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RESEARCH ARTICLE

# Adaptation of leaf morphology of the *Eurycoma longifolia* Jack to different site conditions in the province of Thua Thien Hue, Central Vietnam

*Sự thích nghi của hình thái lá với các điều kiện sống khác nhau ở cây Bách bệnh (Eurycoma longifolia Jack) tại tỉnh Thừa Thiên Huế, miền Trung Việt Nam*

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Leaf area and stomata are important parameters in studies of taxonomic classification since both parameters might reflect the plant adaptation mechanisms to different environmental conditions. This study analyzed *Eurycoma longifolia* Jack leaf traits in different ecological regions (moisture zone: A Luoi, Bach Ma, Nam Dong; dry zone: Phong Dien) and leaves of seedlings in the nursery. The study used scanners and Images to measure leaf area; imagej and the AxioVision SE64 softwares were used to evaluate stomatal density by separating the lower cuticle through a clear nail polish impression. Our results showed that the leaf area of mature trees from the dry zone is smaller (355.7 and 484.1 $cm^2$/leaf), however, the stomata density is higher than that of the humid area (284.4 and 137.9 stomata/$mm^2$). Under the same nursery conditions, the seedlings' leaf area was similar while seedlings' stomatal density, originated from the dry zone, was lower. Results indicate that the stomatal density changes as an adaptation to changing habitat conditions. This study gives indicates that *E. longifolia* is a tree species with high adaptability given the leaf morphology changes under a changing climate. Therefore, this tree species should be a priority for planting in order to increase biodiversity in different ecological regions.

*Đặc điểm diện tích lá và mật độ khí khổng là những thông số quan trọng trong các nghiên cứu phân loại học nhằm phản ánh cơ chế thích nghi của thực vật với điều kiện sống. Vì thế nghiên cứu này đã tiến hành phân tích các đặc điểm của lá cây Bách bệnh (Eurycoma longifolia Jack) từ các vùng khác nhau (A Lưới, Bạch Mã, Nam Đông: vùng ẩm và Phong Điền: vùng khô) và lá cây con của chúng ở vườn ươm. Nghiên cứu đã sử dụng máy quét và ImageJ để đo diện tích lá và phần mềm AxioVision SE64 và ImageJ để đánh giá mật độ khí khổng thông qua  tách lớp biểu bì phía dưới lá qua lớp sơn mỏng Kết quả nghiên cứu cho thấy, diện tích lá của cây trưởng thành từ vùng khô nhỏ hơn (355.7 and 484.1 $cm^2$/leaf) nhưng mật độ khí khổng lại lớn hơn so với vùng ẩm (284.4 and 137.9 stomata/$mm^2$). Tuy nhiên, cùng điều kiện sống ở vườn ươm, diện tích lá của cây con tương tự nhau trong khi mật độ khí khổng của cây con có nguồn gốc từ vùng khô lại thấp hơn. Kết quả này chỉ ra rằng sự thay đổi của mật độ khí khổng như là một sự thích nghi với thay đổi của điều kiện môi trường sống. Từ đó nghiên cứu này cho thấy cây Bách bệnh là một loài cây gỗ với khả năng thích nghi cao nhờ sự thay đổi về đặc điểm hình thái lá khi thay đổi khí hậu cho nên loài cây này nên được ưu tiên trong việc gây trồng nhằm tăng cường tính đa dạng cho các vùng sinh thái khác nhau.*

Keywords: Leaf area, stomatal density, mature trees, seedlings, *Eurycoma longifolia*

## 1. Introduction

*Eurycoma longifolia* Jack is a famous medicinal plant of the Simaroubaceae family (Effendy *et al.*, 2012) which has been topic of research and development not only in Vietnam but also around the world. *E. longifolia* is a shrub or small timber tree that grows up to 10 m in height (Chi, 2012). *E. longifolia* is basically found as an understorey tree, from moist to wet, in the lowland rainforests up to 500 m above sea level (*asl.*) in the Southeast of Asia (Hussein *et al.*, 2005). According to Kuo *et al.* (2003), this species can grow on hillsides and ridges with sandy or clay soils; It can be also distributed in primary and secondary evergreen and mixed dipterocarp forests in Burma, Indo-china, Thailand, Sumatra, Borneo and the Philippines. *E. longifolia* is a light-demanding and drought-tolerant species in Vietnam. The species is distributed at elevations ranging from 200 to

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1,100 m *asl*. (it concentrates mostly on 500 - 900 m *asl*.); it can grow in ferrasols, lavisols, slightly acidic soils or in average particle size distributions. In sandy areas, this plant is mainly distributed in natural forest, in high sand dunes or in sandy shrub areas. Because of the strict environmental conditions with high precipitation, high sunlight intensity, low clay ratio (< 15%) and mainly shoot regeneration, the trees prefer to grow in clumps to support each other for their development (Phuong *et al.*, 2013; Men *et al.*, 2017). *E. longifolia* populations have been observed in areas with an average temperature ranging between 25$^0$C and 30$^0$C. This species requires an annual rainfall range of about 2,000-4,000 mm and 86% of humidity (Effendy *et al.*, 2012).

*E. longifolia* has attracted great attention leading to the great harvest of wild-grown trees for all part of the plant for medicinal purposes. As a consequence, the plant has suffered a rapid decrease in its natural population. Studies regarding its morphology are needed to provide information for propagation, domestication and breeding programs for the conservation of valuable medicinal plant resources. Furthermore, there is a lack of information about the adaptation of this species, in terms of leaf morphology, to the different site conditions (dry or moist sites). Leaf area and stomata are important parameters for studies of taxonomic classification which might reflect the plant adaptation mechanisms to different environmental conditions. We studied those leaf traits of *E. longifolia* autochthones mature trees from contrasting sites (dry to moist site conditions) and of seedlings from those mother trees in the nursery garden in Thua Thien Hue, central Vietnam.

## 2. Material and methodologies

## 2.1. Overview of study site

The Thua Thien Hue province is located in the Northern Central Coast region with a total forest and forestry land area of about 288,334.37 ha. There are three types of forest including protected, special-use and production forests. Moreover, Thua Thien Hue is one of the central provinces with the unique ecological landscape denominated "interior sandy areas". It covers 24,358 ha and occupies 4.82% of the total natural land area (Thao *et al.*, 2015). The field work of this research was conducted in the Bach Ma National Park, the Nam Dong, A Luoi and Phong Dien (a sandy area) districts, located in the Thua Thien Hue province, central Vietnam (Fig. 1). The seedlings from different provenances were propagated in the nursery garden of the Huong Thuy town. The laboratory work was conducted in the Chair of Forest Botany, TU Dresden, Germany.

### 2.1.1. Nam Dong district

The Nam Dong district is situated in the south-west of the Thua Thien Hue province in central Vietnam. The coordinates of the Nam Dong district extend from 16°00' to 16°15' latitude north and 107°27' to 107°53' longitude east. It is surrounded by Phu Loc in the East, by Huong Thuy in the North, by A Luoi districts and the Quang Nam province in the West and by Laos in the South-West. The forests in Nam Dong are classified into three major categories: fractionally logged primary forests, closed secondary forests and open secondary forests (Averyanov *et al.*, 2003). Nam Dong has a tropical climate with two distinctive seasons, a dry and rainy caused by the monsoon. The average annual temperature on the land surface is 25.2°C with a maximum of 29.6°C. Nam Dong is one of the districts with the highest precipitation rates in the Thua Thien Hue province. The average annual precipitation is 310 mm, reaching a peak of 1,047 mm in November; its lowest recorded precipitation was around 63 mm is in March in the period 2013 - 2017 (NS, 2018).

### 2.1.2. Bach Ma National Park

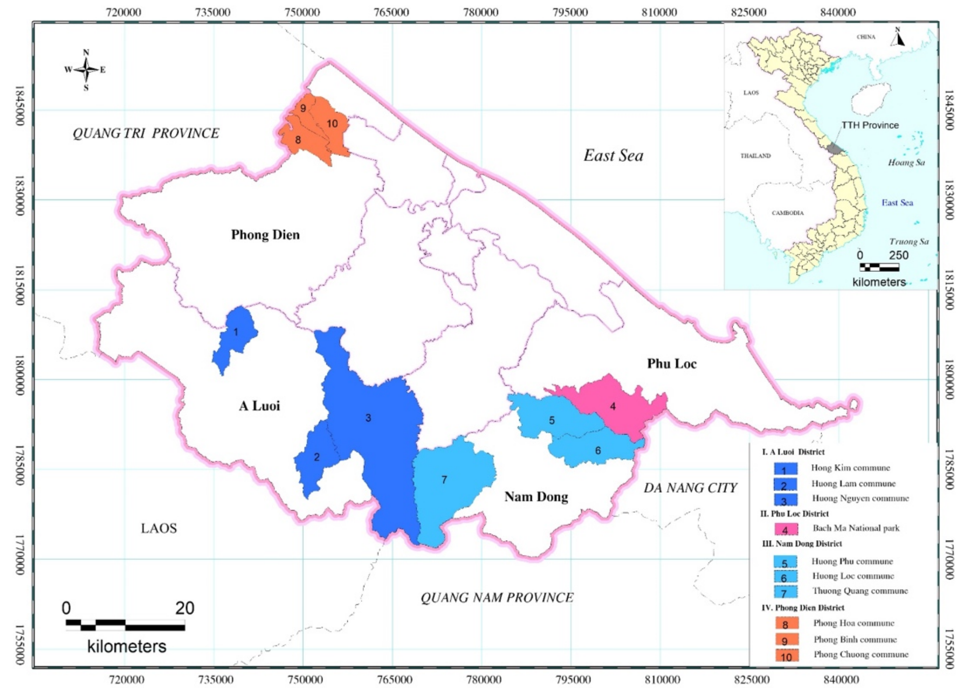
The Bach Ma National Park (BMNP) is located on the southern edge of the Thua Thien Hue Province in central Vietnam (16°05'-16°15' north and 107°43'-107°53' east). It is situated in the central Annamite Mountains and lies on a high mountain ridge that runs from west to east (from the Laotian border to the East Sea) with several high peaks above 1,000 m asl (An *et al.*, 2018). The BMNP has two typical primary forests, including a tropical evergreen closed forest (> 900 m) and a sub-tropical evergreen closed forest (< 900 m). Bach Ma has the highest rainfall volume in the Thua Thien Hue province with an annual average temperature of 23.1°C. Between 1998 and 2000, the total rainfall reached 10,758 mm/year. The total annual precipitation of the whole region is 3,722 mm; at the Bach Ma Mount, precipitation is up to 7,977 mm (BMNP, 2018).

### 2.1.3. A Luoi district

A Luoi is a remote mountain district located in the western terrain of the North Annamite Mountains. The altitude ranges between 156 m and 1,162 m *asl*. The coordinates of the A Luoi district extend from 16°00' to 16°27' latitude north and from 107°03' to 107°30' longitude east (Toai & Dien, 2017). Alike to Nam Dong and Bach Ma, A Luoi has a tropical monsoon climate influenced by the transitions between the north and the south. As displayed in Fig. 3.2, A Luoi has the lowest average temperature (22.3°C) in comparison to other regions, rarely increasing more than 25°C. A Luoi also has the highest annual average precipitation and the highest total annual rainfall with values of 3,319 mm and 3,983 mm, respectively (ALS, 2017).

### 2.1.4. Phong Dien district

The Phong Dien district is located in the north of the Hue city with geographic coordinates from 16°20′ to 16°44′ latitude north and from 107°03′ to 107°30′ longitude east. The East Sea connects the district with the Quang Dien district in the east. It borders the Huong Tra town in the southeast, the Quang Tri province in the northwest and the A Luoi district in the south. The average annual temperature ranges from 20°C to 25°C. In the summer season, temperature can reach up to 40.7°C while it may fall down to only 8.8°C during winter. Compared to other regions such as A Luoi (3,983 mm), Nam Dong (3,722 mm) and Bach Ma (3,610 mm), the yearly total rainfall in Phong Dien (including the mountains) is the lowest with 2,959 mm (Thao *et al.*; PDG, 2019).




*(Source: GFD, 2019 edited by Dien D.)*

**Figure 1. Map of the study sites in four different distribution areas**

## 2.2. Research methodologies

### 2.2.1 Plant material

*Leaf area*

Leaves from mature trees and seedlings were collected from fields and from the nursery garden between July and September of 2018. A total of 440 leaf samples from 88 different mature trees with five replications were randomly harvested to measure leaf area. The samples consisted of: 135 leaf samples (from 27 trees) collected from A Luoi (mountain area); 70 leaf samples (from 14 trees) harvested from Bach Ma (mountain area); 125 leaf samples (from 25 trees) harvested from Nam Dong (mountain area) and 110 leaf samples (from 22 trees) were taken from Phong Dien (sandy soil area).

Regarding the seedlings, 45 leaves from 45 different seedlings, corresponding to 9 mother trees in the nursery garden, were collected to estimate leaf area (also used for counting stomatal density). Those samples included: 30 seedlings from 6 mother trees with provenances from the moist sites (1 mother tree from Bach Ma, 2 mother trees from A Luoi and 3 mother trees from Nam Dong). With provenance from the dry site, 15 seedlings, out of 3 mother trees were collected. The leaves were immediately scanned after harvesting and stored in a fridge at $5^0$C to further estimation of leaf stomata.

*Leaf stomata*

Twelve leaf samples, collected from mature individuals among 88 trees in four studied sites (3 trees per site), were used to estimate stomatal density. From each leaf, five leaflets were taken from five different positions (one leaflet in the top, two leaflets in the middle part and two leaflets at the basis at right and at left). A total of 45 seedling leaves from different individuals (one to two years of age) were used to estimate the leaf area and stomatal density.

### 2.2.2. Methods for evaluating leaf morphology

Leaf area was measured using a Flatbed scanner (Canon Line 300, Vietnam) and saved in A4 formats with a scale. They were evaluated with ImageJ 1.51j8 (Wayne Rasband, National Institutes of Health, USA); leaf area is specified in $cm^2$.

The number of stomata was estimated by preparing slides from the clear nail polish impression at two opposite middle positions of lower surfaces of each leaflet. Ten images were obtained from each slide with a total area of 0.2378 $mm^2$ per picture (at 100µm scale) with the use of AxioVision SE64 Rel. 4.9.1 Software (Carl Zeiss Microscopy, USA). Finally, the number of stomata was counted for 600 images by ImageJ. Moreover, cross sections of stomata were also obtained in order to estimate the size and shade of stomata and trichrome type. In total 2,250 images were captured from 45 leaves of seedlings to estimate the stomatal density.

### 2.2.3. Data analysis

Data were analyzed and assessed using multiple mathematical and statistics methods applied in biology and forestry. Descriptive statistics, including averages, standard deviation, range (minimum and maximum) etc., were applied. Data were imported to Excel 2016 (v.16), Minitab (v.17) was used to analyze the collected data. Excel was used for data management and chart drawing. The "XLSTAT", version 2019.1, was included in Excel to apply *z*-tests in order to check population means (mountainous and sandy areas) when the variances are known and the sample size is large (normal distribution). The non-parametric Kruskal-Wallis test was used to verify mean differences among four populations (A Luoi, Bach Ma, Nam Dong and Phong Dien). The association among variables was tested through Spearman's or Pearson's correlation, which is dependent on normality tests. All hypothesis tests were performed at the $\alpha$ = 0.05 significant level.

# 3. Results and discussion

## 3.1. Leaf characteristics of mature trees

### 3.1.1. Leaf area

This study covers four areas, including A Luoi, Bach Ma, Nam Dong from mountains and Phong Dien from sandy areas. The mountainous areas are often moister, cooler and lower in radiation than the arid environment found in sandy areas. Thus, we may use the terminologies "moist site" to refer to the mountainous areas and "dry site" for sandy areas. Several studies have reported that leaf temperature and water availability are two main variables correlating with leaf size (Pooter & Rozendaal, 2008; Wright *et al*., 2017). However, our research was unable to estimate these parameters.

The leaf area of mature trees differs across sites ($p$ = 0.000, $\chi^2$ = 90.896). A Luoi has the largest area of leaves (501.7 ± 115.1 $cm^2$ on average) followed by Bach Ma and Nam Dong (476.7 ± 118.71 $cm^2$ and 469.2 ± 109.39 $cm^2$, respectively). Phong Dien has the smallest leaf area with 355.7 ± 95.48 $cm^2$ in average (Fig. 2). Regarding to moist and dry sites, Fig. 3 clearly shows that the area of leaves from the moist site is much larger than that from the dry location; the averages range from 253.2 $cm^2$ to 813.4 $cm^2$ in humid environments and from 184 $cm^2$ to 525.8 $cm^2$ in dry ($p$ < 0.0001, $z$-test).

Leaf area differs among the sampled sites. *E. longifolia* leaves from moist sites, which could also be called as shade leaves, were larger than those from dry sites (also known as sun leaves). This plant is often distributed in the lowland rainforests (no full sunlight) while in sandy areas the canopy is more open. Multiple research has concluded that shade-grown leaves are frequently larger, thinner, less deeply loped and have fewer conducting tissues, probably to capture more light and overcome their neighbours (Medina & Mooney, 1983; Pallardy, 2008).

As expected, leaf size responds to humidity, hence, those areas with higher humidity have larger leaves. Parkhurst & Loucks (1972) suggested that sun leaves are smaller than shade ones, probably to mitigate the boundary layer thickness, to enhance convective heat loss, to reduce transpiration and to prevent the leaf overheating. As Mitchell (1998) stated, *Taxus brefolia* have longer leaves in shade and shorter leaves in sun. However, the leaf size is not always larger in the shade-grown sites. For instance, leaves of *Picea sitchensis* are short and narrow in shade-grown (Leverenz & Jarvis, 1980).

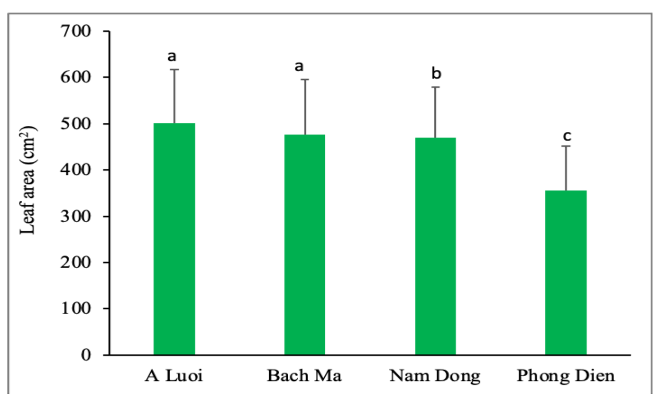



**Figure 2. Leaf area of *E. longifolia* mature trees (Mean with different letter(s) are significantly different among four different sites ($p$ = 0.000, Kruskal-Wallis test and the paired z-test. Error bars indicate the mean ± standard deviation).**

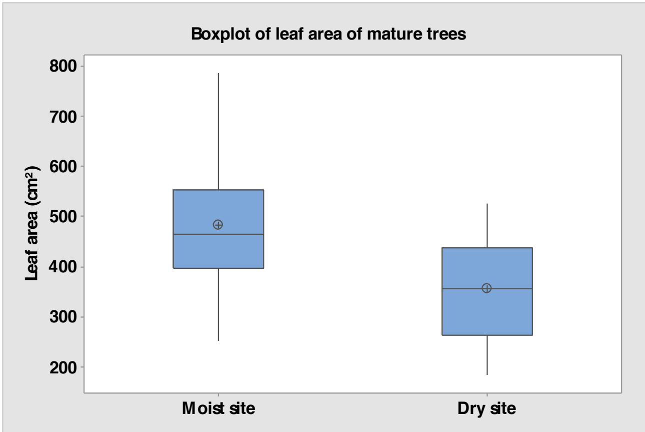



**Figure 3. The difference of leaf area in moist (mountainous) and dry (sandy) sites**

### 3.1.2. Stomatal density

Different environmental conditions, such as water availability, temperature, light exposure or $CO_2$ concentration, may affect stomatal density and size (Xu and Zhou, 2008; Salisbury, 1927). A total of 600 images were captured from 60 leaflets (from 12 individuals), harvested from four different sites. Within sites, stomata density (in stomata/mm$^2$) is highly variable: from 88.3 to 201.9 in A Luoi, from 46.3 to 214.5 in Bach Ma, from 105.1 to 214.5 in Nam Dong and from 197.6 to 386.9 in Phong Dien (Fig. 4). In comparison, Phong Dien is the area with the largest density (almost the double than other sites) with an average of 284.36 ± 38.86 stomata/mm$^2$. Particularly, the stomata density values from both, A Luoi and Bach Ma, are similar but lower than the stomata density from Nam Dong.

The present study results indicate that the average stomatal density values from moist and dry areas were 284 and 138 stomata/mm$^2$, respectively (Fig. 5). These are lower values than those found by Ichie *et al.* (2016), being estimated up to 301.4 stomata/mm$^2$ of *E. longifolia* in lowland tropical rainforest. This is caused due to the difference in ecological conditions which is an important influence on the physiological process of the stomata occurrence (Pekşen *et al.*, 2006). However, their research was conducted among 176 individuals of 136 tree species with from 3 to 5 leaves for each plant; therefore, the average number of samples was only 1.2 individuals per species. This research is a general study of many tree species at once; hence, the collected data was incomplete and insufficient. It is crucial to have a more focused research on stomatal density for this species given the few investigations that can be found. This species can also provide detailed information of stomatal frequency for understorey species estimated as 162.4 stomata/mm$^2$ and for canopy gap (strongest light intensity) with up to 559 stomata/mm$^2$. In comparison with this suggestion, the stomatal density found with this study for the lowland mountains and the sandy area is quite similar.

Stomatal frequency or density can fluctuate within leaves and plants and can be influenced by environmental factors such as water availability, light intensity, temperature and $CO_2$ concentration (Colin & Mark, 1996). We have predicted that the leaves from environments with low humidity would have lower number of stomata/mm$^2$ in order to reduce evapotranspiration. This hypothesis was based on previous studies indicating that plants in insufficient water conditions can increase their stomatal frequency, leading to an increase in their transmission for gas exchange in photosynthesis (Schlüter *et al.,* 2003). A higher density requires more water to be transpired and more $CO_2$ to be taken up. Rajmohan (2014) also showed that, when water is limited in a dry environment, photosynthesis can be affected, leading to dry out. In addition, the stomatal density of *Euonymus europaeus* from sun sites is higher than that from shade sites.

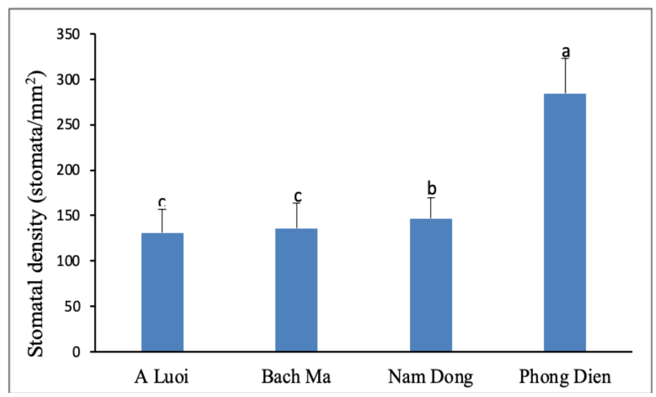



**Figure 4. The difference in stomatal density of mature trees at four sites. (Mean with different letter(s) are significantly different among four different sites, *p* = 0.000, Kruskal-Wallis test and the paired *z*-test. Error bars indicate the mean ± standard deviation).**

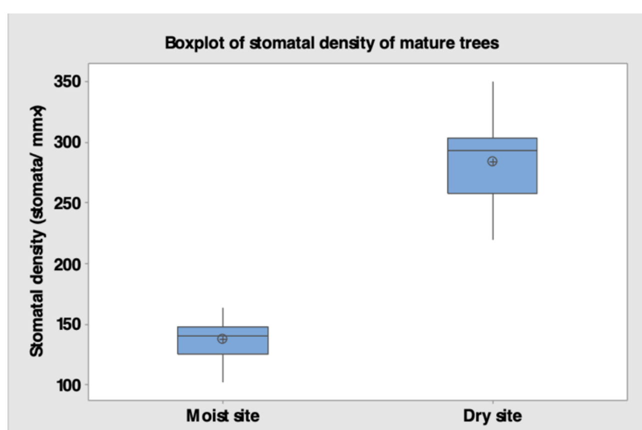



**Figure 5. The difference in stomatal density of mature trees in moist and dry sites**

This study found a higher stomatal density in Phong Dien (dry place) in comparison with that in A Luoi, Bach Ma and Nam Dong (moist places). Leaves in dry conditions are thick and the amount of chlorophyll per unit area of leaf increases because they have to arrange all chloroplasts along the cell surface of mesophyll. Thus, there are strong correlations among photosynthetic ability, leaf thickness and the cell surface area of mesophyll (Jurik, 1986; Nobel, 1975). Recently, Pallardy (2008) confirmed that shade leaves have a reduced stomatal density, superior interveinal areas and a lower ratio of internal/external surface. A study on *Taxus brevifolia* presented by Mitchell (1998) indicates a higher stomatal density under sunny conditions than in shade-grown. Moreover, several studies have also concluded that leaves are often smaller and thicker in individuals that have grown in full sunlight, dry soil or low humidity than those in the shade (Salisbury, 1927; Penfound, 1931; Cooper & Qualls, 1967). Salisbury (1927) recognized that in *Mercuria lisperennis*, the values of stomatal frequency at the top of the plant tend to be gradually reduced, or even disappearing, when this plant grows in moist conditions. In addition, two *Abies* species (*A. alba* and *A. grandis*) that were grown in the shade had lower stomatal frequency than those grown under the sun (Magnussen, 1983). Veit & Hubertus (2018) also concluded that leaves of most *Abies* species in xeric conditions are shorter but have a higher number of stomata than those in moist conditions.

### 3.1.3. The correlation between mature leaf area and stomata density

There is a generally considered strong correlation between the leaf area and the stomata density ($r^2$ = - 0.59 and $p$ = 0.000, Spearman's correlation coefficient). The direction of the relationship is negative (inverse relationship), meaning that the larger the leaves the lower the stomatal density (Fig. 6). The leaf area tends to be small in dry conditions, however, it does not mean that tiny leaves have a low transpiration rate and fewer stomata. Thoday (1931) noted that in some plants with small leaves the transpiration surface is higher than that of similar plants with larger leaves. Thus, the transpiration is minimized by larger leaf sizes. In humid understories, the decrease in stomatal transpiration or leaf temperature is caused by the increment in leaf size; this means that the leaves from under to over temperatures, which can drive up to mitigate the stomatal frequency (Medina *et al.*, 1983).

The lack of water in drought conditions can influence the leaf size by declining the cell size and causing an increase in stomatal frequency (Martínez *et al.*, 2007; Xu and Zhou, 2008). According to Franks & Farquhar (2007), the number of stomata can rise when the leaf area declines because it needs to fit sufficient stomata units for each unit of leaf area to respond to the $CO_2$ flux as desired and to support the photosynthetic capacity. Salisbury (1927) discovered that the stomatal frequency was negatively related to the leaf size given the high number of cells per unit area in smaller leaves. Gay & Hurd (1975) explained that stomata is often differentiated in the early stage of leaf expansion. Their frequency reaches the maximum at young age and declines until it reached a relatively stable density, unconventionally of cell insertion or area. The inverse correlation between the leaf area and the stomatal density was also found for *Taxus brevifolia* (Mitchell, 1998). Humidity and dry conditions have a high effect on the occurrence of stomata and leaf size. However, leaf stomata have a negative correlation with the leaf area, tree height and tree diameter. This may be explained because the leaf area is reduced in a dry area, affecting the photosynthesis and tree growth. Schäfer *et al.* (2000) found that stomatal frequency in *Fagus salvarica* decreases as the tree height increases. Recently, Peel *et al.* (2017) also confirmed that the stomatal density of *Rhizophora mangle* in mangrove forests is inversely related to the tree height and diameter.

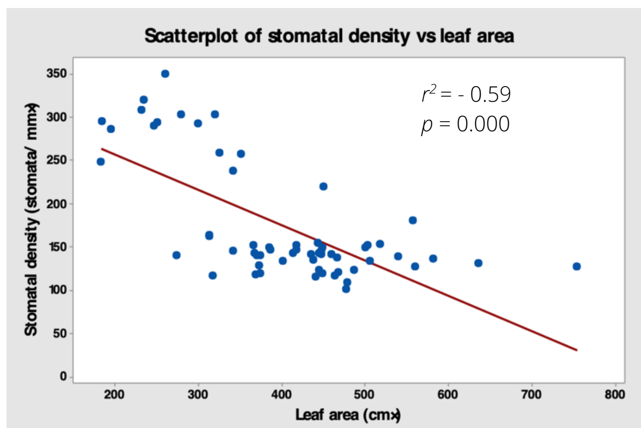



**Figure 6. The negative correlation of leaf area with stomatal density of mature trees**

## 3.2. Leaf traits of seedlings

There are not large differences in the leaf morphological traits of the seedlings, from several provenances, cultivated in the nursery garden. The average leaf area of different origins is similar (110.2 cm$^2$). Although the seedlings were cultivated in the same environmental conditions, their stomatal density differs across four different provenances. Those from A Luoi have the lowest number of stomata (an average of 202.1 ± 4.0 stomata/mm$^2$) followed by Phong Dien (247.0 ± 52.4), Bach Ma's (261.2 ± 9.9) and Nam Dong (280.1 ± 53.1). In general, the stomata density of the seedlings originated from the dry site is similar to that from the moist site (247.0 stomata/mm$^2$) (Table 1).

No significant differences were found between the leaf area with stomatal density and the seedling age, seedling height, seedling collar diameter ($p > 0.05$). The stomatal density of the seedlings has a positive relationship with the seedling age and the seedling collar diameter ($r^2$ = 0.444 and $r^2$ = 0.301 with $p$ = 0.002 and 0.045, respectively). The seedling stomata are not affected by the seedling height ($p$ = 0.703).

**Table 1. Range, mean (±standard deviation) of seedling characteristics from four different provenances**

| Seedling traits | Provenances | | | | p-value | H-value |
|---|---|---|---|---|---|---|
| | A Luoi | Bach Ma | Nam Dong | Phong Dien | | |
| Leaf area (cm²/leaf) | 114.3±32.2$^a$ | 100.6±32.2$^a$ | 111.5±24.0$^a$ | 109.4±25.8$^a$ | 0.686 | 1.482 |
| Stomatal density (stomata/mm²) | 202.1±4.0$^c$ | 261.2±9.8$^{ab}$ | 280.1±53.1$^a$ | 247.0±52.4$^b$ | 0.003 | 14.011 |

*Notes: *Kruskal-Wallis test, ($^{a,b,c:}$ t-test)*

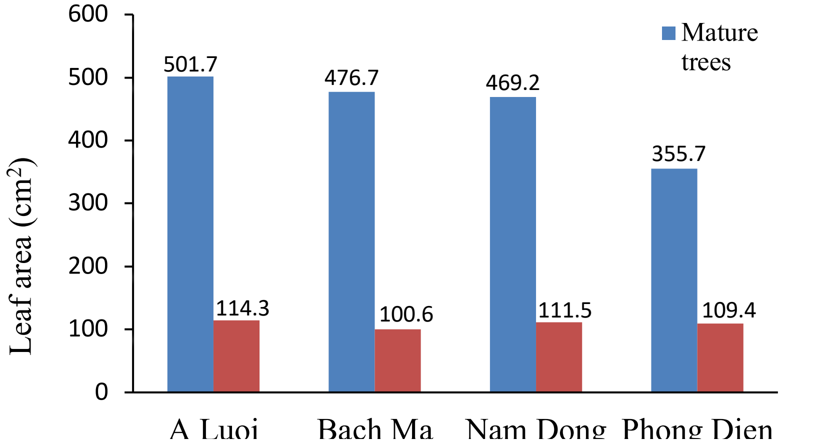
## 3.3. The comparison leaf traits between mature trees and seedlings

As displayed in Fig. 7, the patterns of leaf traits differ among the seedlings and the mature trees. The leaf area of mature trees is nearly 3-5 times larger than that those of the seedlings. The stomatal density displays wide ranges among the leaves of mature trees and those from the seedlings (from 131.3 to 284.4 stomata/mm$^2$ and from 202.1 to 247.0 stomata/mm$^2$, respectively). Although seedlings from different sites have a similar leaf area, they are not the same as those from mature trees, the average number of stomata density from the A Luoi, Bach Ma and Nam Dong provenances (137.9 stomata/mm$^2$) was nearly twice as high as that of mature trees. Even mature trees from the dry site had a smaller leaf area than those from the moist site. The stomata density values for these two locations were 284.4 and 247.0 stomata/mm$^2$, respectively; a significantly higher density than those of the seedlings ($z$-test, $p < 0.0001$).

The pattern of leaf traits differed between mature trees and seedlings. *E. longifolia* seedlings from several provenances were grown under the same conditions in the nursery garden to reduce the environmental variation. Thus, the leaf area of the multiple seedlings was nearly the same, whereas a difference in stomatal adaptability was found under the same environmental conditions. In general, seedling leaves (around two-years-old) were smaller than mature leaves. From a previous study, seedlings from many forest trees, such as *Campnosperma auriculatum* in Malaysia, had larger leaves than those of adult trees. In juvenile plants or heteroblastic, the leaves of seedlings or saplings tend to be larger, thinner and developed without any order, compared to leaves from mature plants of the same species (Whitmore, 1975; Hall & Swaine, 1981).

The comparison between seedlings and mature trees showed unexpected results with seedlings having smaller leaf areas but with a higher number of stomata compared to adult trees (245.6 and 174.6 stomata/mm$^2$, respectively). The stomatal density of seedlings was higher than the stomatal density of mature trees from the moist site but lower than those from the dry site. This result indicates that the stomata can quickly adapt to changes in their living environmental conditions such as open areas with intense sunlight. This study implies that *E. longifolia* is a tree species with an extremely high capacity of adaptation under contrasting environmental conditions. Seedlings from different provenances can be well cultivated under the same conditions in nursery gardens.

a)




b)

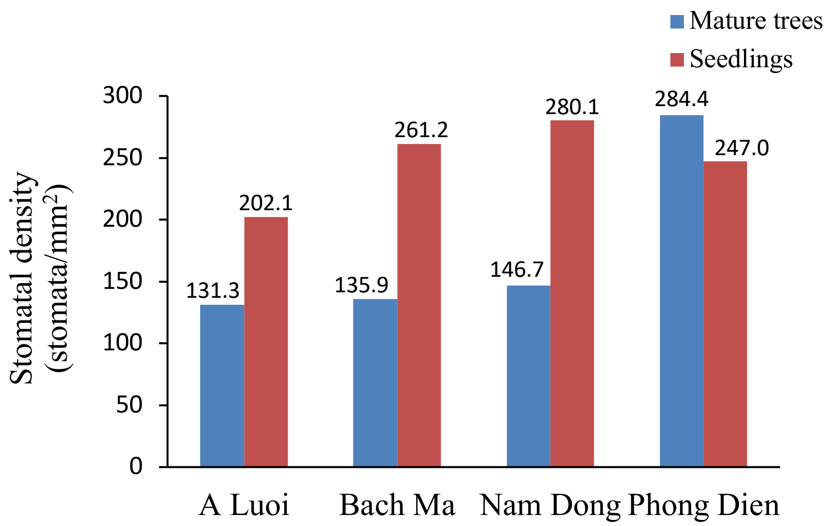



**Figure 7. The differences between mature trees and seedlings in aspects of a) leaf area and b) stomatal density in different sites**

## 4. Conclusions

The leaf area of *E. longifolia* from mountainous locations is larger than those from sandy location. However, its stomatal density is higher in dry sites compared with moist sites. The results of the study confirmed that the leaf area is inversely related to the stomatal density; dry conditions may increase the number of stomata as the leaf size decreases. Even seedlings have a smaller leaf area but a higher number of stomata than the adult trees. Thus, this study implies that *E. longifolia* is a tree species with an extremely high capacity of adaptation in contrasting environmental conditions. Seedlings from different provenances can be well cultivated under the same conditions in a nursery garden.

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