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RESEARCH ARTICLE

Genetic diversity of *Dendrobium* species revealed by simple sequence repeat (SSR) markers

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Abstract Dendrobium is an epiphytic orchid, which is highly valued as a cut flowers. The assessment of genetic diversity is crucial to study the relationship between the species and also to develop the novel hybrids. In this study, 25 cross species simple sequence repeat (SSR) markers were screened for amplification in *Dendrobium* species. Eighteen polymorphic markers were used to estimate the genetic diversity in 15 *Dendrobium* species. The 18 SSR markers generated a total of 55 polymorphic bands, with an average of 3.05 bands per primer. The observed and expected heterozygosity within the species ranged from 0 to 0.62 and 0.30 to 0.86, respectively. Cluster analysis based on the unweighted pair group method with an average led to a dendrogram with three major groups that coincided for morphological characters mostly for flower colour, flower shape and nature of shoot, but did not coincide with their geographical locations. Results revealed that genetic variation exists amongst the 15 *Dendrobium* species, which will be helpful in selecting desirable species as a parent for crossing purpose to develop new interspecific hybrids.

Keywords *Dendrobium* species · Orchidaceae · Geographical locations · Genetic diversity · SSR markers · Cluster analysis

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Introduction

Orchid flowers are highly prized and admired by flower lovers around the world for their unique and diverse characteristics. They come in a wide range of forms, sizes, and colors, making them incredibly appealing and captivating. Orchids are indeed popular as both pot plants and cut flowers. *Dendrobium* is the second largest genera amongst the Orchidaceae family after *Bulbophyllum* (Basavaraj et al. 2020) and gained importance in cut flower, perfume and cosmetic industries. The use of *Dendrobium* orchids in the traditional medicine system have been known in many parts of the world since ancient times (Varma and Rajashekar 2022).

The northeast region of India is rich in genetic diversity and is one of the world's eight mega biodiversity hotspots, hosting nearly 900 species of orchids out of 1350 species present in India; i.e.,

69% of India's orchid taxa (Ninawe and Swapna 2017). Keeping in view the enormity and diversity of indigenous ornamental plant species in India and orchids in particular, a networking approach is essentially required which could share the responsibility of germplasm collection, characterization, conservation, evaluation and maintenance of precious genetic resources.

Characterization of genetic relationship and diversity of orchids is very important for conservation and its utilization in crop improvement programmes (Roy et al. 2017). Molecular markers provide a very precise tool for evaluating genetic diversity of germplasm which helps to select more diverse parents in breeding programme which has led to an advancement in analysis of orchid genetic diversity (Wang et al. 2009). In spite of the evolutionary significance of the *Dendrobium* orchids, the available data of these species using reproducible and reliable markers is very less. With the rampant forest destruction, *Dendrobium* orchids is facing extinction and needs to be conserved through proper plant conservation strategies. At present the research reports on the genetic diversity of *Dendrobium* species in particular is scanty which is essential for genetic conservation and plant breeding programme. Keeping in view the need for conservation and future use in breeding programmes, the present study aimed to characterize and identify the genetic diversity of 15 important species of genus *Dendrobium* by using simple sequence repeat (SSR) markers which will be useful for further crop improvement programmes.

Materials and methods

Plant materials

In the present study, fifteen wild *Dendrobium* species (Fig. 1) were characterized. These orchids were collected from different locations of East Siang District of Arunachal Pradesh (Table 1; Fig. 2) and maintained at College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India. Collected genotypes were grown in pots having 1:1:1 ratio of brick pieces, coconut husk and cocopeat along with few charcoal pieces for purifying the media.

DNA Extraction

DNA was extracted from the young fresh leaves through CTAB method as performed by Doyle and Doyle (1987) with slight modifications. Tender young leaves of 0.5 g from each species were used to isolate genomic DNA. RNA was removed by treating DNA with RNase (Himedia Laboratories Pvt. Ltd. Thane, Maharashtra) at 65 °C for 20 min. The DNA quality was checked on 0.8% agarose gel stained with ethidium bromide (EtBr) and visualized in the gel documentation system.

SSR marker genotyping

The molecular diversity of the 15 *Dendrobium* species were characterized by using 25 SSR markers of *Dendrobium officinale* and *Dendrobium loddigessi* (Cai et al. 2012; Lu et al. 2012).

PCR amplification was carried out with 20 µl reaction mixture containing 2 µl of 50 ng template DNA, 2 µl of 10×PCR buffer, 1.4 µl of 25 mM $MgCl_2$, 0.3 µl of 10 mM dNTPs, 1 µl of 10 µM of each forward and reverse primer (AgriGenome Labs Pvt. Ltd. Kochi, Kerala) and 0.3 µl of Taq DNA polymerase (5U/µl). All the chemicals were procured from Himedia Laboratories Pvt. Ltd. Thane, Maharashtra. Thermal profiles were standardized for each SSR primer pair based on its melting temperature using a master cycler 5331 gradient –PCR machine (Eppendorf, Hamburg, Germany). The standard annealing temperatures of all the SSR primers are given in Table 2. The PCR fragments were resolved on a 3% (w/v) agarose gel with 2 µl ethidium bromide in 1×TAE buffer at a constant voltage (75 V) for 120 min and visualized under UV light using a Fire reader gel documentation system (IGene Labserve Pvt. Ltd. New Delhi). The data were stored for further analysis.

Statistical data analysis

For the molecular analysis, amplification profiles of different primer combinations for all genotypes were analyzed by comparing DNA fragment sizes on agarose gel with 100 bp DNA ladder. The amplified fragments in each SSR marker were scored

Fig. 1 Variability in flower colour and form of 15 *Dendrobium* species used in the study

Table 1 Fifteen *Dendrobium* species collected from various geographical locations of East Siang District, Arunachal Pradesh

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

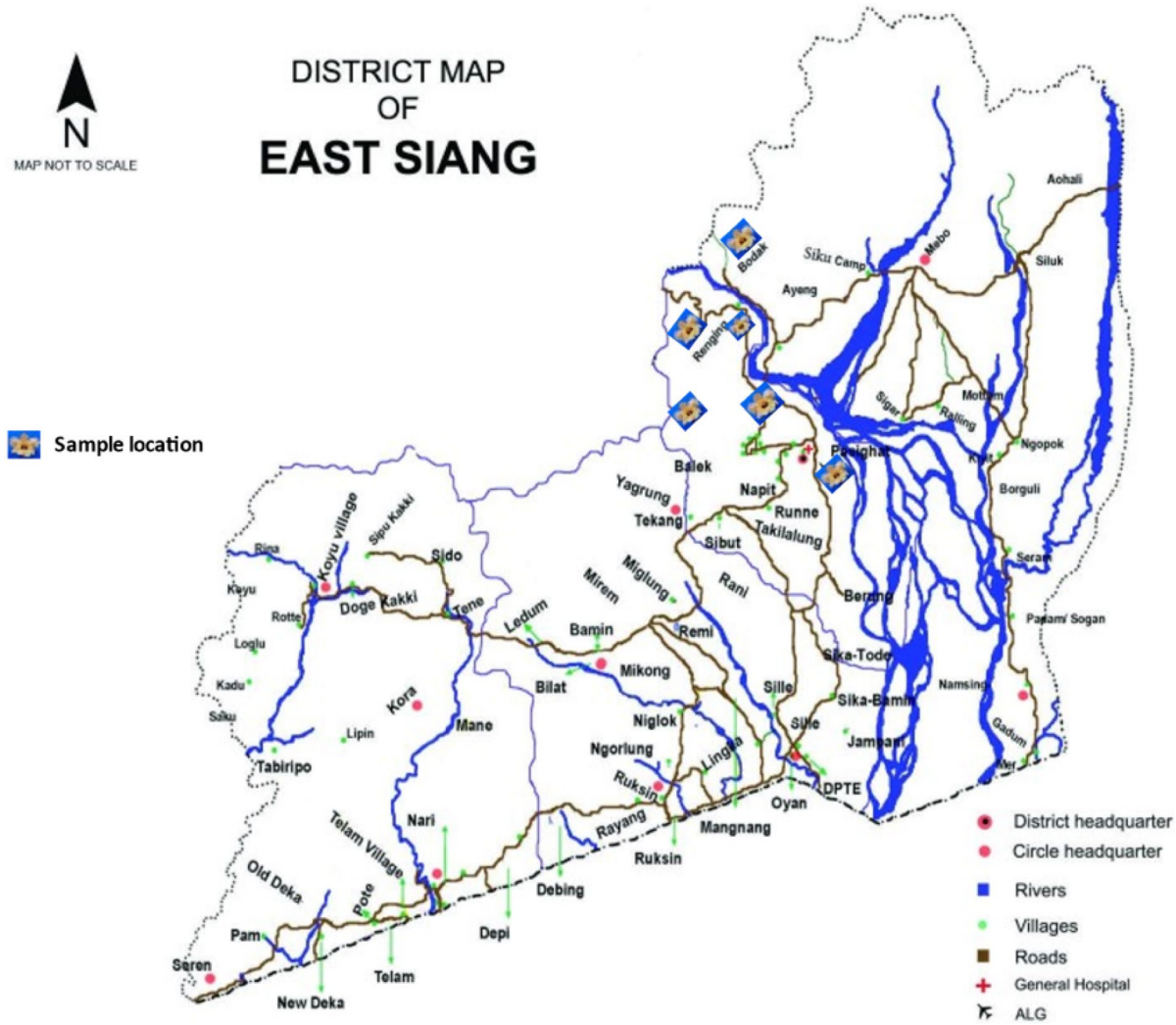

Fig. 2 This map showing sampling location of the *Dendrobium* species

manually for their presence (1) and absence (0) among the 15 *Dendrobium* species. Number of alleles and values of polymorphic information content (PIC), observed heterozygosity and gene diversity (GD)/ expected heterozygosity (He) were calculated in GenAlEx 6.5 software (Peakall and Smouse 2006; Banks and Peakall 2012). The software NTSYS-pc Version 2.02i was used to generate the Jaccard's similarity coefficient matrix (Rohlf 1987). The genetic distance was calculated by deducting the similarity index value from unity, which was used to produce the dendrogram by using Molecular Evolutionary Genetics Analysis v11 (MEGA11) software through Unweighted Pair Group Method with Average (UPGMA) (Tamura et al. 2021). Based on the SSR banding patterns, polymorphism percentages were calculated for different primers.

Results and discussion

Genetic diversity of species can be estimated by using molecular markers. Microsatellite markers have proved to be a powerful tool for cultivar identification, evaluation of the varietal purity and analysis of genetic diversity. SSR markers are useful tool for

Table 2 Details of simple sequence repeat primers used for molecular characterization of 15 *Dendrobium* species

Sl. No	Primer code	Microsatellite primer sequence (5′→3′)	Observed Allele length range (bp)	Annealing temperature (°C)	Total number of bands produced	Number of Polymorphic bands	Percent polymorphism
1	CXY01	F:GATAACGCAAGAGGAAAC pR:ATGGCTCAAACTGTAGGC	174–275	58	5	5	100
2	CXY03	F:TGTCTCCTCCACTCCTCT pR:TGTTACACCACTCGGCAC	168–228	60	3	2	66
3	CXY04	F:CAAGCTGCTCGATCCATT pR:GCTCCAAACAAACCCACAC	105–470	60	6	6	100
4	CXY07	F:TGACCAAGCATCACAAGC pR:CAGCAAAGAACTGAGAAATAAG	148–339	60	5	5	100
5	CXY08	F:GCGAGGTGAGAATGAAGT pR:GCCACCCTGATTACTATG	120–367	58	3	3	100
6	CXY09	F:CAGTGGAGGTCTGTTTGAT pR:AGGAGCAGCGAGTTTAGT	132–445	60	6	6	100
7	DOeSSR6	F: TCCCAATCCTGAAATCTATAA pR: GTAGGAAGAGACGAGGAGAAG	257–639	54	5	5	100
8	DOeSSR19	F: TATCTCAAAGACCTTTGCTTG pR: ATAAGCTAGCCATGCCATGTT	119–151	56	1	1	100
9	DOeSSR21	F: GGCCACTTACCTTTCTTTCTA pR: GAAGAGAAGCGAGAGAAGATT	132–168	55	1	1	100
10	DOeSSR30	F: TAGTCGCCGCTTCTTTACT pR: GTCCGGATGCTGGATAAC	131–157	56	1	1	100
11	DOeSSR36	F: TCTTTCCTCTCTTCCTCCTAA pR: CTTCTCGTTCACGTAAACACT	144–178	54	1	1	100
12	DOeSSR40	F: AGTGGTTACGGTCACTTACCT pR: GAGAAGATTCCAACCATATCC	135–158	55	1	1	100

Table 2 (continued)

Sl. No	Primer code	Microsatellite primer sequence (5′→3′)	Observed Allele length range (bp)	Annealing temperature (°C)	Total number of bands produced	Number of Polymorphic bands	Percent polymorphism
13	DOeSSR42	F: GCTGTTTTCCAGGTT GTAAT pR: TCCTTCTCTTCTTTC TTTTCC	129–394	53	3	3	100
14	DOeSSR48	F: CGCTCTGTTTCTCTC TACCTC pR: AAGACCTTACGA TATTGCACA	105–542	55	5	5	100
15	DOeSSR59	F: GATCTTTAGGGAGAT GCAAAT pR: GTAGCCTTCCTT CTCCTCAT	147–584	55	3	3	100
16	DOeSSR67	F: GGCCTAGAGGAT GAAGAAATA pR: CGAGACTTCTCC TTTGTGAC	138–163	55	1	1	100
17	DOeSSR85	F: AGGAGGTGAAGA ATGGGAGG pR: AACAGCAGCATC AATCAAATAG	131–668	56	5	5	100
18	DOeSSR87	F: ATGACGCCATGTACC ACTCC pR: ACAACGATTCGC ACCAGTTC	268–389	58	2	2	100
	Total				56	55	98.11
	Average				3.11	3.05	

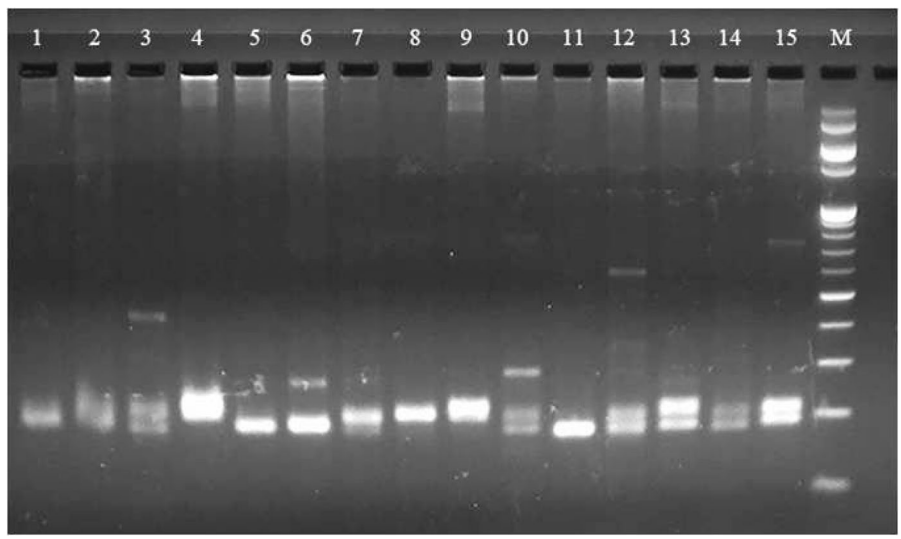

Fig. 3 Simple sequence repeat marker profiles of 15 *Dendrobium* orchid species produced by CXY01. Lanes M, 100 bp DNA ladder; lanes 1–15 amplicons of *Dendrobium* samples

observing genetic diversity and these are PCR based, highly polymorphic, multi-allelic, co-dominant, easily reproducible and widely distributed across the genome (Wu et al. 2021; Li et al. 2022). Comparative genomic mining of SSR markers among closely related species, is one of the most efficient and cost-effective methods for discovering novel DNA markers. Sequence data from a variety of plant species revealed that there is enough homology between the genomes of two or more closely related genera or species. As a result, primer pairs designed for one species could be used to detect SSRs in related species and even other genera within the same family (Kalia et al. 2011).

SSR marker analysis

Twenty-five SSR primers were screened, out of which 18 polymorphic markers were used for molecular characterization and analysis of genetic diversity among the 15 *Dendrobium* species (Fig. 3). Fifty-six alleles with sizes ranging from 105 to 668 base pairs were detected with an average of 3.11 alleles per locus. A number of alleles produced by 18 primers

Table 3 Floral characters of 15 *Dendrobium* orchid species

Species	Nature of shoot	Sepal dominant colour	Petal predominant colour	Lip predominant colour	Dorsal sepal shape	Lateral sepal shape	Petal shape	Lip shape
Dendrobium lituiflorum	Cane (cylindric fleshy)	White group	White group	Purple group	Oblong	Linear	Ovate	Ovate
Dendrobium aphyllum	Cane (woody)	White group	White group	Yellow group	Linear	Triangular	Elliptic	Orbicular
Dendrobium primulinum	Bulbous (round)	Purple group	Purple group	White group	Oblong	Oblong	Oblong	Orbicular
Dendrobium fimbriatum	Cane (cylindric fleshy)	Yellow group	Yellow group	Yellow group	Elliptic	Triangular	Obovate	Orbicular
Dendrobium nobile	Cane (cylindric fleshy)	Purple group	White group	Violet group	Oblong	Oblong	Ovate	Ovate
Dendrobium chrysotoxum	Bulbous (round)	Yellow group	Yellow group	Yellow group	Oblong	Oblong	Ovate	Orbicular
Dendrobium densiflorum	Cane (clavate fleshy)	Yellow group	Yellow group	Yellow group	Elliptic	Elliptic	Elliptic	Orbicular
Dendrobium nobile var. *alba*	Cane (cylindric fleshy)	White group	White group	Purple group	Oblong	Oblong	Ovate	Ovate
Dendrobium macraei	Bulbous (round)	White group	White group	White group	Elliptic	Elliptic	Linear	Oblanceolate
Dendrobium jenkinsii	Bulbous (round)	Yellow group	Yellow group	Yellow group	Elliptic	Elliptic	Elliptic	Orbicular
Dendrobium wardianum	Cane (cylindric fleshy)	White group	White group	Yellow group	Elliptic	Elliptic	Ovate	Orbicular
Dendrobium thyrsiflorum	Cane (clavate fleshy)	Yellow group	Yellow group	Yellow group	Oblong	Triangular	Obovate	Orbicular
Dendrobium devonianum	Cane (woody)	White group	White group	Yellow group	Elliptic	Triangular	Ovate	Orbicular
Dendrobium chrysanthum	Cane (cylindric fleshy)	yellow group	Yellow group	Yellow group	Elliptic	Triangular	Ovate	Orbicular
Dendrobium eriiflorum	Bulbous (round)	white group	White group	White group	Elliptic	Triangular	Ovate	Oblanceolate

ranged from 1 to 6 which is similar to the report in *Dendrobium* orchids by Kang et al. (2015); Lu et al. (2013b) and Lu et al. (2012) and in *Cymbidium* by Li et al. (2014). Similarly in another study conducted in *Phalaenopsis* orchids by Fattmah and Sukma (2011), 16 polymorphic SSR were identified with 2–5 alleles while, 14 SSR polymorphic loci were identified 1–12 alleles in *Cymbidium* by Moe et al. (2010). The obtained results indicated that the selected *Dendrobium* species have higher level of genetic diversity due to enormous phenotypic variation in flower colour, flower length, flower shape and nature of shoot (Table 3 and Table 4).

All the markers have shown 100 percent polymorphism, except CXY03. Out of 56 SSR loci, 55 were polymorphic with 98.11% of polymorphism (Table 2). The possibility for high level of polymorphism in *Dendrobium* species is due to their diverse nature. Liu et al. (2014) reported that the overall level of polymorphism (92.50%) indicated the effectiveness of markers to investigate genetic diversity among the different species of *Dendrobium* germplasm.

Table 4 Quantitative trait variation in 15 *Dendrobium* orchid species

Species	Plant height (cm)	Internode number	Internode diameter (cm)	Inflorescence length (cm)	Flower width (cm)	Lip length (cm)	Lip width (cm)	Flower longevity on plant (days)
Dendrobium lituiflorum	69.20 ± 7.20	18.00 ± 2.65	0.73 ± 0.04	0.90 ± 0.06	4.97 ± 0.06	2.97 ± 0.21	2.13 ± 0.06	14.33 ± 0.58
Dendrobium aphyllum	93.87 ± 5.12	31.00 ± 2.00	0.48 ± 0.00	0.40 ± 0.10	3.97 ± 0.06	2.30 ± 0.10	2.37 ± 0.06	20.67 ± 0.58
Dendrobium primulinum	31.33 ± 3.82	13.33 ± 2.52	0.85 ± 0.06	0.90 ± 0.06	5.03 ± 0.25	2.63 ± 0.25	2.67 ± 0.06	6.33 ± 1.15
Dendrobium fimbriatum	77.13 ± 1.85	28.67 ± 3.06	0.69 ± 0.03	10.10 ± 1.03	4.13 ± 0.12	2.40 ± 0.17	2.23 ± 0.15	9.33 ± 0.58
Dendrobium nobile	54.67 ± 7.37	13.00 ± 2.00	1.18 ± 0.06	3.60 ± 0.15	6.73 ± 0.45	3.43 ± 0.12	3.43 ± 0.12	17.33 ± 0.58
Dendrobium chrysotoxum	15.90 ± 0.95	3.33 ± 0.58	1.36 ± 0.13	16.30 ± 0.20	3.80 ± 0.26	1.83 ± 0.06	1.93 ± 0.06	14.33 ± 0.58
Dendrobium densiflorum	41.27 ± 1.78	6.00 ± 1.00	1.10 ± 0.04	17.70 ± 0.12	2.93 ± 0.06	2.10 ± 0.17	2.03 ± 0.06	9.67 ± 1.15
Dendrobium nobile var. *alba*	35.93 ± 1.30	8.67 ± 1.15	1.22 ± 0.01	3.90 ± 0.06	7.17 ± 0.06	3.67 ± 0.21	2.73 ± 0.12	15.67 ± 1.53
Dendrobium macraei	20.17 ± 2.08	5.33 ± 0.58	1.10 ± 0.16	0.70 ± 0.06	0.67 ± 0.06	1.03 ± 0.06	0.47 ± 0.12	4.67 ± 0.58
Dendrobium jenkinsii	6.40 ± 0.20	2.00 ± 0.00	1.16 ± 0.06	0.50 ± 0.12	1.97 ± 0.06	2.23 ± 0.06	1.73 ± 0.12	4.33 ± 0.58
Dendrobium wardianum	23.40 ± 1.20	6.00 ± 1.00	0.85 ± 0.06	1.10 ± 0.06	7.10 ± 0.10	3.20 ± 0.10	2.77 ± 0.06	12.33 ± 1.15
Dendrobium thyrsiflorum	43.33 ± 2.02	7.33 ± 0.58	1.06 ± 0.09	17.40 ± 0.60	2.17 ± 0.06	2.53 ± 0.06	2.43 ± 0.06	8.33 ± 0.58
Dendrobium devonianum	28.73 ± 1.37	5.67 ± 0.58	0.42 ± 0.02	0.70 ± 0.12	6.03 ± 0.15	3.73 ± 0.12	3.53 ± 0.06	9.00 ± 1.00
Dendrobium chrysanthum	36.57 ± 2.00	12.67 ± 1.53	0.64 ± 0.05	0.90 ± 0.00	3.00 ± 0.10	2.27 ± 0.12	1.63 ± 0.12	8.67 ± 0.58
Dendrobium eriiflorum	15.10 ± 0.89	2.67 ± 0.58	1.31 ± 0.15	8.70 ± 0.30	0.93 ± 0.06	0.37 ± 0.12	0.73 ± 0.06	5.67 ± 0.58

Similar results were obtained by Basavaraj et al. (2020); Kang et al. (2015) and Lu et al. (2013a) having 66.66%, 42% and 85.91% polymorphism in *Dendrobium* orchids, respectively. Huang et al. (2010) and Jantasuriyarat et al. (2012) also reported 90.80% and 64.80% polymorphism, respectively in different orchid genera.

The various genetic diversity parameters such as, polymorphic information content (PIC), observed heterozygosity and gene diversity (GD)/expected heterozygosity (He), were analyzed (Table 5). An average PIC value serves as the ideal index for measuring polymorphism. PIC values above 0.5 denote high polymorphism loci, between 0.25 and 0.5 which denotes intermediate polymorphism loci, and below 0.25 denoted low polymorphism loci (Ge et al. 2013; Bhargav et al. 2021). All the selected primers were polymorphic and the polymorphic information content ranged from 0.33 to 0.90 with an average of 0.74 which indicated that the loci displayed intermediate to high polymorphism and showed the occurrence of a broad gene pool amongst the species. The high levels of polymorphic markers can be used to track the introduction of genes into desirable genetic backgrounds that are advantageous to find species that are suitable for varietal development. The results are in concurrence with findings of Cai et al. (2012) where PIC value ranged from 0.36 to 0.84 with a mean of 0.64 in *Dendrobium loddigesii.* Similarly, in *Cymbidium* and *Phalenopsis* also reported PIC value ranging from 0.17 to 0.89 and 0.18 to 0.72 with a mean of 0.63 and 0.67, respectively (Moe et al. 2010; Fattmah and Sukma 2011).

The observed heterozygosity (Ho) ranged from 0.00 to 0.62 and found to be highest for the primers namely, CXY04 (0.62), followed by DOeSSR6 (0.50) and CXY09 (0.44) which reflects their ability to provide unique genetic profiles across the species. The expected heterozygosity (He) or gene diversity (GD) ranged from 0.30 to 0.86 with an average of 0.72. The values obtained are in agreement with the findings of Cai et al. (2012) in *Dendrobium loddigesii* where the observed heterozygosity ranged from 0.00 to 0.70 and the expected heterozygosity from 0.45 to 0.85. Estimates of genetic diversity helps in organizing germplasm, identifying cultivars, and choosing parents for hybridization. In plant breeding programmes, genotypes that exhibit a sufficient level of polymorphism based on genetic diversity are

Table 5 Genetic diversity measurements in15 *Dendrobium* orchid species using simple sequence repeat data

Sl. No	Primer name	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Polymorphism information content (PIC)
1	CXY01	0.40	0.76	0.53
2	CXY03	0.25	0.30	0.33
3	CXY04	0.62	0.85	0.67
4	CXY07	0.20	0.81	0.88
5	CXY08	0.18	0.86	0.89
6	CXY09	0.44	0.86	0.90
7	DOeSSR6	0.50	0.81	0.72
8	DOeSSR19	0.00	0.64	0.81
9	DOeSSR21	0.21	0.71	0.68
10	DOeSSR30	0.00	0.54	0.71
11	DOeSSR36	0.00	0.72	0.82
12	DOeSSR40	0.00	0.64	0.69
13	DOeSSR42	0.08	0.72	0.77
14	DOeSSR48	0.27	0.78	0.84
15	DOeSSR59	0.00	0.76	0.89
16	DOeSSR67	0.00	0.60	0.65
17	DOeSSR85	0.20	0.78	0.74
18	DOeSSR87	0.07	0.78	0.75
	Average	0.19	0.72	0.74

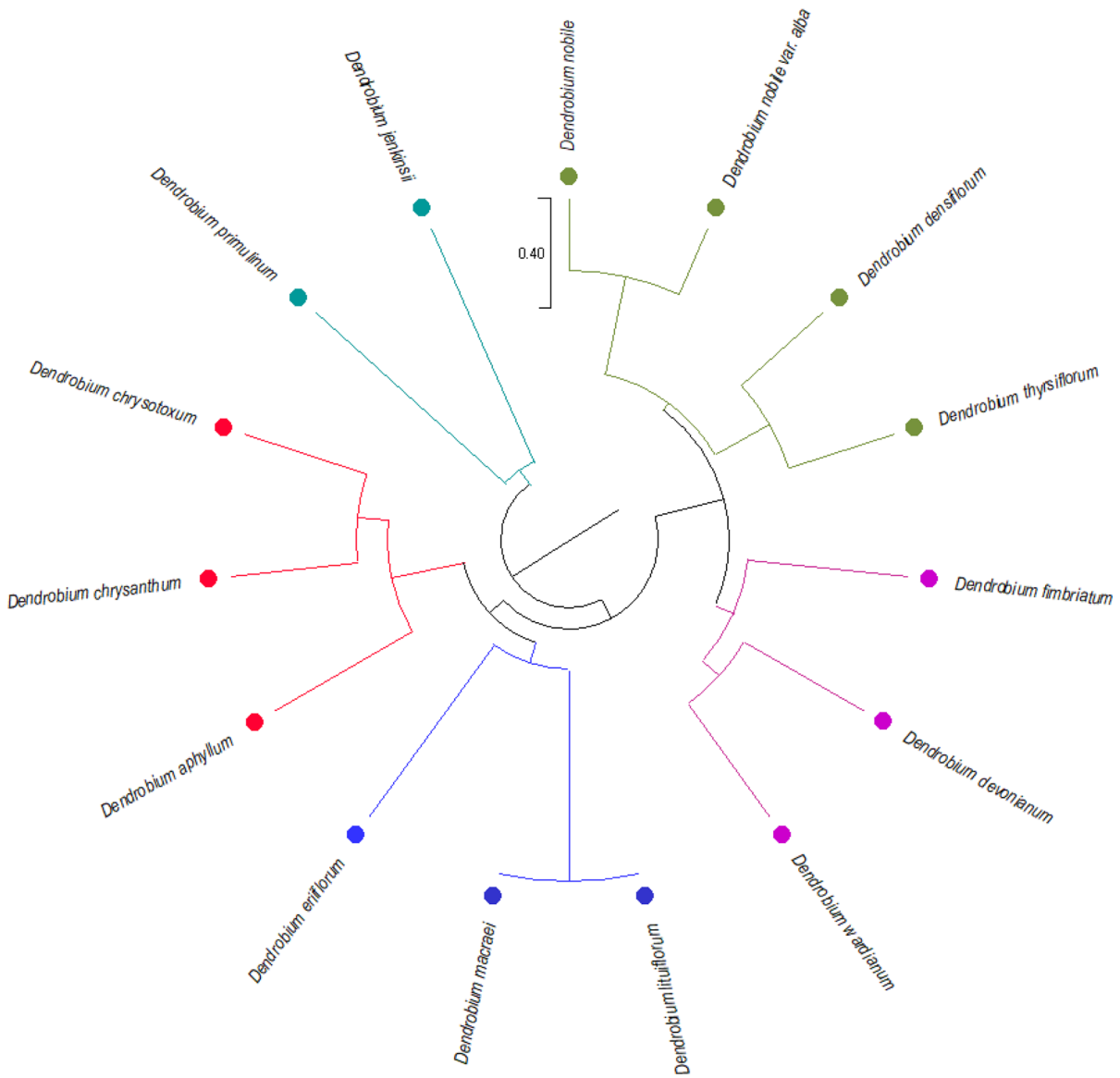

Fig. 4 Dendrogram showing genetic relationship among 15 *Dendrobium* species based on simple sequence repeat markers allelic data

chosen (Joshi et al. 1997; Louarn et al. 2007; Ren et al. 2012).

Genetic relationship among *Dendrobium* species

Results of diversity analysis revealed that 15 genotypes have been grouped in 3 different clusters (Fig. 4), in which cluster-I comprised of 7 genotypes (*Dendrobium densiflorum*, *D. devonianum*, *D. fimbriatum*, *D. nobile*, *D. nobile* var. *alba, D. thyrsiflorum* and *D. wardianum*) Cluster-II comprised of 6 genotypes (*D. aphyllum*, *D. chrysanthum*, *D. chrysotoxum*, *D. eriiflorum*, *D. lituiflorum* and *D. macraei*) and Cluster-III comprises of 2 genotypes (*D. jenkinsii* and *D. primulinum*. Similar clustering pattern was observed by Lee et al. (2020), Kang et al. (2015), Li et al. (2014), Liu et al. (2014), Lu et al. (2013b)

and Moe et al. (2010) using SSR markers in different orchid genera.

Cluster I comprised of white, purple and yellow species of tall stature and big sized flowers. Cluster II represents the tall plants and small sized flowers, respectively. The genotypes in Cluster III are white and yellow flower coloured with bulbous shoot and less flower longevity (4 days) (Tables 3 and 4). Species located in Cluster I, II and III indicated more phenotypic variations. Similar findings were reported by Kang et al. (2015) and Lu et al. (2013b) in *Dendrobium* species. The results of the present findings indicated that the species from different regions genetically varied amongst the genus and conservation of the germplasm will be helpful for crop improvement. Since SSR markers produced sufficient information for classification of *Dendrobium* species into different groups, showing diversity for flower colour, flower shape and nature of shoot; the findings of this research can be efficiently used for genetic diversity assessments in *Dendrobium* genus.

Conclusion

The investigation revealed the successful utility of SSRs from closely related species in fingerprinting and estimation of genetic diversity amongst the *Dendrobium* species. Results revealed genetic variation that exists amongst the *Dendrobium* species, which will help in desirable species selection for crossing purpose to develop novel hybrids, with various flower forms and colours and to identify markers which are linked to desirable traits for marker-assisted selection (MAS).Thus the present study opens up new opportunities for targeted hybridization and advanced breeding techniques to enhance their aesthetic and economic value.

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Data availability Raw data are available upon request from the corresponding author.

Declarations

Conflict of interest All the authors declare that there are no conflicts of interest to disclose.

Consent to participate All author's have given consent to participate.

Consent for publication All authors have given consent to publication.

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