Differences in Leaf Functional Traits of *Canarium vulgare* Leenh. Between Two Growth Stages: Mature vs. Seedling

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Abstract. This comparative study aimed to understand the life strategies adopted by the tropical tree species: *Canarium vulgare* Leenh by measuring functional leaf traits in two different developmental stages. This study found that there was a noticeable increase in Leaf Mass per Area (LMA), Leaf Dry Matter Content (LDMC), Leaf Density (LD), Stomatal Density (SD) and Leaf Vein Density (LVD), but not in Leaf Length (LL), Leaf Width (LW), Leaf Slenderness (LS), Leaf Thickness (LT) and Leaf Area (LA). In which the LMA, LDMC, LD, SD and LVD were found higher in the leaf of adult trees compare to seedlings. These changes indicate there was a shifting in the resource allocation to the leaf, in which the adult tree invests more construction costs in the leaf compared to seedlings. This strategy would benefit the adult tree to cope with the environment, for instance, increasing the hydraulic rate and increasing its ability to defend against herbivory as plants develop.

Keywords: *Canarium vulgare* · leaf functional traits · seedling · tree

1 Introduction

Plants evolved various life-history strategies to maximize fitness. Most plants start their life cycle from seed germination and then develop into adult plants. In contrast to many animals, in plants, the transition from a juvenile to an adult usually does not coincide with the attainment of final body size. In plants, especially trees, the hydraulic limitation proposed could be scaling the height of the mature tree. As the plants grow, they increase their height and at the same time, the hydraulic resistance from the soil to the canopy leaves was increased [1, 2].

Canarium vulgare Leenh. is a fast-growing tropical plant species that is naturally distributed in Java island, Lesser Sunda island, Maluku, New Guinea, Salomon island and Sulawesi (https://powo.science.kew.org). The mature tree of C. vulgare has a large and dense canopy and also a big stem diameter [3]. The previous study of an anatomical feature of C. vulgare suggested that foliar structure largely varies across Canarium

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species [4]. The species of C. vulgare appears naturally in tropical primary and secondary rainforests at low and medium altitudes [5].

In a tropical forest, the environmental condition is the crucial factor that could determine the plant's development. The canopy layer, for instance, affects the plants' growth and forest regeneration [6]. The climate, biogeography, soil and water condition and also growth form significantly affect the functional priorities of the plants, which in turn influence the plants' survival. Leaf functional traits are one of the fundamental traits that play important roles in plant functioning. In the previous decades, botanists usually used leaf morphology characteristics mainly as tools in systematics. However, in recent years, scientists found that variations in leaf morphological and leaf veins patterns are influenced at both the ontogenetic and evolutionary levels by a variety of climatic and environmental parameters. The foliar characteristics are important traits to study the plant's adaptation and response to environmental conditions [7–9].

During development, the different plant species have various patterns of resource allocation strategy, either to allocate the energy in vegetative, regenerative or defence. The allocation strategy during development was reflected in the ontogeny change of plants. The increased growth in size and the alteration in functional demands as plants grow are the two main processes connected to resource allocation strategy. Plants could produce organ that requires more energy, for instance having a larger area of foliage or root during the development. In addition, as plants age and develop, their carbon-nutrient balance, storage capacity and shoot-to-root ratio increase, while their growth rate and metabolic activity decrease. Along with the development of the plants, the functional priorities of growth, resistance, storage, and reproduction also alter, and these modifications can potentially influence resource allocation [10]. The leaf is the adaptive organ in plants, and as the primary assimilation organ, it determined the plant's productivity. Leaf morphological traits are related to resource allocation through the cost of energy expenditure needed to construct its feature. The resources would be allocated neither to build the high nor low-cost leaf feature.

In the leaves, the energy of leaf construction is influenced by photosynthetic, herbivory defence and environmental disturbance. These conditions affect the energy expenditure needed by the plants to build costly leaf structures to adapt and survive. The changes in the functional leaf traits reflect the adaptation strategy of the plant species. For instance, the shade-tolerant plant species have larger but thinner leaves compared to the plants that require high light intensity. The leaves that adapt under light intensity is thicker because it was provided by the thicker cuticle and has longer and multiple layers of palisade cell. Meanwhile, the shade-tolerant plant species tend to have a larger area of leaves for absorbing light energy to increase the photosynthesis rate when the light intensity is low. In addition, the smaller leaf area of the sun-plants species also benefits to reduce water loss through transpiration. The sun leaves also have a higher stomatal density but are smaller than the shade-tolerant leaves [11, 12]. Besides the different light exposure, the leaves of the plants also have various strategies to cope with the water limitation. The variation of the leaf morphology and physiology is also found in a different forms of the plants. Understanding how the leaves change their morphology and physiology is crucial to study their adaptation to survive during the juvenile to adult phase. Therefore, the study of alteration in leaf traits is important to study the growth strategy of plants and also its functional ecology. The objective of this study is to understand how the changes in leaf functional traits of one of the tropical tree species: C. vulgare by comparing two developmental stages: seedling and mature trees.

2 Methods

2.1 Study Site and Plant

The study took place in Purwodadi Botanical Garden - National Research and Innovation Agency (BRIN), Pasuruan, East Java (Indonesia) at coordinates of the site are latitude 112.44 and longitude -7.57 with an altitude of 300mdpl. Two trees and three seedlings of *Canarium vulgare* species were selected for this study. The life span of the seedlings used in this study was 4 months and the adult tree is 64 years old (the age of the tree was collected from the Purwodadi Botanical Garden Registration Department). Due to the difficulty of accessing the upper leaves of the mature tree, the leaves exposed to the sun on the relatively low branches were carefully selected. To avoid developmental bias, only the mature leaves were used in this study.

2.2 Leaf Morphology

For each individual at two different life stages (seedlings and tree), ten leaves were randomly selected and measured for the leaf length (LL), leaf thickness (LT) and leaf width (LW) of the leaf blade. All the selected leaves were collected and then scanned with a scanner, and their surface leaf area (LA) was determined using digital pixel-counting software (Image J) [13]. The fresh mass leaves were weight using scales. Thereafter, the leaves were oven dried for 48h at 65 °C and weight again for their dry mass. From these measurement data, the following leaf traits were measured [14, 15]: Leaf dry matter content (LDMC), leaf density (LD), leaf slenderness (LS) and leaf mass per area (LMA).

2.3 Stomatal Density

The stomatal observation was conducted on the lower (abaxial) epidermis of the leaves where the abundance number of stomata is present. The abaxial part of the leaves was cleaned and then coated with clear nail varnish. The imprint of clear nail varnish was removed from the leaf surface after drying for app. 15 min and placed under a light microscope Olympus to observe. The number of stomata was then counted and calculated using the Image J software. The stomata density was expressed on an mm–2 bases.

2.4 Leaf Veins Observations

A 2cm2 leaf fragment was excised from each leaf leave then cleaned with 10% NaOH and stained with Safranin (2%) [16]. The fractions of leaves were then examined under a microscope (Olympus) and photos were taken. All veins present in the leaf under unit area (a) were traced manually. The total length of present veins (Lv) were measured using the digital image analysis and then calculated using the formula in Table 1..

2.5 Data Analysis

The differences in leaf traits among different development growth were tested with one-way ANOVA with two groups using Statistica 64 software version 13.3.

3 Results and Discussions

The seedlings and mature trees of *C. vulgare* had not significantly vary in LW, LL, LT, LA, and LS. Significant differences were found in the five leaf functional traits: LDMC, LD, LMA, SD and LVD**.** The leaf size plays important role in plant physiological processes. It varies due to environmental factors, especially climate variation [17, 18]. In the two different developmental stages, the morphological leaf size only showed slightly different in LW, LL, LT, LA, LS. An earlier study using *Macaranga gigantea* found that the leaf area of the seedlings was significantly lower compared to the sapling and tree, where the tree and sapling had similar LA (Ishida et al., 2005). In my result, the variation of the morphometric leaf character in length, width, area, slenderness and thickness were not varied. Probably not all the plant species have the same pattern in the leaf morphological change. For instance, the study found that leaf area, length and width were found low in seedlings, and it attained maximum value in the sapling stages and decreased with the increase of tree age [19].

In contrast with in LW, LL, LT, LA, and LS, the LD of the seedlings was found three times lower than trees. In line with the LD, the LMA was also found significantly increase with the increase of growth of *C. vulgare*. The LMA is the product of LD and LT, however previous papers mentioned that the LMA is correlated with the variation in LD, not the thickness [20, 21]. The LMA is the traits that could indicate the plant's functional aspects such as photosynthetic rate, decompositions rate, leaf herbivore defence and the potential growth rate [1, 22, 23]. High LMA shows a higher tissue density and indicates the larger resource investment in mass per unit area.

Leaf mass is the combination of both LDMC and LMA. Both LDMC and LMA is the indicator of the plant's species resource allocation strategies. The plants' growth-related allocations of resources should be viewed as a part of the strategy to maximize fitness. Body size is a matter of the fitness of organisms**.** The bigger body of a mature tree can also benefit plants in competition for light and nutrients, but this trait is not always beneficial as it can lead to a higher risk of extrinsic mortality (e.g. falling trees during storms). Two drivers have a role in tree mortality: biotic and abiotic. Biotic drivers of mortality, such as herbivores, pathogen and nutrition competitions, and the abiotic factor such as drought due to high transpiration. These factors strongly regulate the mortality of larger trees [24]. In the mature tree, there are hydraulic limitations to transfer the water and nutrients from the ground to the top compared to the small seedling. Hence the adult trees will suffer more due to the high demand for transpiration [25, 26]. Thus to cope with the abiotic factors, as the *C. vulgare* grows up, it allocates more resources to the leaf than when it is still in the seedlings stages. This allocation causes the increment in the leaf dry matter. The mature tree of *C. vulgare* invests more resources in the leaf traits could also be seen in the LVD.

Leaf veins have higher construction costs in comparison to other tissue leaf tissue [9]. Leaf veins are the major structures for physical support and water/nutrient transport within the leaf, which play an important role in maintaining leaf growth and development. It also transports photosynthate and signal molecules from the mesophyll to the rest of the plant [9, 27, 28]. High LVD gives several benefits to the tree and enables greater stomatal density (Fig. 2) and it could increase the phloem transport efficiency [9]. The higher LVD benefit the mature tree to transport the nutrients and increase its hydraulic efficiency. At the same time, the higher LVD also gives the advantage to increase the ability against herbivory. It prevents mechanical damage to the leaves because the herbivore will spend more energy on the leaves with higher LVD. Thus, the seedling stage is more vulnerable to herbivore attack during its development. Probably the seedling during the seedling stages has to allocate more of the resources to grow and produce more leaves to do photosynthesis which need more energy. As the resources are limited there are not enough resources for resistance [29]. This suggest that the risk of predation of *C. vulgare* will higher in the seedling stage compare to mature tree. It may during development of *C. vulgare*, it increase the resources allocation to the leaves to cope with the hydraulic limitation, at simultaneously increasing its defense in the mature stage.

4 Conclusions

Overall, this study found that several leaf traits were changing as *C. vulgare* develops. Significant changes were found in the LMA, LD, LDMC, SD, and LVD, but not in LL, LW, LT, LA and LS. The changes in the leaf traits are related to the resource allocation strategy, where the mature tree invests more in the construction costs of the leaf compared to seedlings. It benefits the adult tree to cope with the environment, for instance, increasing the hydraulic rate and increasing its ability to defend against herbivory as plants develop. Meanwhile, the seedling would allocate more energy to growth. This is a preliminary study to understand the ontogenetic changes of the leaf traits. Investigating how tropical plant species change their functional traits is very interesting yet also challenging. Further study regarding leaf functional traits across plants type (e.g. evergreen, deciduous) in the tropical area at their complete development stages is needed, to understand the functional ecology of plants and to address the life strategy adopted by tropical plant species (Fig. 1).

5 Figures and Tables

Table 1. The leaf traits parameter in this study, data collection and the abbreviation

Trait	Abbreviation	Data Collection	Unit
Leaf Length	LL	direct measurement	cm
Leaf Width	LW	direct measurement	cm
Leaf Thickness	LT	direct measurement	µM
Leaf Area	LA	using Image J software	cm^2
Leaf Density	LD	$\frac{Leaf\ Dry\ Mass}{LA \times LT}$	$g.cm^{-3}$
Leaf Slenderness	LS	$\frac{LL}{LW}$	$cm.\ cm^{-1}$
Leaf Dry Matter Content	LDMC	$\frac{Leaf\ Dry\ Mass}{Leaf\ fresh\ Mass}$	$g.g^{-1}$
Leaf Mass per Area	LMA	$\frac{Leaf\ Dry\ Mass}{LA}$	$g.m^{-2}$
Leaf Veins Density	LVD	$\frac{total\ length\ of\ leaf\ veins(Lv)}{unit\ area(a)}$	$mm.mm^{-2}$
Stomatal Density	SD	$\frac{number\ of\ stomata}{unit\ area}$	mm^{-2}

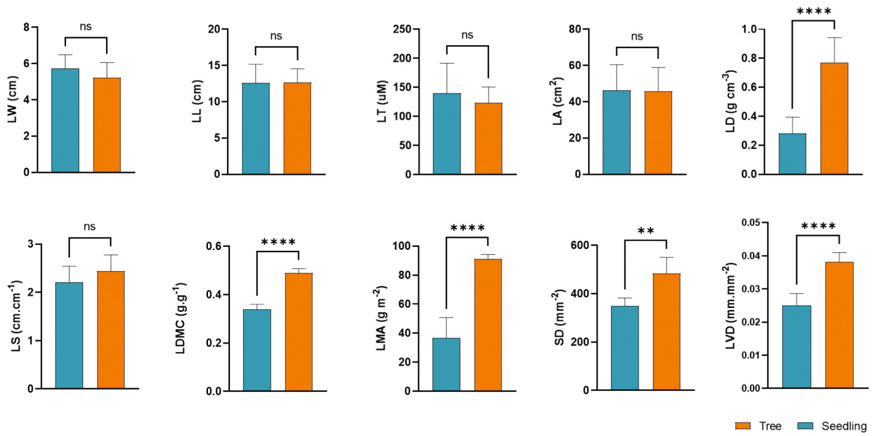

Fig. 1. Differences in leaf functional traits of *Canarium vulgare*. Level of significance is expressed as follow: ns P > 0.05; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001.

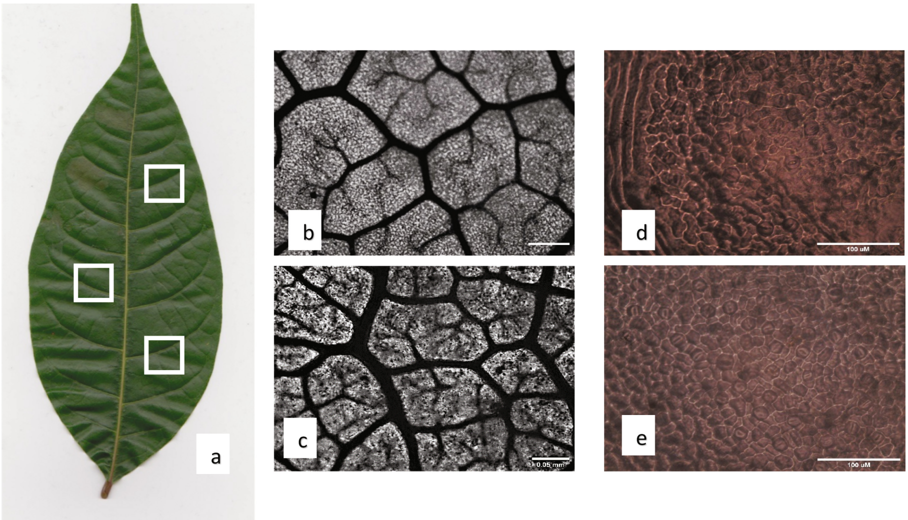

Fig. 2. Three parts of leaf fragments were excised for leaf veins observation (a); Photo of leaf veins of the seedling (b) and mature tree (c); Photo of stomatal of seedlings (d) and adult tree (e), the SD was found higher in the tree compared to the seedling.

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