

## LEAF TRAITS OF SAL (*SHOREA ROBUSTA* GAERTN.) POPULATIONS SELECTED FROM DIFFERENT REGIONS OF BANGLADESH

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### Abstract

This study investigated the variation in leaf traits of Sal (*Shorea robusta* Gaertn.) by collecting leaf samples from the natural populations of Sal distributed in Madhupur National Park, Cumilla and Dinajpur areas of Bangladesh. Soil samples were collected at 0 - 10 cm depth near the base of the tree trunk of the respective plants selected for the collection of leaf samples. Soils were analyzed for moisture content, pH, electrical conductivity, available N, total P, and organic C contents. Data showed a range of variation in the responses of leaf traits of the three populations. However, none of the morphological and physiological traits showed significant differences except chlorophyll content ( $p = 0.0047$ ). Conversely, significant difference appeared in most of the leaf anatomical properties such as stomatal pore index ( $p = 0.0369$ ), open-close behavior ( $p < 0.0001$ ) and stomatal density ( $p = 0.0008$ ) among the three forests. Stomatal density, pore index, and percent open stomata were higher in the Madhupur forest, while closed stomata were significantly higher in the Dinajpur forest. Thus, the present study indicated that although all leaf traits did not show a similar trend of response, leaf stomata were more responsive to different geographical distribution, which could be attributed to the variation in soil moisture conditions.

### Introduction

Plants show various strategies to adapt to their environment. Among these strategies, leaf traits are important since they vary significantly in different environmental conditions and indicate climate change<sup>(1,2)</sup>. To adapt to the environmental changes, plants adjust leaf traits, including morphological, physiological, and anatomical properties showing more remarkable plasticity, reflecting the adaptability and indicating the role of plants to the external environment<sup>(3)</sup>. Therefore, study on plant leaf traits is critical because they reflect the adaptation of plants and help link global climate changes with the dynamic changes of plant population.

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Under the family Dipterocarpaceae, Sal (*Shorea robusta* Gaertn.) is the dominant tree species in the deciduous forests of the tropics. The species is found in dry and moist deciduous forests in the south and south-eastern Asia<sup>(4)</sup>. Sal forests show a patchy distribution in Bangladesh, with the main occurrence in the central and north-western parts with varying soil and climatic conditions<sup>(4,5)</sup>. The variation in the climatic and edaphic features of these regions is likely to be reflected by the leaf traits of Sal plants. However, there has been limited data on the leaf traits of Sal in the deciduous forests of Bangladesh<sup>(5)</sup>. Therefore, the objective of the present study was to investigate whether the leaf traits (morphological, physiological, and anatomical) of Sal differed among populations selected from different regions with different soil and climatic conditions of Bangladesh.

### Materials and Methods

*Description of study sites:* Leaf samples were collected from three natural populations of Sal situated in Madhupur National Park (24° 45' 00" N and 90° 05' 00" E), Dinajpur (25° 37' 0.12" N and 88° 45' 0.00" E) and Cumilla (23° 27' 0.0036" N and 91° 11' 59.99" E) (Fig. 1). A total of nine locations, each three from these forests, were selected to collect leaf and soil samples. The average precipitation, humidity and temperature of the three forests sites were 124.4 mm, 71% and 27°C for Madhupur National Park, 103.0 mm, 79%, and 26°C for Cumilla and 149.6 mm, 79%, and 25°C for Dinajpur<sup>(5)</sup>.

*Collection of leaf and soil samples:* Fully expanded youngest fresh leaves were collected from Sal plants to study leaf properties. The soil sample was collected at 0 - 10 cm depth near the base of the tree trunk of the Sal plant which was selected for the collection of leaf samples. Leaf and soil samples were collected into separate plastic bags and then labeled. For analysis, collected samples were brought to the Ecology and Environment Laboratory, Department of Botany, University of Dhaka. For the analysis of leaf properties such as fresh leaf weight, dry leaf weight, turgid leaf weight, leaf length, leaf breadth, leaf area, specific leaf area (SLA), 5 fresh leaves were used from the samples collected from each plot. For the measurement of leaf length and breadth, each leaf was spread over a plain sheet, and then a millimeter-scaled ruler was placed along the mid-rib to determine the leaf length and breadth. Dry leaf weight was measured after drying the leaf in the oven at 80°C for 24 hours. Leaf chlorophyll content was determined by using a chlorophyll meter (SPAD-502Plus, Minolta, Japan). Specific leaf area (cm<sup>2</sup>/g) was calculated as the leaf area of the sampled leaves divided by the dry weight of the leaf. Leaf dry matter content (mg/mg) was calculated as leaf dry mass divided by fresh leaf mass; leaf water content per unit area (mg cm<sup>-2</sup>) as fresh leaf mass minus dry mass, divided by leaf area. Stomatal length (µm) was measured using five randomly selected stomata in each leaf of five leaves per plot. For measuring stomatal density (mm<sup>-2</sup>), the number of stomata per unit area was counted from the images at a magnification of 40 by

impression technique. Stomatal pore index (SPI) was calculated by following the formula,  $SPI = \text{Stomatal density} \times \text{Stomatal pore area}$ . Soil properties such as pH, electrical conductivity, moisture content, available N, total P (%), and total organic C (%) were determined immediately after collection from the field. Soil pH was determined from soil suspension: water (2 : 1, v : w) using a pH meter (Hanna pH meter, pHeP). Soil conductivity was determined in suspension with distilled water (5 : 1, v : w) using a conductivity meter (Hanna conductivity meter). Soil moisture content (%) was determined from 10 g fresh soil after oven-drying at 80°C for 24 hrs. Organic carbon content (%) was determined by Walkley and Black method using 1.0 g soil<sup>(6)</sup>. Total N content was determined by the Kjeldahl method following extraction from 1.0 g soil with conc. H<sub>2</sub>SO<sub>4</sub><sup>(6)</sup>. Soil P was determined by following the colorimetric method<sup>(7)</sup>.



Fig. 1. Map of Bangladesh showing the distribution of Sal (*Shorea robusta* Gaertn.) forests selected for the present study.

**Statistical analysis:** One-way ANOVA was performed to compare soil properties as well as leaf morphological, physiological and anatomical traits among the three Sal forests selected in the present study. Tukey-Kramer HSD tests were done to compare the level of significant difference among the sites. Analyses were done by using JMP 4.0 software (SAS Institute, Carry, NC, USA).

## Results and Discussion

**Soil properties:** The soil physico-chemical properties of the three forest sites were shown in Fig. 2. The soil of the three forest stands showed significant difference in electrical conductivity ( $p = 0.04$ ), moisture ( $p = 0.038$ ) and phosphorus ( $p = 0.026$ ) contents. The highest values were recorded for electrical conductivity ( $\mu\text{S}/\text{cm}$ )  $20.66 \pm 2.09$ , moisture content (%)  $25.9 \pm 1.56$  and P contents (%)  $0.22 \pm 0.004$  in Madhupur, Cumilla, and Dinajpur, respectively. On the other hand, the lowest values of electrical conductivity and moisture content were in Dinajpur ( $12.03 \pm 1.32 \mu\text{S}/\text{cm}$  and  $19.82 \pm 1.78 \%$ , respectively) and that of P (%) was in Madhupur ( $0.012 \pm 0.001$ ). Although there were no significant differences in pH, organic C and available N among the three study areas, Dinajpur showed the highest value for pH ( $6.03 \pm 0.02$ ). In contrast, the lowest was found in Cumilla ( $5.41 \pm 0.09$ ), indicating that the soil of Cumilla is slightly more acidic than Dinajpur soil. The highest amount of soil organic carbon was found in Dinajpur and the lowest in Madhupur forests. However, the values for soil nitrogen content varied slightly between Cumilla ( $0.021 \pm 0.0052 \%$ ) and Dinajpur ( $0.021 \pm 0.0028 \%$ ).

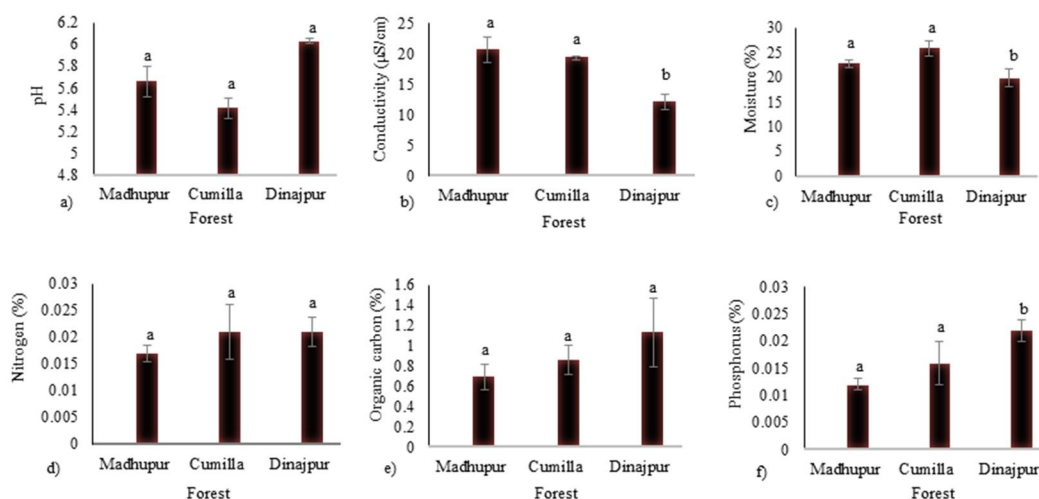


Fig. 2. The physico-chemical properties of soils collected from Madhupur National Park, Cumilla and Dinajpur Sal forests; pH (a), electrical conductivity ( $\mu\text{S}/\text{cm}$ ) (b), moisture (%) (c), nitrogen (%) (d), organic carbon (%) (e) and phosphorus (%) (f).

**Leaf morphological properties:** Fig. 3 showed the mean values of leaf length, leaf breadth, leaf area, and SLA of Sal plants among the three study sites. Although there was variation in mean values, significant differences did not appear among the three forest sites. The highest leaf length ( $19.37 \pm 0.23 \text{ cm}$ ), leaf breadth ( $10.55 \pm 0.49 \text{ cm}$ ), and area ( $154.293 \pm 9.75 \text{ cm}^2$ ) were recorded in Cumilla. On the contrary, the lowest leaf length ( $18.47 \pm 0.92 \text{ cm}$ ), leaf breadth ( $10.39 \pm 0.63 \text{ cm}$ ), and leaf area ( $146.317 \pm 16.15 \text{ cm}^2$ ) were

recorded in Madhupur. The lowest value of SLA ( $150.862 \pm 3.061 \text{ cm}^2/\text{g}$ ) was found in Dinajpur.

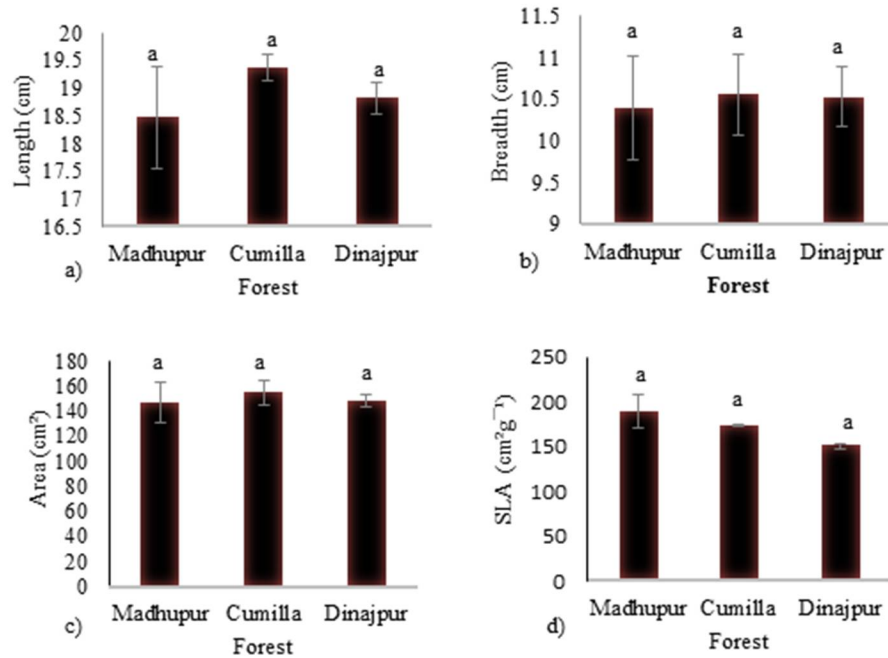


Fig. 3. The morphological properties of the leaf of Sal of Madhupur National Park, Cumilla and Dinajpur Sal forests; leaf length (cm) (a), breadth (cm) (b), area (cm<sup>2</sup>) (c), and specific leaf area (cm<sup>2</sup>/g) (d).

*Leaf physiological properties:* Except for leaf chlorophyll content, none of the leaf physiological properties showed significant differences among the three Sal populations (Fig. 4). The highest amount of chlorophyll content ( $33.76 \pm 1.16 \mu\text{g}/\text{cm}$ ) was in Dinajpur, and the lowest ( $26.77 \pm 1.23 \mu\text{g}/\text{cm}$ ) was in Madhupur forest. Chlorophyll content was widely influenced by environmental conditions<sup>(8,9)</sup>. Other studies also showed that chlorophyll was significantly associated with temperature and moisture<sup>(10,11)</sup>. However, greater soil P content might also influence the higher chlorophyll content in the leaf of Sal plants in the Dinajpur site.

*Leaf anatomical properties:* Leaf anatomical properties such as stomatal length, stomatal breadth, % open stomata, % close stomata, total stomata, SPI and stomatal density were shown in Fig. 5. Except for stomatal length and breadth, all other stomatal traits such as percentage of open and closed stomata, SPI, and stomatal density showed significant differences among the three forests. The highest % open stomata ( $66.06 \pm 3.65 \%$ ) and the lowest percent of closed stomata ( $33.93 \pm 3.65 \%$ ) were recorded in Madhupur. The lowest values were recorded for SPI ( $0.000019 \pm 0.0000045$ ) and density ( $46 \pm 1.23$ ) in Cumilla.

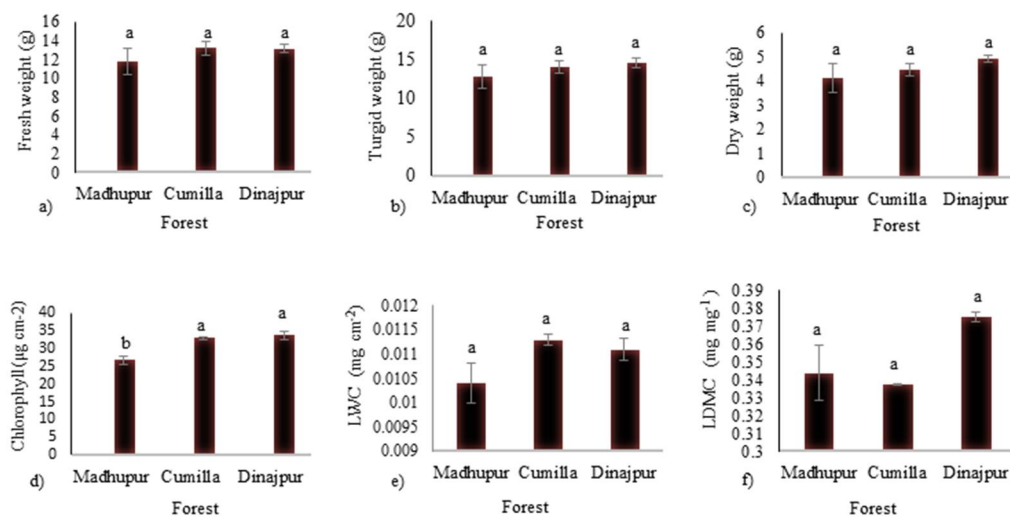


Fig. 4. The physiological properties of leaf of Sal of Madhupur National Park, Cumilla and Dinajpur Sal forests; fresh weight (g) (a), turgid weight (g) (b), dry weight (g) (c), chlorophyll content (d), leaf water content (mg cm<sup>-2</sup>) (e), and leaf dry matter content (mg/mg) (f).

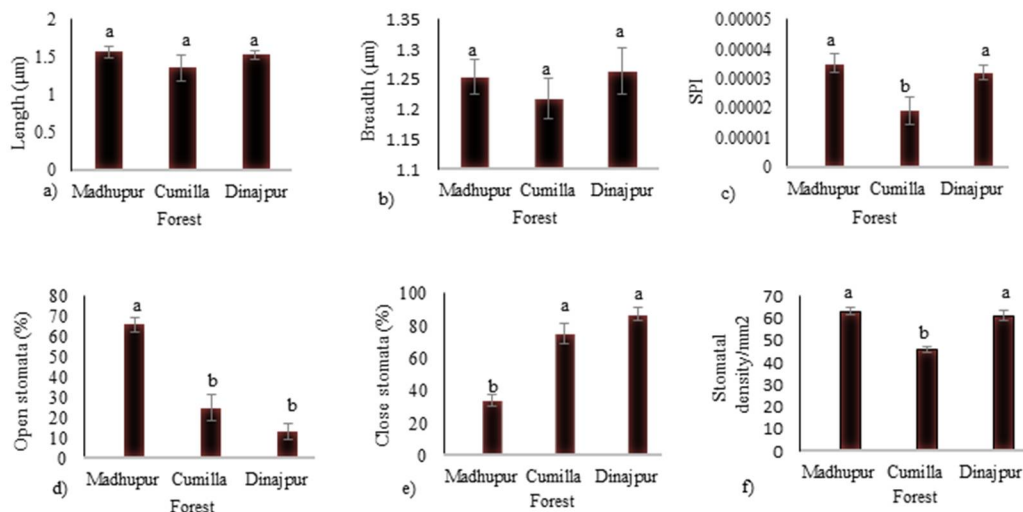


Fig. 5. The anatomical properties of the leaf of Sal of the forests of Madhupur National Park, Cumilla and Dinajpur; stomatal length (µm) (a), stomatal breadth (µm) (b), SPI (stomatal pore index) (%) (c), open stomata (%) (d) closed stomata (%) (e), and density of stomata (f).

On the contrary, the highest values for SPI ( $0.000035 \pm 0.0000032$ ) and stomatal density ( $63.17 \pm 1.47$ ) were found in Madhupur. Data of the present study revealed that compared to morphological and physiological traits, leaf anatomical (e.g., stomatal) traits were more responsive to environmental changes. Similar results were also found by other

studies<sup>(9,11)</sup>. The highest number of closed-stomata in Dinajpur forest might be associated with the mechanism of adaptation of this plant with the dry condition of this forest since under dry conditions, and plants have to reduce transpiration to retain moisture content in plant cells during the drought condition. Dinajpur Sal forest is regarded as dry deciduous, while that of the Madhupur forest is regarded as moist deciduous in Bangladesh. Other studies also reported the role of water in soil and plant tissues in regulating stomatal conductance<sup>(12,13)</sup>. Similar to the other findings, data of the present study revealed that leaves of Sal plants responded to changing environmental conditions by adjusting their stomatal behavior exhibiting long-term adaptations of plants through stomata<sup>(14)</sup>.

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