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GENETIC VARIABILITY AND HERITABILITY IN GROWTH AND LEAF ASSOCIATED TRAITS OF SELECTED *DALBERGIA SISSOO* GENOTYPES

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Dalbergia sissoo Roxb. Ex. DC. is a multipurpose, high-value timber tree species native to the Indian subcontinent. The present study aimed at accessing variability among selected 12 genotypes of *D. sissoo* from Himachal Pradesh and Jammu and Kashmir, India. Branch cuttings from the genotypes were grown under nursery conditions and various plant growth traits like plant height, collar diameter, number of branches per plant, and leaf associated traits such as rachis length, petiole length, leaflet length, length of mid-rib, leaflet width and distance between leaflets, were recorded at six months of age. The genotype DSHH1 (*D. sissoo*, HFRI, Himachal Pradesh 1) showed better leaf characters *viz.*, rachis length, leaflet length, midrib length and leaf width. However, DSHJ4 (*D. sissoo*, HFRI, Jammu and Kashmir 4) performed better for collar diameter and the number of branches per plant. A higher Phenotypic Coefficient of Variance (PCV) value than the Genotypic Coefficient of Variance (GCV) for most of the studied traits, with lesser differences between both parameters, indicated that the selected traits were not much under environmental influence. High heritability was recorded for rachis length 68%, the number of branches per plant 75.96%, length of midrib 79.08%, and leaflet length 82.09%. However, moderate heritability was recorded for collar diameter 46.91% and plant height 58.21%. Based on the studied traits, shisham genotypes were grouped into two major clusters. Cluster A was comprised of seven genotypes (DSHH1, DSHJ4, DSHJ1, DSHH4, DSHH5, DSHJ6 and DSHH3) and five in Cluster B (DSHH6, DSHH2, DSHJ5, DSHJ3 and DSHJ2). Thus, there is significant variability in the selected shisham genotypes and they can be utilised for breeding and improvement in traits with better genetic estimates.

Keywords: Shisham, morphometric traits, genotypic coefficient of variation, phenotypic coefficient of variation, Principal Component Analysis, Cluster analysis

INTRODUCTION

Dalbergia sissoo (Shisham) is an important high value timber tree species of India, known for its hard, strong and durable wood (Singh & Singh 2003). In addition to being used as timber, it is also used for its medicinal values and as a livestock fodder (Mukherjee et al. 1971, Hassan et al. 2016). It is an early successional species of the riverine forests, and possesses potential for atmospheric nitrogen fixation due to diazotrophic bacterial association with roots (Singh & Pokhriyal 2002). It is native to the Himalayan foothills and is mostly found in India, Nepal, and Pakistan. It is widely distributed in the northern part of India, up to elevations of 900–1500 m above mean sea level in sub-Himalayan regions. Due to its high economic value, the species has been planted worldwide as an exotic species in countries including China, Cuba, Brazil, and Honduras (Wang et al. 2011). It thrives well in tropical and sub-tropical climatic conditions, with optimum temperature ranging between 10 to 40 °C, and 760–4570 mm annual precipitation (Tewari 1994). It prefers to grow on porous soil of sand, pebbles and boulders with adequate moisture and avoids the clayey soil due to possible threat of diseases (Troup 2008).

In the past few decades, the decline in area under shisham forest has been an alarming issue worldwide. *D. sissoo* mortality has been reported from many parts of India (Baghchee 1945, Bakshi 1954, Dayaram et al. 2003, Negi et al. 2003, Shukla 2002). The cause of shisham decline has been attributed to root rot and wilt disease caused by the fungi *Ganoderma lucidum* and *Fusarium solani* respectively (Baghchee 1945, Bakshi & Singh 1959, Mukerjee et al. 1971, Harsh et al. 2011, Sibounnavong et al. 2012, Pant et al. 2023). However, the role of abiotic factors and climate change in shisham mortality has also been reported (Afzal et al. 2006). Many researchers are working on the development of ecofriendly management strategy to control phytopathogens using botanicals and biocontrol agents (Tapwal et al. 2017, Sheel et al. 2022, Kumari & Tapwal 2023). Among these, *Trichoderma* species have been extensively studied for their effectiveness against phytopathogens such as *Fusarium.* Additionally, plant extracts, such as the aqueous extract of *Berberis lycium,* have shown promising potential in controlling pathogenic *Fusarium* isolates (Pant et al. 2023). Despite the advantages of plant- and microbe-based biocontrol agents over chemical pesticides, several challenges are also associated with their use. The efficacy of these agents can vary significantly depending on environmental factors such as temperature, humidity, and soil pH, as well as biotic factors including microbial competition, leading to inconsistent results across different conditions (Pirttila et al. 2021, Sabbahi et al. 2022, Lahlali et al. 2022). Therefore, it is crucial to overcome these problems by devising suitable tree improvement strategies for *D. sissoo*, to ensure better productivity and sustainability of this species.

Selection is the first and key step in any tree improvement program. Genetic variability within a species is crucial for the effective selection and improvement of desired traits (Zobel & Talbert 1984). In order to exploit the existing natural variations and establish a diverse base for *D. sissoo*, superior genotypes were selected from Himachal Pradesh and Jammu and Kashmir, U.T. India, based on traits such as height, stem diameter, straightness, and overall health. Germplasm from these selected genotypes was collected and is currently being maintained in a nursery in Dharampur, Mandi district, Himachal Pradesh, India. Germplasm is frequently utilised to meet the evolving needs of tree species, making it essential to ensure that the collected germplasm exhibits substantial variability in economically important traits. Consequently, it is vital to screen and evaluate various genotypes for growth and adaptability traits before formulating any breeding strategies

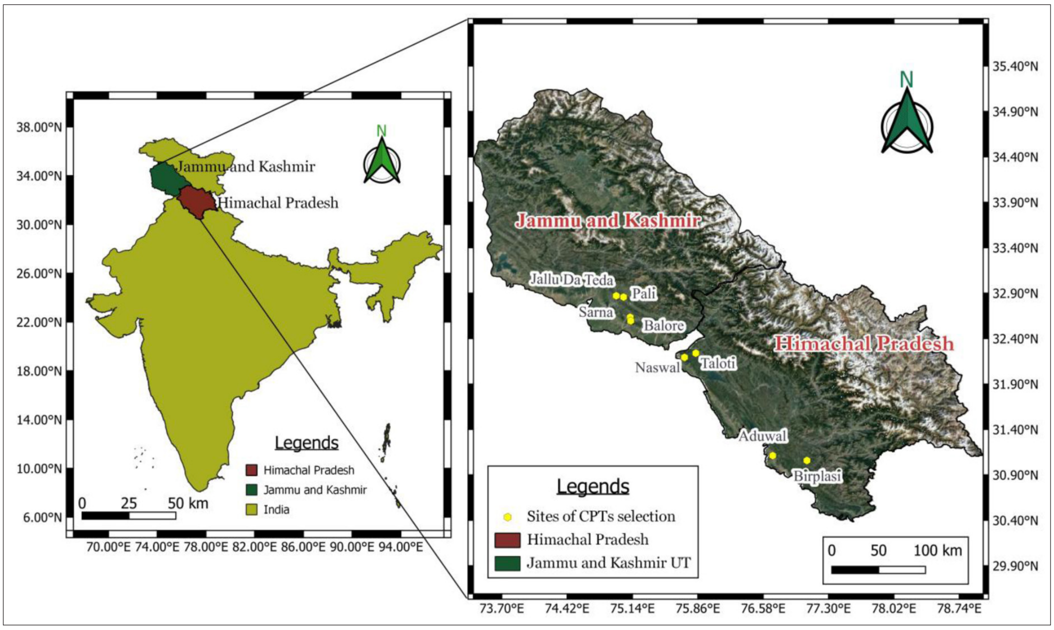

Figure 1 Location map of selected *D. sissoo* genotypes

for improvement. In this context, genetic parameters such as the Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), and Heritability (H^2) are critical for understanding the extent of variability and its genetic control within the germplasm (Sharma & Bakshi 2011, Terfa & Gurmu 2020). Thus, this study was conducted to assess genetic variability by evaluating GCV, PCV, and the heritability of growth and leaf morphological traits among selected shisham genotypes. The insights gained from this assessment will be pivotal for guiding future breeding and tree improvement programs.

MATERIALS AND METHODS

Study site

The target regions of our study were the Union Territory of Jammu and Kashmir and the state of Himachal Pradesh in India. Various populations of *D. sissoo* were identified in these regions based on Forest Working Plans and field surveys. In order to select superior genotypes of *D. sissoo*, natural populations and plantations were evaluated using the criteria established by Clark & Wilson (2005). In the Shivalik region of the Himalaya, variations in topography due to different river systems and chains of low hills create unique habitats and variations in vegetation (Champion & Seth 1968, Rawat & Bhainsora 1999). Therefore, river systems and hill barriers were also considered in selecting populations. Finally, eight populations were selected, each comprising at least 30 trees that exhibited superior growth characteristics, particularly in terms of height and girth (Figure 1, Table 1). The germplasm of selected genotypes was raised in the nursery at Dharampur, Mandi, Himachal Pradesh to investigate the variability among them based on morphological traits.

Selection of *Dalbergia sissoo* genotypes

Genotypes of *D. sissoo* were selected by following the comparison tree method (Penny et al. 1982). The selection was primarily made for the characters of growth (plant height, diameter at breast height (DBH) and straightness) and vigour (health). Initially, a potential candidate plus tree (CPT) with good phenotype was identified and marked with one red ring. Simultaneously, ten comparison trees with good phenotype (relatively better in terms of height, stem diameter and straightness) around the marked CPT were also enumerated. The CPT and the comparison trees were scored based on the maximum points allotted to various traits i.e., plant height (15 points), DBH (15 points), clear bole length (15 points), stem characteristics (15 points) and vigour (diseases resistant = 30 points). Finally, the CPTs scoring more than 90% of the total score were designated as plus tree and marked with two red rings. Stem cutting of plus trees were collected and propagated under nursery conditions.

Experimental design for nursery trial

The experiment was conducted in nursery using Randomized Block Design (RBD) and each treatment was replicated five times. Ten cuttings per genotype were planted in each replication.

Parameters recorded

The nursery parameters such as plant height (cm), collar diameter (mm), number of branches per plant (NBP), rachis length (cm), petiole length (cm), leaflet length (cm), length of mid-rib (cm), leaflet width (cm) and distance between leaflets (cm) were observed at six months age.

Statistical analysis

The data recorded was subjected to statistical analysis to study the variation between genotypes collected from different sites. Analysis of Variance (ANOVA) was performed for the data recorded using software IBM SPSS Statistics, version 29.0.0.0 (241). Genetic parameters such as variability (Burton & De-Vane 1953, Pillai & Sinha 1968) and heritability (Lush 1940) were computed using formulas given below:

Genotypic coefficient of variation (GCV)

$$\text{GCV}(\%) = \frac{\sqrt{\text{Genotypic variance } (\sigma_g^2)}}{\text{Population mean of character } (\bar{x})} \times 100$$

Table 1 Locations of selected *D. sissoo* genotypes in Himachal Pradesh (H.P.) and Jammu and Kashmir Union Territory (J&K UT)

Genotypes	Locations	Latitude (N)	Longitude (E)	Altitude (m)	DBH (cm)	Height (m)	Clear bole height (m)	Disease
DSHH1	Birplasi, Nalagarh, Solan, H.P.	31°02.217’	076°37.438’	288 m	42.5	14.5	3.5	0
DSHH2	Birplasi, Nalagarh, Solan, H.P.	31°02.217’	076°37.438’	296 m	43	11.5	3.75	0
DSHH3	Naswal, Nurpur, Kangra, H.P.,	32°11’36.8”	075°42’49.2”	346 m	49.36	20	5	0
DSHH4	Aduwal, Nalagarh, Solan, H.P.	31°06’42.72”	076°41’09.32”	395 m	27.7	10.7	4.3	0
DSHH5	Aduwal, Nalagarh, Solan, H.P.	31°06’42.72”	076°41’09.32”	395 m	28.7	12	3.2	0
DSHJ1	Sarna, Jammu, J&K	32°38’07.5”	075°06’53.5”	450 m	58.91	27	5	0
DSHJ2	Jallu Da Teda, Jammu, J&K	32°52’17.1”	074°57’41.8”	644 m	66.87	23	4	0
DSHJ3	Pali, Jammu, J&K	32°51’25.8”	075°02’32.2”	679 m	43.94	17	3.5	0
DSHJ4	Jallu Da Teda, Jammu, J&K	32°52’17.1”	074°57’41.8”	644 m	38.12	15.5	4.5	0
DSHH6	Taloti, Nurpur, Kangra, H.P.	32°14’22.0”	075°50’17.5”	469 m	81.84	31	4.5	0
DSHJ5	Sarna, Jammu, J&K	32°38’07.5”	075°06’53.5”	450 m	61.78	24.5	4.5	0
DSHJ6	Balore, Jammu, J&K	32°35’44.9”	075°06’54.3”	336 m	39.8	18	3	0

Phenotypic coefficient of variation (PCV)

$$\mathrm{PCV}(\%) = \frac{\sqrt{\text{Phenotypic variance } (\sigma_p^2)}}{\text{Population mean of character } (\bar{x})} \times 100$$

Broad Sense Heritability (H_{BS}^2)

$$H_{BS}^2(\%) = \frac{\text{Genotypic variance } (\sigma_g^2)}{\text{Phenotypic variance } (\sigma_p^2)} \times 100$$

The estimates of PCV and GCV were classified into low (less than 10%), medium (10 to 20%) and high (more than 20%) following (Sivasubramanian & Madhavamenon 1978). Heritability was divided into low (less than 30%), medium (30 to 60%) and high (more than 60%) according to (Robinson 1966). Hierarchical cluster analysis and Principal Component Analysis (PCA) were performed and dendrogram as well as biplot were generated using software Past 4.03.exe. version.

RESULTS AND DISCUSSION

Phenotypic variation

The mean collar diameter and plant height for selected genotypes ranged from 8.82 mm to 19.39 mm and 33.2 cm to 173.6 cm, respectively (Figure 2). The highest mean collar diameter was recorded for DSHJ4 (*D. sissoo*, HFRI, Jammu and Kashmir 4) followed by DSHJ6, DSHJ3, DSHH1 (*D. sissoo*, HFRI, Himachal Pradesh 1) and the lowest for DSHH2, while the mean plant height was highest for DSHH5 followed by DSHJ6, DSHH3, DSHH1 and lowest for DSHJ2. The clonal variation for plant height and stem radial growth traits in shisham has been reported in various studies performed under nursey and field conditions (Vidakovic & Siddiqui 1968, Nautiyal et al. 2003, Husen 2008). Both traits constitute a very important component for the growth and yield estimation of any timber tree species and are widely in practice for the selection of suitable genotypes for breeding (Ulak et al. 2022). Analysis of variance for

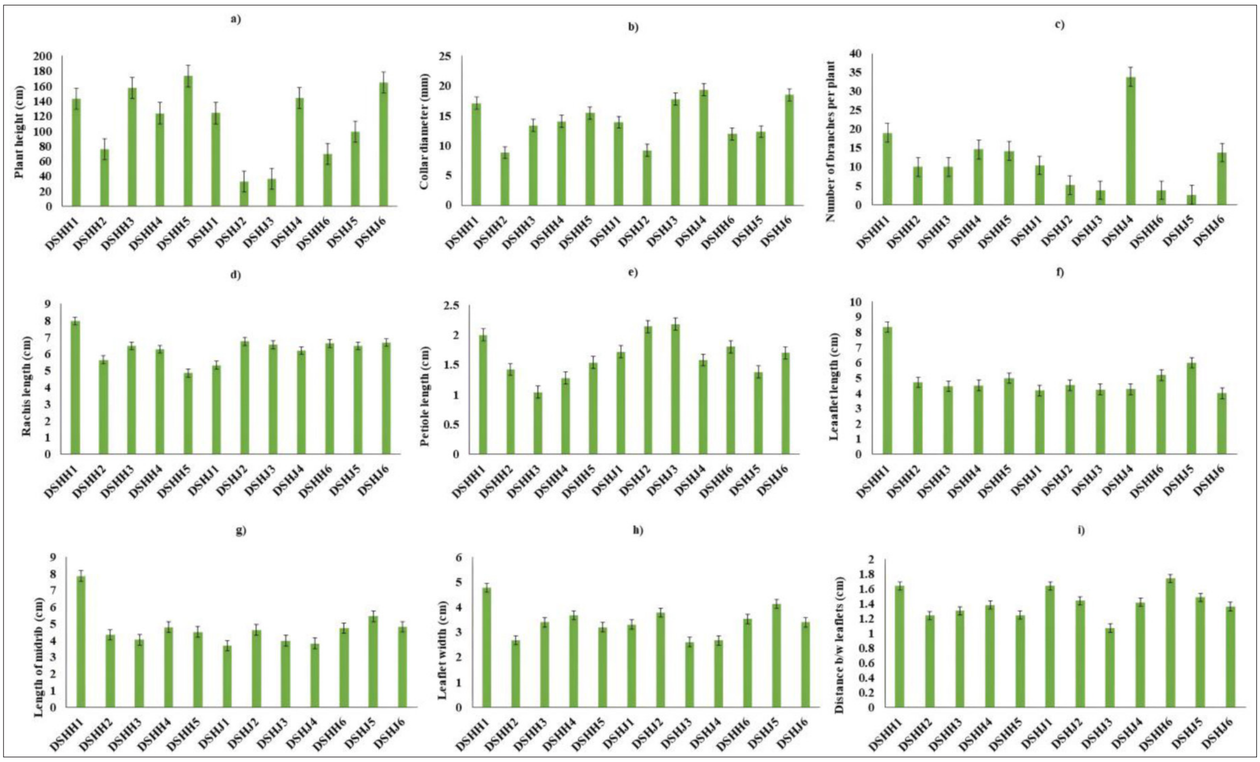

Figure 2 Growth and leaf morphological traits of the studied *D. sissoo* genotypes represented in the form of bar diagram

Table 2 Analysis of variance (ANOVA) table for growth and leaf associated traits among *D. sissoo* genotypes

Source	df	Mean squares of traits								
		Plant height	Collar diameter	No. of branch per plant	Rachis Length	Petiole length	Leaflet length	Length of midrib	Leaflet width	Distance between leaflets
Genotype	11	11883.180***	59.804***	371.848***	3.140***	0.602***	7.177***	6.133***	2.006***	0.186**
Replication	4	1060.067	11.110	20.225	2.234	0.230	2.185	0.729	1.447	0.110
Error	44	1491.930	11.037	22.125	0.270	0.121	0.300	0.308	0.155	0.070

different phenotypic traits revealed significant differences among the studied *D. sissoo* genotypes (Table 2). In all the studied genotypes, the leaf was compound with five leaflets, but exhibited significant variations in studied leaf traits (Figure 2). The leaflet length was maximum for DSHH1 (8.34 cm) and the minimum for DSHJ6 (3.99 cm). Leaflet width was highest for DSHH1 (4.78 cm) and lowest for DSHJ3 (2.60 cm). Other leaf traits such as rachis length and length of midrib were highest for DSHH1 with values 8.00 cm and 7.86 cm respectively, whereas their respective lowest values were recorded in DSHH5 (4.86 cm) and DSHJ1 (3.69 cm). Furthermore, the mean number of branches per plant was highest in DSHJ4 (33.80), followed by the minimum in DSHJ5 (2.60). Many plants functional traits, like photosynthesis, transpiration, resilience to various abiotic stresses, nutrient requirements, etc., are determined by leaf traits. Trait values of leaves vary within species and depend upon genotype variations and environmental gradients (Guet et al. 2015, Dong et al. 2020). Therefore, leaf traits are widely used to evaluate the variability in the populations or the selected germplasms (Singh et al. 2012, Guet et al. 2015). The data analysis of recorded data revealed the existence of sufficient variability among the studied genotypes and they can be selected as potential candidates in the genetic improvement programme of this species.

Genotypic and phenotypic coefficient of variation

For most of the studied traits, the genotypic coefficient of variance was slightly lesser than the phenotypic coefficient of variance (Table 3). It indicates that the studied traits were less influenced by environmental factors. Rawat & Nautiyal (2007) reported similar results for the growth traits of *D. sissoo* under field trails. The highest GCV and PCV values were recorded particularly for number of branches (71.0% and 81.55%), plant height (40.32% and 53.21%), collar diameter (21.78% and 31.81%), leaflet length (23.42% and 26.15%) and length of midrib (26.7% and 28.94%). The high GCV and PCV values indicate the existence of high variability in these particular traits. High variability specifies the possibility of effective selection for the improvement of these traits. However, the moderate GCV and PCV were found for the rachis length (11.96% and 14.50%), while moderate GCV and high PCV was recorded for petiole length (18.82% and 28.27%), leaflet width (17.75% and 21.14%) and distance between leaflets (10.76% and 21.59%). High PCV and low GCV values indicate environmental influence on the trait. Generally, the moderate to low variability depict the need for enrichment or improvement in breeding material (Terfa & Gurmu 2020).

Heritability

Heritability is considered as an indicator of transmissibility of traits from parents to progeny. It can be classified as low, medium and high Heritability based on its value < 30%, 30%–60% and > 60% respectively. These estimates help the plant breeder in identification of most suitable traits for genetic improvement. Mostly the traits with high and moderate heritability are selected for the genetic improvement of plants. Most the traits taken into consideration for this study were manifested from moderate to high heritability. Of the tested traits high heritability was recorded for leaflet length (82.09%), length of midrib (79.08%), number of branches per plants (75.96%), leaflet width (70.48%), and rachis length (68%). While, the moderate heritability

Table 3 Estimates of variability and heritability in traits of *D. sissoo*

Characters	Range	Mean ± SE	σ^2_g	σ^2_p	GCV (%)	PCV (%)	H2BS (%)
Plant height (cm)	33.2–173.6	112.283 ± 4.987	2078.25	3570.180	40.60	53.21	58.21
Collar diameter (mm)	8.82–19.39	14.332 ± 0.429	9.753	20.790	21.79	31.81	46.91
No. of branches per plant	2.6–33.8	11.767 ± 0.607	69.944	92.069	71.07	81.54	75.96
Rachis length (cm)	4.86–8	6.3333 ± 0.067	0.573	0.843	11.96	14.50	68.00
Petiole length (cm)	1.04–2.18	1.648 ± 0.045	0.096	0.217	18.82	28.27	44.30
Leaflet length (cm)	3.99–8.34	4.9504 ± 0.071	1.375	1.675	23.69	26.14	82.09
Length of midrib (cm)	3.69–7.86	4.725 ± 0.072	1.169	1.472	22.84	25.68	79.08
Leaflet width (cm)	2.60–4.78	3.428 ± 0.510	0.370	0.525	17.75	21.14	70.48
Distance b/w leaflets (cm)	1.07–1.74	1.413 ± 0.034	0.023	0.093	10.76	21.59	24.85

was recorded for petiole length (44.30%), collar diameter (46.91%) and plant height (58.21%). Distance between leaflets shows the lowest heritability at 24.85%. These findings are in line with earlier findings as high heritability for the growth traits like plant height and DBH has been reported in *D. sissoo* (Sharma & Bakshi 2014). Basically, heritability indicates about the proportion of variations in traits controlled by the genetic difference among the individuals of a population. Higher heritability means higher genetic control of a particular trait and greater the response to the selection.

Genetic divergence

The clustering pattern reveals the relatedness among genotypes and most closely associated genotypes are grouped together in a cluster based on the studied traits (Yeshitila et al. 2023). Two major clusters, consisting cluster A and B (Figure 3) were formed in a hierarchical clustering of genotypes using Unweighted Pair Group Method with Arithmetic mean (UPGMA). Cluster A was smaller and consisted of five genotypes (DSHH6, DSHH2, DSHJ5, DSHJ3, DSHJ2) while cluster B was larger including seven genotypes (DSHH1, DSHJ4, DSHJ1, DSHH4, DSHH5, DSHJ6, DSHH3). In both the clusters genotypes belonging to both Himachal Pradesh and Jammu & Kashmir were present indicating the geographical distribution had played no any significant role in clustering pattern. In many studies, it has been observed that genetic diversity and their geographic dispersions are not always correlated and genotypes from different regions grouped together in hierarchical clustering based on similarity in their traits (Singh & Sofi 2012). Cluster A and B both consisted of two distinct sub-clusters. The sub-cluster I under major cluster A consists of two genotypes such as DSHJ2 and DSHJ3 and both were from higher elevations respectively at 644 and 679 m in Jammu and Kashmir. The cluster mean of studied traits for both the clusters indicated the major role of plant height, collar diameter, and number of branches per plant in grouping of genotypes. The cluster mean of these three traits demonstrated significantly higher difference among both the clusters than that of other studied traits (Table 4).

Principle Component Analysis (PCA) was also used to investigate connections between selected genotypes and studied morphological characters. Four principal components (PCs) with Eigen value greater than one were obtained

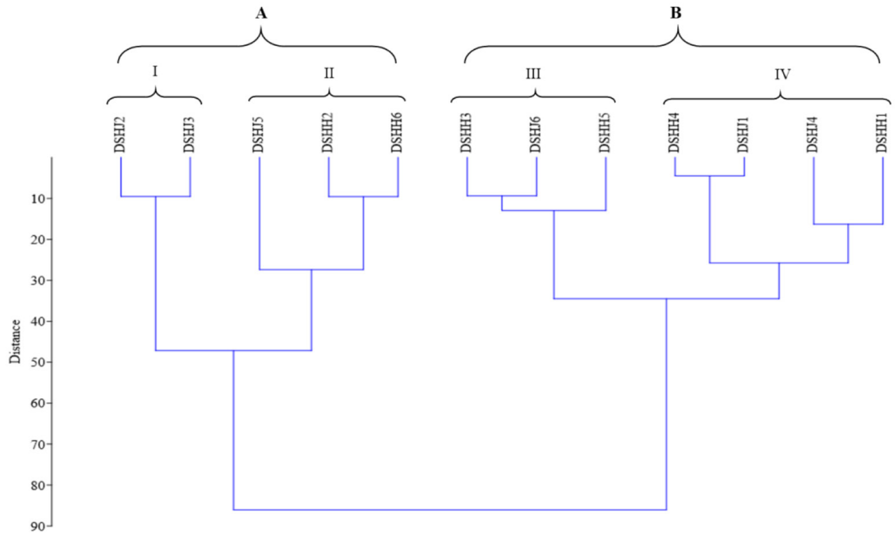

Figure 3 Cluster dendrogram of *D. sissoo* genotype

Table 4 Cluster mean of the traits of *D. sissoo*

	Cluster means for different traits								
Cluster No.	Plant height (cm)	Collar diameter (mm)	No. Of branches per plant	Rachis length (cm)	Petiole length (cm)	Leaflet length (cm)	Length of midrib (cm)	Distance b/w leaflets (cm)	Leaflet width (cm)
1	147.49	15.98	16.54	6.27	1.55	4.96	4.79	1.43	3.49
2	63.00	12.02	5.08	6.42	1.78	4.94	4.63	1.39	3.34

in the analysis (Table 5). Principal component 1 (PC1) had highest Eigen value ($\lambda 1 = 2704.19$) followed by the PC2 (49.03), therefore the PCA biplot between both the components (PC1 and PC2) was used for the interpretation of result. The significant role of plant height, collar diameter, and number of branches per plant in grouping of genotypes was also evident from their loading values in PC1 and PC2. The loading value for the traits in PC1 was maximum for plant height (0.993), followed by number of branches per plant (0.107) and collar diameter (0.0369). However, PC2 displayed maximum loading value for number of branches (0.9714) followed by collar diameter (0.2060), petiole length (0.0127) and length of rachis (0.0175). The genotype DSHJ4 was present as an outlier on the PCA biplot which was distinct in terms of number of branches per plant. It displayed very high number of branches per plant than the other genotypes. Genotypes DSHH3, DSHH5 and DSHJ6 showed positive correlation for plant height and they were also grouped together in sub-cluster III in cluster analysis (Figure 4). In terms of plant height, these three genotypes were at par maximum among the studied genotypes. Furthermore, genotypes DSHH4, DSHH1 and DSHJ1 were also close to each other in terms of various leaf traits, collar diameter and plant height. The genotypes DSHJ3 and DSHJ2 grouped together and both were at par in most of the studied traits. Both the genotypes attained lowest plant height and lesser number of branches per plant. Three genotypes DSHH2, DSHH6 and DSHJ5 which were part of same sub-cluster II under Cluster A also showed closeness in PCA biplot. DSHH6 and DSHJ5 both were negatively related to number of branches per plant and possessed least number of branches. It is very evident from the hierarchical clustering and PCA that plant height, collar diameter, and numbers of branches per plant are highly weighted variables and are extremely important for grouping and hybridization of *D. sissoo*

Table 5 Principal Component analysis for morphometric traits among different genotypes

Parameters	PC 1	PC 2	PC 3	PC 4
Length of Rachis (cm)	-0.00261	0.01755	0.091527	0.33055
Petiole length (cm)	-0.00378	0.012752	0.060021	0.05111
Leaflet length (cm)	0.00261	-0.00422	0.002638	0.64892
Length of Midrib (cm)	0.003192	-0.00636	0.033396	0.6078
Distance b/w leaflets (cm)	0.000261	-0.00072	-0.0151	0.052107
Leaflet Width (cm)	0.001832	-0.02664	-0.01247	0.30363
Collar diameter (mm)	0.036969	0.20608	0.97069	-0.04836
Plant height (cm)	0.9935	-0.11255	-0.01303	-0.00336
No. of branches per plant	0.10744	0.9714	-0.21	0.018393
Eigen Value	2704.19	49.03	6.98	3.14
Percentage Variance	97.58	1.98	0.28	0.12
Cumulative % of Variance	97.58	99.56	99.84	99.96

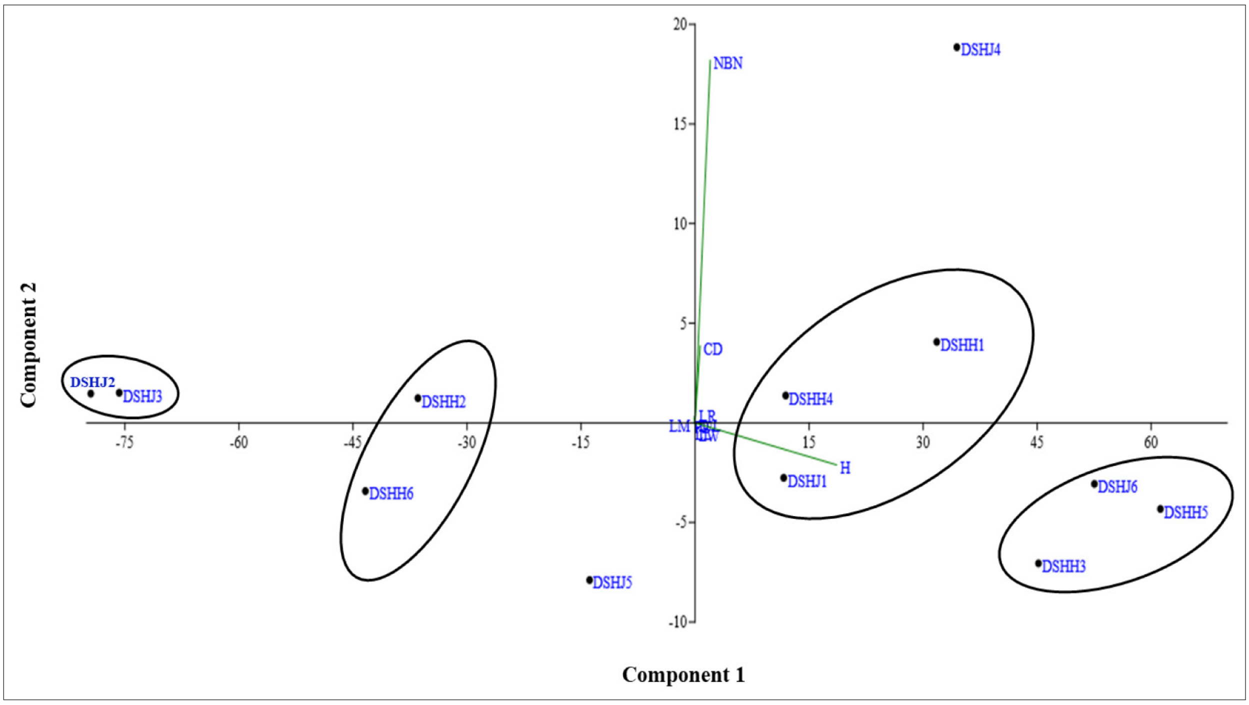

Figure 4 Principal Component Analysis (PCA) based on morphological traits of different genotypes

genotypes. Sharma and Bakshi (2014) have also reported maximum contribution of plant height and collar diameter growth in divergence of *D. sissoo*.

CONCLUSIONS

Understanding of variability in traits and their inheritance pattern is very crucial for selection and improvement in desired traits of a species. In this investigation sufficient variation in the phenotypic traits like plant height, collar diameter and leaf morphology were observed in the selected *D. sissoo* genotypes. Traits such as height and diameter growth are crucial for the selection and improvement of timber tree species. Among the selected genotypes DSHJ6, DSHJ4 and DSHH1 demonstrated significantly better performance in both traits, making them strong candidates for future breeding and plantation activities. Due to high to moderate heritability and high contribution on grouping of individual variables like plant height, diameter, leaf associated traits (rachis length, leaflet length, length of midrib) and number of branches per plant can be used in screening reliable genotypes of shisham in breeding program. Furthermore, crossing individuals from different clusters and sub-clusters can facilitate the combination of desired traits and lead to substantial genetic gains. Thus, the findings of this study are instrumental in enhancing parent selection and breeding strategies by providing more accurate genetic estimates and promoting inter-cluster hybridisation of shisham genotypes.

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