# Phenotypic Variation in Leaves of Santalum album L. 

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#### Abstract

Considerable variation have been noticed in the leaf traits of sandalwood in 1997. Until now, only a limited number of studies have been made to describe the leaf phenotypic variation. The present study was conducted in a compact sandalwood population at the Institute of Wood Science and Technology, Bengaluru, India in June 2022 for phenotypic variation in the leaf of sandalwood. The 20 mature trees with 20 leaf parameters for the assessment of phenotypic variation in leaves were selected. Leaves were categorized into nine phenotypic traits, based on their variation in size and shape, such as big ovate, big lanceolate, big elliptic, medium ovate, medium lanceolate, medium elliptic, small ovate, small lanceolate, and small elliptic. The leaf parameters varied significantly, the leaf length ranged from 2.50 to 11.50 cm , and the breadth from 1.00 to 4.50 cm . Leaf parameters were very useful for better understanding the sandalwood phenotypic variation at the taxonomic level.


Keywords: Leaf, Phenotypes, Sandalwood, Variation, Traits

Indian sandalwood is the world's second most valuable tropical heartwood species (Arun Kumar 2016). The population of sandalwood is dwindling due to the selective removal of mature trees in the wild and it is categorized as a "vulnerable" species by the IUCN (Arun Kumar 2019). As quantification of variation and selection of superior phenotypes are important steps in the improvement of any tree species, the IWST (erstwhile Sandalwood Research Centre), Bangalore in 1977-78 has initiated studies to document variation in leaf, heartwood, and oil. Badami and Venkata Rao (1930) made a study to classify the variation into several varieties to evolve a spike diseases resistant strain of sandalwood. The phenological characteristics of sandalwood especially leaf traits have been deciphered by Kulkarni (1994). Initially, some of the leaf variation were suspected of spike disease, which induces different leaf modifications at various stages of infection (Badami and Venkata Rao 1930). Plants are sessile organisms and can adjust their phenotypic and physiological characteristics with environmental heterogeneity (Matesanzet al 2010). This adaptation mechanism occurs in two possible ways: local adaptation and phenotypic plasticity (Palacio-López et al 2015). Phenotypic plasticity is one of the most considerable mechanisms in plants, responding to various climatic conditions (Matesanz et al 2010, Stotz et al 2021), as evolutionary mechanisms cannot always keep up with climate change (Vázquez et al 2015).

Effects of these plastic responses in several important ecological traits Viz., morphology, physiology, anatomy, and
phenology are well documented (Sultan 2000, Valladares et al 2007). One of the prevalent results of phenotypic plasticity is prominent variation in leaf shape and size (Chitwood 2016, Tsukaya 2018). Phenotypic plasticity is the ability of a genotype to produce different morphological and physiological responses when exposed to different environmental conditions (La'zaro-Nogal et al 2015, Aranda 2017). These different environmental conditions can result in morphological and physiological leaf trait differences (Niinemets and Kull 2001, Sanches et al 2010, Matheus et al 2018). Accordingly, many studies have noted leaf phenotypic responses along various environmental factors, such as temperature (Atkin 2005, McKee et al 2019), precipitation (McDonald et al 2003, Meier and Leuschner 2008) and light (Grassi 2001, Coble 2014). Classification based on leaf types is subjective and may also depend on individual preference. Moreover, a single parameter alone does not adequately reflect the overall phenotypic expression. For these reasons, phenotypic characterization based on numerical criteria has become a common tool to identify and classify morphological characters in taxonomy (Rakonjac et al 2010). Characterization of leaf morphology with many parameters may add additional knowledge to numerical taxonomy. Hence, this study was conducted to describe the phenotypic diversity of leaves in sandalwood.

## MATERIAL AND METHODS

Study area: The present study was conducted in a compact sandalwood population at the Institute of Wood Science and

Technology, Bengaluru, India (N 13 $3^{\circ} 00^{\prime} 67.5^{\prime \prime}$ and $E$ $77^{\circ} 34^{\prime} 20.6^{\prime \prime}$ ) in June 2022. The elevation of the study site is 874 m . The mean maximum and minimum temperature range from 21.0 to $33.0^{\circ} \mathrm{C}$. The annual rainfall is 102.9 cm (Fig. 1).
Sampling and data collection: The sandalwood population is distributed in about 7ha of land. In the 1990s, it was considered one of the best natural population in India. The age of trees is> 10 years old, and the mean girth and height of sandalwood trees are 42.58 cm (GBH) and 6.63 m respectively. In the present study, 20 trees with uniform canopies were randomly selected based on crown shape and size. From each selected tree, leaves were collected in 5 replications, of which 5 leaves in each replication and totally 25 leaves in the middle portion of the crown, from all directions, for morphometric characterization. The mature leaves were categorized into three groups, based on the size of the leaf Viz., Big, Medium, and Small. In each leaf size class, three types were considered based on their leaf shapes Viz., Ovate, Lanceolate, and Elliptic. The morphological variation in 20 parameters were presented in Table 1, Figure 2 (Abdus et al 2011, Runan et al 2022).
Data analysis: An MS Office Excel version of 2021 was used to analyse the recorded data. Descriptive statistics (mean, standard deviation) for quantitative parameters and one-way analysis of variance were used to test the significant difference among leaf traits for all the quantitative parameters. Coefficients of variation (CV) were calculated to compare relative variation in each leaf parameter and correlation matrix among the parameters was calculated. QGIS version of 3.24.0-Tisler was used to make the study map and to document good-quality pictures we used a DSLR- D90 (NIKON) camera.

Table 1. List of the quantitative parameters used for characterization of sandalwood leaves

| Parameters | Measurement unit and description |
| :---: | :---: |
| Leaf Length (LL) | Measuring scale in cm |
| Leaf Middle Width (1/2) (LMW) | Measuring scale in cm |
| Leaf Upper Quarter Width (3/4) (LUQW) | Measuring scale in cm |
| Leaf Down Quarter Width (1/4) (LDQW) | Measuring scale in cm |
| Leaf Tip Angle (LTA) | Protractor in degree |
| Leaf Tip Type (LTT) | Taxonomy books and monographs |
| Leaf Base Angle (LBA) | Protractor in degree |
| Leaf Base Type (LBT) | Taxonomy books and monographs |
| Petiole Length (PL) | Measuring scale in cm |
| Petiole Type (PT) | Taxonomy books and monographs |
| Leaf Area (LA) | Graph sheet in $\mathrm{cm}^{2}$ |
| Leaf Perimeter (LP) | Thread in cm |
| Leaf Margin (LM) | Taxonomy books and monographs |
| Leaf Shape (LS) | Taxonomy books and monographs |
| Leaf Color (LC) | Visual observation |
| Leaf Thickness (LT) | Digital caliper in mm |
| Petiole Color (PC) | Visual observation |
| Vein Color (VC) | Visual observation |
| Leaf Area and Perimeter ratio (A/P) | Mathematical formula |
| Leaf Length and Breadth ratio (L/B) | Mathematical formula |



Fig. 1. Sandalwood population in IWST Bengaluru, Karnataka

## RESULTS AND DISCUSSION

Variation in leaf size: Wide range of morphological variation were observed in the leaves of the sandalwood for all the parameters (Table 2, 3). The leaf length ranged from 2.50 to 11.50 cm and the breadth from 1.00 to 4.50 cm . Based on leaf size, sandalwood leaves were grouped into three classes, Viz., big (9.0-11.5 cm length; 3.6-4.5 cm breadth), medium ( $5.0-8.0 \mathrm{~cm}$ length; 2.0 to 3.5 cm breadth) and small (2.5-4.0 cm length; 1.0 to 1.7 cm breadth). $60 \%$, $25 \%$, and $15 \%$ were medium-sized, smaller-sized and bigger-sized leaf trees were recorded. The LUQW of the standard leaf varies from 0.53 to 2.76 cm , the LDQW varies from 0.64 to 4.12 cm , the LTA varies from $15.93^{\circ}$ to $69.80^{\circ}$, LBA varies from $18.72^{\circ}$ to
$86.52^{\circ}$, PL varies from 0.26 to 1.56 cm , LA varies from 3.43 to $28.84 \mathrm{~cm}^{2}$, LP varies from 3.59 to 21.74 , LT varies from 0.06 to 0.26 mm (Fig. 3).
Variation in leaf shape: 35\% were ovate and lanceolateshaped, and the remaining $30 \%$ were elliptic-shaped (Fig. 4 and Table 2, 3).
Variation of leaf phenotypic traits: The big elliptic leaves recorded the highest mean LL and the big lanceolate leaves recorded the highest mean of LMW and PL. The highest mean of LUQW, LDQW, LA, and LP was recorded in the big ovate leaves and the highest mean LTA, LT and LBA was recorded in medium ovate leaves. The mean lowest value of LL was recorded in small ovate leaves, the mean LMW in

Table 2. Leaf phenotypic traits of Sandalwood (Mean $\pm$ SD)

| Leaf size Leaf shape |  | LL | LMW | LUQW | LDQW | LTA | LTT | LBA | LBT |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Big | Ovate | $9.24 \pm 1.05$ | $3.65 \pm 0.44$ | $2.76 \pm 0.32$ | $4.12 \pm 0.50$ | $64.52 \pm 4.89$ | Acute Subacute | $82.24 \pm 15.95$ | Equilateral, Round |
|  | Elliptic | $10.25 \pm 1.35$ | $3.73 \pm 0.37$ | $1.77 \pm 0.15$ | $3.90 \pm 0.27$ | $59.20 \pm 11.64$ | Acute | $83.68 \pm 15.02$ | Oblique, Round |
|  | Lanceolate | $9.86 \pm 0.82$ | $3.76 \pm 0.26$ | $1.96 \pm 0.22$ | $2.47 \pm 0.31$ | $66.28 \pm 9.53$ | Round Mucronate Acute Subacute | $66.96 \pm 8.72$ | Equilateral, Acute |
| Medium | Ovate | $5.42 \pm 0.73$ | $2.89 \pm 0.42$ | $2.50 \pm 0.43$ | $3.26 \pm 0.48$ | $69.80 \pm 12.43$ | Acute Round Mucronate Obtuse Subacute | $86.52 \pm 9.68$ | Equilateral Oblique, Round, Cuneate |
|  | Elliptic | $7.26 \pm 0.54$ | $2.54 \pm 0.20$ | $1.48 \pm 0.12$ | $2.64 \pm 0.24$ | $58.92 \pm 10.31$ | Acute | $72.64 \pm 7.57$ | Equilateral |
|  | Lanceolate | $6.20 \pm 0.57$ | $3.09 \pm 0.25$ | $1.70 \pm 0.20$ | $2.10 \pm 0.25$ | $60.40 \pm 6.38$ | Acute Subacute | $71.00 \pm 9.11$ | Acute |
| Small | Ovate | $2.51 \pm 0.53$ | $1.12 \pm 0.25$ | $0.93 \pm 0.20$ | $1.19 \pm 0.24$ | $28.03 \pm 5.72$ | Acute Mucronate Retuse | $31.72 \pm 6.78$ | Round, Obtuse |
|  | Elliptic | $2.97 \pm 0.38$ | $1.04 \pm 0.16$ | $0.70 \pm 0.13$ | $1.90 \pm 0.15$ | $20.94 \pm 3.81$ | Acute | $24.36 \pm 4.45$ | Round, Oblique |
|  | Lanceolate | $2.55 \pm 0.19$ | $1.68 \pm 0.09$ | $0.53 \pm 0.07$ | $0.64 \pm 0.08$ | $15.93 \pm 2.05$ | Acute | $18.72 \pm 10.97$ | Acute |

Table 3. Leaf phenotypic traits of Sandalwood (Mean $\pm$ SD)

| Leaf size | Leaf shape | PL | PT | LA | LP | LM | LC | LT | PC | VC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Big | Ovate | $0.97 \pm 0.20$ | Straight <br> Twisted | $28.84 \pm 5.03$ | $21.74 \pm 2.25$ | Entire nonwavy | Green | $0.20 \pm 0.04$ | Yellowish green | Yellowish green |
|  | Elliptic | $1.26 \pm 0.22$ | Straight <br> Twisted | $18.04 \pm 4.38$ | $18.94 \pm 2.76$ | Entire nonwavy | Dark green | $0.18 \pm 0.05$ | Yellowish green | Yellowish green |
|  | Lanceolate | $1.56 \pm 0.18$ | Straight <br> Twisted | $13.52 \pm 2.95$ | $15.66 \pm 1.60$ | Entire wavy | Green | $0.14 \pm 0.02$ | Yellowish green | Yellowish green |
| Medium | Ovate | $1.46 \pm 0.21$ | Straight <br> Twisted | $17.52 \pm 3.88$ | $17.22 \pm 1.58$ | Entire nonwavy | Yellowish green | $0.26 \pm 0.06$ | Yellowish green | Yellowish green |
|  | Elliptic | $1.12 \pm 0.12$ | Straight <br> Twisted | $9.28 \pm 1.47$ | $12.30 \pm 1.21$ | Entire wavy | Medium green | $0.15 \pm 0.06$ | Yellowish green | Yellowish green |
|  | Lanceolate | $1.13 \pm 0.33$ | Straight <br> Twisted | $11.54 \pm 2.15$ | $14.76 \pm 1.44$ | Entire wavy | Yellowish green | $0.17 \pm 0.02$ | Yellowish green | Yellowish green |
| Small | Ovate | $0.44 \pm 0.09$ | Straight <br> Twisted | $4.56 \pm 1.3$ | $4.14 \pm 1.29$ | Entire wavy | Light green | $0.10 \pm 0.02$ | Yellowish green | Yellowish green |
|  | Elliptic | $0.35 \pm 0.06$ | Straight <br> Twisted | $4.65 \pm 0.82$ | $4.69 \pm 0.88$ | Entire nonwavy | Green | $0.08 \pm 0.02$ | Yellowish green | Yellowish green |
|  | Lanceolate | $0.26 \pm 0.03$ | Straight <br> Twisted | $3.43 \pm 0.45$ | $3.59 \pm 0.46$ | Entire nonwavy | Yellowish green | $0.06 \pm 0.01$ | Yellowish green | Yellowish green |

small elliptic leaves, and the lowest mean value of LUQW, LDQW, LTA, LBA, PL, LA, LP, and LT was recorded in small lanceolate leaves (Table 2, 3). The highest $L / B$ was recorded in small and big elliptic leaves and the highest $A / P$ was recorded in big ovate leaves. The lowest value of $L / B$ and $A / P$ was observed in small elliptic and medium lanceolate


Fig. 2. Diagrammatic representation of leaf parameters of sandalwood

respectively. One way analysis of variance confirmed that, there is a significant difference among some of the quantitative parameters of leaf phenotypic traits in sandalwood.
Big Ovate (BO): The length/breadth ratio was 2.53, and the leaf area/perimeter ratio of 1.32 was recorded (Fig. 3 b, e).
Big Elliptic (BE): The length/breadth ratio was 2.74, and the leaf area/perimeter ratio of 0.95 was recorded (Fig. 3 a , d).
Big Lanceolate (BL): The length/breadth ratio was 2.62, and the leaf area/perimeter ratio of 0.86 was recorded (Fig. 3 c).

Medium Ovate (MO): The length/breadth ratio was 1.87, and the leaf area/perimeter ratio of 1.01 was recorded (Fig. 3 h , g).

Medium Elliptic (ME): The length/breadth ratio was 2.85 , and the leaf area/perimeter ratio of 0.75 was recorded (Fig. 3 $\mathrm{f}, \mathrm{i}, \mathrm{n}$ ).
Medium Lanceolate (ML): The length/breadth ratio was 2.00, and the leaf area/perimeter ratio of 0.78 was recorded (Fig. 3j, k, l, m, o).
Small Ovate (SO): The length/breadth ratio was 2.24, and the leaf area/perimeter ratio of 1.10 was recorded (Fig. 3t).
Small Elliptic (SE): The length/breadth ratio was 2.85 , and the leaf area/perimeter ratio of 0.99 was recorded (Fig. 3p, q).
Small Lanceolate (SL): The length/breadth ratio was 1.51, and the leaf area/perimeter ratio of 0.95 was recorded (Fig. $3 r, s)$. Detailed nine phenotypic leaf traits were explained in Tables 2, 3.
Variation in leaf colour: Sandalwood leaf shows distinct colour variation from dark green to yellowish green (Fig. 5).
Variation in tip and base of leaves: A total of six types of variation were observed in both the tip and base of sandalwood leaves. Tip types were Acute, Retuse, Round, Subacute, Obtuse, and Mucronate. Base types are Equilateral, Acute, Cuneate, Round, Obtuse, and Oblique (Fig. 6, 7). All six types of leaf tip were present in ovate leaf, four types of leaf tip (Round, Mucronate, Acute, and Subacute) were recorded in lanceolate and only one type was presented in elliptic (Acute). Five types of leaf bases were present in ovate (Round, Equilateral, Cuneate, Oblique,


Ovate


Elliptic


Lanceolate

Fig. 4. Variation in leaf shapes of sandalwood

Obtuse), three were in elliptic (Round, Equilateral, Oblique), and two in lanceolate (Equilateral, Acute).
Variation in petiole and margin of leaves: Two types of petioles Viz., straight and twisted were observed. The leaf margin showed wavy and non-wavy types. Both veins and petiole were yellowish-green in colour (Fig. 8, 9).
Correlation among leaf phenotypic traits: The correlation matrix (Pearson) of sandalwood leaf parameters showed a significantly positive correlation (Table 4). The highest correlation ( $r=0.96$ ) was between LTA and LBA; the lowest correlation ( $r=-0.02$ ) was observed between LBA and A/P. Thus, all leaf parameters were associated positively with each other.
Extent degree of leaf phenotypic traits: The highest CV was recorded in leaf base angle (LBA) (10.42-58.60\%) and the lowest in leaf middle width (LMW) (5.36-22.32\%.) The coefficient of variation (CV) for all the quantitative traits shown in Table 5.
Unique characteristics feature in leaf traits of
sandalwood: Some of the leaves showed unique characteristic feature in their leaves. The apex of the leaf comes inward direction, this type of observation were recorded in medium-sized elliptic leaves (Fig. 3 g ) and folded leaves (Fig. 3 h ). Some of the small ovate leaves were (margins of the leaf slightly folded inward direction) cupshaped (Fig. 3 t ) and the branches of these trees were a drooping pattern. Small-shaped lanceolate leaves (Fig. 3 m ) were also found in dropping branches of sandalwood trees.


Fig. 5. Colour variation in sandalwood leaves

Table 4. Pearson's correlation matrix among the leaf phenotypic traits

|  | LL | LMW | LUQW | LDQW | LTA | LBA | PL | LA | LP | LT | L/B | A/P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LL | 1 |  |  |  |  |  |  |  |  |  |  |  |
| LMW | 0.937792 | 1 |  |  |  |  |  |  |  |  |  |  |
| LUQW | 0.723431 | 0.814578 | 1 |  |  |  |  |  |  |  |  |  |
| LDQW | 0.810435 | 0.771937 | 0.862085 | 1 |  |  |  |  |  |  |  |  |
| LTA | 0.816756 | 0.883298 | 0.905624 | 0.793295 | 1 |  |  |  |  |  |  |  |
| LBA | 0.815684 | 0.871804 | 0.901077 | 0.878105 | 0.969475 | 1 |  |  |  |  |  |  |
| PL | 0.796597 | 0.856632 | 0.78797 | 0.684421 | 0.951964 | 0.893758 | 1 |  |  |  |  |  |
| LA | 0.759738 | 0.809195 | 0.931588 | 0.910574 | 0.767969 | 0.820255 | 0.610624 | 1 |  |  |  |  |
| LP | 0.879376 | 0.935765 | 0.938618 | 0.921168 | 0.922368 | 0.951816 | 0.827237 | 0.933592 | 1 |  |  |  |
| LT | 0.567908 | 0.698735 | 0.912957 | 0.809332 | 0.881559 | 0.920947 | 0.793978 | 0.790935 | 0.860557 | 1 |  |  |
| L/B | 0.491529 | 0.181495 | 0.142505 | 0.467367 | 0.226803 | 0.239985 | 0.216571 | 0.199161 | 0.243362 | 0.018202 | 1 |  |
| A/P | -0.03572 | -0.03713 | 0.310077 | 0.293106 | -0.09547 | -0.02228 | -0.27121 | 0.490281 | 0.158355 | 0.143907 | 0.03719 | 1 |

Correlation is significant at a 0.05 probability level

Table 5. Coefficient of variation in leaf phenotypic traits (\%)

| CV | LL | LMW | LUQW | LDQW | LTA | LBA | PL | LA | LP |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| BO | 11.36 | 12.05 | 11.59 | 12.14 | 7.58 | 19.39 | 20.62 | 17.44 | 10.35 | 20.00 |
| BL | 13.17 | 9.92 | 8.47 | 6.92 | 19.66 | 17.95 | 17.46 | 24.28 | 14.57 | 27.78 |
| BE | 8.32 | 6.91 | 11.22 | 12.55 | 14.38 | 13.02 | 11.54 | 21.82 | 10.22 | 14.29 |
| MO | 13.47 | 14.53 | 17.20 | 14.72 | 17.81 | 11.19 | 14.38 | 22.15 | 9.18 | 23.08 |
| ML | 7.44 | 7.87 | 8.11 | 9.09 | 17.50 | 10.42 | 10.71 | 15.84 | 9.84 | 40.00 |
| ME | 9.19 | 8.09 | 11.76 | 11.90 | 10.56 | 12.83 | 29.20 | 18.63 | 9.76 | 11.76 |
| SO | 21.12 | 22.32 | 21.51 | 20.17 | 20.41 | 21.37 | 20.45 | 28.51 | 31.16 | 20.00 |
| SL | 12.79 | 15.38 | 16.46 | 7.89 | 18.19 | 18.27 | 17.14 | 17.63 | 18.76 | 25.00 |
| SE | 7.45 | 5.36 | 13.21 | 12.50 | 12.87 | 58.60 | 11.54 | 13.12 | 12.81 | 16.67 |



Fig. 6. Variation in leaf base of sandalwood


Fig. 7. Variation in leaf tip of sandalwood


Fig. 8. Variation in leaf petioles of sandalwood


Fig. 9. Variation in leaf margin of sandalwood

The taxonomic importance of leaf variation is reported in several tree species namely Populus simonii and P. nigra (Jingshan Ren et al 2020), Pyrus pyraster (Antonio et al 2022) and Carpinus tschonoskii (Runan et al 2022). In general, leaf morphology characteristics can be varied between species, different populations of the same species, or within an individual at different development stages. It has been considered that leaf size is influenced by environmental as well as genetic factors (Hay and Tsiantis 2006). But when it comes to sandalwood leaf diversity did not exhibit any correlation with geographical locations or climate (Kulkarni and Srimathi 1998). The occurrence of intermixed types in a forest account for polygenic inheritance. The segregation of these types was also observed in the progeny trials showing a heterogeneous nature of the species. Similar findings were recorded by Kulkarni (1995) categorized sandalwood leaves into six biotypes Viz., (1) ovate (2) elliptic (3) lanceolate (4) linear (5) big and (6) small. In the ovate biotype two sub-types Viz., (a) ovate -lanceolate (b) ovate-elliptic were found with similarities in leaf length, breadth (width), length/breadth ratio and leaf area (De Candolle 1857).

## CONCLUSION

In the Santalum album, L. leaves were categorized into nine types based on phenotypic traits, mainly on their variation in size and shape, such as big ovate, big lanceolate, big elliptic, medium ovate, medium lanceolate, medium elliptic, small ovate, small lanceolate and small elliptic. In each leaf category, studied the twenty leaf parameters such as LL, LMW, LUQW, LDQW, LTA, LTT, LBA, LBT, PL, PT, LA, LP, LM, LS, LC, LT, PC, VC, A/P, and L/B. The variation in sandalwood trees can be utilized the selection and improvement of superior genotypes. Leaf parameters were
very useful for better understanding of the sandalwood phenotypic variation at the taxonomic level. IWST, Bengaluru is conserving phenotypically diverse sandalwood population, that can be further utilized for tree improvement programmes.

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