

DIFFERENCES IN LEAF PIGMENTS CONTENT AND STOMATA DISTRIBUTION ACROSS EIGHT *Hevea* SPECIES

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ABSTRACT

Photosynthesis is very important to plants in order to produce their own food. The study of this process involves wide range of field including morphology, physiology, and biochemical studies. In this study the analysis of leaf pigments content including chlorophyll and anthocyanin content and stomata distribution that directly and indirectly involve in photosynthesis were conducted. Eight *Hevea* species that are *H. brasiliensis*, *H. camargoana*, *H. guinensis*, *H. benthamiana*, *H. rigidifolia*, *H. nitida*, *H. spruceana*, and *H. pauciflora* were analyzed. Between these eight species, *H. brasiliensis* has the highest chlorophyll content while *H. rigidifolia* with the lowest chlorophyll content. Meanwhile, the anthocyanin content was found to be the highest in *H. guinensis* (36.747 mg/L) and lowest in *H. camargoana* (15.1 mg/L). Stomata distribution that directly involves in gas exchange during photosynthesis was found to be high in *H. camargoana* (190.55/mm²) and low in *H. rigidifolia* (92.25/mm²). As a conclusion, this study can be used as a guideline to indicate the physiological state and functional of the plant leaves as a result from analysis of chlorophyll and anthocyanin content and also the distribution of stomata.

Key words: *Hevea* species, leaf pigment, stomata

INTRODUCTION

Conventional breeding and selection of *Hevea brasiliensis* may take into account some physiological characteristic of the clones in designing further breeding strategies. Considerable effort and continuous attempt are being made through breeding and selection to improve rubber productivity and to meet increasing demand from the stakeholders. There are many *Hevea* clones that are constantly being bred to get a better and superior genotype. An improvement of the genetic composition of *Hevea* species is being conducted (de Souza *et al.*, 2015). *Hevea* species are another source of genetic reservoir that may be exploited by breeders for that matter (Goncalves *et al.*, 2006).

Morphology, physiological characteristic and biochemical information derived from the *Hevea* clones can assist future *Hevea* breeding program. Leaf pigment content for instance can provide

valuable insight into the physiological performance and function of leaves (Merzlyak *et al.*, 1999), which at the end can be correlated with the yield, and productivity of each rubber tree. Chlorophylls absorb light energy and transfer it into the photosynthetic apparatus. Carotenoids (yellow pigments) can also contribute energy to the photosynthetic system. Anthocyanins (pink, purple, and red pigments) may also protect leaves from excess light or from UV light (Fini *et al.*, 2011). Reports show that anthocyanin in leaves is mainly located in the mesophyll and lower epidermis than in the upper epidermis where they would be more effective as light screen (Gould & Quinn, 1999). Anthocyanins may also serve as scavengers of reactive oxygen intermediates (Hatier & Gould, 2008; 2009) and significant effect to human health as well by preventing diseases such as tumor and cardiovascular (Chen, 2016).

Since that pigments are very important for leaf function, variations in pigment content may provide information concerning the physiological

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state of leaves. Anthocyanin content tends to be high in young leaves that also have low photosynthetic rates (Gould *et al.*, 2000) in leaves of plants where growth has been limited by low temperature of other stresses and pollinators attack (Chalker-Scott, 1999; Winkel-Shirley, 2001; Hughes *et al.*, 2005).

In *Hevea*, clonal variations in photosynthesis rate (Samsuddin, 1986; Nugawela *et al.*, 1995; Miguel *et al.*, 2007) and stomata distribution (Bastiah *et al.*, 2009) have been observed. Previously, positive correlation was found between leaf water potential and stomatal and also leaf water potential and photosynthetic rate of two clones of *Hevea brasiliensis* (Sittichai & Sdoodee, 2014). The identification of stomata distribution is important for better clone selection. The stomatal closure that occurred during water stress or drought can reduced the carbon dioxide uptake and thus reduce photosynthesis rate (Sangsing *et al.*, 2004).

To assess the leaves photosynthetic efficiency between *Hevea* species, we studied some of the parameters that are directly or indirectly associated with the physiological function of the leaves such as the pigment content, stomata distribution and morphology character of leaves across eight native *Hevea* species namely *H. brasiliensis*, *H. benthamiana*, *H. pauciflora*, *H. camargoana*, *H. guinensis*, *H. rigidifoli*, *H. nitida* and *H. spruceana*.

MATERIALS AND METHODS

Plant materials

Leaf samples were taken from *Hevea* 1995 germplasm at Bt. Arang, Selangor in the morning between 0900 h to 1100 h when weather conditions were good and no rain prior to the sampling day. Small branches were cut at random with the help of a pruning knife attached to the pole. The leaves were washed and blotted dry and used for extraction and determination of leaf pigments.

Determination of chlorophyll content

The chlorophyll content of leaf was measured following the method suggested by Shaw *et al.* (1965). One-gram sample (fresh weight) of leaf tissue was ground with 50 ml (v/v) cold ethanol (80% v/v) in a mortar. After filtering the leaf extract, 1 ml of the filtered solution was diluted to 5 ml with ethanol. The relative chlorophyll concentrations were determined by measuring the optical-absorption at 665 mu using a Perkin Elmer spectrophotometer Lambda EZ201. Distilled water was used as a blank (reference material) in the spectrophotometer reading.

Determination of anthocyanin content

Leaves were cut into approximately 1 mm strips and 1 g of this was placed into a beaker containing 10 ml of 0.1 M HCl. The mixture was stirred occasionally and placed in a bell jar for 4 hours. The extract was then filtered and the filtrate was read at 520 mu in the spectrophotometer Lambda EZ201. The anthocyanin extract was measured in terms of pigment unit per gram of leaf tissue (Thimann & Edmunson, 1949) following the equation:

$$\text{Pigment units} = 520 \times v(\text{HCl})/10$$

Where V is volume in millilitres.

Stomata distribution

Fresh leaves were washed with distilled H₂O and air-dried for several minutes. A thin layer of cutex was applied and spread evenly on the lower surface of the foliar and allowed dry. The dried cutex was carefully peeled off from the foliar and the shadow cast replicas were mounted onto glass slides in Canada balsam to be examined under an optical microscope (Leica DM750) at 10x magnification with a field of view approximately 0.936mm x 0.702mm. Image analysis of the stomata was carried out using an image analyser system (Leica Application suite ver. 3.7).

Scanning electron microscopy (SEM) of foliar

Fresh leaves were cut into small pieces of 10 cm x 10 cm and mounted on a copper stub smeared with carbon paste for adhesion. Uncoated leaves samples were immediately introduced into the low-pressure column and examined using a FEI Quanta 180 ESEM at electron microscopy laboratory, HUKM.

RESULTS AND DISCUSSION

Chlorophyll contents in the leaves of eight *Hevea* species were given in Figure 1. The results indicate that the mean for *H. brasiliensis* is significantly larger than the means of all the other groups. There are four means that are not found to be significantly different *i.e.* *H. nitida*, *H. spruceana*, *H. camargoana* and *H. guinensis*. The mean for *H. rigidifolia* however is found to be significantly smaller than the means of the other species. There is a significant difference in mean of chlorophyll content between species (p-value = 0.0004). With this variation in chlorophyll content across the species it is quite plausible to carry out another experiment in order to see the correlation between some of the other reported parameters directly related to the photosynthesis of the leaf such as the palisade thickness, spongy layer thickness, number of cells

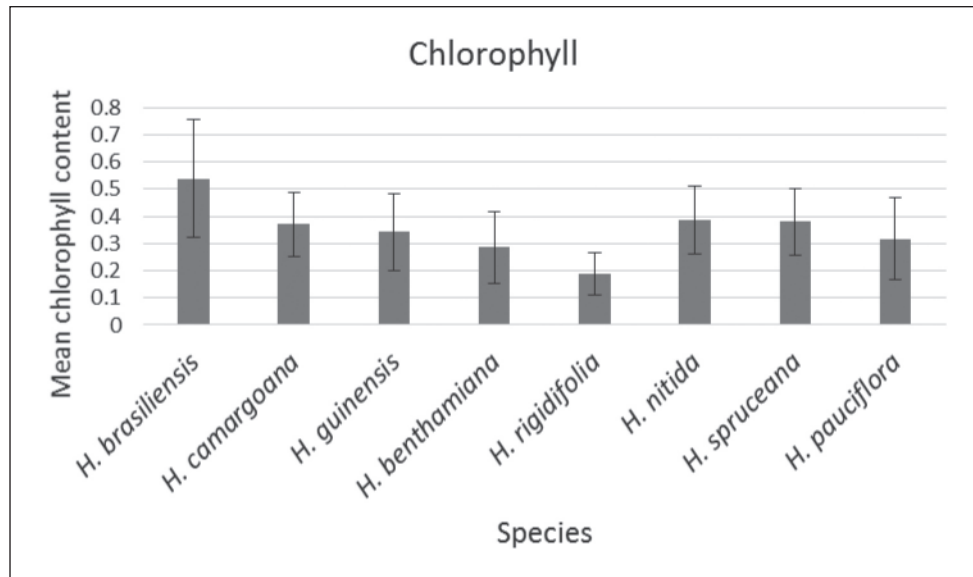


Fig. 1. Chlorophyll content of eight *Hevea* species.

in palisade unit length and number of cells in spongy layer unit across the species (Figure 2). Those parameters were reported diverse in their nature in different growth stages of *Hevea brasiliensis* (Engku Azlin Rahayu Engku Ariff *et al.*, 2011). With a comprehensive data that include all those parameters, it will give a more meaningful interpretation and in depth information on the *Hevea* spp. foliar physiological performance and function in relation to their photosynthetic activity.

Figure 3 shows the content of anthocyanin in leaves of eight *Hevea* species. Result shows that

the highest mean for anthocyanin content is in *H. guinensis* and the lowest is in *H. camargoana*. However, there is no significant different in mean of anthocyanin content between the species (p -value = 0.5289). It was reported that anthocyanin content tends to be high in the young leaves and reduced as they become matured. Young leaves also have low chlorophyll content and exhibit low photosynthetic rate (Zhang *et al.*, 2016).

Another sampling is going to be carried out for anthocyanin content in order to see the relation with different sampling time and also on trees with

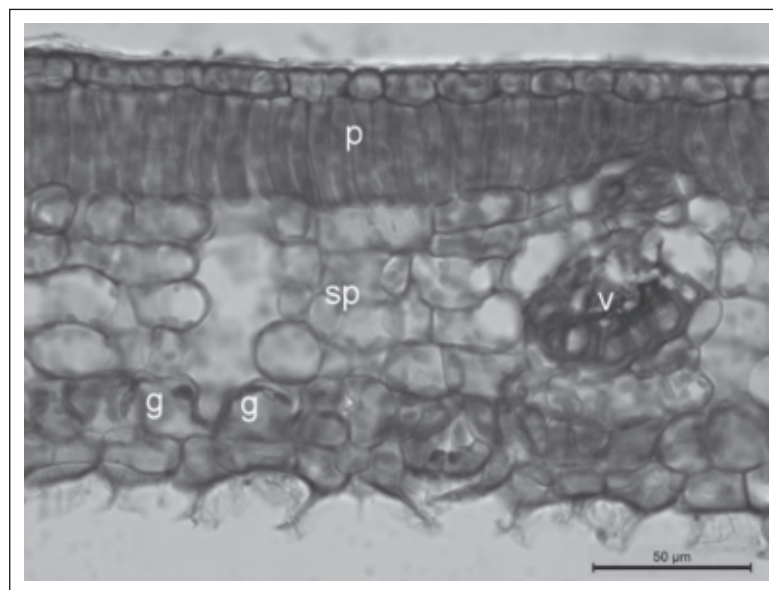


Fig. 2. Optical micrograph of *H. pauciflora* leaf lamina. Two guard cells (g) of a closed stoma leading to a cavity in the leave lamina. There is also evidence of foreign pathogens on the lower epidermis (stained red). p = palisade cells, sp = spongy cells, v = vascular bundle, g = guard cell. Magnification x 10.

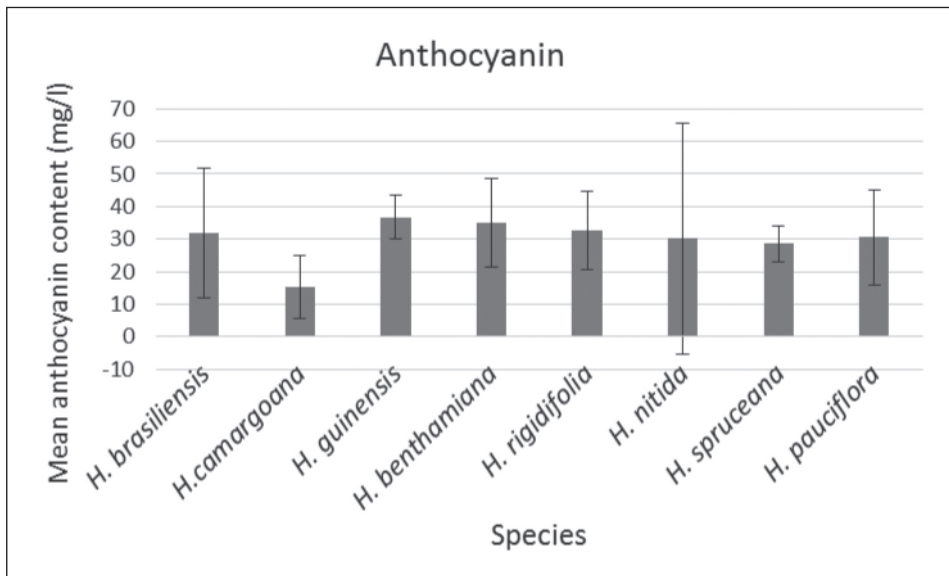


Fig. 3. Mean of anthocyanin content of eight *Hevea* species.

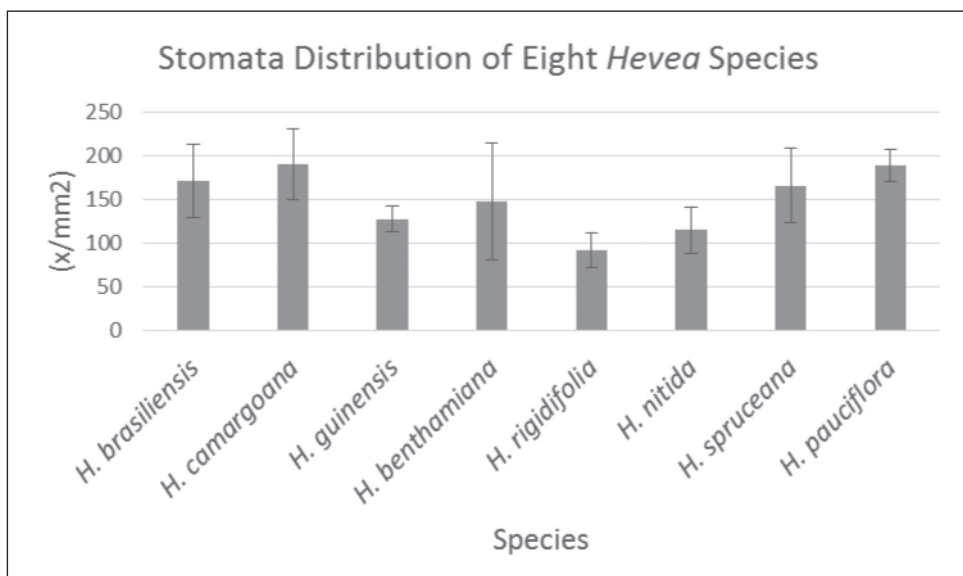


Fig. 4. Stomata distribution per unit area on leaves of eight *Hevea* species.

different ages. Reflectance index factor of the foliar has been reported to be one of the interference factors in getting the precise reflectance reading from the spectrophotometer (Sims & Gamon, 2002). Even though anthocyanins do not participate in photosynthesis directly, these pigments often help to protect the leaves against excessive sunlight that can damage some leaf tissues (Steyn *et al.*, 2002; Hughes *et al.*, 2005).

The analysis of number of stomata per unit area is shown in Figure 4. *H. camargoana* has the highest mean while *H. rigidifolia* with the lowest mean. There are two groups of means. *H. brasiliensis*, *H.*

camargoana, *H. spruceana*, *H. pauciflora* fall in the group with the higher mean whereas *H. rigidifolia*, *H. nitida* and *H. guinensis* in another group of the lower means. The distribution of stomata is different in all eight *Hevea* spp. This is similar with previous finding, where the difference in number of stomata in Amazonia tree species is influenced by the genetic background, including the tree height (Carmago & Marengo, 2011). Stomata numbers in the lower epidermis range from 92 to 190 per square millimeter for the eight *Hevea* species were investigated.

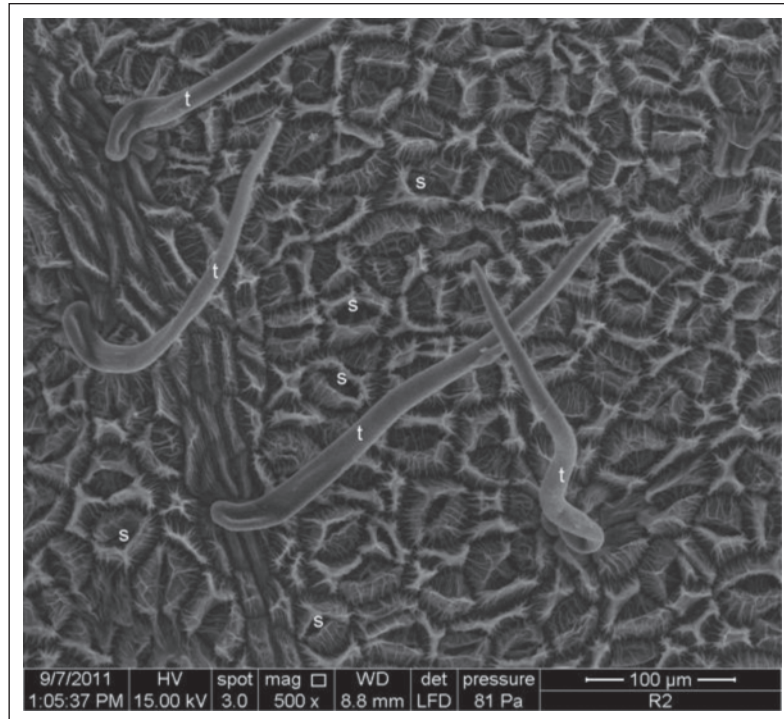


Fig. 5. Scanning electron micrographs of *H. rigidifolia* leaf abaxial. Most of the stomata seems to be closed indicate the turgor pressure of the guard cell decline (possibly because of time taken during the transport) t = trichome, s = stomata. Magnification 500x.

Scanning electron microscopy (SEM) micrographs of the *Hevea* abaxial area also revealed that in certain *Hevea* species such as *H. camargoana*, *H. pauciflora* and *H. rigidifolia* the existence of specialized appendage called trichome (Fig. 5). The role of trichome is involves in water balance on the surface of the leaf. They help increase the rate of water absorption and reduce the rate of water loss due to evaporation in order to keep the leaf surface cool. As temperature is another important factor affecting photosynthesis (Kositsup *et al.*, 2009), having trichomes on the leaves of certain *Hevea* species may assist the tree to function more effectively during photosynthesis. This extra appendage can also be considered as a unique feature in those species and can be used as species identification in *Hevea* taxonomy purposes.

The brief observations made in this report regarding the pigments content, stomata distribution and morphology of foliar across eight *Hevea* species indicate that all the variations shown in the selected parameters that contribute directly or indirectly to photosynthesis may be exploited further in breeding and selection of *Hevea*. The present study was done with the assumption that all the randomly selected *Hevea* species are in the normal and healthy condition, without any nutrition deficiencies, which would lead to sub-normal photosynthesis.

CONCLUSION

This study reported on some of the parameters that have direct or indirect effect on the leaves of eight *Hevea* species in relation to *Hevea* photosynthesis function. *H. brasiliensis* is found to have the highest mean for chlorophyll content whereas *H. rigidifolia* the lowest. Anthocyanins are highest in *H. guinensis* and lowest in *H. camargoana*. Despite having the lowest anthocyanins content among all the species, *H. camargoana* shows to have the most number of stomata per unit area and *H. rigidifolia* has the lowest. Trichomes are found on the leaves abaxial surface of *H. camargoana*, *H. pauciflora* and *H. rigidifolia*. The trichomes help in improving the photosynthesis by reducing the water loss especially in high temperature.

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