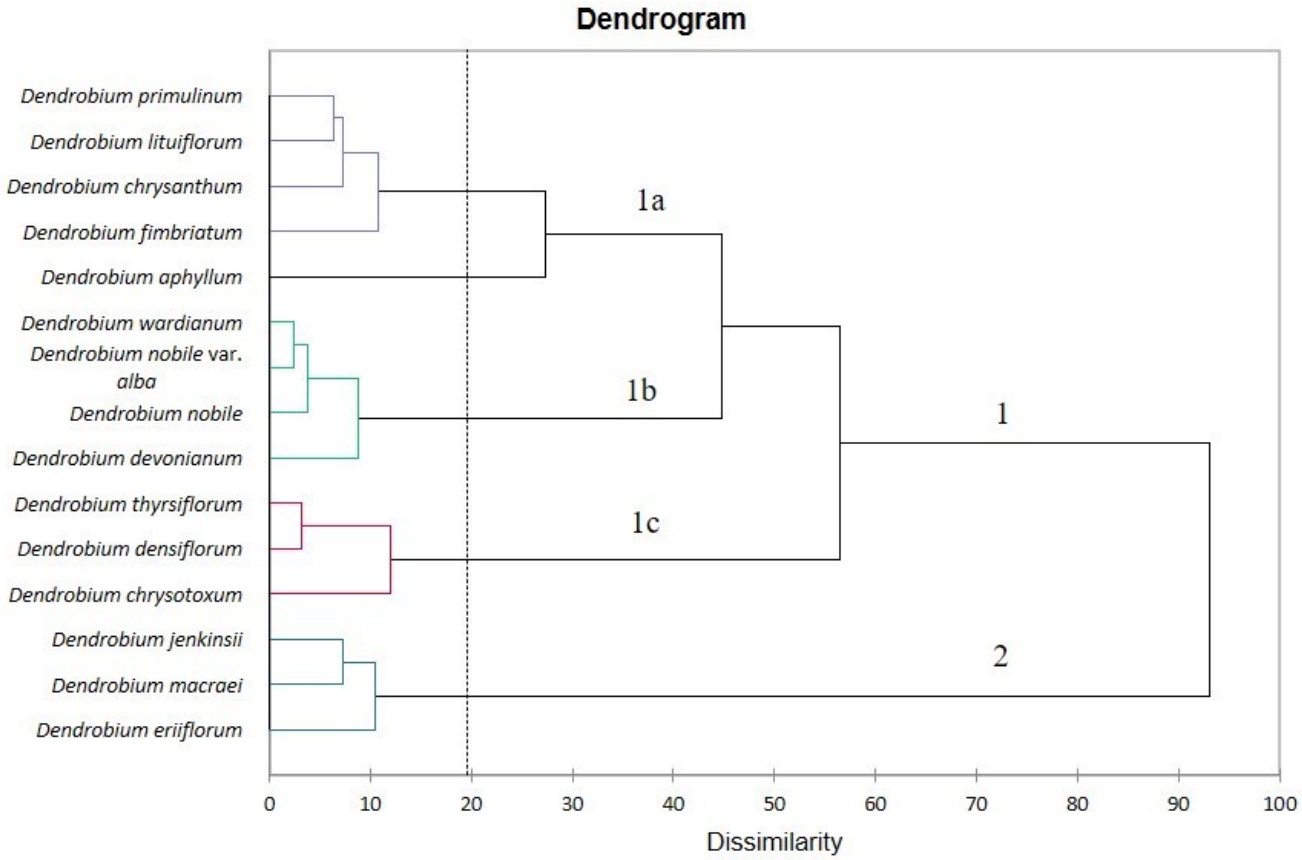

Fig. 2. Dendrogram for morphological traits of Dendrobium species.

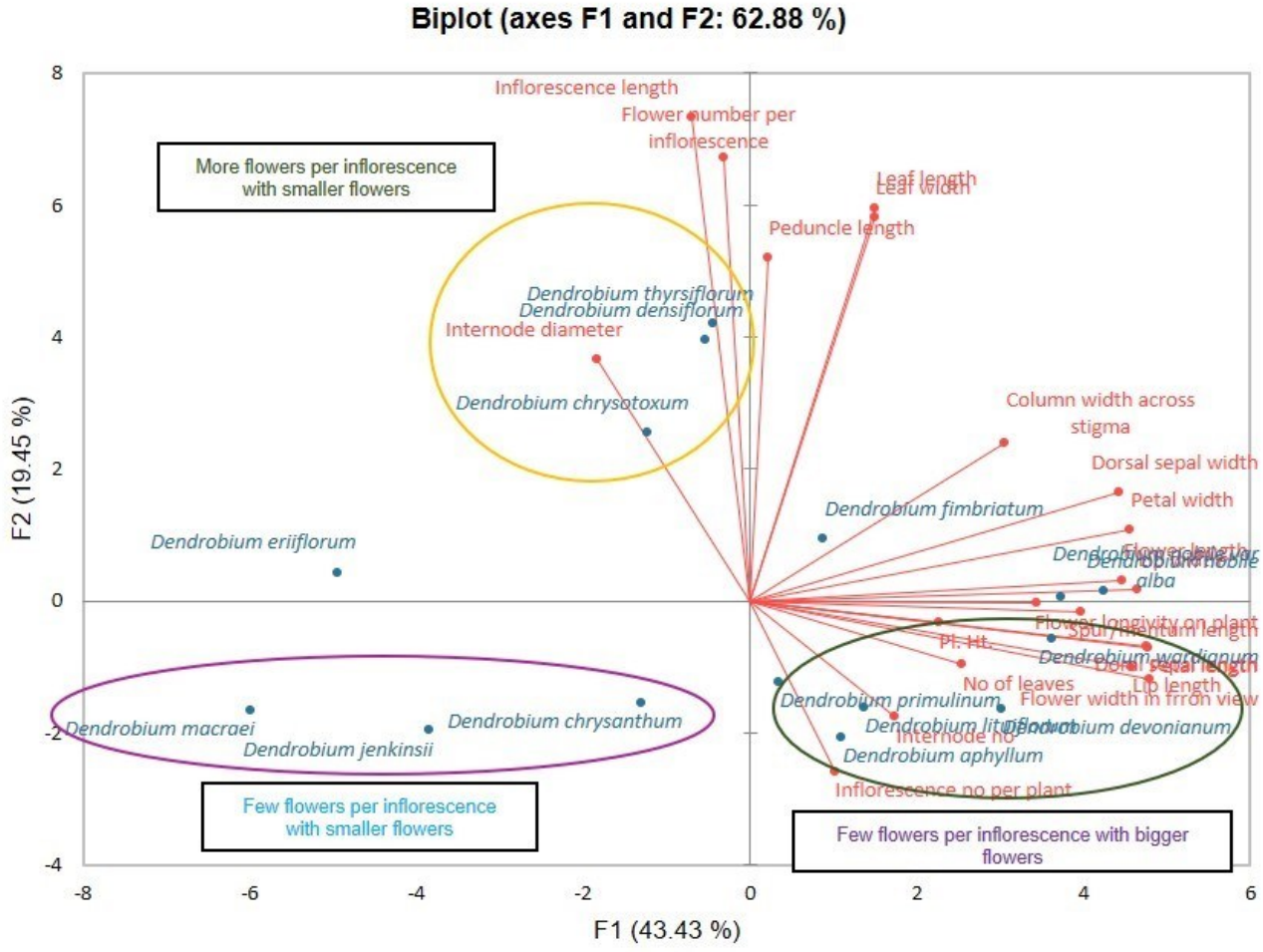

Fig. 3. Scatter plot of Dendrobium species based on first two principal components.

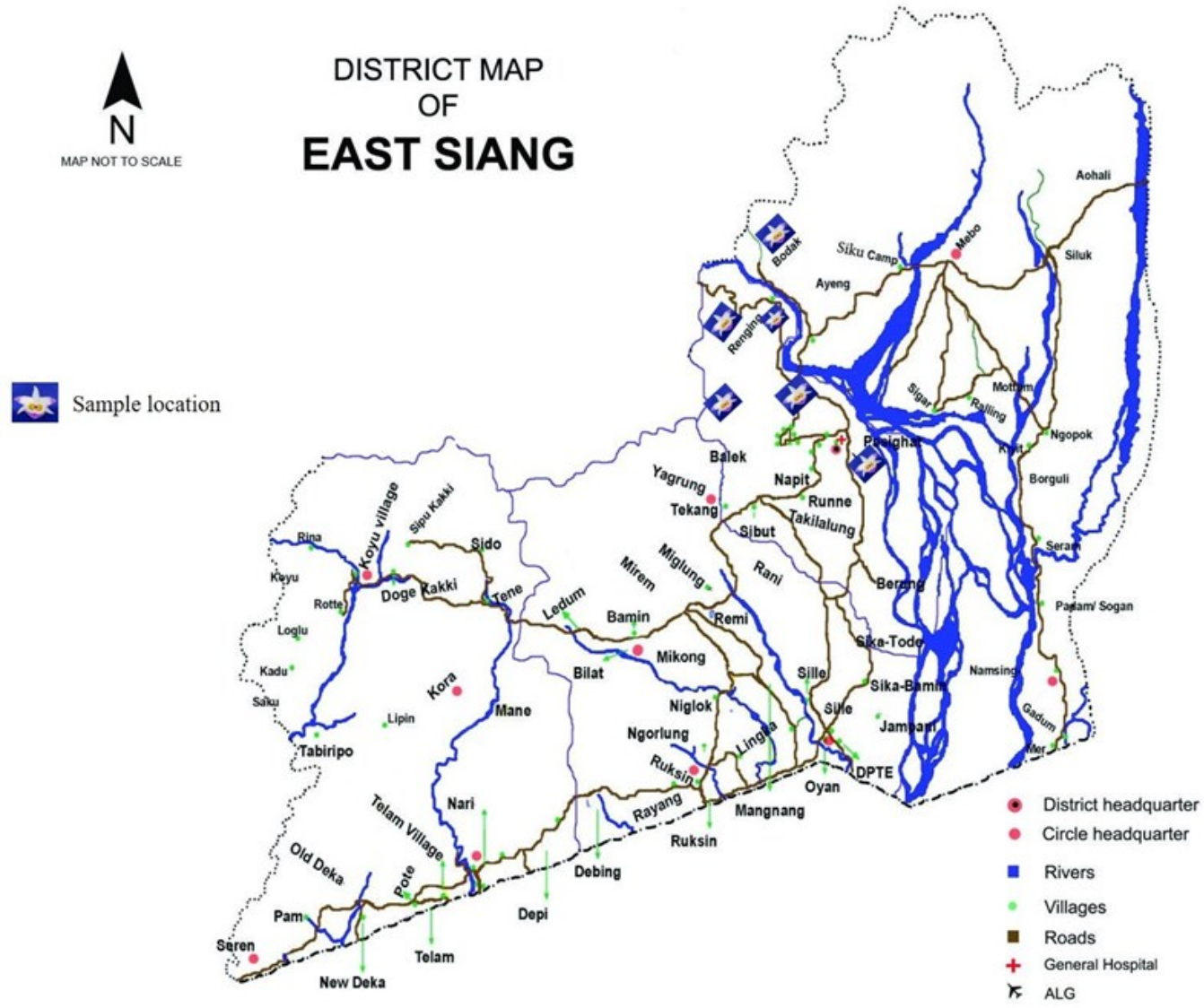

Fig. 4. This map depicts the sample locations of *Dendrobium* species.

has a minimal effect on that principal component (34). In the first principal component of the five considered, flower width in front view (+ 0.314) and Petal length (+ 0.313) were the most essential traits to contribute to the total phenotypic variation and internode diameter had a negative loading of - 0.120 (Table 3). The most important characteristics for the second principal component were inflorescence length (+ 0.481), flower number per inflorescence (+ 0.440) and inflorescence number per plant, with a negative loading of - 0.169. Significant variation was recorded for traits such as inflorescence length, flower number per inflorescence, leaf length, leaf width, petal width and dorsal sepal width, as mentioned by the relative length of the vectors in the biplot diagram. Species like *Dendrobium densiflorum*, *Dendrobium thyrsiflorum* and *Dendrobium chrysotoxum,* which had more flowers per inflorescence with smaller flowers, formed a group in one quadrant; species with a smaller number of flowers per inflorescence and relatively smaller sizes formed another group and remaining all the species with lesser number of flowers per inflorescence with bigger size formed another group in other quadrants. Most morphological characters contributed equally to the grouping of *Dendrobium* orchid species. The result of PCA is consistent with that of the cluster analysis. Various flower crops also observed a similar pattern (36-38).

Conclusion

The fifteen *Dendrobium* species characterized for 52 vegetative and floral traits using DUS testing guidelines in the present study revealed considerable variation in the morphological characters collected from different places in the East Siang District of Arunachal Pradesh. This information can efficiently identify and characterize *Dendrobium* species that have not been described. This will be helpful for farmers/breeders to recognize and differentiate *Dendrobium* species and seek protection under PPV and FR, New Delhi. Clustering and principal component analysis for quantitative traits revealed completely different significant groups. Heterosis can be exploited for qualitative and quantitative characteristics by crossing the most compatible species with desirable traits.

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Table 3. Principal Component Analysis for 21 quantitative traits of Dendrobium species

Variable	F1	F2	F3	F4	F5
Eigenvalue	9.121	4.084	3.663	1.222	0.785
Cumulative variance (%)	43.434	62.880	80.324	86.141	89.879
Plant height (cm)	0.148	-0.022	0.427	-0.129	0.251
Internode number	0.114	-0.115	0.413	-0.116	0.335
Internode diameter (cm)	-0.120	0.240	-0.209	0.531	-0.133
Number of leaves	0.167	-0.064	0.381	0.188	-0.091
Leaf length (cm)	0.099	0.390	0.090	-0.042	0.497
Leaf width (cm)	0.098	0.382	0.126	-0.222	-0.325
Inflorescence number per plant	0.066	-0.169	0.430	0.103	-0.393
Inflorescence length (cm)	-0.045	0.481	0.048	0.030	0.041
Peduncle length (cm)	0.014	0.342	0.232	0.181	-0.169
Flower number per inflorescence	-0.021	0.440	0.063	-0.319	-0.146
Flower length (cm)	0.293	0.020	-0.165	-0.061	0.126
Flower width in front view (cm)	0.314	-0.078	-0.099	0.111	-0.010
Doral sepal length (cm)	0.312	-0.044	-0.127	0.082	0.045
Dorsal sepal width (cm)	0.290	0.108	-0.160	-0.028	0.150
Petal length (cm)	0.313	-0.047	-0.127	0.078	0.009
Petal width (cm)	0.299	0.071	-0.104	0.052	0.152
Lip length (cm)	0.300	-0.065	-0.101	-0.156	-0.138
Lip width (cm)	0.305	0.012	-0.036	-0.087	-0.247
Column width across stigma (cm)	0.201	0.157	-0.044	0.355	-0.006
Spur/mentum length (cm)	0.261	-0.010	-0.075	-0.329	-0.311
Flower longevity on plant	0.226	-0.002	0.255	0.397	-0.028

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Authors' contributions

DMR performed the experimental work. DMR and VB carried out the data analysis and interpretation. KCM designed and supervised the study. SK, AKM and AKP conducted the data curation and formal analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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