RELEVANCE OF FOLIAR EPIDERMAL CHARACTERS IN THE DELIMITATION OF THREE FORMS OF *ELAEIS GUINEENSIS* (JACQ.)

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ABSTRACT

The leaf epidermal morphology of *Elaeis guineensis* (Jacq.) was studied in other to differentiate between its three forms: dura, tenera and pisifera. Stomata were present only at the abaxial leaf surfaces of the three forms studied. Tetracytic stomata as well as stomatal complexes were observed in all the forms. However, Siamese stomata was observed only in tenera form. Tabular epidermal cells with straight walls were observed on the abaxial epidermis of all the forms. Trichomes were present on both adaxial and abaxial epidermes but more on the abaxial epidermis. Although glandular trichomes were prominent in all the forms, there were higher morphological diversity of glandular trichomes at the abaxial epidermis of all the three forms. Occurrence of Siamese trichome in tenera is considered very diagnostic in taxonomic characterization of the taxon. A dichotomous key of identification taxa within the species was presented.

{**Citation:** Osuji, Julian Onyewuonyeoma, Ajah, Obiageri Florence. Relevance of foliar epidermal characters in the delimitation of three forms of *Elaeis Guineensis* (*Jacq.*). American Journal of Research Communication, 2016, 4(9): 180-189} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Elaeis guineensis Jacq., a pleonanthic species belonging to the family Arecaceae, subfamily Arecoideae, tribe Cocoeae, subtribe Elaidinae and synonyms: *Elaeis melanococca* J. Gaertn, comonly known as the African oil palm was first illustrated by Nicholaas Jacquin in 1763, hence the name *Elaeis guineensis* Jacq. The Arecaceae family is large, comprising of five subfamilies (Dransfield *et al*., 2005). The Arecoideae forms the largest subfamily of the Arecaceae and has been classified into 190 genera and 2,364 species (Govaerts and Dransfield, 2005). *Elaeis* comprises of two known species, the African *Elaeis guineensis* and the Tropical American *Elaeis* *oleifera* (Kunth) Cortes *ex* Prain (synonym: *Coroza oleifera* (Kunth) L.H. Bailey (Vossen *et al*., 2007). It is however believed that the *Elaeis guineensis* is a native of West Africa. Vossen *et al.*, (2007) reported that the greatest genetic variation is found in south-eastern Nigeria and western Cameroon and there is also fossil evidence that the Niger delta is its most likely origin.

Elaeis guineensis occurs naturally in three forms which can be differentiated by their pigmentation and structural characteristics. The *dura* form has a 2-8 mm thick endocarp which contributes about 25-55 % of weight of fruit such that the mesocarp occupies 35-65 % of the fruit. The kernels are large, 7-20 % of fruit weight. The pisifera form has no endocarp and about 95 % mesocarp (Latiff, 2000), having small kernels in fertile fruits that often rot prematurely. The fruit to bunch ratio is very low, the infertile palms show strong vegetative growth but of little commercial value. They are instead used for crossing with the dura palm to produce the tenera (DxP) hybrid after M. Beirnaert discovered the single gene inheritance of shell thickness in 1939 in the then Belgian Congo (Zaire) (Hartley, 1988). This discovery was the cornerstone for the oil palm producing industry and has paved a way for breeding, selection and production of high yielding DxP planting materials. The tenera form is a hybrid obtained from crossing dura and pisifera (Corley and Tinker, 2003). The tenera has a 0.5-3 mm thin endocarp, which contributes 1-32 % of fruit weight with the mesocarp occuping 60-95 % of fruit weight; having larger number of bunches than the dura form but lower mean bunch weight and lower fruit to bunch ratio.

Studies carried out by many authors have shown that cuticular characters are of high taxonomic value. Gill and Karatela, (1983a and 1983b), Gill and Nyawuame (1990), Ugborogho *et al*. (1992), Osuji (2005) have emphasized the value of micromorphological features including stomata in the taxonomic consideration of plant taxa. Ugborogho *et al*. (1993) used cuticular studies in the delimitation of three species of *Dioscorea* L. Luis *et al*. (2010) revealed the plasticity of the foliar epidermal morphology by studying the effects of different growth conditions on the oil palm seedlings.

Although much work have been done on *Elaeis guineensis*, none has attempted to differentiate between the three forms of the species using cuticular characterization as an enabling tool. The works of Stace (1965), Pereira-Sheteolu (1992), Ugborogho *et*. *al* (1992 and 1993), Osuji (2005) among others have shown that characters derived from epidermis could aid taxonomic identification and classification because of their stability on one hand and high structural diversity on the other. In this report, the epidermal micro-morphology of the three forms of *E. guineensis* has been investigated to deepen taxonomic elucidation of the species.

MATERIALS AND METHODS

Materials of *Elaeis guineensis* used for this research work were collected from the National Institute for Oil Palm Research (NIFOR), Benin City in Edo State, Nigeria. Rectangular pieces were cut from the mid portions of mature leaves of dura, tenera and pisifera collections. The pieces were treated with concentrated nitric acid following the method of Obute and Ugborogho,

(1994) until air bubbles appeared indicating the separation of the epidermes. Forceps and mounted needles were used to remove the adaxial epidermis while the abaxial epidermis was bleached with 3.5 % sodium hypochlorite solution (NaOCl) for 5 minutes to clear the epidermis completely. Each peel of the epidermis was washed with deionized water, stained with 0.1 % safranin solution and mounted in 100 % glycerine on a clean glass slide and under No.1 cover slips. Each slide preparation was examined microscopically. Distribution of foliar trichomes were visually scored at X 100 field of view under a microscope. Number of epidermal cells and stomatal distribution were scored at X 400 field of view of the microscope in order to achieve the stomatal index. Sizes of the stomata and trichomes were assessed with an ocular micrometer at X 400 magnification. Micrographs of clear cepidermal peels were obtained manually using Cannon Power Shot digital camera.

RESULTS

Epidermal cells

Four subsidiary cells surrounded each stoma in all the forms under investigation. The shapes of the cells varied from isodiametric to polygonal. The anticlinal walls were straight for all the forms studied. Tabular epidermal cells with straight walls (Plate 1) were prominent in only the abaxial epidermal tissue of the 3 forms of *E. guineensis* studied.

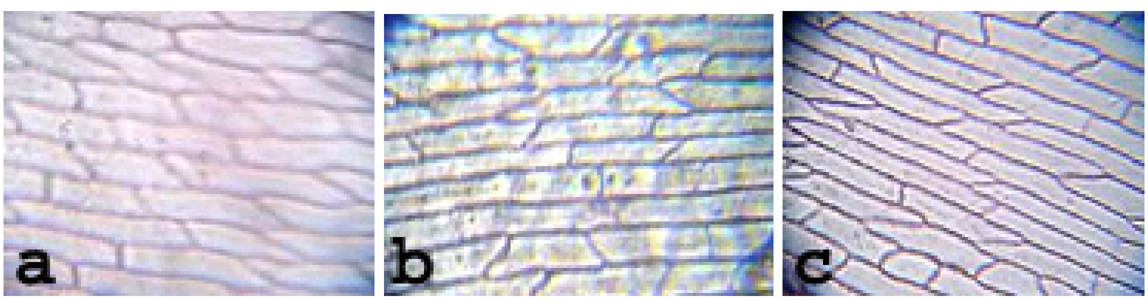

Plate 1: Tabular epidermal cells of the adaxial epidermis. a) dura, b) pisifera and c) tenera at X 400 magnification.

Stomatal features

The dura, tenera and pisifera forms of *E. guineensis* had nearly round stoma each with narrowly elliptic stomatal pore. In all the 3 forms, only the abaxial epidermis had stomata. The stomatal counts per field of view at the magnification of X 400 varied in all three forms being 31 for dura, 29 for tenera and 33 for pisifera. The stomatal indices were 7.11 % for dura, 8.01 % for tenera and 7.93 % for pisifera. Table 1 shows that the variability in the number of stomata is highest in the dura and lowest in the pisifera. Figure 1 revealed the increase in the stomatal length of tenera over the others.

Table 1: Abaxial mean stomatal distribution, area (length and breadth in μm) and stomatal indices (%)

Taxa	Number of Stomata (χ±S.d)	Length of Stomata	Breadth of Stomata	CoV of Stomatal Number	Stomatal Index
Dura	31.3±4.2	7.42	3.18	13.4	7.11
Tenera	28.6±3.5	9.54	3.18	13.4	7.11
Pisifera	33.0±2.0	8.48	4.24	6.06	7.93

χ = mean, S.d = standard deviation, C.O.V. = Coefficient of Variation. Size in eyepiece micrometer units: 1 unit = 1.06μm (Haskell and Wills, 1985)

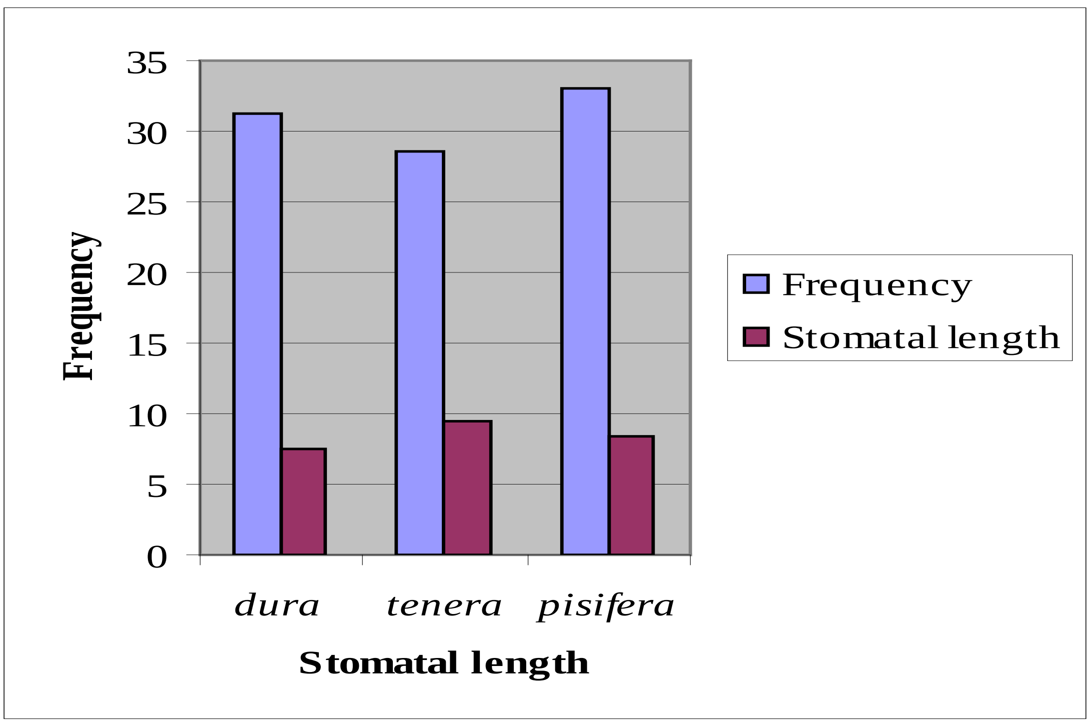

Figure 1: Frequency distribution of stomatal lengths in dura, tenera and pisifera.

Tetracytic stomata were prominent in all the 3 forms. The stomata were close to each other having multiple stomata complexes of 3 - 4 (Plate 2). Stomatal abnormalities such as the Siamese stomata were observed in only the tenera form (Plate 3).

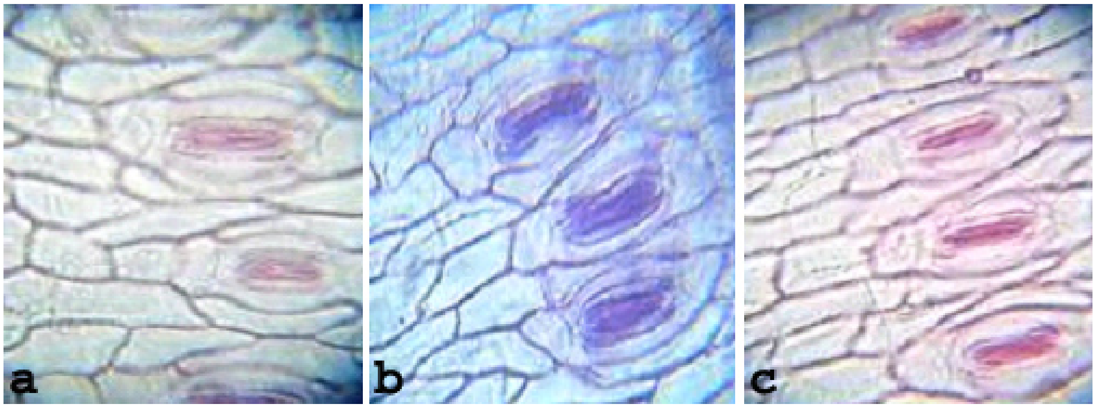

Plate 2: Stomatal complexes of a) dura, b) pisifera and c) tenera at X 400 magnification.

Plate 3: Siamese stomata of tenera at X 400 magnification.

Trichomes

All the three forms of the *E. guineensis* under investigation revealed that both the adaxial and the abaxial epidermes had trichomes. The trichomes were distributed more on the abaxial than the adaxial epidermis. The tenera form had the least presence trichome in both the adaxial and the abaxial epidermes.

The trichome counts per X 100 field view of the adaxial epidermis were 17 for dura, 14 for tenera and 16 for pisifera. For the abaxial epidermis, the counts per X 100 field of view were 35 for dura, 32 for tenera and 36 for pisifera. However, statistical evidence revealed that the observed difference in mean number of trichomes in the adaxial and abaxial epidermes was highly significant ($p < 0.05$) for the dura, tenera and pisifera.

The glandular trichome was prominent in all the 3 forms of the *E. guineensis*. The glandular trichome of dura, tenera and pisifera on the adaxial epidermis were the same, having only the gland though with slight variation in the number of its component cells (Plate 4). Table 3 showed the measurements of the length and breadth of the trichomes in both the adaxial and abaxial epidermes of the forms being investigated.

Table 2: Mean values of trichome distribution and trichome area (length and breadth in µm) of the dura, tenera and pisifera

Taxa	Epidermal Surface	Number measured	Min.	χ±S.d.	Max.	CoV	Length	Breadth
Dura	Upper	30	14	16.7±2.3	18	13.8	7.42	5.30
Dura	Lower	30	31	34.7±3.2	37	9.2	19.08	9.54
Tenera	Upper	30	13	13.7±0.6	14	4.4	7.42	5.30
Tenera	Lower	30	30	32±3.2	35	10.0	16.96	9.54
Pisifera	Upper	30	12	15.7±4.7	21	30.1	8.48	5.30
Pisifera	Lower	30	32	35.3±3.1	38	8.8	10.6	8.48

χ = mean, S.d. = standard deviation, CoV = coefficient of variation. Size in eyepiece micrometer units: 1 unit = 1.o6 µm (Haskell and Wills, 1985)

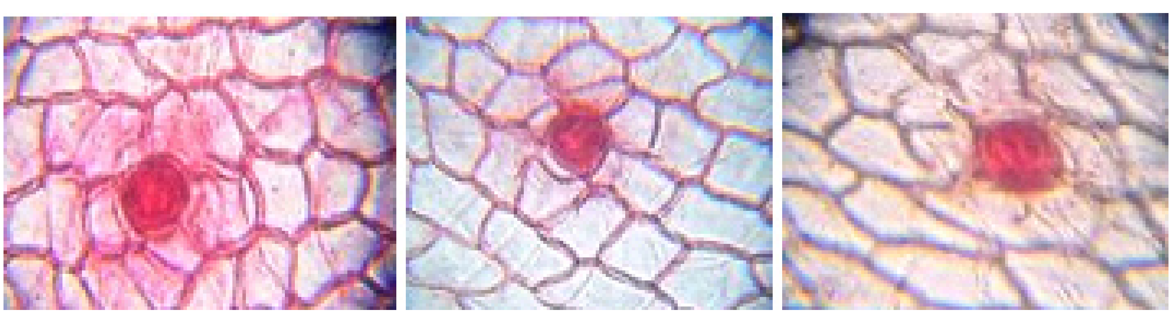

Plate 4: Adaxial epidermal surface showing the trichome of *dura* (A), *pisifera* (B) and *tenera* (C) at X40 magnification.

However, there were variations in the glandular trichomes present at the abaxial epidermis of all the forms studied. Dura had two different types of glandular trichome: one with very long blunt stock and another that consisted of a gland with a short stock which had 3 - 4 cells (Plate 5a).

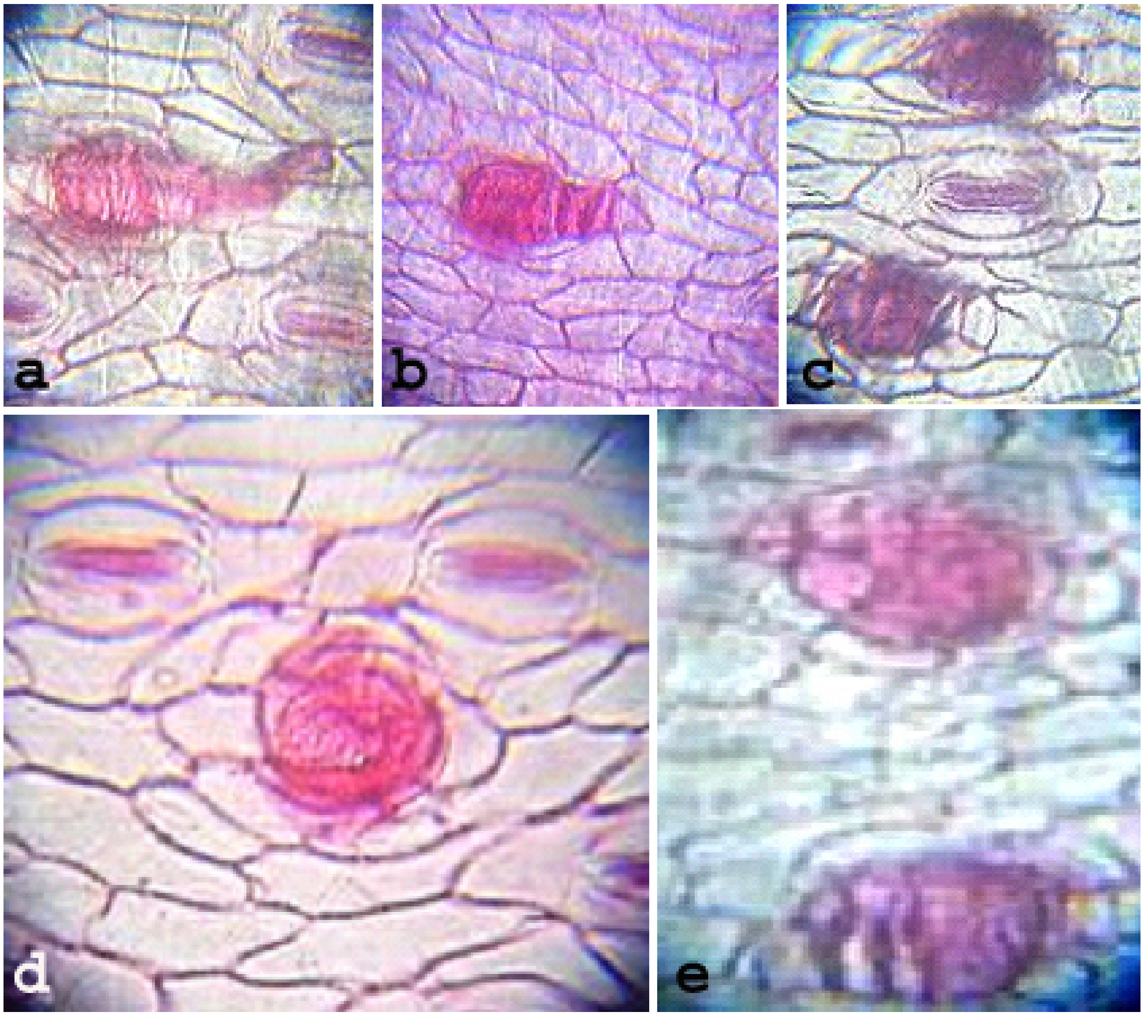

Plate 5: Abaxial epidermal surface showing presence of glandular trichomes and their variations, a-b) Dura, c) Pisifera and d-e) Tenera. Magnification = X 400.

Pisifera had one type of glandular trichome (Plate 5b) while tenera had two types of glandular trichomes: one with only a gland (Plate 5c, left) and another that had 2 - 3 linearly arranged cells at the base of the gland and comprises a very short stock (Plate 5c, right). Additionally, Siamese trichomes were also observed in the tenera foliar epidermis (Plate 6).

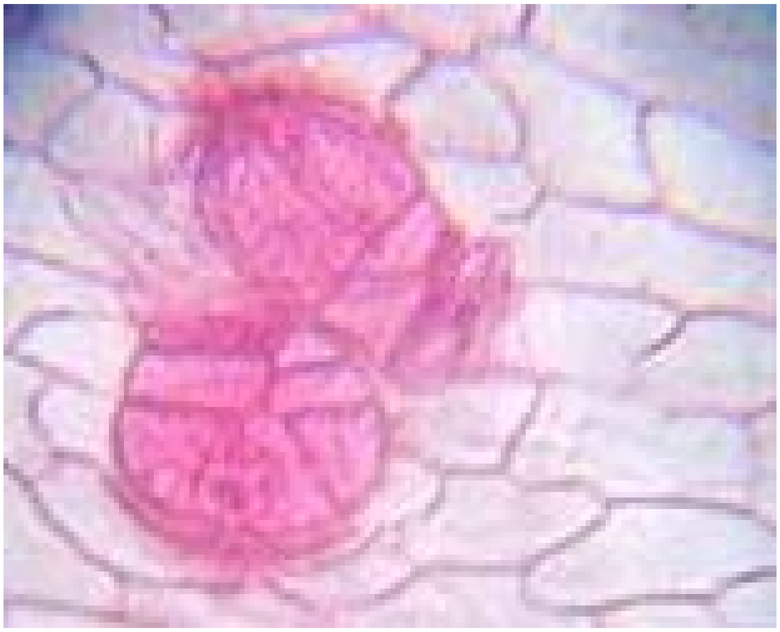

Plate 6: Siamese trichomes of tenera at X 400 magnification.

DISCUSSION

The presence of stomata only on the abaxial epidermes of dura, tenera and pisifera is in contrast with the observation of Luis *et al*., (2010). Their report stated that stomata occur on both the abaxial and adaxial epidermes but more on the abaxial epidermis. However, occurrence of stomata only on the abaxial epidermis has also been reported by Shah and Gopal, (1972); Onwueme, (1978) and Ugborogho *et al*., (1993), Osuji and Nwala (2015). The absence of stomata on the adaxial epidermis may indicate further controls water loss and gaseous exchange.

Futhermore, stomatal type is of no diagnostic value in this species. Tetracytic type of stomata occurs in all 3 forms, hence it cannot be used in differentiating these sub-taxa of *E. guineensis*. However, stomatal index could be used in differentiating them. The stomatal length and index of tenera, which is believed to be a hybrid was the highest value of these. This could be an indication of improved vigour due to its hybrid origin. Thus, the combination of stomatal index and size constitute a useful taxonomic tool for discrimination plant forms within this species.

It is believed that variation in stomatal size in different cultivars of the same species may be associated with variation in chromosome number or ploidy levels. Stace (1965) also emphasized the usefulness of stomatal complex in taxonomy especially in the identification of small fragments, i.e. sub-taxa. The tetracytic stomatal type, stomata aligned with the tabular epidermal cells observed in this research are in agreement with Luis *et al.* (2010). According to Tomlinson (1990), these stomatal types are peculiar to few Liliopsida including Arecaceae and Cyperaceae.

Intrestingly, a stomatal abnormality such as the Siamese stomata observed in this research has been reported by Ugborogho (1982); Gill and Karatela, (1983a) and (1983b); Karatela and Gill, (1986); Gill and Nyawuame, (1990) and Ugborogho *et al*., (1992). Ugborogho (1982), on Sida L. has observed that such rare character as the Siamese stomata could be of taxonomic value.

The frequency of trichomes on both the adaxial and the abaxial epidermis is very significant in this species. The tenera form has the least pubescence in both epidermes. Metcalfe and Chalk (1979) hold that trichome frequency and size are environmentally controlled while Stace (1965) believes that pubescens is constant in a species when present and showed a constant range of form and distribution useful in diagnosis. Interestingly, trichome abnomalities such as the contiguous trichome as observed in tenera is *novel* and very unique and has not been reported. This trichome morphology is a useful diagnostic tool in distinguishing tenera form of *E. guineensis* from dura and pisifera. The basic type of trichome in this species is the glandular, however, differences exist that can be used in differentiating the 3 forms under study. The *tenera* form has the type of trichome that is intermediate between pisifera and dura as well as the trichome type of pisifera. The trichome morphology of this plant species is a useful taxonomic tool that seems to confirm the hybrid status of the tenera.

Conclusively, it is very obvious that the different forms of *Elaeis guineensis* have other differences deeper than their macro-morphological delimitations. Information and data presented in this work is expected to correct the erroneous belief that the three forms of *E. guineensis* can only be differentiated by their fruit forms. Moreover, further investigation is required to establish further tools necesary to facilitate selection and improvement of oil palm production.

KEYS TO FORMS OF *ELAEIS GUINEENSIS*

1. Stomatal index greater than 8 %, abaxial trichome count per field of view less than 32, presence of Siamese stomata and trichomes -- -- tenera
1. Stomatal index less than 8 %, abaxial trichome count per field of view greater than 34 -- -- 2

2. Adaxial trichome length greater than 8, abaxial trichome length less than 11, one type of glandular trichome present at the abaxial epidermis -- pisifera
2. Adaxial trichome length less than 8, abaxial trichome length greater than 19, two types of glandular trichomes present at the abaxial epidermis -- dura

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