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Leaf Functional Traits Are Related to Tree Size and Location in Cotton Trees *Bombax malabaricum*

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Abstract

Exploring intraspecific variation of functional traits of different sizes and ecogeographical regions is important to understand the adaptation strategies of tree populations to their environments. In this study, we explored the variation and bivariate relationships of 16 functional traits of 30 trees of *Bombax malabaricum* across 5 geographical regions in Hainan Island and between large- and small-sized tree populations. Principal component analysis showed that leaf thickness (LTh), guard cell length, and lower epidermis (LE) thickness were the key functional traits implicated in varying ecological strategies of *B. malabaricum*. A significant variation was found in the key functional traits including LE thickness, LTh, and guard cell (GCL) in populations across different ecogeographical regions. However, the LE and LTh vary significantly between the large- and small-sized trees. The LTh and LE thickness also showed an allometric relationship across different geographical regions and tree sizes. Hence, it was concluded that trees vary their ecological strategy according to their ontological developments across environments. Moreover, adaptation strategies of large-sized trees differing from small-sized ones highlight the fact that priority should be taken to conserve the trees with high age.

Keywords

Hainan Island, *Bombax malabaricum*, tree size, functional traits, adaptation strategies

Functional traits that include morphological (Mott, Gibson, & O'Leary, 2010), intrinsic physiological, and biochemical traits (McIntyre et al., 1999) are core attributes shared by plants (Cornelissen et al., 2003). These traits are closely related to plant morphology, physiology, and life cycle characteristics and can automatically reflect the adaptive mechanism of plants to the environments (Violle et al., 2007). Although it was generally recognized that interspecific variation in functional traits helps plants pass through an environmental filter (Burns, 2010; Cornwell & Ackerly, 2009; McGill, 2008), recent studies found that intraspecific variation is comparable with interspecific variation for some traits (Albert et al., 2010). Within-species variability may allow species to adjust performance in the face of environmental heterogeneity as a consequence of plastic shift in trait values (Paine et al., 2011). The intraspecific variation in leaf morphology, anatomical structure, and water use was observed to help plant populations survive

in different geographical regions (Gratani et al., 2003). Temperature, precipitation, and soil properties are thought to be the main factors leading to variation in these functional traits (Meng, Ni & Wang, 2007; Miyazawa & Lechowicz, 2004).

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Variation in functional traits with developmental stages can help the plant adapt to changing environments (Ackerly et al., 2002; Niinemets et al., 2007; Poorter et al., 2010). For example, the specific leaf area may be related to the structure formation of the leaf skeleton, which often decreases with the progress in developmental stage of plants (Niinemets et al., 2007). At the seedling stage, plants cannot support large-area construction costs and long revenue cycles due to the deficiency in resources; thus, the leaf area of the plant is smaller, but the specific leaf area is larger. At the tree forming stage, water potential at the top of the plant is generally lower than that of the seedlings because of its difficulty of transportation, resulting in smaller specific leaf area at the top of the plant (Kenzo et al., 2015; Niinemets, 2002).

Nutrient allocation in different plant structures and physiological activities often differ in limited environmental resource conditions (Weiner, 2004) so that plants may increase growth, survival, and reproduction (Wright et al., 2007; Wright & Westoby, 1999). Such allocation strategies can be explored by assessing the slope of pairwise trait relationships (Wright et al., 2004). A slope that significantly differs from +1.0 or -1.0 (i.e., allometric relationships, log scale) indicates that plants disproportionately allocate resources to specific plant functions. Plant functional trait pairs help species in designing competitive positions along ecological strategy axes. For example, the allometric correlation between leaf vein density (VD) and leaf area in dry environments may suggest that plants have tried to increase investments in leaf VD, whereas they might have reduced it in leaf area (Nardini et al., 2010; Sack & Scoffoni, 2013).

Large-sized trees, especially old trees that often have grown for a long time and record local geography, climate, history, and culture with high ecological and economic values, would deserve a high level of conservations (Xu & Zhang, 2017). The large-sized trees were older compared with the small-sized trees, and both the types differed in their functional traits across environmental conditions (Long et al., 2011). *Bombax malabaricum* is a native species and has a long history of planting in Hainan Island. Large-sized *B. malabaricum* has a high ornamental and economic value and has a significant potential for the understanding of history, culture, and climate of Hainan Island. Therefore, the present study was designed to explore the variation in functional traits of *B. malabaricum* between large and small sizes and across geographical distributions and to assess the bivariate relationships among these traits. We hypothesized that (a) there would be significant difference in functional traits between large- and small-sized *B. malabaricum* and across different geographical regions, and (b) there would be an allometric relationship between functional trait pairs of *B. malabaricum*.

Materials and Methods

Study Sites

Hainan Island is located at the northern edge of the tropics, with an Eastern longitude of 108°36'43" to 111°2'31" and Northern latitude of 18°10'04" to 20°9'40" (Figure 1). The area of Hainan Island is 33,920 ha (Wang, 2008). Precipitation distribution at the island is related to topography and the monsoon climate. The highest annual rainfall occurs in its central part and exceeds 2,300 mm, while the lowest occurs in

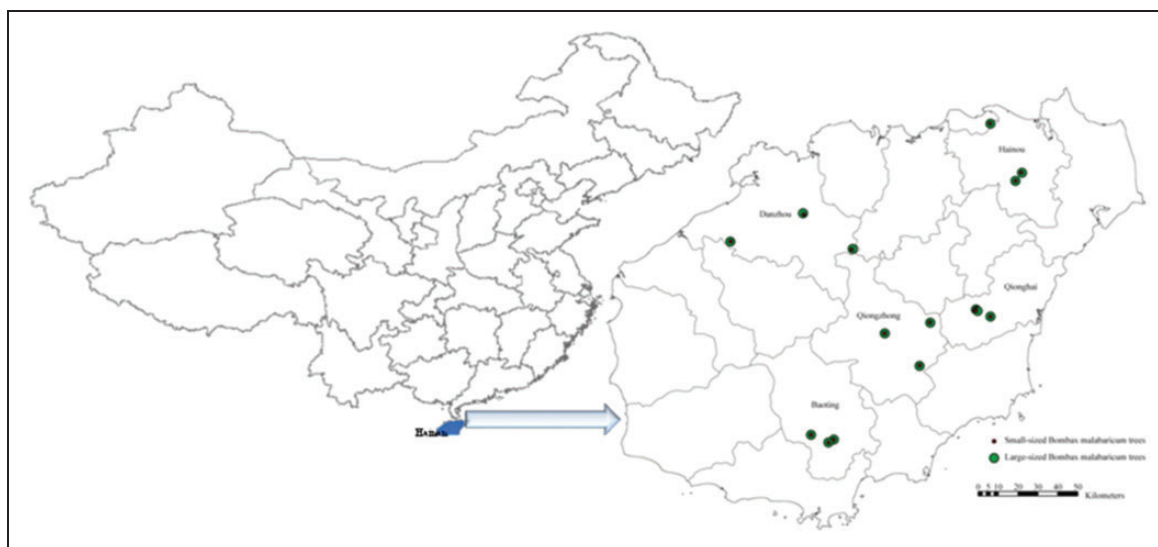


Figure 1. Overview of the Research Area.

the Western coastal areas and is lower than 1,000 mm (Gao et al., 1981). Soil types include coastal sand, brick red soil, and yellow soil (Gong, 2004), and the natural forest vegetation types include tropical monsoon forest, lowland rainforest, the mountain rainforest, coniferous forest, cloud forests, and mangroves (Gao, 2009).

B. malabaricum is widely distributed in rural areas of Hainan Island and has high ornamental and economic value. According to the climate data of 18 meteorological stations in Hainan Province, samples of *B. malabaricum* were collected from 5 counties having different climatic factors, including Haikou, Qiongzong, Qionghai, Danzhou, and Baoting (Table S1; Che et al., 2014).

Data Collection

We first measured the diameter at the breast height (dbh) of all the *B. malabaricum* trees in rural areas of the five regions and selected the large-sized trees with dbh value around 120 cm and as well as the small-sized ones having a dbh value about 20 cm. Then, 3 *B. malabaricum* individuals of each tree size were sampled in each county, and there were a total of 30 tree individuals for analyses. We measured a total of 16 functional traits for the large- and small-sized trees (Table 1). These traits are related to the water utilization and photosynthesis ability of the plants (Cornelissen et al., 2003), which can reflect adaptation strategies of *B. malabaricum* trees across the five geographical regions. The adaptation strategies of this species in different regions may lead to the micromorphological variation in the sizes, stomatal density (SD), stomatal complex, leaf Vein density (VD), leaf epidermal thickness, palisade tissue thickness, sponge tissue thickness, sponge tissue thickness ratio, lower epidermis (LE) thickness, leaf thickness (LTh), chlorophyll content, specific hydraulic conductivity of leaf, specific hydraulic conductivity of branch, sapwood area (SA), sapwood volume, and specific leaf area.

Branch Trait Measurements

We collected the annual sunward branches with a diameter of 7 to 15 mm and a length of 20 to 40 cm of the *B. malabaricum* trees to measure the branch-specific hydraulic conductivity (KS) and leaf-specific hydraulic conductivity (KL). About 10 cm of the length of each branch was inserted into a bucket containing half water to prevent air from entering from one end of the catheter. Black plastic bags were used to shield plants from light. The bark at both ends of the branch was cut with a blade and was connected to a device to determine the hydraulic conductivity (Sperry et al., 1988; Zimmermann, 1978). To determine the flow velocity,

lower ends of the branches were connected to 0.2-ml diameter pipette. After stabilization for 10 to 20 minutes, the value of the pipette scale and time were recorded and was repeated 5 times. To calculate the KS and KL, we used the following formula, respectively ($\text{kg} \cdot \text{m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and KL ($10^{-4} \text{kg} \cdot \text{m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$). When measuring the SA, branches with a diameter of 7 to 15 mm were inserted into the bucket, which were protected with black plastic bags. A Vernier caliper was used to measure the distance from the core to the outer edge of the sapwood to the heartwood in four different directions, and the SA (cm^2) was calculated by using the formula of ellipse area.

Leaf Trait Measurements

Three to five recently expanded sunward leaves (current year's growth) of each individual were collected, and the leaf functional traits were measured.

The stomatal density (SD) was determined using nail oil blotting. The clear nail oil was first spread evenly on the back of a 2×2 cm leaf, avoiding the main vein of the leave. After 1 minute, the leaves were peeled off with a pair of tweezers and spread on a prepared glass slide. The SD was observed with a 40-fold optical microscope, and images were taken (Sperry et al., 1988) and were analyzed using the Image J 1.8 software (Image J, National Institutes of Health). SD (per/mm^2) equals the ratio of stomata number and leaf area. The guard cell length (GCL; per/mm^2) was calculated as the total GCL divided by number of the guard cell. Based on the SD and GCL, the stomatal area index (%) was calculated using the published protocol (Sack et al., 2003), which is related to the maximum stomatal conductance.

To measure the Vein density (VD), we took a 2×2 cm leaf sample with no main veins. The samples were then observed under a 20-fold optical microscope, and the micrographs were taken. The photos were then analyzed using the Image J image processing software. The VD (mm/mm^2) equals to the vein length divided by the total area of the visual field (Brodribb & Jordan, 2011). The leaf area was measured using the photoelectric leaf area meter (WDY-500A). Then, the leaves were dried to constant weight at 70°C for >3 days and weighted to the nearest 0.01 g. The specific leaf area (mm^2/mg) was calculated from the measurements for each tree sampled. Leaves of 2 to 4 cm in size were sliced by clipping them between radishes while avoiding the main vein of the leaves. Next, LThs (μm), upper epidermis (μm), sponge tissue (μm), palisade tissue (μm), and LE (μm) leaves were measured using a 40-fold microscope, and 3 micrographs per sample were taken and calculated with Image J 1.8 software. The chlorophyll content was measured by

using a chlorophyll meter SPAD-502 (portable chlorophyll detector, KONICA MINOLTA, Japan).

Data Analysis

All the functional trait data were log10 transformed to achieve a normal distribution before further analysis. Principal component analysis and Pearson's correlation were used to identify the key functional traits of *B. malabaricum*. Variations in the functional traits across the tree size and geographic regions were assessed using two-way analyses of variance. The bivariate relationships among the functional trait pairs within each tree size and geographic region were examined, using the Model type II regression analyses (Warton et al., 2006). The slope of bivariate relationships was estimated using a standardized major axis regression (Warton et al., 2006). Data analysis and visualization were performed using R 3.5.2 software.

Results

Key Functional Traits of *B. malabaricum* Trees

The first, second, and third principal components of principal component analysis accounted for 30%, 18%, and 15% variability. Variables with loading exceeding 0.80 were LTh (0.94), LE (0.89), and GCL (0.81) of the first principal component, SA (0.82) of the second principal component, and KS (0.85) of the

third principal component (Table 2). Correlation analyses showed that LTh, GCL, and LE were significantly associated with other traits (Figure 2). These results suggested that LTh, GCL, and LE are the key functional traits reflecting the adaptation strategies of *B. malabaricum* trees to environments.

Variations in Functional Traits With Geographical Regions and Tree Sizes

The GCL, LTh, and LE of *B. malabaricum* tree populations were significantly affected by geographical regions, $F_{(4, 20)} = 7$, $p < 0.05$; $F_{(4, 20)} = 13.67$, $p < 0.05$; $F_{(4, 20)} = 10.51$, $p < 0.05$, with the highest values in Baoting country (Figure S1). LTh and LE significantly increased in larger sized *B. malabaricum* trees, $F_{(1, 20)} = 8.01$, $p < 0.05$; $F_{(1, 20)} = 8.13$, $p < 0.05$. The variation in LTh and LE trait values was also explained by a significant interaction between tree size and geographic region, $F_{(4, 20)} = 17.10$, $p < 0.05$; $F_{(4, 20)} = 8.58$, $p < 0.05$, respectively (Table S2, Figure S1).

Allometric Growth Relationships of Functional Traits Across Geographical Regions and Tree Sizes

Standard major axis regression showed that LE–LTh of *B. malabaricum* trees had a significant relationship across four geographical regions (Danzhou, $p = 0.04$, slope = 1.57; Qionghai, $p = 0.01$, slope = 1.19; Qiongzong, $p = 0.02$, slope = 3.21; Haikou,

Table 1. Basic Information of Functional Traits.

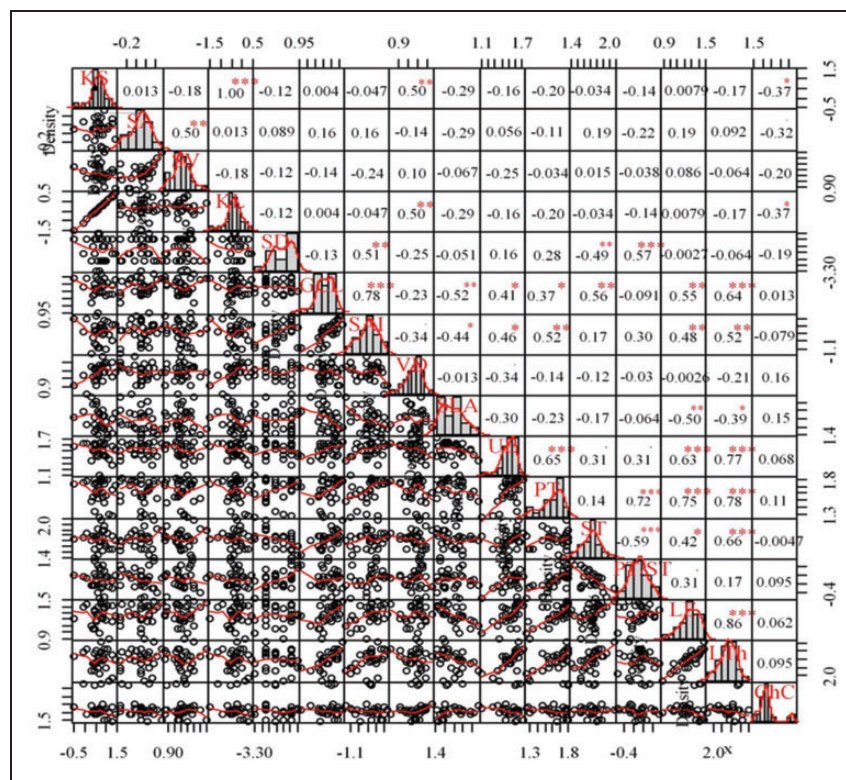
Functional traits	Abbreviation	Unit	Ecological description
Specific water conductivity of branches	KS	$\text{kg} \cdot \text{m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$	Water transfer from stem to leaf and water use efficiency of plants
Sapwood area	SA	cm^2	Water conduction of leaf branches
Sapwood volume	SV	mm^3	Water conduction of plant branches
Specific water conductivity of leaf	KL	$10^{-4} \text{kg} \cdot \text{m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$	Leaf water transfer efficiency
Stomatal density	SD	per/mm^2	Control photosynthetic and transpiration rates
Vein density	VD	g/cm^2	Leaf moisture transport
The length of guard cell	GCL	per/mm^2	Related to respiration and transpiration
Stomatal pore area index	SAI	%	Comprehensive index of stomatal conductance
Specific leaf area	SLA	mm^2/mg	Comprehensive index of photosynthesis
Upper epidermis	UE	μm	Leaf defense and protection
Palisade tissue	PT	μm	Main sites of chloroplast distribution
Spongy tissue	ST	μm	Gas exchange
Palisade tissue/spongy tissue	PT/ST	%	The value is big; the light absorption is more
Lower epidermis	LE	μm	Leaf defense and protection
Leaf thickness	LTh	μm	Leaf resource acquisition and defense
Chlorophyll content	ChC	SPAD	Measuring photosynthetic production potential of plants

Note. KS = branch-specific hydraulic conductivity; SA = sapwood area; SV = sapwood volume; KL = leaf-specific hydraulic conductivity; SD = stomatal density; VD = vein density; GCL = guard cell length; SAI = stomatal area index; VD = vein density; SLA = specific leaf area; UE = upper epidermis thickness; PT = palisade tissue thickness; ST = spongy tissue thickness; PT/ST = ratio of the thickness of spongy tissue to palisade tissue; LE = lower epidermis; LTh = leaf thickness; ChC = chlorophyll content.

Table 2. Results of the Principle Component Analysis (PCA) of Functional Traits of *Bombax malabaricum* in Hainan Island.

Functional traits	Principle Component 1	Principle Component 2	Principle Component 3
KS ($\text{kg} \cdot \text{m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	-0.07	0.10	0.85
SA (cm^2)	0.28	0.82	0.36
SV (mm^3)	0.05	0.77	0.37
KL ($10^{-4} \text{kg} \cdot \text{m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	-0.05	0.03	-0.11
SD (per/mm^2)	0.06	-0.41	0.75
GCL (per/mm^2)	0.81	0.05	-0.22
SAI (%)	0.70	-0.31	0.23
SLA (mm^2/mg)	-0.60	-0.16	-0.12
UE (μm)	0.76	-0.24	-0.10
PT (μm)	0.75	-0.38	0.28
ST (μm)	0.57	0.42	-0.60
PT/ST (%)	0.14	-0.46	0.74
LE (μm)	0.89	0.12	0.01
LTh (μm)	0.94	-0.01	-0.20
ChC (SPAD)	0.10	-0.32	-0.26
Proportion rate (%)	0.30	0.18	0.15
Cumulative variance (%)	0.30	0.48	0.63

Note. KS = branch-specific hydraulic conductivity; SA = sapwood area; SV = sapwood volume; KL = leaf-specific hydraulic conductivity; SD = stomatal density; GCL = guard cell length; SAI = stomatal area index; SLA = specific leaf area; UE = upper epidermis thickness; PT = palisade tissue thickness; ST = spongy tissue thickness; PT/ST = ratio of the thickness of spongy tissue to palisade tissue; LE = lower epidermis; LTh = leaf thickness; ChC = chlorophyll content.

**Figure 2.** Pearson Correlation Analyses of Functional Traits of *B. malabaricum* in Hainan Island.

KS = branch-specific hydraulic conductivity; SA = sapwood area; SV = sapwood volume; KL = leaf-specific hydraulic conductivity; SD = stomatal density; GCL = guard cell length; SAI = stomatal area index; VD = vein density; SLA = specific leaf area; UE = upper epidermis thickness; PT = palisade tissue; ST = spongy tissue; PT/ST = ratio of the thickness of spongy tissue to palisade tissue; LE = lower epidermis; LTh = leaf thickness; ChC = chlorophyll content.

$p < 0.01$, slope = 0.55; Figure 3), except the Baoting country ($p = 0.08$, slope = 2.82). However, other functional trait pairs did not show significant correlations (Figure 2). The LE was significantly related to LTh across the two tree sizes (large-sized tree, $p < 0.01$, slope = 1.46; small-sized tree, $p < 0.01$, slope = 0.78; Figure 4).

Discussion

Adaptation Strategy of *B. malabaricum* Across Geographical Regions

A significant variation was observed in the functional micromorphological traits of *B. malabaricum* that

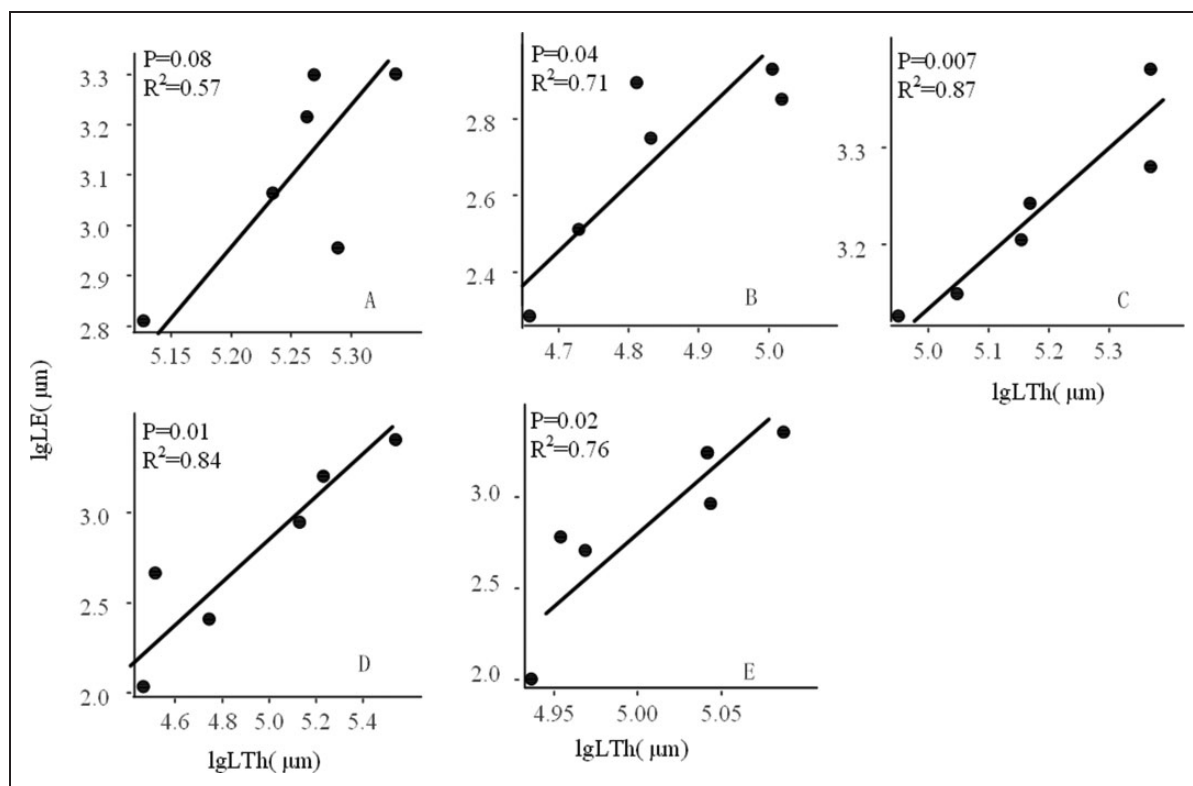


Figure 3. Standardized Major Axis Regression of Functional Trait Pairs of *B. malabaricum* Across Geographical Distributions: (A) Baoting, (B) Danzhou, (C) Haikou, (D) Qionghai, and (E) Qiongzong. LE = lower epidermis; LTh = leaf thickness.

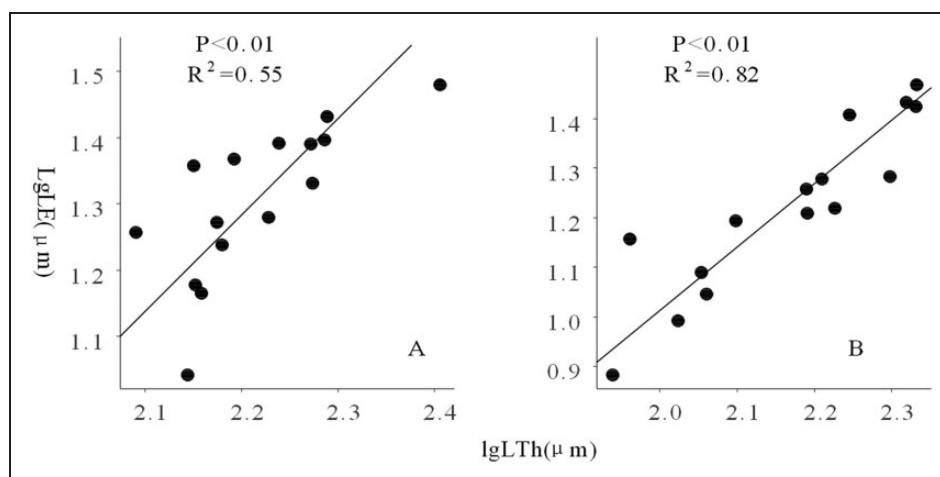


Figure 4. Standardized Major Axis Regression of Functional Trait Pairs Between *B. malabaricum* Trees With Varied Tree Sizes: (A) large-sized diameter and (B) small-sized diameter. LE = lower epidermis; LTh = leaf thickness.

included LE, LTh, and GCL across five different geographical regions (Table 3, Figure S1). The LE thickness and the LTh were higher in the tree populations in the Haikou and Baoting countries, followed by Qionghai and Qiongzong (Table S1). The LE and LTh reflect the water retention capacity of the plant to some extent and affect the water balance of the leaves (Hetherington & Woodward, 2003). Among the five geographical regions, Haikou has the least amount of precipitation (Table S1). The water supply of *B. malabaricum* was limited such that the plant leaves were thickened to increase their water storage capacity. Baoting had higher temperatures throughout the year and so was the plant transpiration rate. Therefore, we infer that *B. malabaricum* have thick leaves to achieve the required water retention potential.

The GCL of *B. malabaricum* collected from Haikou and Baoting were comparatively larger, followed by those collected from Qionghai and Qiongzong. The GCL in leaves collected from Danzhou was the smallest. Overall, the GCL is associated with gas exchange during photosynthesis (Hetherington & Woodward, 2003). Haikou and Baoting have fewer sunshine hours per year (Table S1), thus limiting the photosynthesis of *B. malabaricum*. The *B. malabaricum* trees may enhance photosynthetic efficiency by increasing their gas exchange rate. The traits of *B. malabaricum* may also be affected by many other factors such as water, temperature, light, CO₂ concentration, and physical and chemical properties of the soils (Li & Bao, 2005). The adaptability of *B. malabaricum* to various environmental factors and their synergistic effects in different geographical regions should be assessed in future studies.

Adaptation Strategies of *B. malabaricum* Across Tree Sizes

The *B. malabaricum* trees showed variation in functional traits during the developmental and growth stages. For example, the sizes of LE and LTh of large-sized trees were significantly higher than the small-sized trees (Table 2, Figure S1), which was consistent to the previous findings (Steppe et al., 2011). The LE thickness partially reflects the water retention capacity of plants.

Thicker the LE, the stronger the capacity of preventing water dispersion. Therefore, the water retention capacity of large *B. malabaricum* trees is higher than that of small trees. The roots of large *B. malabaricum* trees often rot with increasing age, resulting in a decrease in water absorption by the tree roots and insufficient water supply (Volder et al., 2005). The water retention is achieved by increasing the leaf LTh and LE thickness. Similarly, Steppe et al. (2011) also reported that the leaf and stratum corneum thickness increased with the increasing age of the tree. The LTh is associated with the exchange of matter and energy through photosynthesis (Dong & Zhang, 2001; Scoffoni et al., 2011). The large *B. malabaricum* trees often consume more resources due to their large size. Therefore, *B. malabaricum* tree enhanced the photosynthesis rate by increasing the number of mesophyll cells and accumulating more energy for the formation and maintenance of the tree structure and increasing the LTh.

Bivariate Relationships Among Functional Traits

The allometric relationships were found statistically significant between the thickness of LE and LTh in different geographical regions (Figure 3). For example, the slopes of the LE–LTh regression relationships were higher than 1.0 in Danzhou, Baoting, Qionghai, and Qiongzong, suggesting that the *B. malabaricum* trees in these four regions invest more resources in their LE thickness than the LTh. The thickness of LE typically reflects the water retention capacity of the plant. Owing to the dry season in these areas and the roots of large size *B. malabaricum* trees often rot, the lower thick epidermis of *B. malabaricum* trees provide the advantage by preventing water from dispersion. Tree populations of the *B. malabaricum* from Haikou have a LE–LTh regression slope less than 1.0 (Figure 4), suggesting that resources utilized in LTh could be higher than the LE thickness. The LTh is associated with exchanges of matter and energy during photosynthesis (Scoffoni et al., 2011). The vascular tissue in the mesophyll cells ensures the smooth progress of photosynthesis, while the palisade and spongy tissues are closely related to the plant photosynthetic rate. The latitude of Haikou is high with

Table 3. Results of the Two-Way Analyses of Variance (ANOVAs) of Functional Traits of *B. malabaricum*.

Functional traits	Tree size		Geographical region		Tree size: geographical region	
	F value	p value	F value	p value	F value	p value
GCL (per/mm ²)	0.03	.88	7.00	<.05	2.04	.13
LE (μm)	8.13	<.05	10.51	<.05	8.58	<.05
LTh (μm)	8.01	<.05	13.67	<.05	17.10	<.05

Note. GCL = guard cell length; LE = lower epidermis; LTh = leaf thickness.

lower light intensity when compared with the other four regions. Increasing the number of chloroplasts could ensure the optimum photosynthesis rate of *B. malabaricum* trees and therefore increase the LTh as well.

Allometric relationships were also statistically significant between LE and LTh in both the large and small-sized *B. malabaricum* trees (Figure 4). This suggests that there is an invariant LE–LTh relationship across the different plant development stages. *B. malabaricum* trees tend to utilize more resources to the construction of the leaf lower epidermal thickness. *B. malabaricum* is a heliophilous species, which grows under intensive sunlight, resulting in high transpiration rates and a higher tendency of water loss. Therefore, *B. malabaricum* trees achieve water retention by thickening their LE of the leaves. The regression slopes of large-sized *B. malabaricum* (1.46) were higher than the small-sized trees (1.28), indicating that large-sized trees need more resources in thickening the LE than the smaller ones. Our findings suggested a disproportional resource allocation in LE and LTh throughout the developmental and growth stages. This probably results from the fact that the roots of large-sized *B. malabaricum* trees are vulnerable to decomposition (Volder et al., 2005) because of old ages, which limits the water absorption. The large-sized *B. malabaricum* trees, therefore, achieve water retention by thickening the LE of the leaves.

Implications for Conservation

We noted that large-sized *B. malabaricum* trees have higher functional traits such as thicker epidermis and thicker leaf compared with smaller ones. This study provides evidence that there is an ontological change in ecological strategies to the environment for *B. malabaricum* throughout life. This highlights the fact that conservation of these large-sized trees should be prior to protect in the future. The consistent allometric relationships between LE–LTh of large-sized *B. malabaricum* trees across different geographical regions also suggested that water physiology plays an important role in old-aged trees. Some measures such as artificial transplanting, improving soil moisture, and water-absorbing abilities are recommended for further studies.

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Supplemental Material

Supplemental material for this article is available online.

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