

Stomatal density of sago palm (*Metroxylon sagu Rottb.*) with special reference to positional differences in leaflets and leaves, and change by palm age

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Abstract This study was undertaken to investigate the stomatal density in leaflets of sago palm, a tropical starch crop, to gain some fundamental knowledge related to photosynthesis of the leaves. 1) To investigate the stomatal density at different parts on leaflets attached to lower, middle and upper positions in one leaf, leaves on the lower, middle and upper parts of plants were sampled from the palms at trunk formation stage (about 5-years old) in a farmer's sago garden in Tebingtinggi Island in Riau, Indonesia. The tip part of a leaflet had lower stomatal density than basal or middle part, and abaxial surface had higher stomatal density (550~750/mm²) than adaxial surface (100~250/mm²). Stomatal density of both sides on a leaflet showed a tendency to decrease from base to tip part with the decrease in thickness. Little difference was observed in the stomatal densities among leaves and leaflets at different positions in a plant and in one leaf, respectively. 2) To investigate the change of stomatal density by palm age, leaflets on the middle position in leaves of middle part of 1- to 5-years old palms were sampled in a farmer's sago garden in Muka, Sarawak, Malaysia. Stomatal density of abaxial surface markedly increased from 1- to 3- years old (400~900/mm²), however, it gradually increased thereafter and attained almost the definite value (1000/mm²) at 5-years old, trunk formation stage. In adaxial surface, the stomatal density also increased with aging of palms, but the density (50~120/mm²) is significantly smaller than that of abaxial surface (400~1000/mm²), irrespective of palm age.

Key words: leaf position, leaflet, palm age, sago palm, stomatal density, stomatal length

サゴヤシ葉身の気孔密度について

——小葉部位, 小葉着生位置, 葉位並びに樹齢による差異——

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要約 東南アジア原産のデンプン資源作物であるサゴヤシについて, その葉の気孔密度を葉位別, 小葉位別および小葉の部位別に調べ, さらに気孔密度が樹齢によってどのように変化するかについて検討した。

実験 I (小葉の着生位置別および部位別気孔密度): インドネシア, リアウ州トゥピンティング島の農家サゴヤシ園に生育する幹立ち直後の個体について, 小葉を葉位(上位, 中位, 下位)別, 小葉位(上位, 中位, 下位)別に採取し, 各

小葉の先端部、中央部、基部の気孔密度および葉厚を測定した。気孔密度は小葉の表側で 100~250 個/mm²、裏側で 550~750 個/mm² と裏側で顕著に高かった。小葉内の部位別気孔密度をみると、表側では基部 > 中央部 > 先端部の順に高く、裏側では中央部、基部に比べて先端部で低かった。小葉の同一部位の気孔密度には、小葉位間および葉位間で差が認められなかった。また、小葉上の気孔密度と葉厚は表側、裏側ともに有意な正の相関を示し、葉厚の薄かった小葉の先端部ほど気孔密度が低かった。

実験 II (樹齢による気孔密度の変化): マレーシア、サラワク州ムカの農家サゴヤシ園において、サッカー発生後 1 年目から幹立ち期までの 1 年ずつ樹齢の異なる個体から、主幹の中位葉の中位小葉を採取し、小葉中央部について気孔密度および気孔長を測定した。幹立ち期までの樹齢別気孔密度は裏側ではサッカー発生後 1~3 年目にかけて顕著に増加 (400~900 個/mm²) したが、その後は漸増して 5 年目 (幹立ち期) にはほぼ一定 (約 1000 個/mm²) になった。表側では幹立ち期まで増加し続けた (約 50~120 個/mm²)。気孔長は表側で 12~14 μm、裏側で 9~13 μm であり、気孔密度の高い裏側の方が小さく、また樹齢に伴い減少する傾向を示したために、気孔密度と気孔長は裏側では高い負の相関関係 ($r = -0.970^{**}$) を示した。以上の結果より、サゴヤシの葉の気孔密度は、1) 表側に比べて裏側で顕著に高いこと、2) 葉位間、小葉位間に差はなく、小葉内では先端部で低いこと、3) 幹立ち期までは増加するが、その後の変化は小さいこと、が明らかになった。

キーワード 気孔長、気孔密度、サゴヤシ、樹齢、小葉、葉位

Introduction

Metroxylon species are indigenous to the lowlands of Southeast Asia and Melanesia, located between 10°N and 10°S, up to an altitude of 700 m (Flach, M. 1977). The palms accumulate the starch in their trunk as a reserver. In Southeast Asia and Melanesia, sago palm had been used traditionally, and still now it is one of the staple foods in some areas in these districts. Sago palm is the only crop that can grow in deep peat soil area where the pH is extremely low, groundwater level is high in most of year round and mineral content is very low. According to Fukui (1984) and Kyuma (1992), the peat soil area in Southeast Asia is estimated about 20 to 30 million ha. In addition, sago palm can maintain the sustainable production without intensive management once it became ready to harvest, because the plant can propagate vegetatively by suckers emerged from the base of mother palm. By these benefits, the huge exploitation of sago palm plantation has started in peat soil areas in Indonesia and Malaysia in recent years.

Flach (1977) reported that the starch production of sago palm is remarkably higher than the world average of other starch crops such as root and tuber crops. Starch production of sago palm is based on the leaf photosynthesis, therefore studies on the photosynthesis is very fundamental and important, although very few reports on the photosynthesis in

sago palm are available. Flach (1977) and Uchida et al. (1990) reported that leaf photosynthetic rate of young sago palms before trunk formation stage is 9~13 mg CO₂ dm⁻² h⁻¹ and 13~15 mg CO₂ dm⁻² h⁻¹, respectively. Moreover, Uchida et al. (1990) reported the photosynthesis of young sago palm seemed to be mainly regulated by stomata with a low transpiration rate due to the low stomatal density. For the stomatal density of sago palm leaf, Yamamoto et al. (1994) reported that it varied by varieties, palm-ages and leaf positions on plant, etc., but there were only few detailed studies about stomatal density of sago palm leaf. This study was undertaken to investigate the stomatal density in sago palm leaf in Indonesia and Malaysia.

Materials and Methods

1. Stomatal density on leaflets attached to different positions in one leaf

This study was carried out in a farmer's sago palm garden on shallow peat soil in Tebingtinggi Island in Riau, Indonesia in 1998. Upper, middle and lower leaves were collected from the three palms at trunk formation stage estimated about 5-years old after sucker emergence. These palms have about 30 to 50 cm trunk length with 40 to 50 cm diameter. Numbers of adaxial and abaxial stomata were observed at tip, middle and basal part of the leaflets attached to the upper, middle and lower positions in each col-

lected leaf. The length and the maximum width of each leaflet were measured by a ruler and the thickness of all the position where the number of stomata was observed was also measured by a vernier caliper (Digimatic, Mitutoyo Co., Japan). To take the replica of stomata, an adhesive synthetic paste (Cemedine C in commercial name) was spread thinly on the both surface of leaflet by a finger. After drying for 2 to 3 minutes, separate it from the leaflet by using the adhesive tape, and put the adhesive tape on the slide glass. Numbers of stomata were counted at 3 microscopic fields randomly chosen from each slide (Fig. 1). Each field consisted of 0.75 mm^2 . Stomatal lengths were determined by measuring guard cell length of about 20 stomata per replica with video micrometer (VM-30, Olympus Co., Japan).

2. Change of stomatal density by palm age

The change of the stomatal density in sago palm

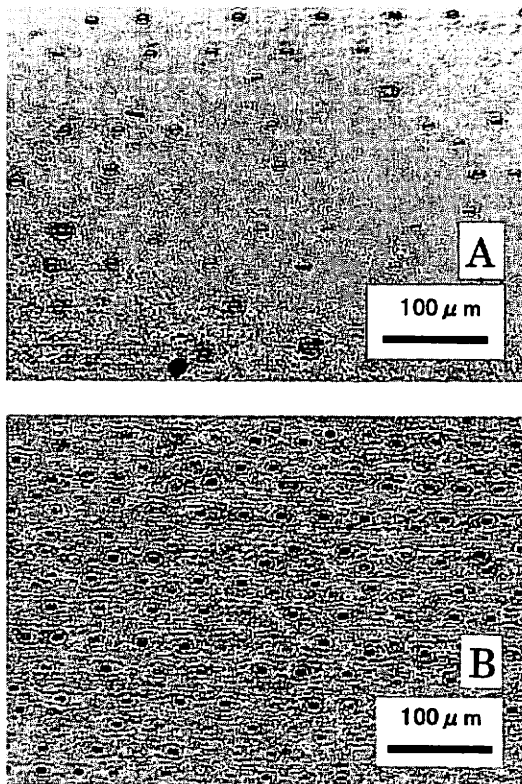


Fig. 1 Photomicrographs of adaxial surface (A) and the abaxial surface (B) of sago palm leaflet at trunk formation stage ($\times 130$).

leaves by plant age was investigated in a farmer's sago garden on mineral soil at Muka, in Sarawak, Malaysia in 1998. For this investigation, 1, 2, 3, 4 and 5 years old sago palm emerged directly from their mother palms were chosen by owner of the garden. The middle leaflet of the middle leaf in each palm was collected. The length, maximum width and thickness of the each leaflet, and stomatal densities on both abaxial and adaxial surfaces were measured and observed following the same procedures and methods described above.

Result and Discussion

1. Stomatal density on leaflets attached to different positions in a leaf

The length and maximum width of leaflets were shown in Fig. 2. Both of the length and the maximum width were longer and wider in order of middle > lower > upper leaflets, irrespective of the leaf positions. Significant difference in both length and width of the leaflets was not observed among leaves attached to the same position in a leaf. Stomatal density ranged from 550 to 750/mm² on abaxial surface and from 100 to 250/mm² on adaxial surface of leaflets, and little difference was observed among leaflets and leaf positions, although the densities at tip, middle and basal part in a leaflet varied significantly (Fig. 3). The stomatal density on adaxial surface increased from tip to basal part, while on abaxial surface, tip part showed lower stomatal density than middle and basal parts. Abaxial surface showed approximately 4 to 6 times higher stomatal density than adaxial surface. The stomatal density and leaf thickness showed a significant positive correlation in both surfaces (Fig. 4). This result implies that stomatal density on a leaflet was decreased from base to tip part with decrease of leaf thickness, and this tendency was more clearly observed on adaxial surface than on abaxial surface.

The results mentioned above indicate that little difference was observed in the stomatal densities among leaves and leaflets at different positions in a plant and in one leaf, respectively, although the tip part in a leaflet had lower stomatal density than middle or basal part, and abaxial surface had higher sto-

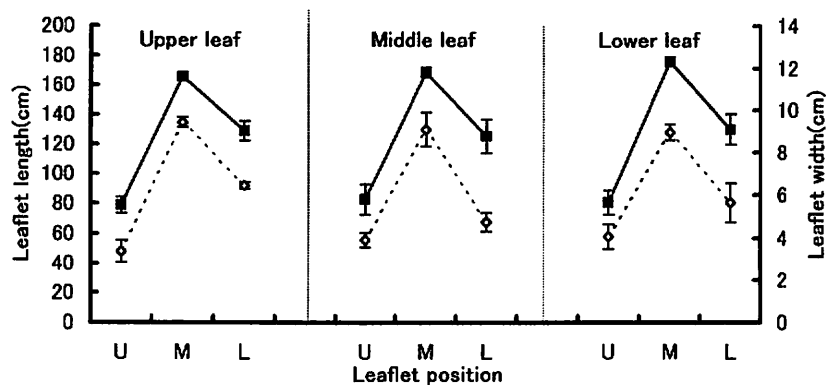


Fig. 2 Length and maximum width of leaflets attached to the upper, middle, and lower leaves of sago palm at trunk formation stage in Riau. Values are means of 3 replications. Standard errors ($n = 3$) are indicated with vertical bars on their symbols. ■ and ◇ indicate leaflet length and maximum width of leaflets, respectively. U, M and L indicate the leaflets attached to upper, middle, and lower positions in a leaf.

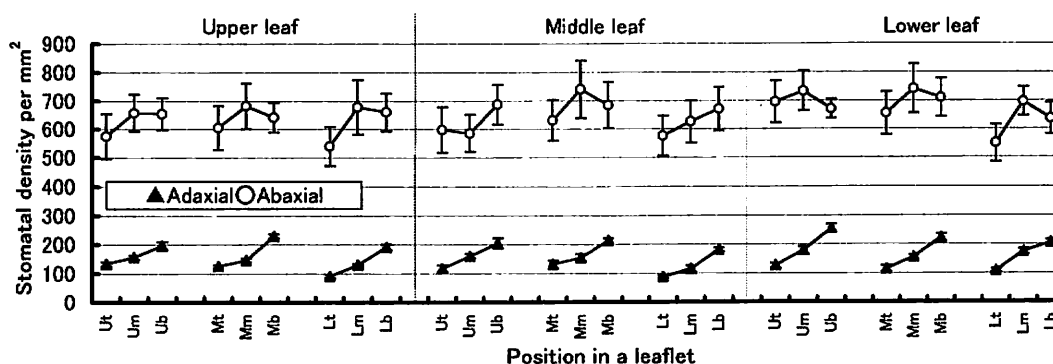


Fig. 3 Stomatal density on leaflets in the leaves attached to the upper, middle and lower positions at trunk formation stage in Riau. U, M and L indicate the leaflets at upper, middle and lower positions in each leaf, respectively. And t, m and b indicate the tip, middle and basal parts in a leaflet, respectively. Values are means of 3 replications. Standard errors ($n = 3$) are indicated with vertical bars on the symbols.

matal density than adaxial surface. These results coincide with the report of Yamamoto et al. (1994). According to the reports on the difference of stomatal density by leaf position, in monocotyledonous plants such as rice, wheat, barley and maize, etc., the stomatal densities of upper leaf and on abaxial surface in each leaf had higher stomatal densities than those of lower leaf and on adaxial surface, respectively (Hoshikawa 1971), however, in sago palm little differences of stomatal densities in leaf positions were observed. This result may be associated with the small

difference of leaf and leaflet sizes at the same position after trunk formation stage in sago palm (Osozawa 1990).

In one leaflet as we showed above, the stomatal density in the tip part was lower than that in the middle or basal part on both surfaces, while we did not find out significant difference of it in each part of leaflets within one compound leaf. It is reported that in the leaf of blue panic grass (*Panicum antidotale* Ratz.) (Dobrenz et al. 1969), grain sorghum (*Sorghum bicolor* L.) (Liang et al. 1975), and smooth bro-

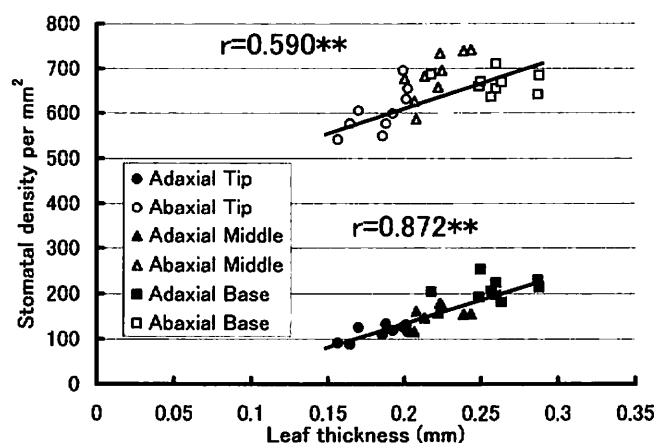


Fig. 4 Relationship between leaf thickness and stomatal density. The data are the same with those in Fig. 3. Different leaf and leaflet positions are included in same symbol. Each symbol indicates the average of 3 replications. **: $p < 0.01$.

megrass (*Bromus inermis* Leyss.) (Tan and Dunn 1975), the stomatal density in the tip part is lower than that in the basal part. However, in the leaf of rice, the stomatal density in the middle part is higher than those of other parts (Yoshida 1978). From the dorsiventral point of view, the stomatal distribution pattern of sago palm leaflets corresponded with the pattern of blue panic grass, grain sorgam or smooth brome grass for adaxial surface and with rice for abaxial surface.

2. Change of stomatal density by palm age

Some growth characters of young palms, differing in age from 1- to 5-years old were shown in Table 1. All characters increased in number with increasing the palm age after emerging. The plant height was

around 8 m in 5-years old palms. Stomatal density on abaxial surface was significantly higher than that on adaxial surface, irrespective of palm ages, and increased from 400/mm² in 1-year old palm to 900/mm² in 3-years old palm (Fig. 5). Stomatal density gradually increased thereafter and attained almost the definite value (1000/mm²) at trunk formation stage, i.e. 5-years old after sucker emerged. In adaxial surface, the stomatal density also increased with aging of palms as 122/mm² at the trunk formation stage, but it is significantly lower than that of abaxial surface. The stomatal density of 5-years old palms was higher on the abaxial surface, but lower on adaxial surface than those of sago palms at about same age growing in experiment 1, in Tebintinggi, Riau, In-

Table 1 Some growth characters of young sago palms at different ages in Mukah, Sarawak, Malaysia.

Year after sucker emergence	Palm height (m)	No. of leaves	Middle leaf length (m)	Leaflet No. per leaf*	Leaflet length (cm)	Leaflet max. width (cm)	Leaflet thickness (mm)**
1	2.1 ± 0.2	4.0 ± 1.2	1.9 ± 0.1	12.3 ± 0.88	41.7 ± 2.33	3.33 ± 0.20	0.123 ± 0.0040
2	3.6 ± 0.6	5.3 ± 0.9	2.9 ± 0.6	20.7 ± 3.38	57.0 ± 7.37	4.53 ± 0.48	0.155 ± 0.0040
3	5.5 ± 0.3	4.6 ± 0.9	5.3 ± 0.5	37.3 ± 1.45	87.6 ± 1.70	6.10 ± 0.26	0.170 ± 0.0038
4	6.0 ± 0.5	7.0 ± 0.6	5.7 ± 0.1	47.0 ± 1.52	96.2 ± 4.38	6.33 ± 0.52	0.180 ± 0.0029
5	7.8 ± 1.2	7.3 ± 0.3	7.8 ± 0.9	51.0 ± 2.00	123.1 ± 3.18	7.27 ± 0.29	0.194 ± 0.0029

Values are means of 3 replications ± SE. Leaflet characters (length, maximum width and thickness) were measured for the middle leaflets of each middle leaf. *Shown by the number of leaflet pairs. **Mean values measured at tip, middle, and basal parts of the leaflet.

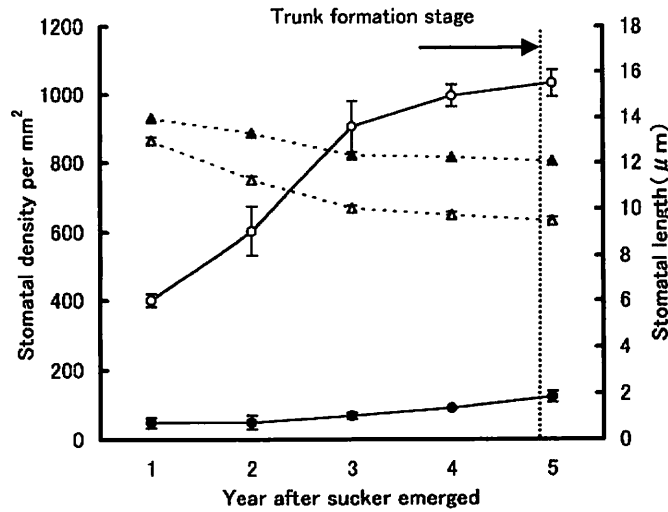


Fig. 5 Difference of stomatal density in young sago palms growing in Muka. Stomatal densities and length were measured at middle portion of middle leaflet attached to middle leaf in each palm. Values are means of 3 replications. Standard errors ($n = 3$) are indicated with vertical bars on the symbols. ● and ○ indicate stomatal densities of adaxial and abaxial surfaced, respectively. ▲ and △ indicate stomatal length of adaxial and abaxial surfaces, respectively.

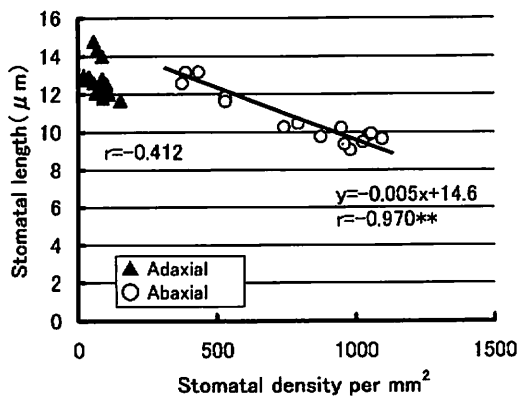


Fig. 6 Relationship between stomatal density and stomatal length. The data are the same with those in Fig. 5, but all the replication values are shown in this figure. **: $p < 0.01$.

donesia. These differences may be associated with the difference of soil types, varieties and meteorological conditions, etc. Factors associated with the change of stomatal density of sago palm should be met further investigation.

As for the length of stomata, adaxial surface showed longer ($12 \sim 15 \mu\text{m}$) than those on abaxial surface ($9 \sim 14 \mu\text{m}$) through the all palm ages. The stomatal length of both surfaces decreased by plant age from 1- to 3-years old, but was not so changed thereafter. The stomatal length of sago palm leaves are shorter compared with those of rice ($23 \sim 26 \mu\text{m}$), maize ($48 \sim 56 \mu\text{m}$), and barley ($23 \sim 35 \mu\text{m}$) leaves (Jodo 1986). Significant negative relationship between stomatal density and stomatal length was observed on abaxial surface ($r = -0.970^{**}$), but it was not observed on adaxial surface ($r = -0.412$) (Fig. 6). The total stomatal length per mm^2 including both surface i.e., the sum of stomatal density \times stomatal length in both surface, was $10309 \sim 12334 \mu\text{m}/\text{mm}^2$ at trunk formation stage. These values are higher than those of maize ($6000 \mu\text{m}/\text{mm}^2$), barley ($4000 \sim 5000 \mu\text{m}/\text{mm}^2$), and almost same with rice ($12000 \sim 14000 \mu\text{m}/\text{mm}^2$) (Jodo, 1986).

According to Hoshikawa (1971), the stomatal density of plants already investigated, including crops such as rice, wheat, maize, etc., ranged from 50 to $300/\text{mm}^2$ and there is a tendency of higher density

on abaxial surface than that on adaxial surface. Compared with those cases investigated, the stomatal density of sago palm on the abaxial surface was remarkably higher, and the stomatal densities of leaflet of sago palm at different growth stage from emergence of sucker to trunk formation stage changed much from 400 to 1000/mm² on abaxial surface, while the density on the adaxial surface showed rather unchanged values, ranging from 50 to 120/mm². The variation of stomatal density in the abaxial surface was more noticeable in young sago palm up to 3-years old, while its variation in the adaxial surface was larger in young sago palms of 2- to 5-years old. These results well agree with previous report of the variation of stomatal density in 1- to 2-years old suckers (Yamamoto et al. 1994).

Uchida et al. (1990) reported that the stomatal density in the leaflet of the 4-years old sucker of spiny type sago palm was 94/mm² on adaxial and 159/mm² on abaxial surface, and the photosynthetic rate was about 13~15 mg CO₂ dm⁻² h⁻¹. The 4-years old sucker they used in the study was only 1.2 m in height that was raised from seed in the green house in Japan. In the present study, the suckers we observed were of non-spiny type sago palm and naturally grown in the tropics, which were 4-years old and about 6 m in height. The difference of the variety (or the species), and especially the propagation method and the growth conditions such as soil, temperature, light intensity precipitation and humidity etc. may be associated with the conspicuous growth difference.

Higher stomatal density may be benefit to accelerate the photosynthesis, considering the stomatal limitation of photosynthetic rate in sago palm leaf (Uchida et al. 1990). Therefore, the results mentioned above suggested that the photosynthetic rate of sago palms growing in the tropics may be higher values than those of Uchida's report (1990). Further studies on photosynthetic rate of sago palms in relation to the stomatal density should be proceeded in the tropics.

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