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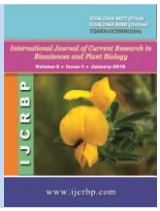

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Effect of Shade at Agrisilviculture System on Characteristics of Stomatal Abaxial Leaf Surfaces in Toraja Highland, Indonesia

Resti Ura'[1]*, Samuel Arung Paembonan[2] and Daud Malamassam[2]

[1]Postgraduate, Faculty of Forestry, Hasanuddin University, Makassar 90245, Indonesia
[2]Faculty of Forestry, Hasanuddin University, Makassar 90245, Indonesia

***Corresponding author.**

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ABSTRACT

Agrisilviculture was a mixture of forestry and agricultural crops in various canopy strata. This study aims to analyze the effect of shade on the agrisilviculture system on the stomatal characteristics at abaxial leaf surfaces in Toraja Highland Indonesia. The method used is to apply acetone on the abaxial leaf surfaces longitudinally to obtain stomatal characteristic data and environmental factor measurements. Data analysis descriptive. The results showed that stomatal density at abaxial leaf tissue was influenced by environmental factors (light intensity, air temperature, humidity, soil pH, and soil moisture), site index and plant species. Tree shade affects on the light intensity received by the leaf canopy on the lower layers. Types of stomatal at abaxial leaf surfaces on *Elmerrillia ovalis* and *Coffea canephora* are anisocytic and cyclocytic type on the leaf of *Colocasia esculenta*. The highest stomatal density above 500 stomatal/mm^2 was found on the leaf of *Elmerrillia ovalis* and *Coffea canephora* and the lowest on the leaf of *Colocasia esculenta* 110 stomatal/mm^2. The stomatal closing cells in leaf all three plant species were kidney-shaped.

Introduction

The agrisilviculture system was a dynamic and ecologically-based natural resource management system that combines tree species on a land where the system not only provides socio-economic benefits to farmers, but also provides its own environmental benefits. Tolerance of a tree species to produce under shade with environmental factors where it grows such as light, water, temperature, humidity, soil pH, soil moisture, wind, and nutrients (Zwieniecki et al., 2016; Tambaru, 2012; Salisbury and Ross, 1992). The ability of a tree species to carry out the process of photosynthesis in the absorption of sunlight and water under shade was used as a measure of the relative tolerance of the plant (Santrucek et al., 2014). Tree plays a very important role in nature because it can reduce CO_2 gas in the air and produce O_2 in the process of photosynthesis (Hoshika et al., 2013; Watanabe et

al., 2010; Fitter and Hay, 1981). Carbon dioxide plays a very important role for photosynthesis in plants, this was influenced by the concentration, if too high concentration, then stomatal close (Beerling, 2017; Kim et al., 2013; Wardhana, 2004; Larcher, 1995). The endurance of each tree species to grow is also influenced by several factors such as: leaf morphology, leaf anatomy, stomatal density, stomatal size, and stomatal location on leaf surface. The high density of stomatal at abaxial leaf surfaces was a process of adaptation of plants to environmental conditions (Campbell et al., 2003).

Sereale District is located in the highland of North Toraja Regency South Sulawesi, Indonesia, which located at an altitude of ± 750-2604 m above sea level. North Toraja is one of rural areas that have a diversity of flora that has not been much studied (Tambaru, 2012). Based on the above description, we conducted a study on the effect of shade at agrisilviculture system on characteristics of stomatal abaxial leaf surfaces.

Materials and methods

Plant materials used in this study were: *Elmerrillia ovalis*, *Coffea canephora* and *Colocasia esculenta*. Chemicals used acetone to make stomatal leaves.

Leaf measurements and observations included leaf morphology, stomatal type characteristics, stomatal size, stomatal index, the number of stomatal, and number of epidermis cells in Agrisilviculture plants. Analysis of longitudinal stomatal characteristics of leaf plants on the surface of the lower leaves (abaxial) smeared with acetone on leaves still in the tree. The dried stomatal mold is then covered with clear tape and then drawn and placed on top of the glass object. Stomatal preparations are observed under a microscope with 200x magnification. observations of leaf anatomical characters include the number of stomatal, epidermis cells and stomatal index. Observation of length, width, stomatal opening, and stomatal type using 400x magnification. Samples were then photographed using Bino Microscope and Photo model DS.Fi I Nikon ECLIPSE 80i with 400x magnification. The calculation of Stomatal Index (SI) based on the formula (Sunarti et al., 2008; Damayanti, 2007 *cit.* Tambaru, 2015; Santrucek et al., 2014; Obembe, 2015) was as follows:

$$SI\% = \frac{S/L}{(S+E)\,L} x\ 100\%$$

Where,
S = number of stomatal; E = number of epidermis cells; L = unit leaf area

Environmental factors that influence plant growth were measured are light intensity (light meter model LX-130), temperature and humidity (thermometer/hygrometer). Measurements done at 10 am at the time of sunlight intensity in optimal state. In addition, measurements were also done on pH and soil moisture (soil tester model DM-5).

Results and discussion

Description of plant species in the agrisilviculture system in Sereale North Toraja are:

a. *Elmerrillia ovalis* (Miq.) Dandy

Habitus is a tree, tap root system, round stem, the direction of growing stem was erectus, monopodial branching, branch skew up (patens), single leaf spread, elongated leaf shape, pinnate leaves, flat leaf edge, pointed leaf tip, base of pointed leaves, green leaf color, chartaceus leaf meat, leaf surface wrinkled. The shape of the canopy of *Elmerrillia ovalis* is pyramidal, including Classis Dicotyledoneae and Family Magnoliaceae.

b. Robusta Coffee *Coffeacanephora* Pierre ex Froehner var. robusta

Shrub habit, tap root system, round stem, the direction of growing stems is erectus, sympodial branching, direction of growing branches was drooping (declinatus), single leaf lying opposite, oval leaf shape, pinnate leaves, leaf edges, leaf meat like paper, pointed leaf tip, base of leaves rounded-dull, slick shiny surface of the leaf, the color of the

leaves on the upper surface was dark green and lower light green. The shape of the canopy of *Coffeaca nephora* was semi-globular included in the Class Dicotyledoneae and Family Rubiaceae.

c. Taro *Colocasia esculenta* (L.) Schott.

Habitus herbs-shrubs, fiber root system, stem/ stems tuber are in the soil, the direction of growing stems is erectus. Root rosette was the leaves grow near the surface of the land. Single leaf, leaf shape in the form of a shield (peltatus), leaf edges, pointed leaf tip, leaf base grooved, palminerved leaves. Upper leaf surface was slippery and coated with wax, leaves are green, leaf meat was soft and contains water. The shape of the canopy of *Colocasia esculenta* was semi-globular, included in the Class Monocotyledoneae and Family Araceae.

The result of data analysis characteristic of stomatal at abaxial leaf surfaces the longitudinal cross section from the study of the shade effect on the agrisilviculture system in Sereale North Toraja was shown in Table 1 and Figs. 1-4.

The results of the study in Table 1 and Fig. 1 show the type stomatal at abaxial leaf surfaces *Elmerrillia ovalis* and *Coffea canephora* are anisocytic. Type anisocytic are these stomatal remain encircled by three subsidiary cells of which one was distinctly smaller or large in size than the other two (Chachad and Vaidya, 2016; Sreelakshmi et al., 2014; Mulyani, 2006; Pandey and Chadha, 1996). The irregular stomatal distribution on the leaf surface is a hallmark of Classis Dicotyledoneae (Nugroho et al., 2006). Type stomatal abaxial of *Colocasia esculenta* was a cyclocytic type with four or more subsidiary cells arranged in a closed ring around the stoma. The cyclocytic type was a regular dispersal of stomatal in a longitudinal series characteristic of Class Monocotyledoneae (Nugroho et al., 2006; Pandey and Chadha, 1996).

Table 1. Stomatal characteristics based longitudinal cross-section of leaf on agrisylviculture system in Sereale North Toraja.

Characteristics	*Elmerrillia ovalis*	*Coffea canephora*	*Colocasia esculenta*
Location I:			
Stomatal type	Anisocytic	Anisocytic	Cyclocytic
The spread of stomatal	Irregular	Irregular	Arranged longitudinally
Cell shape of closing stomatal	Kidney-shaped	Kidney-shaped	Kidney-shaped
Stomatal length (µm)	24.00	14.4 – 28.80	19.20 – 26.40
Stomatal width (µm)	26.40 – 28.80	24.00 – 36.00	21.60 – 26.40
Stomatal opening (µm)	10.20 – 12.00	7.20 – 12.00	7.20 – 7.80
Stomatal size (µm)	500.54 – 546.40	273.02– 819.07	400.44 –550.60
Stomatal index (%)	30.999 – 38.567	20.095 – 27.273	5.237 – 7.469
Stomatal density (mm^2)	772 – 1012	416 – 612	68 – 84
Number of epidermis cells (mm^2)	1612 – 2128	1532 – 2020	892 – 1520
Location II:			
Type Stomatal	Anisocytic	Anisocytic	Cyclocytic
The spread of stomatal	Irregular	Irregular	Arranged longitudinally
Cell shape of closing stomatal	Kidney-shaped	Kidney-shaped	Kidney-shaped
Stomatal long (µm)	24.00 – 33.60	24.00 – 31.20	28.80 – 40.80
Stomatal width (µm)	24.00 – 33.60	33.60 – 36.00	28.80 – 33.60
Stomatal opening (µm)	8.40 – 12.00	9.60 – 13.20	6.60 – 17.40
Stomatal size (µm)	637.06 – 700.76	682.56 – 828.17	764.47 – 1083.00
Stomatal index (%)	36.949 – 38.361	21.208 – 26.695	5.398 – 12.308
Stomatal density (mm^2)	804 – 936	504 – 632	84.00 – 256.00
Number of epidermis cells (mm^2)	1372 – 1548	1384 – 2348	1472 – 1824

Description: Location I in Pongmela Sereale and Location II in Buntu Sereale North Toraja.

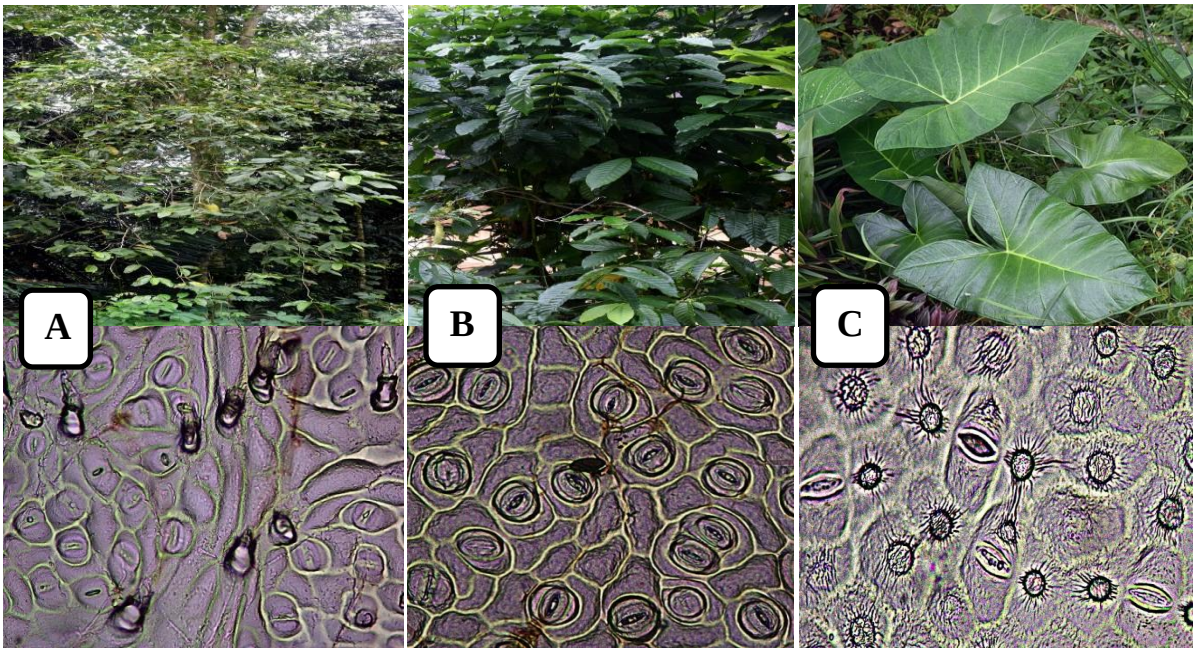
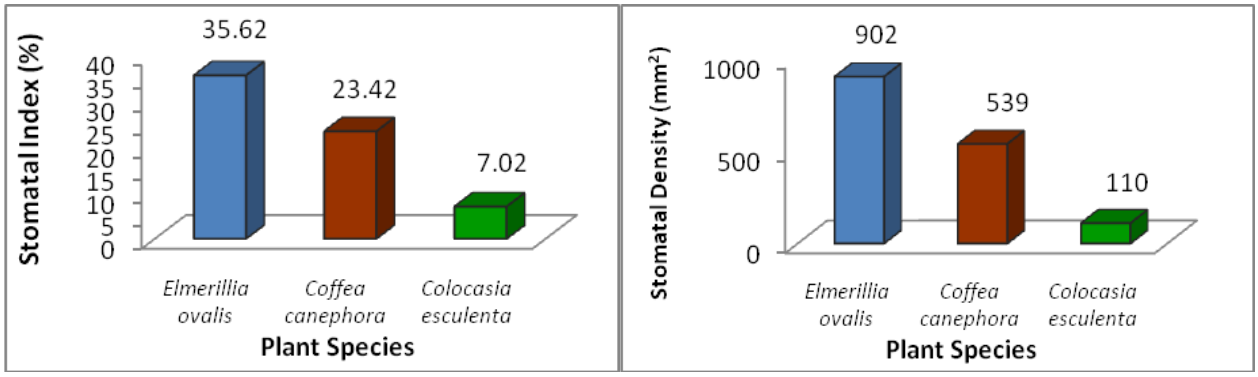
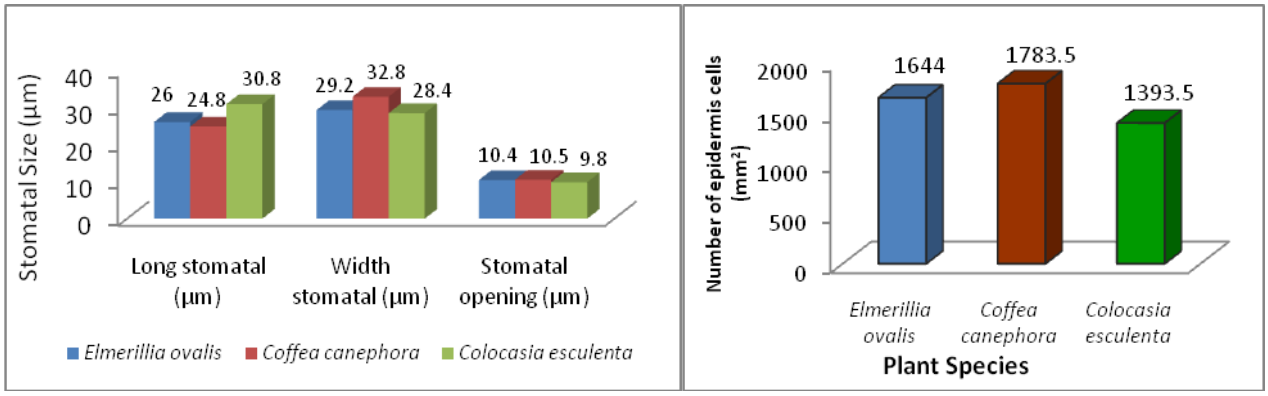


Fig. 1: Plant morphology and stomatal magnification (400x): (A) *Elmerrillia ovalis*, (B) *Coffea canephora*, (C) *Colocasia esculenta.*



A **B**

Fig. 2: A. Histogram of average stomatal index (%); B. Histogram of average stomatal density (mm^2).



A **B**

Fig. 3: A. Histogram of average number of epidermis cells (mm^2); B. Histogram of average stomatal size (µm).

The average stomatal index in Fig. 2A shows the highest stomatal index found on *Elmerrillia ovalis*

leaves 35.62% and the lowest on the leaves of *Colocasia esculenta* 7.02%. The density of stomatal at abaxial leaf surfaces in Fig. 2B shows that in *Elmerrillia ovalis* averages of 902 stomatal/mm^2 and the average *Coffea canephora* 539 stomatal/mm^2 including high stomatal density amounts as the amount above 500 stomatal/mm^2. At *Colocasia esculenta* the averages 110 stomatal/mm^2, including low stomatal density for less than 300 stomatal/mm^2 (Tambaru, 2017). The stomatal closing cells in all three plant species were kidney-shaped (Mulyani, 2006; Pandey and Chadha, 1996).

The results of the study in Fig. 3A show the highest average number of epidermis cells found in the *Coffea canephora* 1783.5 epidermis cells/mm^2 and the lowest in the *Colocasia esculenta* 1393.5 epidermis cells/mm^2. The result of stomatal size at abaxialin Fig. 3B shows that the longest average stomatal in *Colocasia esculenta* is 30.8 µm and the shortest *Coffea canephora* is 24.5 µm. The widest stomatal of *Coffea canephora* is 32.8 µm and is narrower in *Colocasia esculenta* 28.4 µm. The largest stomatal opening of *Coffea canephora* 10.5 µm and *Colocasia esculenta* 9.8 µm. According to Hidayat (2009) *cit*. Tambaru, (2017), that the stomatal size is said to be less long (<20 µm), long (20-25 µm) and very long (> 25 µm). Where large stomatal actually gave low stomatal density and small stomatal gave high density in some plant species (Tambaru, 2012; AbdulRahaman and Oladele, 2003). Stomatal density of abaxial leaves more than the leaf of adaxial, this was a mechanism of plant adaptation to terrestrial environmental conditions (Campbell et al., 2003), to reduce transpiration on the leaf surface (Kumekawa et al., 2013; Larcher, 1995; Salisbury and Ross, 1992). According to Nugroho et al. (2006), there are two types of leaves on the plant is dorsiventral leaves usually grow horizontally, on the top of the leaf surface the color is brighter than the lower leaf surface was found in Class Dicotyledoneae. Type of leaf isobilateral if the leaves grow vertically, so both sides of the leaf surface receive sunlight with the same intensity found in Classis Monocotyledoneae. The results of Tambaru (2012) show that the decrease in the number of stomatal can be influenced by the location where it grows, the type of plants and environmental factors.

Salisburry and Ross (1992), Fitter and Hay (1981) stated that the leaves exposed to full sunlight have a narrow leaf surface area and thick, while the leaves are shaded leaf size was wider and thinner.Leaves in shaded plants have the ability to absorb far-red-light with a 700 µm wavelength, the leaves contain a lot of chlorophyll b. Sun exposed leaves can directly absorb red-light wavelength 680 µm from leaves containing chlorophyll a (Tambaru, 2012; Salisburry and Ross, 1992).

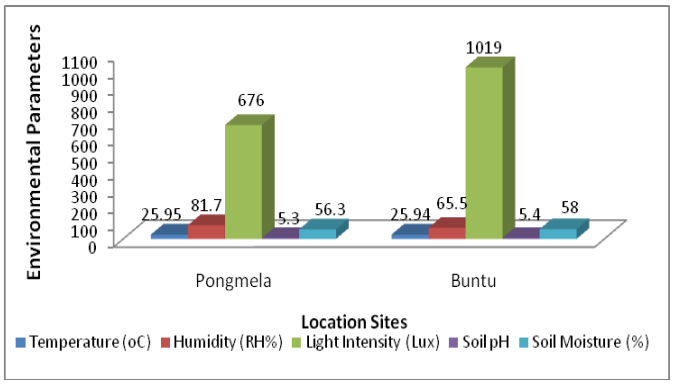


Fig. 4: Histogram of average comparisons of environmental parameters at research sites shade trees in Sereale North Toraja, Indonesia.

The result of environmental factor measurement in Figure 4 at 2 research sites, I Pongmela location: temperature 25.95°C, humidity 81.7 RH%, soil pH 5.3, and light intensity 676 lux. Location II Buntu: temperature 25.94°C, humidity 65.5 RH%, soil pH 5.4, and light intensity 1019 lux. The results of this study indicate that the density of stomatal was influenced by environmental factors such as: the intensity of sunlight, temperature, humidity, and soil pH, site index and plant species. Tree shade affects the intensity of sunlight received on the leaf canopy in the lower layers, thus affecting the activity of plant photosynthesis. Each type of plant has a different response to the high and low intensity of light for photosynthesis activity (Wijayanto and Aziz, 2013). According to Holland and Richardson (2009), there is an increase in stomatal density based on altitude, especially in

mountain areas.

The research location in Sereale, North Toraja was a mountainous area that has a diversity of flora with cold environmental conditions and high humidity conditions are very favorable for the growth of plants, especially *Elmerrillia ovalis*, *Coffea canephora* and *Colocasia esculenta.* This type of plant also has more stomatal in abaxial leaf surfaces, potentially absorbing more carbon dioxide from the environment.

Conclusion

Stomatal density of abaxial leaf surfaces was influenced by environmental factors (sunlight intensity, air temperature, soil moisture pH, and soil moisture), site index and plant species. Tree shade affects the intensity of sunlight received by the leaf canopy on the lower layers. The stomatal types at abaxial leaf surfaces on *Elmerrillia ovalis* and *Coffeaca nephora* are: anisocytic and cyclocytic type on the leaf of *Colocasia esculenta*. The highest stomatal density above 500 stomatal/mm^2 was found on *Elmerrillia ovalis* and *Coffea canephora* leaf and the lowest 110 stomatal/mm^2 on *Colocasia esculenta* leaf. The stomatal closing cells in leaf of all the three plant species were kidney-shaped.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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