



A COMPARATIVE STUDY OF STOMATAL CHARACTERISTICS OF THE NINE *Pandanus* SPECIES FROM NIAS ISLAND, NORTH SUMATRA PROVINCE, INDONESIA

STUDI KARAKTERISTIK STOMATA SEMBILAN JENIS *Pandanus* ASAL PULAU NIAS, SUMATERA UTARA, INDONESIA

Helmin Parida Zebua, Nursahara Pasaribu*, Etti Sartina Siregar

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Indonesia

*Corresponding author: nursahara@usu.ac.id

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Abstract

The identification of *Pandanus* species generally relies on morphological characteristics and requires confirmation from other identification features, such as stomata. A comparative study of stomatal characteristics among nine Pandan species originally from Nias Island, namely *Pandanus atrocarpus*, *P. auranticus*, *P. labyrinthicus*, *P. militaris*, *P. odoratissimus*, *P. penangensis*, *P. tectorius*, and *P. utilis* has been investigated. Anomocytic stomata without papillae on subsidiary cells were observed on both leaf surfaces, with significant interspecific differences in adaxial and abaxial stomatal frequencies. *Pandanus tectorius* exhibited the highest adaxial (30.71 ± 0.81) and abaxial (1.87 ± 0.12) stomatal frequencies. *Pandanus labyrinthicus* showed the highest stomatal index (adaxial 16.61 ± 2.51 , abaxial 0.87 ± 0.11), while *P. penangensis* had the largest stomatal size ($137.54 \pm 6.66 \mu\text{m}$). Overall, the stomatal parameters, including frequency, index, and size, were higher on the adaxial surface than the abaxial surface, emphasizing interspecific variations. These findings contribute valuable supportive data for the botanical systematics of *Pandanus* spp. in the region, enhancing our understanding of morphological characteristics crucial for species identification.

Keywords: Anomocytic; Stomatal index; *Pandanus*; Nias Island; North Sumatra

Abstrak

Identifikasi jenis dari *Pandanus* cenderung menggunakan ciri morfologi dan memerlukan konfirmasi dari karakter lainnya, salah satunya stomata. Studi perbandingan stomata di antara sembilan spesies Pandan di Pulau Nias, Sumatera Utara telah dilakukan, yaitu *Pandanus atrocarpus*, *P. auranticus*, *P. labyrinthicus*, *P. militaris*, *P. odoratissimus*, *P. penangensis*, *P. tectorius*, dan *P. utilis*. Hasil penelitian menunjukkan bahwa keseluruhan jenis *Pandanus* memiliki tipe stomata berupa anomositik pada kedua permukaan daun atau amfistomatous tanpa adanya papilosa pada sel tambahan. Frekuensi stomata adaksial dan abaksial memiliki perbedaan yang nyata secara statistik lintas jenis. Frekuensi stomata tertinggi pada daun adaksial/abaksial diamati berturut-turut dari *P. tectorius* ($30,71 \pm 0,81$) dan *P. tectorius* ($1,87 \pm 0,12$). Indeks stomata daun tertinggi diamati berturut-turut berasal dari *P. labyrinthicus* ($16,61 \pm 2,51$) untuk adaksial dan *P. labyrinthicus* ($0,87 \pm 0,11$) untuk abaksial. Ukuran stomata terbesar diamati berturut-turut berasal dari *P. penangensis* ($137,54 \pm 6,66 \mu\text{m}$) dan *P. odoratissimus* ($64,56 \pm 3,96 \mu\text{m}$). Secara umum, tipe stomata pada semua jenis adalah anomositik tanpa adanya papila pada sel penjaga. Parameter stomata lainnya, yaitu frekuensi, indeks, dan ukuran pada bagian adaksial cenderung lebih tinggi dibandingkan permukaan abaksial dengan variasi nilai secara interspesifik.

Kata kunci: Anomositik; Indeks stomata; *Pandanus*; Pulau Nias; Sumatra Utara

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INTRODUCTION

Pandanaceae is one of the tropical plant families in Indonesia with an estimate of more less than 900 species, with grouped into five genera, namely *Benstonia*, *Freycinetia*, *Marterllidendron*, *Pandanus*, and *Sararanga* (Angiosperm Phylogeny Group, 2009). Sumatra is considered as a potential habitat to explore undescribed pandan species compared to another island such as Kalimantan, Celebes and others (Keim, 2011). In comparison, numerous findings on other pandan species, e.g *Freycinetia*, have been reported progressively from Sumatran provinces along with its species revisions (Pasaribu, 2010a).

A new species, *F. berbakensis* was first described as originally from Jambi province, Sumatra, Indonesia (Widjaja et al., 2008). This study reported nine species of *Freycinetia* from Jambi, with the finding of *F. scabrosa*, being a newly described species (Pasaribu & Widjaja, 2009). Additional two new species, namely *F. dewildeorum* and *F. leuserensis* were described and reported from Sumatra (Pasaribu, 2010b). Although the reports on *Freycinetia* are more updated, the possibility on finding other pandan species, e.g *Pandanus* is still high in Sumatra. Genera *Pandanus* represent the largest member in the *Pandanaceae* family, however, still few of them are reported from Sumatra (Vaughan & Wiehe, 1953).

Five species of *Pandanus* were reported from Aceh Singkil, Aceh Province, i.e *Pandanus atrocarpus*, *P. labyrinthicus*, *P. militaris*, *P. odoratissimus*, and *P. tectorius* (Marpaung et al., 2013). Two *Pandanus* species, namely *P. amaryllifolius* and *P. furcatus* were reported from Muara Enim, South Sumatra (Keim, 2017). Exploration by assuming the existence of unique or new species in the least explored habitats may be initiated, especially in other small islands of Sumatra. During an exploration in Nias Island, we collected nine species of *Pandanus* (unpublished data) following the data on its anatomical characterization. Identification efforts of *Pandanus* are mainly through its flowering parts or floral morphologies. In particular cases, some individuals may not produce floral parts which is impossible to identify the sterile plants (Kam, 1971). In this study, we described the stomatal variation in nine species of Nias *Pandanus* as additional information on anatomical varieties for identification purposes. The results may then support the data in systematics botany, especially limited to *Pandanus* of Sumatra.

MATERIALS AND METHODS

Nine species of *Pandanus* were collected during an exploration on 2016–2017 in Nias Island, North Sumatra, Indonesia. The species comprised of *Pandanus atrocarpus*, *P. auranticus*, *P. labyrinthicus*, *P. militaris*, *P. odoratissimus*, *P. penangensis*, *P. tectorius*, *P. utilis*, and an unidentified *Pandanus* sp. The voucher specimens were deposited to Herbarium MEDA, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia.

Anatomical features observed in this study are stomatal size, stomatal frequency, and stomatal index. Microscopy examination was performed by making a semi-permanent paradermal slice based on modified simple scraping technique (Metcalfe, 1960). Fresh specimens were boiled in water for 5–10 min, and divided into abaxial and adaxial samples for each examination. The softened leaf parts were sliced into smaller parts and placed on top of an object glass. A drop of 1% safranin was added into the slide, following a drop of glycerine solution. The slide was covered and examined using a light-compound microscope under 400× magnification.

The stomatal frequency was calculated as the number of stomata per unit area by direct observation. Stomatal size was calculated from the guard cells region with the stoma (pore) based on Franco (Franco, 1939) using the following formula, guard cell areas= $a \times b \times 0.7854$. Where, a= the length, b= the width of stomata. Stomatal index was determined according to Salisbury (1927) using the following formula, stomata index= $S/(E + S) \times 100$. Where, S is the number of stomata per unit leaf area and E is the number of epidermal cells per unit leaf area. Specimens were observed randomly for 25 selected microscopic field areas. Minitab ver. 16.0 was used to the statistical significance (P values) between the differences in all mean values of ada-/abaxial stomata using One-way ANOVA between each stomatal parameters within *Pandanus* species.

RESULTS

The leaf surface examination on stomata of *Pandanus* species from Nias Islands, revealed the dominant stomatal type of paracytic, in which one or more subsidiary cells are placed parallel to the pore among the guard cells (Figure 1). Stomata were observed both from adaxial and abaxial leaf parts. The mean stomatal frequency, index, and size of each *Pandanus* species were calculated and the data are presented in Figures 2, 3, and 4. The parameters measured were greatly different between adaxial and abaxial stomata. Based on stomatal frequency, the highest frequency was observed from *P. tectorius* (30.71 ± 0.81) while the lowest was from *P. militaris* (8.23 ± 0.75) in adaxial leaf. Meanwhile in abaxial leaf, the highest frequency was also observed from *P. tectorius* (1.87 ± 0.12), while the lowest from both *Pandanus* sp. and *P. odoratissimus* (1.00 ± 0.00).

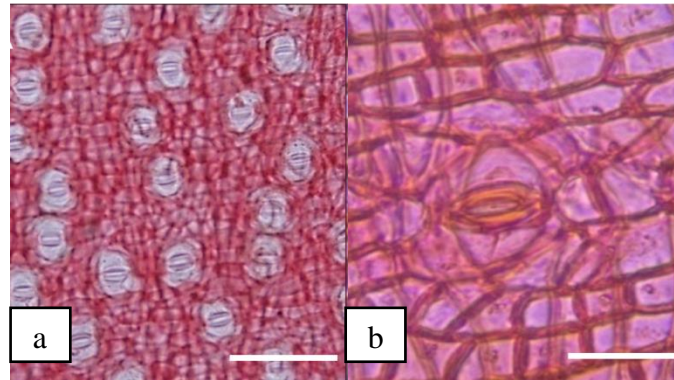


Figure 1. Light micrographs of *Pandanus penangensis* showing the paracytic type of stomata are scattered abaxial stomata (a) and adaxial stomata (b). Magnification at 400x. Scale bar for a= 100 μ m, b= 20 μ m

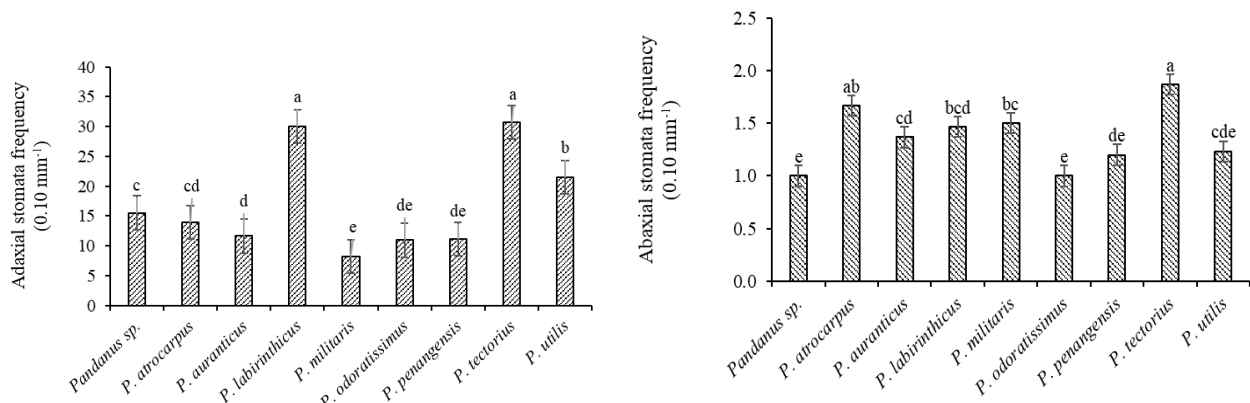
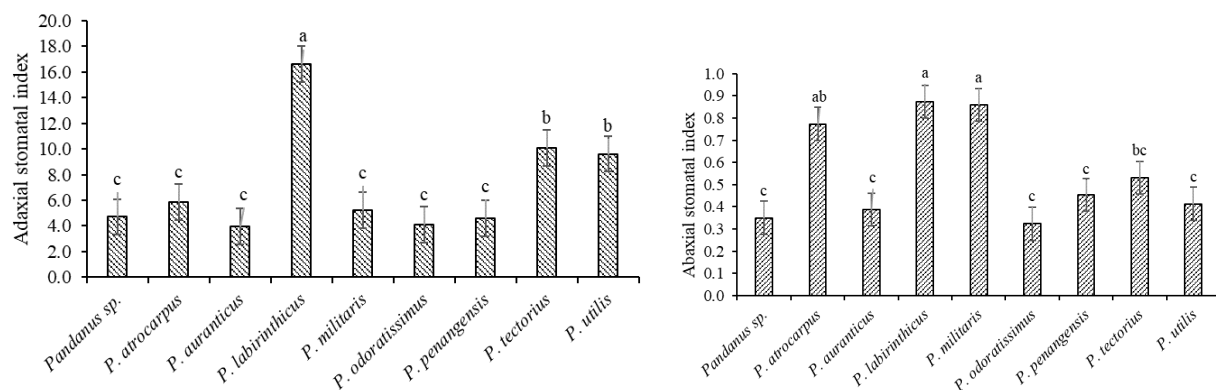


Figure 2. Stomatal frequency of nine *Pandanus* species from Nias island, North Sumatra Province, Indonesia. Different annotations represent a statistical significance ($P \leq 0.05$) based on F-test



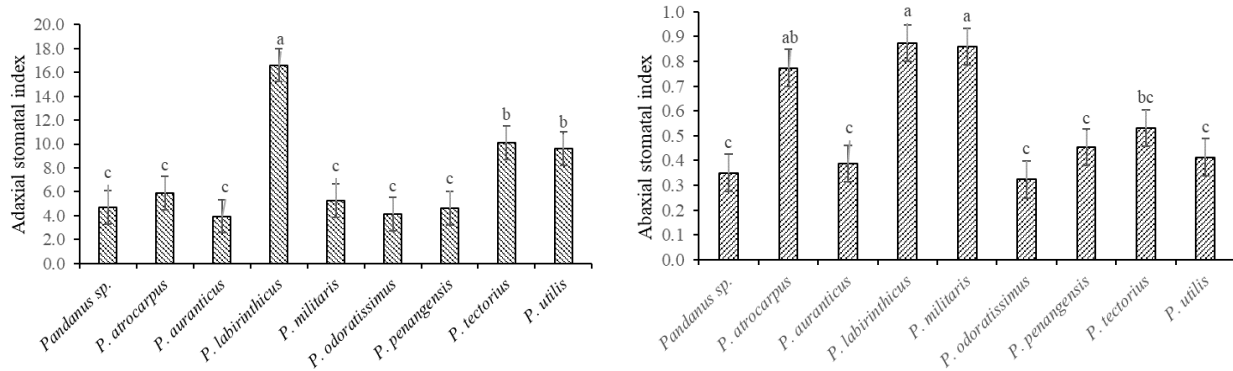


Figure 3. Stomatal index of nine *Pandanus* species from Nias Island, North Sumatra Province, Indonesia. Different annotations represent a statistical significance ($P \leq 0.05$) based on F-test

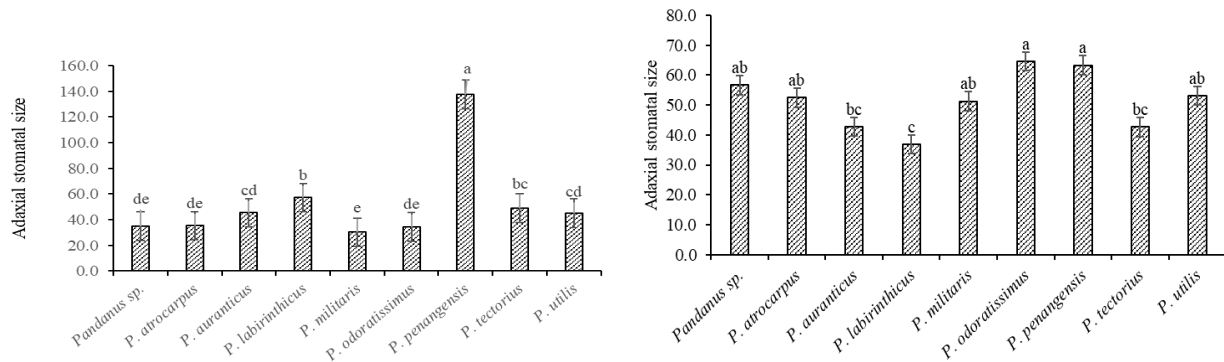


Figure 4. Stomatal size of nine *Pandanus* species from Nias Island, North Sumatra, Indonesia. Different annotations represent a statistical significance ($P \leq 0.05$) based on F-test

Based on stomatal index, the highest index was observed from *P. labyrinthicus* (16.61 ± 2.51), while the lowest from *P. auranticus* (3.95 ± 0.53). Meanwhile in abaxial leaf, the highest index was also observed from *P. labyrinthicus* (0.87 ± 0.11), while the lowest from *P. odoratissimus* (0.32 ± 0.01). Based on stomatal size, the largest stoma was observed from *P. penangensis* ($137.54 \pm 6.66 \mu\text{m}$), while the smallest from *P. militaris* ($30.36 \pm 1.57 \mu\text{m}$). Meanwhile in abaxial leaf, the largest size was observed from *P. odoratissimus* ($64.56 \pm 3.96 \mu\text{m}$), while the smallest from *P. labyrinthicus* ($36.91 \pm 4.72 \mu\text{m}$). Statistical test was also conducted to support the significant variabilities on stomatal characteristics among *Pandanus* species ($P \leq 0.05$) based on F-test.

DISCUSSION

Stomata serve as exchange pores in transpiration with many associated physiological functions. The main function of stomata, e.g. stomatal index and size, are directly related to the carbon dioxide and water vapor resistance (Lea et al., 1977). Different physicochemical environmental factors will lead to differences in stomatal frequency, but not to the stomatal index, in which the latter have been used to screen plant cultivars with improved water and gas exchange capacities (Mishra, 1997). The parameters are then useful in the field of agriculture and plant breeding. In this study, we present the stomatal characteristics of each *Pandanus* species which may also be affected by the environmental condition in Nias Island (Rahayu et al., 2011). Based on the leaf anatomy characteristics, fifteen *Pandanus* species from Java displayed some considerable diagnostic values to be used for its systematic study. The diagnostic characteristics have been formulated in a dichotomous key based on the epidermis, hypodermis, mesophyll, raphides, and stomata characteristics. The latter showed that the stomata of the *Pandanus* species can be grouped into unspecialized stomata (Class 1), papillose lateral subsidiary cells (Class 2), papillose terminal and lateral subsidiary cells (Class 3), papillose neighboring and subsidiary cells (Class 4), and overarching papillae lobed or dendritic (Class 5). The groupings were based on five arbitrary classes proposed by Kam (Metcalf, 1960) and Tomlinson (Tomlinson, 1965). In this study, all the

stomatal types from Nias *Pandanus* can be recognized as an example from Class 1 (unspecialized stomata), without any finding on papillose subsidiary cells. Hence, most of the diagnosed and measured stomatal characteristics are majority from adaxial stomata (paracytic). Different result was reported from a study of *Pandanus* specimens which revealed the tetracytic type and the presence of papillose. The similar result was only regarding the amphistomatous (stomata in both upper and bottom leaf surface) feature to our specimen's study (Santika et al., 2014). In contrary, the stomatal characteristics regarding the frequency, index, and size varied greatly compared to the *Pandanus* species from Aceh region, the Northern Sumatra (Marpaung et al., 2013). The study reported a higher stomatal value in abaxial than adaxial surface, which is different from our result. All the anatomical characteristics from Nias Island may be further investigated for the formulation on a better tentative identification key in the future, by adding more features as the compared characteristics.

CONCLUSION

Nine species of Nias *Pandanus*, namely *Pandanus atrocarpus*, *P. auranticus*, *P. labirinthicus*, *P. militaris*, *P. odoratissimus*, *P. penangensis*, *P. tectorius*, *P. utilis*, and an unidentified *Pandanus* sp., were observed for its stomatal characteristics. In general, the stomata type on all species is anomocytic without any presence of papillose(s) on its guard cells. Stomatal parameters, i.e. frequency, index, and size are higher in the adaxial than the abaxial surface. Emphasizing the adaxial stomatal features as a key diagnostic trait in future studies and identification keys for Nias *Pandanus* species will contribute to a more accurate and reliable taxonomic classification.

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REFERENCES

- Angiosperm Phylogeny Group. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, 161, 105-121
- Franco, C. M. (1939). Relation between chromosome number and stomata in *Coffea*. *Botanical Gazette*, 100, 817-827.
- Kam, Y. K. (1971). Comparative systematic foliar anatomy of Malayan *Pandanus*. *Journal of the Linnean Society of London. Botany*, 64, 315-353.
- Keim, A. P. (2011). *Pandanus* (Pandaceae) in Sumatra & Kalimantan, Indonesia. *Reinwardtia*, 13(3), 255-262.
- Keim, A. P. (2017). Flora pandan kawasan Semende, Muara Enim, Sumatera Selatan. *Indonesian Journal of Biotechnology and Biodiversity*, 1 (2) 38-47.
- Lea, H. Z., Dunn, G. M., & Koch, D. W. (1977). Stomatal diffusion resistance in three ploidy levels of smooth bromegrass. *Crop Science*, 17, 91-93.
- Marpaung, D. R. A. K., Pasaribu, N., & Aththorick, T. A. (2013). Taxonomic study of *Pandanus* (Pandaceae) in swamp area, Aceh Singkil. *Jurnal Natural*, 13(2), 55-63.
- Metcalf, C. R. (1960). *Anatomy of the monocotyledons i. Gramineae*. London: Oxford University Press.
- Mishra, M. K. (1997). Stomatal characteristics at different ploidy levels in *Coffea* L. *Annals of Botany*, 80, 689-692.
- Pasaribu, N., & Widjaja, E. A. (2009). Notes on *Freycinetia* (Pandaceae) from Jambi, Sumatra, with the description of a new species. *Reinwardtia*, 13(1), 87-92.
- Pasaribu, N. (2010a). *Freycinetia* (Pandaceae) of Sumatra (Doctoral dissertation, IPB University, Bogor, Indonesia). Retrieved from <https://repository.ipb.ac.id/handle/123456789/46565>.
- Pasaribu, N. (2010b). Two new species of *Freycinetia* (Pandaceae) from Sumatra, Indonesia. *Reinwardtia*, 13(2), 147-150.
- Rahayu, S. R., Kartawinata, K., Chikmawati, T., & Hartana, A. (2011). Leaf anatomy of *Pandanus* species from Java. *Reinwardtia*, 13(3), 305-313.

- Santika, Y., Tihurua, E. F., & Triono, T. (2014). Comparative leaves anatomy of *Pandanus*, *Freycinetia*, and *Sararanga* (*Pandanaceae*) and their diagnostic value. *Reinwardtia*, 14(1), 163-170.
- Salisbury, E. J. (1927). On the causes and ecological significance of stomatal frequency with special reference to the woodland flora. *Philosophical Transactions of the Royal Society*, B216, 848-852.
- Tomlinson, P. B., (1965). A study of stomatal structure in *Pandanaceae*. *Pacific Science*, 19(1), 38-54.
- Vaughan, R. E., & Wiehe, P. O. (1953). The genus *Pandanus* in the Mascarene Islands. *Journal of the Linnean Society of London. Botany*, 55, 356-133.
- Widjaja, E. A., Pasaribu, N., & Hidayat, A. (2008). A new species of *Freycinetia* (*Pandanaceae*) from Jambi, Sumatra, Indonesia. *Reinwardtia*, 12(5), 441-442.