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Rubber Tree Cultivation and Improvement in Malaysia: Anatomical and Morphological Studies on *Hevea brasiliensis* and *Hevea camargoana*

Ong Chin Wei (Corresponding Author)
Rubber Research Institute of Malaysia, Sungai Buloh, Malaysia
Email: ocwmay1717@gmail.com

Shamsul Bahri Abd Razak
Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia

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Abstract

Rubber trees are among the important cultivated crops in Malaysia, and have contributed to the country's overall economic growth since the 1950s. However, the existing anatomical and morphological studies are relatively insufficient. Currently, *Hevea brasiliensis* has been cultivated and planted commonly as a commercial planting clone, while *Hevea camargoana* remains to be a non-cultivated and underutilized rubber species. For many years, there only exists little information both in private plantations and government agencies that have carried out anatomical and morphological assessments on these underutilized species. There is little information about the characteristics of *H.camargoana*, thus raising the issue among plant breeders on how to best use this underutilized rubber species. This study attempts to investigate the taxonomic values and characteristics of *Hevea brasiliensis* and *Hevea camargoana* through anatomical and morphological studies.

Keywords: *Hevea brasiliensis*; *Hevea camargoana*; Underutilized.

1. Introduction

The rubber tree originates in the rain forests of South America, and is normally distributed in the tropics near the equator. In South America, rubber trees are found in Brazil, Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname and Venezuela [1-3]. There are 10 species in the genus *Hevea* [2], and most of them can survive in a range of environments including wetlands, low altitude moist forests, elevations above sea level, riparian zone, river banks and well-drained areas. The common features for rubber trees are their obvious trifoliate leaves, separated female and male flowers formed at the same inflorescence, and a trilocular capsule fruit pod that holds three seeds which contain latex in almost every portion of the tree. They begin to flower five years after planting, but they are seldom open together, which encourages cross pollination [4]. Latex produced from rubber trees has many qualities that make it an important raw material for numerous products. Latex is harvested by cutting the outer layers of the bark structure of a rubber tree, where a group of specific cells called laticifers or latex vessels are located on the inner bark [5-8]. The bark structure is formed from several distinct soft and hard layers. Soft bark is the thin layer of cells that produces rows of cells as concentric cylinders of parenchyma tissues and tube cells that eventually form the laticifers. *H. brasiliensis* has an average height at 25-30 meters, but some trees can grow higher than 30 meters [2, 9]. Most of them have a high canopy and typical conical shape crown, a trunk with a cylindrical shape, and a normally swollen base. The bark thickness is variable within this species, and the bark surface colour ranges from pale brown to brown, and from reddish purple to dark purple. Sometimes, immature rubber trees even have brown-green mixed colour bark. Seeds are variable in size, but frequently have grey-brow or dark brown mottling [1]. Moreover, this species has thick and green fruit capsules, which are composed of three lobes with seeds [10]. *H. camargoana* is described as a small tree with a relatively small trunk that survives in low scrub forests and woodland swamps, and has a height that ranges between 2-12 meters [2, 9]. Seeds are small in size, no longer than 1.5 cm, with irregular brown mottling. Despite the frequently planted *Hevea brasiliensis*, other rubber species remain underutilized and neglected for breeding and improvement programmes. One of the underutilized rubber species, *Hevea camargoana*, could be assessed for its biological potential and its possibility to be incorporated into breeding and improvement programmes in the near future.

2. Material and Method

2.1. Leaves

Fresh leaves were collected from mature *Hevea* trees found in Selangor, Malaysia. Healthy and mature leaves were collected from randomly selected branches of the lower canopy of trees, about five metres above ground. Ten leaves from five different trees of each species were sampled for this study. These leaves were formed for at least eight weeks after the first season of defoliation in the beginning of a year. In this study, only healthy leaves without an unusual shape nor any deformity by disease or infection were collected as the standard reference for comparison between the species.

2.2. Bark Surface

The smoothness of the bark surface was observed visually at 1.5 m height above ground, from five different trees of each species. The bark surface was in its original condition, without disturbance from harvesting practices that would injure the bark surface. Representative images were then captured using a digital camera.

2.3. Seeds

Seeds from five different trees of *H. brasiliensis* and *H. camargoana* were collected in bags made of netting which were tied around the fruit capsules on trees. This procedure would ensure that the seeds were collected properly, where fruit dehisced were fresh and free of pest infestation. Furthermore, the netting bags were necessary to save the seeds from being consumed by rodents and other wild animals. Next, they were cleaned with distilled water to remove any dirt from their surface, prior to characterization by visual observations using a digital camera.

2.4. Leaf Venation and Petiole

Ten healthy mature leaves were examined from both *H. brasiliensis* and *H. camargoana*. Leaves which were infected by disease were not examined, due to the possibility of distorted venation caused by infection. The pattern of leaf venation between species was examined with light microscopy study under an Olympus BH2 Light Microscope. The same leaves examined for leaf venation for their petiole and vascular bundle, were examined under a light microscope using a free-hand sectioning technique. In this sectioning technique, these leaf samples were softened with distilled water before cutting them with a 10 µm thickness razor blade, focusing at their midrib. These leaf samples were collected from three individual trees per species, where the best sample for each species was further cut into cross sections. Later, sections of samples were stained with Sudan III, and then mounted on glass slides. At the end of the sectioning, these leaf samples with petiole and vascular bundles were placed under an Olympus BH2 Light Microscope.

2.5. Stomata

Ten fully-developed leaves from both *H. brasiliensis* and *H. camargoana* were collected and preserved in a laboratory freezer, prior to Scanning Electron Microscope investigation. Three leaves from five different trees of each species were sampled. These leaves were cut into 4 cm x 4 cm pieces that were positioned onto a copper stub smeared with carbon paste for adhesion. After that, the stomata on these leaf samples were examined under a low-pressure column in the FEI's Scanning Electron Microscope Quanta 180 at 10 kV.

2.6. Laticifers

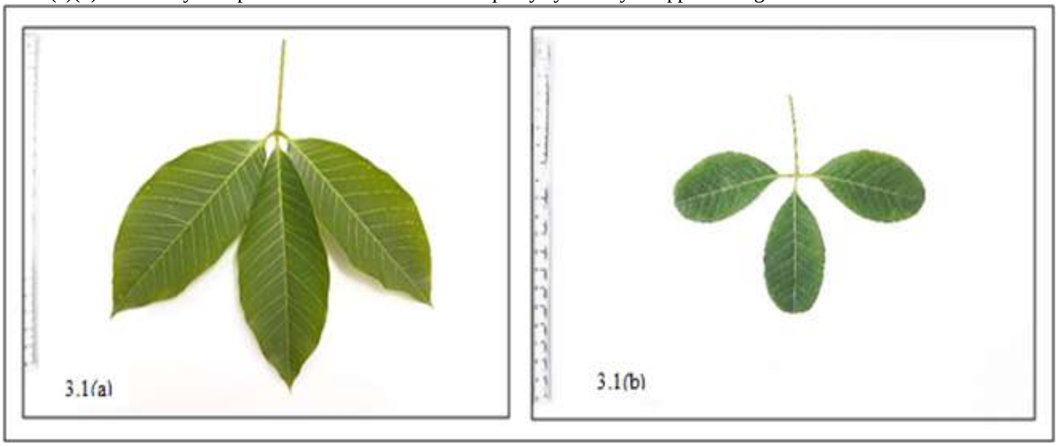
Bark samples from *H. brasiliensis* and *H. camargoana* were collected from the tree trunk at a 1.5 m height above ground using a bark borer that penetrated at least 6 mm into the bark. The samples were then soaked in 10% chromic acid and 10% nitric acid for bleaching purposes, before being transferred to an oven where they were incubated at 60°C for 24 hours. Small glass beads were added into a bottle containing the bark samples, and the bark-bead-solution mixture was gently shaken using a maceration technique until the fibres separated after becoming loose. The mixture was then rinsed three times with 50% ethanol. Next, Sudan III was added, and the mixture was left to stand for three minutes. The solution was re-rinsed with 50% ethanol, until it became clear and free of stain. Sterilized forceps were used to pick up the stained, loose latex vessels, which were then mounted onto a glass slide sealed with glycerine jelly. The slides were examined under an Olympus BH2 light microscope.

3. Results

3.1. Leaves

Both *H. brasilie*nsis and *H. camargoana* ensembled a compound leaf structure, where each leaf blade itself was actually divided into three different leaflets, and of which has their own stalks or petioles. Also, the leaflets were attached and extended outwards altogether, at the end of the same petiole, which is an important characteristic to distinguish from the pinnately-compound leaves derived from the leaf midrib or rachis (*i.e.* continuing of the petiole). These leaflets often have short petiolules, the leaf structure that extends from the petiole, and were occasionally folded back and seemed like they were in a drooping position in the mature stage. The leaf type observed in this study was more accurate to be coined as "palmately-compound trifoliate leaves with equally symmetry at opposite angles" for both species, as shown in Picture 3.1(a) and 3.1(b).

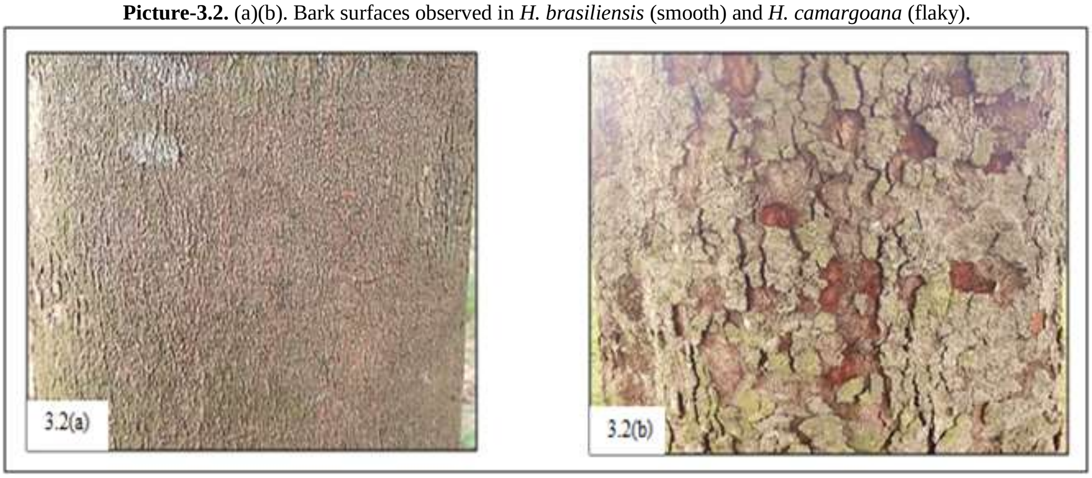
Picture-3.1. (a)(b). Palmately-compound trifoliate leaves with equally symmetry at opposite angles in *H. brasiliensis* and *H. camargoana*.



3.2. Bark Surface

Smooth bark surface was observed in *H. brasiliensis*, whilst flaky bark surface was noticed in *H. camargoana* in Picture 3.2(a) and 3.2(b). Normally, the bark surface in many tree crops is an important protective layer that is initiated from the inner part of the stem cambium. In rubber trees, laticifers in the bark layer are relatively important for latex harvesting. Therefore, the smooth bark surface is preferable to be explored as a commercial planting material. In addition, limited information exists on bark smoothness through the focus of anatomical and morphological studies on rubber species. The results obtained from this study could thus fill the gap and provide important information for future rubber improvement programmes.

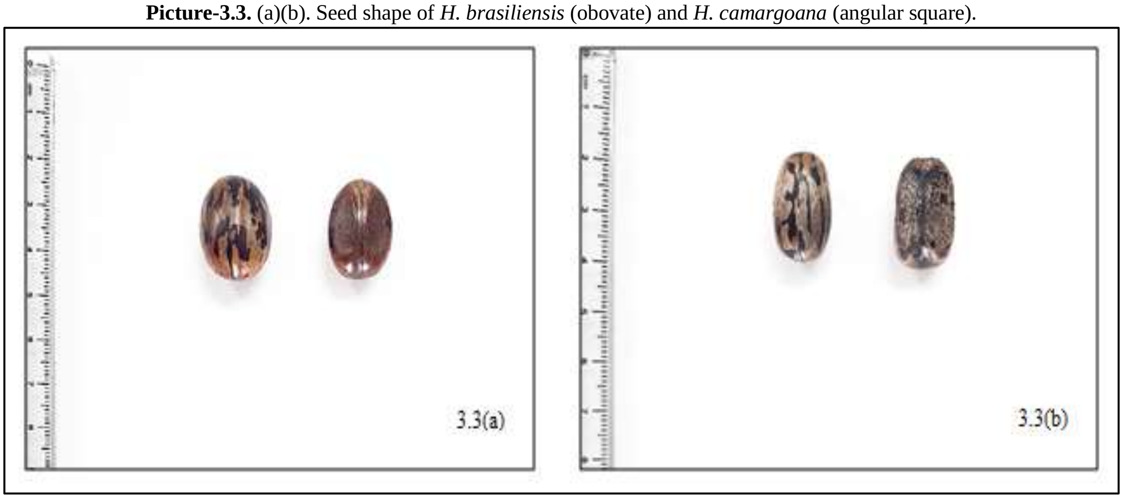
Picture-3.2. (a)(b). Bark surfaces observed in *H. brasiliensis* (smooth) and *H. camargoana* (flaky).



3.3 Seeds

H.brasiliensis resembled a rounded and slightly obovate shape, and the ventral was slightly compressed, while dorsal was slightly rounded with a glossy brown colour seed coat, rich with large brown mottling. Meanwhile, *H. camargoana* showed an obvious angular and recognizable square shape, and the ventral was regularly flat with a rounded dorsal, light-grey seed coat. It was covered with black mottling, as showed in Picture 3.3(a) and 3.3(b).

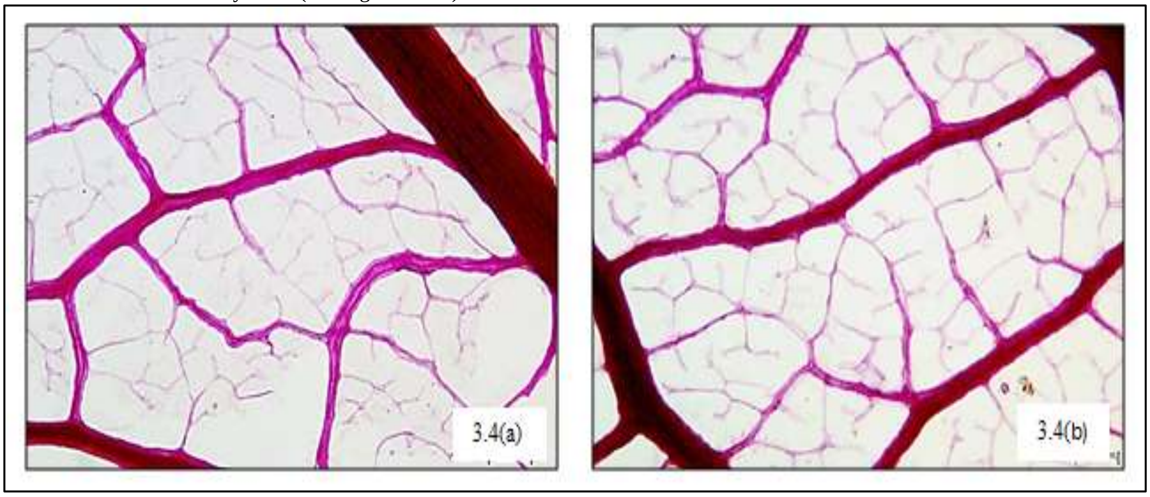
Picture-3.3. (a)(b). Seed shape of *H. brasiliensis* (obovate) and *H. camargoana* (angular square).



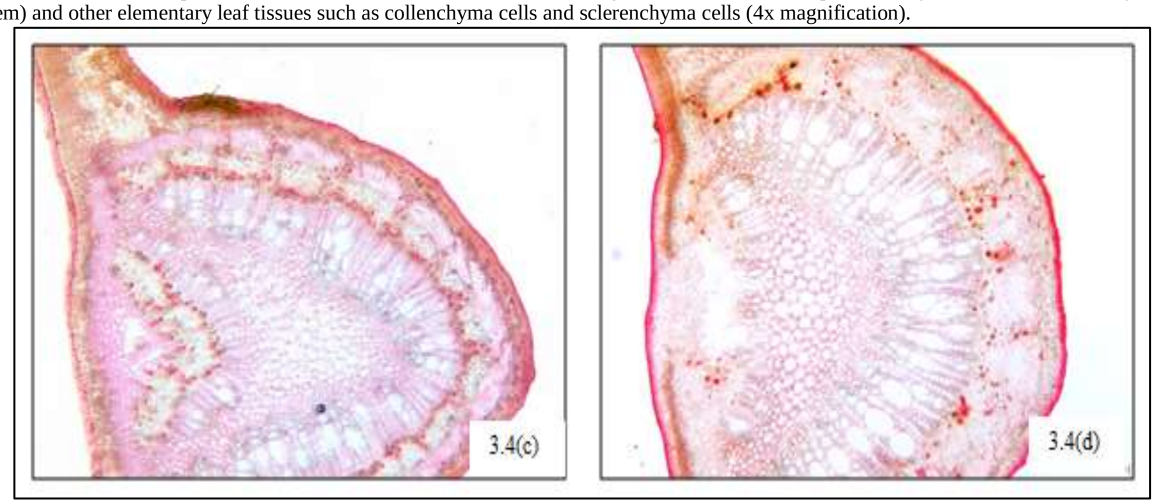
3.4. Leaf Venation and Petiole

Both *H. brasiliensis* and *H. camargoana* with leaves that appeared identical only with primary and secondary veins with visual observation, but light microscope observation further revealed there were smaller tertiary veins extended from the secondary veins, and veinlets extended from the tertiary veins. Many noticeable tertiary veins were branched from the secondary veins, while many veinlets were branched from the tertiary veins, where these veinlets were not able to be detected by visual observation, as shown in Picture 3.4(a) and 3.4(b). The middle vein divides the leaf region into two sections of fairly bilateral symmetry, but the secondary and tertiary veins were not formed as mirror images. The veinlets often terminate in between the secondary and tertiary veins, creating the areole in between veins, which is the area without veins on the leaf surface. Meanwhile, there were no obvious laticifers detected in the leaf petiole in all leaf samples, if compared to the bark structure. All samples showed of petiole, where their leaves consisted of a distinct epidermis layer, vascular tissues (xylem and phloem) and other elementary leaf tissues such as collenchyma cells and sclerenchyma cells, as expected in a dicotyledonous plant in Picture 3.4(c) and 3.4(d). Also, all the samples formed the typically supporting tissues that consist of collenchyma cells, sclerenchyma cells and vascular tissues that could be observed in the majority of perennial crops.

Picture-3.4. (a)(b). Leaf venation pattern showed in *H. brasiliensis* and *H. camargoana* tertiary veins extended from the secondary veins, and veinlets extended from the tertiary veins (4x magnification)



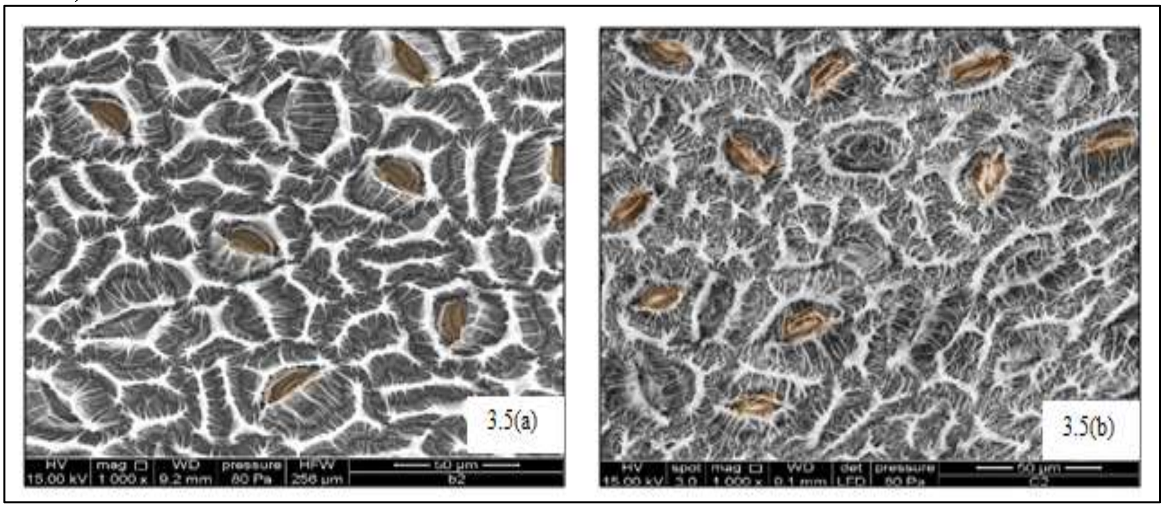
Picture-3.4. (c)(d). Leaf petiole showed in *H. brasiliensis* and *H. camargoana* with a distinct epidermis layer, vascular tissues (xylem and phloem) and other elementary leaf tissues such as collenchyma cells and sclerenchyma cells (4x magnification).



3.5. Stomata

The existence of stomata was hardly revealed at the upper side of the leaf (adaxial), but they were detected in abundance at the underside of the leaves (abaxial). Evidently, the guard cells in both *H. brasiliensis* and *H. camargoana* in this research were found to be kidney-shaped during the preservation procedure, with fixation reagents in SEM. This research also concludes that rubber species would exhibit the same stomata arrangement as the paracytic type, where each guard cell is accompanied by one or multiple subsidiary cells at their longitudinal axes that are parallel to the guard cells in Picture 3.5(a) and 3.5(b). The elongated and kidney-shaped stomata that are normally found on monocotyledon plants did not appear on any of the leaf samples in both rubber species. Similarly, it is interesting to discover that several samples of *H. camargoana* showed specialized hair-like appendages commonly known as trichomes on the underside of the leaf surface when observed under SEM examination. Nevertheless, this characteristic of having trichomes was not detected in all *H. brasiliensis* samples collected. To date, there exists no report about this characteristic in rubber trees or rubber species, especially in the context of Malaysia.

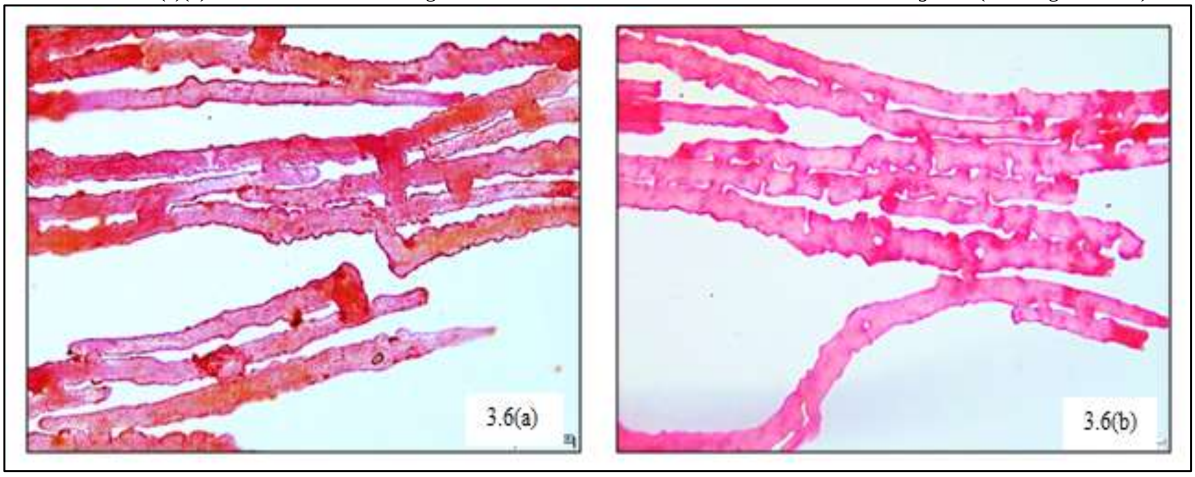
Picture-3.5. (a)(b). Stomata arranged as the paracytic types (highlighted) were observed in *H. brasiliensis* and *H. camargoana* (1000x magnification)



3.6. Laticifers

The result show that the maceration technique of laticifers in both *H. brasiliensis* and *H. camargaona* would resemble a very complex articulated and anastomosing laticifer type. The complexity of these anastomosing laticifers was due to the fact that enzymatic activities take place in the formation of new laticifers by the constant addition of new primordial cells to the existing ones, and not by the growth of individual cells. At the end of the formation processes, the laticifers could be seen as connected as a whole. The anastomosing laticifers were revealed in the longitudinal section by means of tissue maceration. The examination of longitunal section of bark and articulated anastomosing laticifers with light microscope in *H. brasiliensis* and *H. camargoana* is presented in Picture 3.6(a) and 3.6 (b).

Picture-3.6. (a)(b). Articulated anastomosing laticifers were observed in *H. brasiliensis* and *H. camargoana* (10x magnification)



4. Discussion

There was a negative correlation between the age of the tree and leaf length, as mentioned by Chandrasekhar [11]. In the present study, however, no marked phase of acceleration in leaf elongation was observed in mature trees of *Hevea* species between the time of leaf emergence and its full expansion. Nevertheless, leaf shape is a very useful morphological characteristic for species identification and authentication, since there exists no standard reference for leaf shape or leaf expansion during the different stages of development of a rubber tree. In different circumstances**,** the development of the embryo sac and seed coat is under the influence of the female gametophyte as a maternal effect, as the shape of seed and coat colour, shape (ventral and dorsal) and coat appearance (colour and mottling) of the different *Hevea* species are inherited entirely from the female parent. The colour or shape of the seed hardly provide useful information on the male parent for the purpose of improvement. However, colour and shape of seeds are distinctive between *H. brasiliensis* and *H. camargoana*, and this character is useful as one of the taxonomic values. In general, uneven bark surface could cause exuded latex to be drawn away from the collection cup during harvesting. Also, there is an indirect relationship between latex yield and surface smoothness of the bark. Whenever a tapping knife cannot cut cleanly into an uneven bark surface and a deeper cut was forced, this would easily injure the cambium tissue and cause unsatisfactory bark regeneration. The general venation architecture of these *Hevea* species was the same for both *H. brasiliensis* and *H. camargoana*, where they had the obvious primary, secondary, tertiary veins and even veinlets that formed a reticulate pattern, which is a typical venation formation for most dicotyledonous species. When observations were made on their petioles, *H. brasiliensis* and *H. camargoana* demonstrated similar morphology in the epidermis layer, palisade mesophyll, spongy mesophyll, xylem, phloem, collenchyma, and sclerenchyma tissues. This study correspondingly supports the findings of Senanayake and Samaranayake [12], Gomez and Samsidar [13] and Gomez [14], who conclude that rubber leaves often show a high number of stomata on the underside (abaxial) leaf surface, but the number of stomata on the upper side (adaxial) leaf surface remains low. In the meantime**,** the articulated and anastomosing laticifers revealed in all samples showed that limitless laticifer cells were fused together, forming an elongated intrusive branching when viewed under a light microscope; this affirmed the findings by Blaser [15], Vertrees and Mahlberg [16] and Shamsul Bahri Abd Razak [17].

5. Conclusion

One common problem facing by many natural rubber producing countries is the inbreeding depression of rubber trees, due to the cross-breed with closely-related parent trees. Inbreeding depression and the narrow genetic base in rubber trees not only affects the direction of breeding programmes for yield improvement, but also the availability of gene resources to incorporate any useful morphological and biochemical characteristics. To overcome these problems, effort must be made to expand the genetic base by way of an organized infusion of underutilized *Hevea* species such as *H. camargoana*. Once a new pool of genetic resources is assembled, these materials can then be utilized for wide crossing. For *Hevea*, novel materials should be utilized in breeding for the improvement of yield and other important characteristics, which should be undertaken cautiously without upsetting the conservation of the existing pool of available genetic resources. Therefore, broadening the *Hevea* genetic base is an important strategy in future breeding programmes, in order to produce improved planting materials and in turn befit rubber growers for many decades to come.

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