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STOMATAL COMPLEX TYPES, STOMATAL DENSITY, AND THE STOMATAL INDEX IN SOME SPECIES OF *DIOSCOREA*

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Abstract — *Dioscorea alata* L. has three stomatal complex types, namely, paracytic, anisocytic, and tetracytic stomata, with percentage frequency values of 50, 18, and 32, respectively. *Dioscorea bulbifera* has paracytic and anisocytic stomata, with percentage frequency values of 87.60 and 12.40, respectively. *Dioscorea cayenensis* has anisocytic stomata, with a percentage frequency value of 100. *Dioscorea dumetorum* has tetracytic and paractytic stomata, with percentage frequency values of 91.05 and 8.95, respectively. Both *D. esculenta* and *D. rotundata* have paracytic stomata, with a percentage frequency of 100. The range of variation of stomatal density is from 10 (lowest value) in *D. alata* and *D. dumentorum* to 27 (highest value) in *D. bulbifera*. The stomatal index also varies, from 24 in *D. alata* to 47 in *D. cayenensis*. The size of stomata in all species is small, varying in length from 0.74 μm in *D. alata* to 1.79 μm in *D. dumentorum*. An indented dichotomous key based on stomatal features was constructed to distinguish and identify the species.

Key words: Stomatal index, stomatal types, *Dioscorea,* Nigeria

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INTRODUCTION

The yams, *Dioscorea* L. of the family Dioscoreaceae, are monocotyledonous tropical plants which produce underground or aerial tubers and are cultivated on a wide scale in West Africa, the Carribbean and Southeast Asia as a major source of calories for the peoples of these tropical areas (Ikediobi and Igboanusi, 1983). Yam is the common name applied to plants of about 500 species (Stephens, 2003) or 600 species (Milne-Redhead, 1975; Okeke, 2001) of the genus *Dioscorea*. Other terms for yam are true yams, greater yam and tropical yam (Stephens, 2003). Of the 20 species of the genus *Dioscorea* recorded for West Tropical Africa (Miege, 1968), only *D. burkilliana* J. Miege, *D. lecardii* De. Wild, and *D. sagittifolia* Pax have not been recorded for Nigeria. The species under cultivation in Nigeria include *D. alata* L., *D. bulbifera* L., *D. cayenensis* Lam., *D. dumetorum* (Knuth) Pax, *D. esculenta* (Lour.) Burkill, and *D. rotundata* Poir.

In addition to having food value, many species of *Dioscorea* contain sapogenin, a compound having medicinal value. Some wild yams have strikingly variegated leaves and are of interest as ornamentals (Stephens, 2003). Many characters of morphology, physiology, anatomy, cytology, phytochemistry, ecology, and molecular biology have been used to understand the taxonomy of the genus (Lamarck, 1789; Poiret, 1813; Chevalier, 1936; Burkill, 1939; Hutchinson and Dalziel, 1954; Waitt, 1965; Miege, 1968; Ayensu, 1970; Martin and Rhode, 1978; Onwueme, 1978; Akoroda and Chheda, 1983; Onyilagha and Lowe, 1986; Teraurchi et al., 1993; Okeke, 2001, 2004; Hamoni and Toure, 2004; Yuji, 2004; Schols et al., 2008). Not much has been elucidated about the anatomy of yam stomata, with particular reference to stomatal complex types, stomatal density, and the stomatal index. Hence the present study attempts to address this issue. This is with a view to providing baseline data that may be useful for further studies on the genus.

MATERIALS AND METHODS

Collection of study materials

Fresh specimens were collected from the Grain Research Unit (GRU) of the International Institute of

Tropical Agriculture (IITA), Ibadan. Specimens were identified at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Table 1. List of some species of Dioscorea.

Species	Common name
Dioscorea alata L.	Water yam
Dioscorea bulbifera L.	Aerial yam
Dioscorea cayenensis Lam.	Yellow yam
Dioscorea dumetorum (Kunth) Pax	Bitter yam
Dioscorea esculenta (Lour.) Burkill.	Chinese yam
Dioscorea rotundata Poir.	White yam

Specimen preparation

Leaf segments with an area of 1 cm^2 were cut from the leaves of six species of *Dioscorea* (Table 1). They were cut and immersed in 20% chromium trioxide for cuticle maceration (Alvin and Boulter, 1974). A small portion of macerated cuticle was stained in 1% aqueous solution of safranin for about 3 min. Excess stain was rinsed off with water. The stained specimen was then mounted in glycerin for observations in an Olympus microscope.

Determination of frequency of stomatal complex types

Using the field of view at objective magnification of 40x as a quadrate, the frequency of each stomatal complex type was expressed as percentage occurrence of each complex type in a total of 35 fields of view (Obiremi and Oladele, 2001). Terminologies used with respect to stomatal complex types follows those of Dilcher (1974) and Metcalfe and Chalk (1988).

Determination of stomatal density and stomatal index

The stomatal density was determined as the number of stomata per square millimeter. The index was determined as the number of stomata per square millimeter divided by the number of stomata plus number the of epidermal cells per square millimeter multiplied by 100. The lengths of stomata were measured to determine the stomatal size.

RESULTS AND DISCUSSION

Stomatal complex type

Based on the occurrence of stomata on the leaf surface, two types of leaves were recognized, namely, epistomatic leaves (i.e., stomata occurring on the adaxial surface only) and hypostomatic leaves (i.e., stomata occurring on the abaxial surface only). The

Table 2. Stomatal anatomy in some species of *Dioscorea*.

Species	Leaf surface	Stomatal complex types	Frequency of stomatal complex type (%)	Stomatal size (μm)	Stomatal density (mm^{-2})	Stomatal index (%)
Dioscorea alata	Abaxial	Paracytic Tetracytic Anisocytic	50.00 32.00 18.00	0.74±0.01	10.57±2.06 (3-20)	28
	Adaxial	--				
Dioscorea bulbifera	Abaxial	Paracytic Anisocytic	87.60 12.40	1.64±0.02	27.83±4.23 (12-55)	40
	Adaxial	--				
Dioscorea cayenensis	Abaxial	Anisocytic	100.00	1.47±0.04	17.75±4.46 (7.42)	47
	Adaxial	--				
Dioscorea dumetorum	Abaxial	Tetracytic Paracytic	91.05 8.95	1.79±0.03	10.85±2.29 (4-20)	24
	Adaxial	--				
Dioscorea esculenta	Abaxial	Paracytic	100.00	1.38±0.02	21.25±1.52 (15-28)	44
	Adaxial	--				
Dioscorea rotundata	Abaxial	--		1.76±0.01	20.66±1.66 (12-28)	42
	Adaxial	Paracytic Diacytic	90.00 10.00			

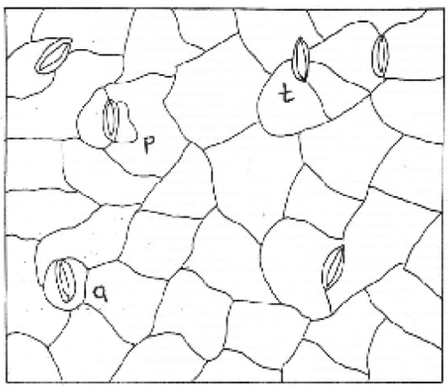

Fig. 1. Leaf surface (abaxial) of *D. alata* showing paracytic (p), anisocytic (a), and tetracytic (t) stomata complex types (600x).

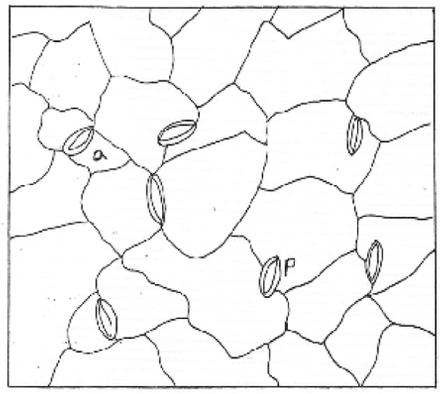

Fig. 2. Leaf surface (abaxial) of *D. bulbifera* showing paracytic (p) and anisocytic (a) stomatal complex types (600x).

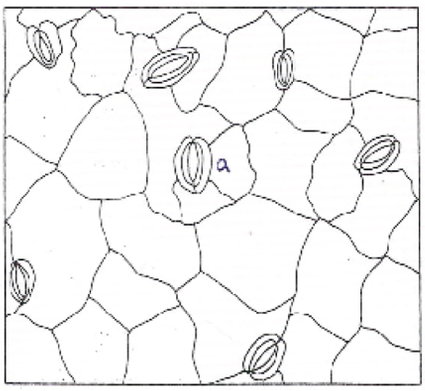

Fig. 3. Leaf surface (abaxial) of *D. cayenensis* showing anisocytic (a) stomatal complex type (600x).

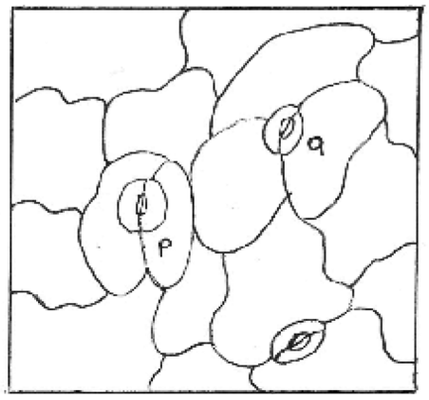

Fig. 4. Leaf surface (abaxial) of *D. dumetorum* showing paracytic (p) and anisocytic (a) stomatal complex types (600x).

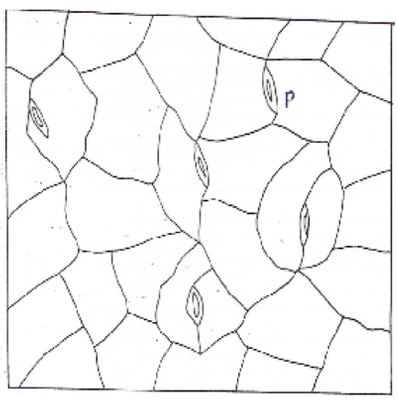

Fig. 5. Leaf surface (abaxial) of *D. esculenta* showing paracytic (p) stomatal complex type (600x).

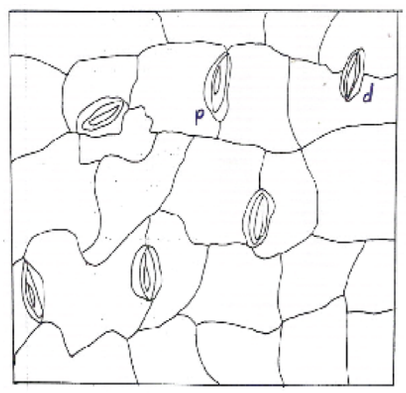

Fig. 6. Leaf surface (adaxial) of *D. rotundata* showing paracytic (p) and diacytic (d) stomatal complex types (600x).

hypostomatic leaf type was found in five species (*D. alata*, *D. bulbifera*, *D. cayenensis*, *D. esculenta*, and *D. dumetorum*), while the epistomatic type was found in *D. rotundata*. Occurrence of stomata on either

one or both sides of the leaves was previously reported by Metcalfe and Chalk (1988) and Qiang et al. (2007). Three types of stomatal complex were identified, namely, paracytic, anisocytic, and tetracytic stomata. *Dioscorea rotundata* possessed paracytic and diacytic stomata; *D. esculenta* possessed only the paracytic stomata complex type; *D. cayenensis* had only anisocytic stomata; *D. bulbifera* possessed paracytic and anisocytic stomata; *D. dumetorum* had paracytic and tetracytic stomata; and *D. alata* possessed all three types, i.e., paracytic, anisocytic, and tetracytic stomata (Table 2).

Stomatal density and stomatal index

Stomatal density and the stomatal index varied from one species to another. High stomatal density (27.83 mm^{-2}) was found in *D. bulbifera*, while lower stomatal density (10.57 mm^{-2}) was found in *D. alata*. Many workers considered stomatal density to be a useful character for distinguishing species when comparable areas of leaf are used (Noggle and Fritz, 1976). Okeke (2004) also used stomata per square millimeter as one of the diagnostic features distinguishing between three species of *Dioscorea* species, namely, *D. cayenensis*, *D. rotundata*, and *D. pruinosa*. He observed that *D. rotundata* had more stomata per millimeter than *D. cayenensis*. This finding was in conformity with the present work (Table 4). Stomatal index (i.e., percentage spread of stomata) values in *D. alata* and *D. dumetorum* were lower than in other species (Table 2). The stomatal index, which indicates the proportion of stomata relative to leaf surface, is also a reliable taxonomic character. This is because it is independent of the changes in epidermal cell size brought about by environmental factors (Metcalfe and Chalk, 1988).

Stomatal size

Based on the classification criterion of Pataky (1969), stomata in the species of *Dioscorea* belong to the small category. Pataky classified stomata with guard cells measuring less than 15 μm as "small" and those with guard cells measuring more than 38 μm as "large". All stomata in the six *Dioscorea* studied were small in size (Table 2). Metcalfe and Chalk (1988) and Beerling and Woodward (1997) reported that large stomata usually gave low stomatal density, while small stomata gave high stomatal density. This statement did not hold true for *D. bulbifera* and *D. alata*, where stomata of *D. bulbifera* gave high stomatal density and stomata of *D. alata* give low stomatal density (Table 2).

Leaf cuticular study is becoming more important because taxonomists, drug industry workers, animal nutritionists, animal toxicologists, and police investigators have all found it useful in plant identification (Daniel, 2005). A number of workers have used leaf features to reclassify many species within a genus or genera within a family (Olowokudejo and Pereira-Sheteolu, 1988; Adegbite, 1995; Abubakar and Yunusa, 1998; Ogunkunle and Oladele, 2000; AbdulRahaman and Oladele, 2005).

An indented dichotomous key based on stomatal features in these species is given below:

1a. Frequency of paracytic stomata, 0-50% 2
  2a. Low or zero frequency of anisocytic stomata; high frequency of tetracytic stomata..... *D. dumentorum*
  2b. High frequency of anisocytic stomata; low or zero frequency of tetracytic stomata .. *D. cayenensis*
1b. Frequency of paracytic stomata, 51-100%..... 3
  3a. Stomatal density, 10 or less ..... *D. alata*
  3b. Stomatal density, 20 or more 4
    4a. Stomatal index, 0-40 ..... *D. bulbifera*
    4b. Stomatal index, 41 and above 5
      5a. Stomatal length, 1.38 mm ... *D. esculenta*
      5b. Stomatal length, 1.76 mm ... *D. rotundata*

REFERENCES

AbdulRahaman, A. A., and *F. A. Oladele* (2003). Stomatal complex types, size, density, and index in some vegetable species in Nigeria. *Niger. J. Bot.* **16,** 144-150.

AbdulRahaman, A. A., and *F. A. Oladele* (2005). Stomata, trichomes, and epidermal cells as diagnostic features in six species of genus *Ocimum* L. (Lamiaceae). *Niger. J. Bot.* **18,** 214-222.

Abubakar, B. Y., and *A. I. Yunusa* (1998). Epidermal structure and stomatal ontogeny as an aid to the taxonomic identification of some species of *Acacia* (Leguminosae – Mimosoideae) from Nigeria. *Niger. J. Bot.* **11,** 117-123.

Adegbite, A. K. (1995). Leaf epidermal studies in three of

Nigerian species of *Aspilia* (Heliantheae – Asteraceae) and two hybrids. *Niger. J. Bot.* **18,** 25-33.

Akoroda, M. O., and *I. R. Chheda* (1983). Agrobotanical and species relationship of Guinea yams. *Trop. Agr.* (*Trinidad*) **60,** 242-248.

Alvin, K. L., and *M. C. Boulter* (1974). A controlled method of comparative study of taxodiaceous leaf cuticle. *Bot. J. Linn. Soc.* **62,** 277-286.

Ayensu, E. S. (1970). Comparative anatomy of *Dioscorea rotundata* and *D. cayenensis*, In: *New Research in Plant Anatomy* (Eds. N. E. Robson, D. F. Cutler, and M. Gregory). *Bot. J. Linn. Soc.* (*Suppl.*) **63,** 127-136.

Beerling, D. J., and *F. I. Woodward* (1997). Changes in land plant function over the Phanerozoic: reconstructions based on the fossil record. *Bot. J. Linn. Soc.* **124,** 137-153.

Burkill, I. H. (1939). Notes on the genus *Dioscorea* in the Belgian Congo. *Bull. Jard. Brux.* **15 (4),** 345-392.

Chevalier, A. (1936). Contribution a l'étude de quelques espèces Africaines du genre *Dioscorea*. *Bull. Mus. Nat. Hist. Bot. Paris, 2 C Ser.* **8 (6),** 520-521.

Dilcher, D. L. (1974). Approaches to the identification of angiosperm leaf remains. *Bot. Rev.* **40 (1),** 1-157.

Hamon, P., and *B. Toure* (2004). The classification of the cultivated yams (*Dioscorea cayenensis – rotundata* complex) of West Africa. *Euphytica* **47 (3),** 179-187.

Hutchinson, J., and *J. M. Dalziel* (1958). *Flora of West Tropical Africa*. Crown Agents for Oversea Government and Administrations, London.

Ikediobi, C. O., and *L. C. Igboanusi* (1983). Identification of yam (*Dioscorea* spp.) species and cultivars by use of electrophoretic patterns of soluble tuber proteins. *Biotropica* **15 (1),** 65-67.

Lamarck, J. (1789). *Dioscorea cayenensis*, In: *Encyclopediae Methodique*, *Vol. III*, p. 233. Paris.

Martin, F. W., and *A. M. Rhodes* (1978). The relationship of *D. cayenensis* and *D. rotundata*. *Trop. Agr.* (*Trinidad*) **55,** 193-206.

Metcalfe, C. R., and *L. Chalk* (1988). *Anatomy of the Dicotyledons*, *Vol. 1*, *2nd Edition*, 100-106. Clarendon Press, Oxford.

Miege, J. (1968). Dioscoreaceae, In: *Flora of West Tropical Africa, Ed. 2, Vol. 3* (Eds. J. Hutchinson and J. M. Dalziel), 144-154. London Crown Agents, London.

Noggle, G. R., and *G. J. Fritz* (1976). *Introductory Plant Physiology*, 322 pp. Prentice-Hall Inc., USA.

Obiremi, E. O., and *F. A. Oladele* (2001). Water conserving stomatal systems in selected *Citrus* species. *S. African J. Bot.* **67,** 258-260.

Ogunkunle, A. T. J., and *F. A. Oladele* (2000). Diagnostic value of trichomes in some Nigerian species of *Ocimum*, *Hyptis* Jazq., and *Tinnea* Kotschy and Peys (Lamiaceae). *J. Appl. Sci.* **3,** 1163-1180.

Okeke, S. E. (2001). The misconception of *Dioscorea cayenensis* sensu stricto (Dioscoreaceae). *Niger. J. Bot.* **14,** 101-114.

Okeke, S. E. (2004). The taxonomic position of members of the so-called *Dioscorea cayenensis – rotundata* complex (Dioscoreaceae). *Niger. J. Bot.* **17,** 95-103.

Olowokudejo, J. D., and *O. Pereira-Sheteolu* (1988). The taxonomic value of epidermal characters in the genus *Ocimum* (Lamiaceae). *Phytomorphology* **38 (2-3),** 147-158.

Onwueme, I. C. (1978). *The Tropical Tuber Crops*, 234 pp. John Wiley and Son, New York.

Onyilagha, I. C., and *J. Lowe* (1986). Studies on the relationship of *Dioscorea cayenensis* and *D. rotundata* cultivars. *Euphytica* **35,** 633-739.

Qiang, L., Long-Jiang, Y., Yang, D., Wei, L., Mao-Tang, L., and *C. Jiang-Hua* (2007). Leaf epidermal characters of *Lonicera japonica* and *Lonicera confusa* and their ecology adaptation. *J. Forest. Res.* **18 (2),** 103-108.

Pataky, S. (1969). Leaf epidermis of *Salix*, In: *Anatomy of the Dicotyledons*, *Vol. 1*, *2nd Edition* (Eds. C. R. Metcalfe and L. Chalk), p. 100. Clarendon Press, Oxford.

Poiret, J. C. (1813). *Dioscorea rotundata*, In: *Encyclopediae Methodique*, *Suppl. III*, p. 130. Paris.

Schols, P., Furness, C. A., Wilkin, P., Huysmans, S., and *E. Smets Fls* (2008). Morphology of pollen and orbicules in some *Dioscorea* species and its systematic implications. *Bot. J. Linn. Soc.* **136 (3),** 295-311.

Stephens, J. M. (2003). Yams – *Dioscorea* spp. University of Florida, IFAS Extension. www.edis.ifas.ufl.edu/MV153

Terauchi, R., Chikaleke, V. A., Thottapilly, G., and *S. K. Hahn* (1993). Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA. *Theoret. Appl. Genet.* **83,** 743-751.

Waitt, A. W. (1965). *A Field Key to Some Nigerian Varieties of Yam* (*Dioscorea* spp.), 127 pp. Agricultural Research Memorandum, No. 60, Ibadan.

Yuji, A. (2004). Classification of edible, indigenous *Dioscorea* species in Japan by leaf morphology and cross-compatibility. *J. Japan. Soc. Hort. Sci.* **73 (4),** 364-373.