Research Article

Variability in Growth, Physiological, and Biochemical Characteristics among Various Clones of *Dalbergia sissoo* in a Clonal Seed Orchard

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Growth and physiological variability among clones of *Dalbergia sissoo* growing in a CSO revealed maximum height and GBH in Gonda clones (C196 and C198) and minimum growth attributes in Rajasthan clones. All biochemical constituents except sugar were also maximum in Gonda clones. Maximum chl. a, total chl., and chlorofluorescence (CF) were recorded in C235 and C123 while chl. b was maximum in C198. Among tested clones, sugar content was maximum in C60 (Chhachhrauli) while C198 (Gonda) revealed maximum protein content. Heritability estimates of 8 characters at 99% revealed strong genetic control of total chls., sugars, proteins, and chl. b; however, maximum genetic gains of 117% and 80% were recorded for sugar and protein content, respectively. Correlation matrix revealed a positive correlation between height and GBH and CF. Among biochemical constituents, chl. a, and chl. b, chl. b, and total chl. were correlated significantly at 0.1% level. Regarding contribution of different parameters to variability, height and GBH were the greatest contributors. Clustering of clones on the basis of all three parameters separated clones in one major and six minor clusters. Average distance from centroid was found to be 22.61 whereas the maximum distance from centroid was 50.75.

1. Introduction

Dalbergia sissoo Roxb. (Shisham) is a multipurpose tree species of India, Bangladesh, Pakistan, and Nepal. It is one of the seven most valuable timber species in Asia and indigenous to India and Burma. It is important timber of Northern India and constitutes 70% of commercial stock used by the timber industry. The heartwood of Shisham is extremely durable and forms a raw material for a variety of wood based industries. The species is widely distributed in sub-Himalayan tract from Indus to Assam and occurs up to an elevation of 900 m extending occasionally up to 1500 m. in the natural habitat. It grows naturally in new alluvium formed deposits but prefers well drained sandy loam soils with adequate moisture supply. Being multipurpose nitrogen fixing tree, it is among the most favored plantation species of Northern India. Under favorable conditions, it attains a height of 30 m and a girth of 2.4 m [1].

Heavy mortality of Shisham registered almost in all Shisham growing areas of India during the last few decades calls for improvement of the species for growth, tree form, and disease resistance. For any tree improvement program, it is foremost important, first, to scan all available genetic variation within/between species and delineation of the best genotype matching to the site for increase in productivity. Screening and evaluation of different genotypes of tree species for adaptability and growth characteristics is essential for tree breeding programs.

Improvement activities in Shisham at FRI were oriented towards selection of candidate plus trees, establishment of vegetative multiplication gardens, seedling and clonal seed orchard, progeny and provenance testing, and so forth. A clonal seed orchard of Shisham was established in year 1996 at Lachhiwala (Dehradun) under World Bank FREE project. Screening of clones showing dominance effects in growth, tree form, disease resistance, and other physiological

attributes in this clonal seed orchard was essential to identify productive clones matching the site.

Physiological parameter like chlorophyll fluorescence is used frequently to determine the state of energy dissipated in the thylakoid membrane, the quantum efficiency of PSII, and the extent of photo inhibition [2]. Husen [3] used this tool to select high quality seedlings or clones for a particular environment. One of the advantages of this method is that it is nondestructive, noninvasive, and rapid procedure to detect leaf perturbation [4]. The ratio of variable fluorescence to maximal fluorescence (FV/Fm) is regarded as the measure of radiation energy absorbed in photosynthesis and is commonly used to assess the relative state of PSII. Fv/Fm is used frequently as an expression of photo inhibition [5, 6]. Greater FV/Fm ratios imply more utilization of absorbed radiant energy and intensification of the Calvin cycle reactions. The capacity of a plant to use and dissipate light energy is a function of both genotype and environmental conditioning. CF has been used for genotypic selection, early selection of clones, or high quality seedlings and seedling growth [3, 4, 7]. The ability of genotype to use a greater portion of the absorbed light energy to assimilate a significantly greater amount of excess energy and thereby reduce the extent of photo-inhibition is variable. In addition to CF, the chlorophyll content of leaves provides supporting evidence in assigning light tolerance of various species/genotypes.

Screening of some clones of *Dalbergia sissoo* for growth, physiological, and biochemical parameters at nursery stage was performed [3] but no detailed report is yet available on variability of clones in the field considering different morphophysiological and biochemical variables which have great significance for improvement of the species. The present work was thus framed out to assess variability in morphological, physiological, and biochemical traits of different clones of Shisham growing in a clonal seed orchard at Lachhiwala (Dehradun) at 13 years of age which could be exploited for future breeding and improvement strategies of the species.

2. Material and Methods

The present investigation was carried out in a clonal seed orchard (CSO) of *Dalbergia sissoo* raised at Lachhiwala, Dehradun (India), which was established in year 1996 under World Bank FREE project.

2.1. Design, Spacing, and Planting Material. The experimental material comprised of 30 clones of *Dalbergia sissoo* and was collected from diverse locations of India and Nepal. The clones were planted at the spacing of 6 m × 5 m in randomized block design (RBD) with three blocks and each block consisted of nine ramets. The clones were 13 years of age at the time of study. The geographical details of *Dalbergia sissoo* clones are given in Table 1.

2.2. Morphological Observation. For morphological studies, observations were recorded on tree height (m) and girth at breast height (cm) on four competitive trees in each block in year 2008 (13 years of age). Tree height was measured with

the help of clinometer (Suunto 37039 Forestry suppliers Inc., Japan) while GBH of the stem was taken at 1.37 m above the ground with the help of measuring tape.

2.3. Physiological Characteristics. Chlorophyll fluorescence emitted by green plants reflects photosynthetic activity of photo system II. A handy plant efficiency analyzer (Handy PEA, Hansatech, UK) was used to monitor chlorophyll fluorescence variable yield (Fv/Fm). Measurements were taken in the month of April at 11:00 AM which was the best time for peak fluorescence. The minimal fluorescence level (Fo) with open PS II induces significant variable fluorescence. The maximum fluorescence level (Fm) of closed PS II centers was determined by providing 1.5 sec saturating pulse at $3000 \mu \text{mol m}^{-2} \text{s}^{-2}$ using dark adapted leaves.

2.4. Biochemical Characteristics. Fresh leaves were collected from three individual trees/clone and brought to plant physiology laboratory in ice boxes. They were stored at -20°C in freezer (Vest frost DFS 345). Quantification of chlorophyll and protein content were done as per protocol developed [8, 9]. For sugar quantification, samples were taken out from the freezer (Vest frost DFS 345), thawed for 20 minutes, and kept at 70°C in oven (YORCO Pvt. Ltd., New Delhi) for two days. The total saccharides moiety in a sample was estimated by the anthrone method [10].

2.5. Statistical Analysis. The data recorded was subjected to *analysis of variance* (ANOVA) to quantify the variation existing among clones for various recorded parameters.

2.5.1. Analysis of Variance (ANOVA). The data was analyzed using *Genstat version 3.2* as per the designed experiment, that is, randomized block design. The sources of factors were clone and blocks with interaction factor. The *F* values thus obtained for different sources of variation were compared with the tabulated values at 0.1% level of significance and respective degree of freedom of source and error. For better interpretation of the significant results, critical differences (CD) and least significant differences (LSD) were calculated. The values of CD and LSD indicate that the treatment (clone) is statistically at par or not.

2.5.2. Computation of Diversity Parameters. Genotypic, phenotypic, and environmental variances were calculated using the following:

$$\text{Genotypic variance } (V_g) = \frac{(M_t - M_e)}{r}, \quad (1)$$

where M_t = mean sum of square of treatment, M_e = mean sum of square of error, and r = block replicates,

$$\text{Environmental variance } (V_e) = M_e, \quad (2)$$

$$\text{Phenotypic variance } (V_p) = V_g + V_e.$$

TABLE 1: Geographical details of *Dalbergia sissoo* clones planted in clonal seed orchard.

S. number	Clone number	State	Forest division	Altitude (meters)	N	E
1	6	Uttarakhand	Chidiyapur	257.88	29°40'58"	78°12'72"
2	10	Uttarakhand	Pathri			
3	18	Uttar Pradesh	Shah Mansoorpur (Saharanpur)	260.27	29°58'43"	78°23'23"
4	19	Uttar Pradesh	Khanpur	—	—	—
5	34	Uttar Pradesh	Tulsipur	—	—	—
6	42	Uttar Pradesh	Tulsipur	—	—	—
7	59	Haryana	Ambala	262.42	30°21'32"	76°52'47"
8	60	Haryana	Ambala	262.42	30°21'32"	76°52'49"
9	66	Haryana	Yamuna Nagar	253.33	30°10'39"	77°28'77"
10	67	Haryana	Yamuna Nagar	253.33	30°10'39"	77°28'77"
11	78	Rajasthan	Sri Ganga Nagar	175.76	29°35'12"	74°21'30"
12	80	Rajasthan	Sri Ganga Nagar	175.71	29°35'08"	74°21'29"
13	84	Rajasthan	Sri Ganga Nagar	175.70	29°35'10"	74°21'31"
14	85	Rajasthan	Sri Ganga Nagar	175.72	29°35'10"	74°21'34"
15	87	Rajasthan	Shergarh	177.16	29°58'52"	74°32'41"
16	88	Rajasthan	Shergarh	177.16	29°58'21"	74°32'23"
17	89	Rajasthan	Shergarh	177.16	29°58'32"	74°32'35"
18	92	Rajasthan	Lakhanwal Village	177.16	29°52'55"	74°29'32"
19	93	Rajasthan	Lakhanwal village	177.16	29°52'55"	74°29'42"
20	94	Rajasthan	Lakhanwal village	177.16	29°52'50"	74°29'55"
21	103	Rajasthan	Hanumangarh	179.12	29°55'23"	74°29'70"
22	123	Nepal	Sagarnath			
23	189	Uttar Pradesh	Gonda	119.98	27°83'12"	74°32'39"
24	192	Uttar Pradesh	Gonda	119.24	27°83'12"	74°32'39"
15	193	Uttar Pradesh	Gonda	119.00	27°83'12"	74°32'39"
16	194	Uttar Pradesh	Gonda	119.86	27°83'54"	74°32'71"
27	196	Uttar Pradesh	Gonda	123.80	27°13'54"	81°93'45"
28	198	Uttar Pradesh	Gonda	123.86	27°13'50"	81°93'23"
29	202	Uttar Pradesh	Gonda	123.82	27°13'54"	81°93'49"
30	235	Uttar Pradesh	Gonda	128.00	27°13'54"	81°93'20"

2.5.3. *Broad Sense Heritability* H^2 . Heritability is the ratio of genetic variance to the total phenotypic variance and was calculated as suggested [11, 12]. Consider

$$H^2 = \frac{V_g}{V_p} * 100, \quad (3)$$

where H^2 = heritability in broad sense.

2.5.4. *Genetic Variability*. Genetic advance (GA) is the expected increase in the magnitude of a particular character when a selection pressure of chosen intensity (i) is applied. This was calculated as per Johnson et al. [12]. Consider

$$GA = \frac{V_g}{V_p} * K * \sqrt{V_p}, \quad (4)$$

where K = selection intensity.

In this study, K was given the value 2.06 which is its expectation in case of 5% selection in large samples from normally distributed population [13].

2.5.5. *Genetic Gain*. Genetic gain expected in percent of mean was calculated using the formula given [12]. Consider

$$\text{Genetic gain} = \frac{GA}{X} * 100, \quad (5)$$

where X = total mean of the clones.

2.5.6. *Correlation Studies*. The linear relationship between and within various morphological, physiological, and biochemical parameters was studied with the help of Minitab release 11.2 using Karl Pearson's simple correlation coefficients.

2.5.7. *Estimation of Genetic Diversity*. For estimation of genetic diversity parameter, clustering analysis of morphological, physiological, and biochemical parameters was carried out with Euclidean method and the Ward linkage cluster analysis using software Minitab release 11.2.

2.5.8. *Contribution of Different Characters towards Divergence*. Percentage contribution of different morphological,

physiological, and biochemical characters towards divergence was estimated with the help of principal component analysis (PCA) using correlation matrix method in Genstat 3.2 software.

3. Results and Discussion

For any tree improvement program, genetic variability studies are prerequisite. Variations within a tree species are due to the effects of heredity and environment. Environmental variations can be reduced by growing the identical genotypes under uniform site and climatic conditions. For genetic variability studies in *D. sissoo*, the present work was carried out in a particular homogenous environmental condition.

The data on morphological, physiological, and biochemical attributes in relation to various clones is cited in Table 2. Clone 196 attained the maximum height (20.17 m) and GBH (74.20 m) while clone 93 and clone 89 showed the minimum height (7.17 m) and GBH (26.80 cm), respectively. High degree of variation (0.1% level) was observed in height and GBH after 13 years of plantation. On the basis of height parameter, genotypes 196, 198, 192, 123, and 235 were identified as superior performing clones while genotypes 196, 123, 194, 198, and 202 were superior on the basis of GBH. These variations in growth characteristics of clones may be attributed to inherent genetic factors as well as the effect of environmental conditions, which may vary from provenance to provenance. Pathak et al. [14] have also reported that the genetic and environmental factors influenced the growth performance of plants. Clones from Gonda and Nepal were performing better than the clones of Rajasthan. This could be due to the fact that clones from these regions were easily adapted being of an origin from the similar ecoclimatic conditions than the clones from Rajasthan. Similar variations in various morphological characteristics were observed earlier by many researchers [3, 15–21]. Rawat and Nautiyal [18] also suggested that variation in growth characteristics of the plants is essentially genetic in nature if it differs in identical environmental conditions.

The analysis of fluorescence ratio (Fv/Fm) after 13 years of growth revealed wide degree of variations among clones (Table 2). Maximum chlorophyll fluorescence was found in clones 123 and 235 (0.760), followed by clones 202 and 192 (0.742), while minimum was observed in clone 89 (0.565), whereas the range was from 0.565 to 0.760 with the mean value of 0.669. All clones were significantly different in chlorophyll fluorescence at 0.1% level with coefficient of variation of 4.8. It has been observed that high Fv/Fm ratio is an indicator of greater production of quantum yield of the photochemical in the clones and hence increase in photosynthetic efficiency. The high value of Fv/Fm is also supported with high values of height and GBH in this study. These remarkable variations in Fv/Fm may be due to effects of genotypes and origin, high photosynthetic pigment contents in leaves, and so forth. Similar clonal variations in Fv/Fm ratio were reported [22] in rubber. Genotypic variations in chlorophyll fluorescence have also been reported [3, 23] in Shisham. Genotype site interactions of Shisham were

reported for photosynthetic rate and pigments in Shisham [18]. Substantial differences in photosynthetic efficiency of different species of *Illium* taxa were also worked [7].

Chlorophyll a and b and total chlorophyll also depicted significant variation (0.1%) with maximum chlorophyll a content (0.92 mg/g fresh weight), chlorophyll b (0.54 mg/g fresh weight), and total chlorophyll (a+b), (1.46 mg/g fresh weight) estimated in clones 235 and 123 belonging to Gonda and Nepal, respectively (Table 2). Devgiri [24] also reported the higher chlorophyll content in *Dalbergia sissoo* seed sources collected from arid and semiarid region. Probably, it could be that the plants in such region might depend on photosynthesis during cooler part of the day, that is, early morning. Maximum chlorophyll ratio a/b was found in clone 198 (1.89), followed by clone 66 (1.85), while minimum ratio was observed in clone 19 (1.49). The range varied from 1.49 to 1.89 with the mean value of 1.68. All clones were significantly different in chlorophyll ratio a/b at 0.1% level (Table 2). Such significant clonal variations in chlorophyll content of Shisham clones in our studies are in confirmation with the work in Shisham [3]. Lewandowska and Jarvis [25] reported that, apart from internal plant factors, other factors, namely light, temperature, and nutrient status of the site, also affect chlorophyll formation in leaves. Lewandowska and Jarvis [25] reported maximum chlorophyll content in summer and minimum in winter for Sitka spruce. Similar observations were recorded in deciduous tree species [26]. In our studies also maximum chlorophyll fluorescence coupled with chlorophyll was observed during summer months (April). Thus, as a part of adaptive strategy, the high chlorophyll content in plant will help in photosynthetic activity even at low light intensity, which supports the present findings.

With regard to biochemical contents, significantly high protein content was found in clone 198 (85.43 mg/gm fresh weight) followed by clone 196 (67.47 mg/gm fresh weight) belonging to Gonda (UP), while minimum content was observed in clone 89 (19.97 mg/gm fresh weight). The range varied from 19.97 to 85.43 mg/gm fresh weight with the mean value of 39.23 mg/gm fresh weight (Table 2). The turnover characteristic of individual protein depends on their intracellular location and accessibility. The probable cause associated with interclonal variation with respect to total soluble protein content may be mainly due to the genetic makeup of the clones as the genetic factors exert more influence in expressing this character than the environmental factors; protein content of different plant parts showed seasonal variation and differed from one species to the other. Chalupa and Durzan [27] showed considerable variation in soluble proteins in *Pinus banksiana* provenances. Significant variations were also reported in protein content among selected clones of *Populus deltoides* and seed sources and clones of *Dalbergia sissoo* [3, 16, 28].

The sugar content significantly ($P < 0.01$) ranged from 4.11 to 58.23 mg/gm dry weight with the mean value of 21.51 mg/gm dry weight. The maximum total sugar content was estimated in clone 60 (58.23 mg/gm dry weight) belonging to Ambala, Haryana, followed by clone 103, Nepal (52.77 mg/gm dry weight), and clone 235 (42.23 mg/gm dry

TABLE 2: Variation in morphophysiological and biochemical characteristics of different clones of *Dalbergia sissoo* (Shisham).

S. number	Clone number	Height (M)	GBH (cm)	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Chlorophyll ratio a/b	Total chlorophyll (mg/g fresh weight)	Chlorofluorescence Fv/Fm (11 hrs)	Sugar (mg/gm dry weight)	Protein (mg/gm fresh weight)
1	6	17.52	60.70	0.859	0.532	1.617	1.391	0.719	26.01	25.50
2	10	17.25	64.40	0.541	0.345	1.568	0.886	0.692	16.79	34.02
3	18	11.30	61.30	0.367	0.205	1.790	0.571	0.642	18.38	29.49
4	19	13.66	48.20	0.450	0.302	1.490	0.753	0.696	24.65	31.01
5	34	7.60	28.00	0.533	0.329	1.620	0.863	0.662	15.04	30.76
6	42	7.73	29.30	0.641	0.352	1.820	0.993	0.642	13.63	28.22
7	59	11.08	42.20	0.729	0.418	1.742	1.147	0.628	17.58	38.84
8	60	10.42	44.30	0.606	0.388	1.560	0.994	0.621	58.23	39.64
9	66	11.68	42.20	0.679	0.367	1.850	1.046	0.674	10.89	42.02
10	67	7.51	36.80	0.492	0.283	1.740	0.775	0.651	21.55	61.65
11	78	8.03	33.70	0.798	0.504	1.583	1.302	0.653	15.72	36.40
12	80	13.32	43.30	0.754	0.449	1.681	1.202	0.652	13.03	24.01
13	84	7.84	34.70	0.583	0.359	1.623	0.942	0.600	7.25	38.95
14	85	12.09	34.70	0.769	0.467	1.646	1.236	0.674	14.66	30.50
15	87	8.24	32.30	0.719	0.417	1.723	1.137	0.683	14.96	61.76
16	88	7.85	27.90	0.851	0.495	1.720	1.346	0.615	36.25	37.08
17	89	7.76	26.80	0.822	0.452	1.817	1.274	0.565	4.11	19.97
18	92	9.85	39.30	0.671	0.433	1.550	1.104	0.593	12.15	39.69
19	93	7.17	36.80	0.637	0.402	1.587	1.039	0.604	17.87	56.52
20	94	12.41	33.70	0.737	0.453	1.627	1.189	0.635	9.07	48.35
21	103	8.18	36.80	0.397	0.217	1.833	0.614	0.653	52.77	24.21
22	123	18.01	69.70	0.920	0.539	1.707	1.458	0.760	25.08	29.63
23	189	14.85	48.60	0.449	0.253	1.777	0.702	0.672	17.65	32.73
24	192	18.32	50.80	0.859	0.532	1.617	1.391	0.742	26.16	25.98
25	193	15.98	53.10	0.491	0.293	1.673	0.784	0.705	18.31	33.74
26	194	15.91	68.70	0.859	0.532	1.617	1.391	0.708	26.26	58.76
27	196	20.17	74.20	0.867	0.500	1.733	1.368	0.730	18.15	67.47
28	198	19.07	65.30	0.573	0.303	1.893	0.876	0.692	18.43	85.43
29	202	15.20	64.80	0.859	0.532	1.617	1.391	0.742	32.57	39.66
30	235	16.84	64.00	0.920	0.539	1.707	1.458	0.760	42.23	24.78
Mean		12.43	46.6	0.681	0.406	1.684	1.087	0.669	21.51	39.23
Standard error difference		1.308	7.81	0.015	0.01	0.02	0.02	0.030	0.80	0.76
Least significance of difference		2.572	15.35	0.03	0.02	0.04	0.05	0.053	1.60	1.53
Coefficient of variation		25.8	41.1	2.9	3.0	1.3	2.9	4.8	4.5	2.4

TABLE 3: Estimation of heritability, genetic advance, and genetic gain in various traits.

S. number	Characteristics	Heritability %	Genetic advance	Genetic gain %
1	Height (m)	86.6	15.71	126.3
2	GBH (cm)	66.6	45.4	97.4
3	Chlorofluorescence	80.33	0.089	13.32
4	Chlorophyll a (mg/gm)	96.66	0.346	55.8
5	Chlorophyll b (mg/gm)	99.13	0.218	59.37
6	Chlorophyll a/b ratio	96.17	0.224	13.16
7	Total chlorophylls	99.46	0.56	56.55
8	Sugar (mg/gm)	99.38	25.38	117.6
9	Protein (mg/gm)	99.26	31.242	79.64

weight) (Table 2). Probably these clones were more efficient photosynthetically and ultimately convert more solar radiation and carbon dioxide into photosynthates in the form of carbohydrates. Chalupa and Durzan [27] showed considerable variation in total soluble sugar and starch contents in *Pinus banksiana* provenances. Singh [29] also reported significant variation in *D. sissoo* seed sources in sugar content. Highly significant differences were observed in total carbohydrate content among seed sources of Shisham [16].

Estimates of heritability (broad sense), genetic advance, and genetic gain were also worked out for various characteristics and cited in Table 3. Heritability estimates in broad sense were more than 75% for height, crown length, and width which is in confirmation with earlier findings in Shisham [16, 18, 21, 30, 31]. Such variation in growth characteristics is essentially genetic in nature if it differs in identical environmental condition [18]. High heritability (broad sense) may be due to nonadditive gene action hence reliable only if accompanied with high genetic gain. High estimates of heritability (above 99%) were observed for total chlorophyll, sugar, protein, and chlorophyll b. Maximum (31.24) of genetic advance was observed for protein and minimum (0.089) for leaf chlorofluorescence whereas genetic gain was maximum (126.3) for height and minimum (13.16) for chlorophyll ratio. High heritability coupled with some intensity of genetic gain was exhibited for height and GBH, while sugars and proteins exhibited more than 99% heritability with good amount of genetic gain. High heritability accompanied with high genetic advance for several growth parameters has earlier been reported [12, 16, 18, 31]. Johnson et al. [12] suggested that high heritability estimates in conjunction with genetic advance are predictable for selecting the best genotype. On the basis of above genetic component, it can be inferred that height, chlorophyll content, protein, and sugar are under strong genetic control and hence can reliably be used as selection criteria for further improvement while GBH and photosynthetic efficiency are more under environmental control hence not reliable for selection.

Expression of a character is the sum total of contribution of other characters and, therefore, screening/selection should be made on the basis of components contributing towards that character. The biometrical tool for helping this is correlation, which depicts the degree and magnitude of relationship

of one character with other characters. The interaction of different characters helps in selection of traits. Improvement of one character leads to simultaneous improvement in other characters. The linear relationship between and within various morphophysiological and biochemical characteristics of *Dalbergia sissoo* clones were studied using Karl Pearson's simple correlation method. Correlation matrix revealed that growth, physiological, and biochemical traits had significant correlations of varying magnitude among themselves. Characteristics, namely, height, GBH, chlorofluorescence, chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll ratio, protein, and sugar exhibited significantly both positive and negative correlation. Clonal height showed highly positive correlation with GBH ($r = 0.881$) at 0.5% level of significance followed by chlorofluorescence ($r = 0.763$) and negative correlation with chlorophyll ratio ($r = -0.036$) (Table 4). The positive correlation of tree height with GBH suggests thus height to be a reliable selection parameter and have positive correlation among both of them [32]. Tree volume is basically a function of tree height and GBH. Thus, it is a reliable selection parameter for tree volume. Khosla et al. [33], in *Pinus roxburghii*, Sharma et al. [34], in *Glaucium flavum*, Dhillon et al. [31], in *D. sissoo*, Rawat [35], in *Pinus wallichiana*, and Gautam [23], in *Dalbergia sissoo*, also reported positive correlation for growth characteristics.

Positive correlations were also discernible between chlorophyll a and total chlorophyll ($r = 0.997$) and chlorophyll a and chlorophyll b ($r = 0.977$) at 1.0% level of significance. Chlorophyll fluorescence and total chlorophyll were positively correlated ($r = 0.314$) (Table 4). Evidences support the highly significant positive correlation between photosynthetic efficiency and chlorophyll content [36]. Sestak and Siffel [37] demonstrated that this linear expression depends on morphological and physiological leaf traits. Significant positive correlation between sugar content and photosynthetic rate was also found in *Terminalia arjuna* [26, 38] to be in confirmation with our findings having significant positive correlation with chlorofluorescence ($r = 0.227$). Negative correlation was observed between protein and sugar ($r = -0.124$) (Table 4).

The contribution of different characters towards divergence was calculated by principal component analysis (PCA) with the following standard correlation matrix method using Genstat 3.2 software. The contribution of different characters

FIGURE 1: Clustering of *Dalbergia sissoo* clones on the basis morphological, physiological, and biochemical parameters with Euclidean method and the Ward linkage cluster analysis.

TABLE 4: Correlation matrix among the various characters of *Dalbergia sissoo* clones.

S. number	Characteristics	1	2	3	4	5	6	7	8	9
1	Height (M)	1.000								
2	GBH (cm)	**0.881	1.000							
3	Sugar (mg/gm)	0.114	0.236	1.000						
4	Protein (mg/gm)	0.124	0.206	−0.124	1.000					
5	Chlorophyll a (mg/gm)	0.304	0.197	0.012	−0.020	1.000				
6	Chlorophyll b (mg/gm)	0.302	0.202	0.043	−0.047	***0.977	1.000			
7	Chlorophyll a/b ratio	−0.036	−0.024	−0.087	0.149	−0.123	−0.328	1.000		
8	Total Chlorophylls	0.305	0.200	0.024	−0.030	***0.997	**0.991	−0.203	1.000	
9	Chloro-florescence	0.763	0.709	0.227	0.046	0.318	0.303	0.021	0.314	1.000

***Correlation at 0.1 % level of significance. **Correlation at 0.5% level of significance.

TABLE 5: Contribution of different leaf morphophysiological and biochemical quantitative characters in creating diversity in Shisham genotypes.

S. number	Characters	Percent contribution
1	Height	44.30
2	GBH	21.17
3	Sugar	12.72
4	Protein	9.65
5	Chlorophyll a	8.07
6	Chlorophyll b	3.01
7	Chlorophyll ratio a/b	1.08
8	Total chlorophylls	0.01
9	Chlorofluorescence	0.0

towards divergence revealed that the maximum contribution was made by height (44.30%) followed by GBH (21.17%), and then sugar (12.72%) and protein (9.65%) and no contribution by chlorofluorescence (Table 5).

Clustering analysis of 30 clones of *Dalbergia sissoo* on the basis of morphological, physiological, and biochemical

characteristics with Euclidean method, and the Ward linkage cluster analysis depicted one major and six minor clusters (Figure 1). Cluster I (major) was comprised of two minor clusters a and b. cluster I a was comprised of ten clones, namely, clones 34, 42, 78, 85, 59, 66, 92, 84, 94, and 80. This group mainly constituted Rajasthan (60%), Gonda (20%), and Haryana (20%). Cluster I b was comprised of nine clones, namely, clones 123, 10, 18, 6, 19, 189, 193, 192, and 202 belonging to Uttar Pradesh (77%), Haridwar (11.5%), and Nepal (11.5%). Cluster II was comprised of clones 67, 93, and 87 belonging to Rajasthan (67%) and Haryana (33%). Cluster III, cluster IV, and cluster V were comprised of single clone in each cluster, namely, clones 88 (Rajasthan), 89 (Rajasthan), and 235 (Gonda), respectively. Cluster VI constituted two clones, namely, clone 60 (Haryana) and clone 103 (Rajasthan). Cluster VII is represented by three clones, namely, clones 194, 196, and 198 all belonging to Gonda. Average distance of clusters from centroid was found to be 22.607 while the maximum distance from centroid was found to be 50.75.

The present study depicted significant interclonal variations in growth, physiological, and biochemical traits in *D. Sissoo* at 13 years of age revealing thus that these traits

could successfully be used to screen reliable clones matching a particular site. Among 30 clones, the clones of Gonda and Nepal revealed maximum Fv/Fm (chlorophyll fluorescence) coupled with maximum growth and hence can be recommended for future plantations and breeding strategies. Productive clones were identified by applying physiological and biochemical tools. The study thus revealed that chlorophyll fluorescence and biochemical markers can enhance selection intensity in addition to growth parameters in tree improvement of *Dalbergia sissoo*.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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