

Leaf Epidermal Micromorphology of Some Land Races of *Dioscorea rotundata* L. Found in West Africa

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Abstract

Anatomical studies of the micromorphology of the leaves of 10 land races of *Dioscorea rotundata* L. found in West Africa were carried out using light microscopy to study the variations in anatomy within the cultivars which would help in the correct identification of the yam cultivars. These land races were Agbocha, Adaka, Alosi, Nwanyieri, Dorban, He-abalo, Dente, Obiaturugo, Ame and Atalibe. Epidermal cells were observed to be polygonal in Agbaochi and Adaka, but sinuous anticlinal epidermal cell walls were observed in the other land races. The leaves of all the plants were hypostomatic. Anisocytic stomata were found in Agbaocha, Adaka, Dorban, He-abalo, Dente and Atalibe. Paracytic and anisocytic stomata were found in Ame while anomocytic stomata were observed in Alosi and Nwanyieri. Mean stomatal index recorded were more in Agbocha (47.37%), Alosi (45.45%), Nwanyieri (44.64%) and less in Dorban (20%), Obiaturugo (20%) and Atalibe (23%). Stomatal length was highest in Dorban ($30.1 \pm 0.95 \mu\text{m}$) and lowest in Nwanyieri ($6.2 \pm 1.1 \mu\text{m}$) while stomatal width was also highest in Dorban ($19.3 \pm 1.1 \mu\text{m}$) and lowest also in Nwanyieri ($4.3 \pm 0.95 \mu\text{m}$). These diagnostic features are taxonomically revealing as observed in the 10 land races studied and can aid in the identification, characterization and description of the plants investigated.

Keywords: *Dioscorea rotundata*; land races; Cultivars; Yam; Micromorphology; Anatomy; Taxonomy.

1. Introduction

Dioscorea is a genus of the family Dioscoreaceae which are monocotyledons. Yam (*Dioscorea sp*) is an economically important starch staple crop in the tropical and subtropical regions of the world especially in Africa, Asia and the Caribbean regions. Among the over 600 species of yam known, only about six are edible while others are used as medicinal plants in the pharmaceutical industry [1-3]. Yam is among the oldest food crops. The family Dioscoreaceae is one of the oldest groups in the angiosperm [4]. Other economically important staple species of yam as food include *D. esculenta*, *D. dumetorium*, *D. bulbifera* (aerial yam), *D. cayenensis* (yellow yam), *D. alata* and *D. trifida*. *Dioscorea rotundata* “the white yam” is native to West Africa. The land races selected for the present study are Agbocha, Adaka, Alosi, Nwanyieri, Dorban, He-abalo, Dente, Obiaturugo, Ame and Atalibe.

Yam is a vine, herbaceous, tuberous with stem twinning clockwise or counter-clockwise, glabrous or sometimes producing prickles, some producing bulbils; branches modified into small aerial tubers. Flowers are staminate, unisexual and pistillate on the plant. White yam tuber is roughly cylindrical in shape. The flesh is usually white with the skin smooth and brown. It is about 1.6m in height.

World annual production of yam is 18 million tons with 15 million from West Africa [5]. Nigeria is the world's largest producer and exporter of yam accounting for over 70% of the total output. Cote d' Ivoire to Nigeria account for over 90% production of *D. rotundata* in Africa. Yam flour is food for over 50 % of the population of Benin Republic. Yam is an important dietary component for Nigerians and West Africans, contributing over 200 calories per person per day for over 160 million people in West Africa. It provides income for farmers and are available all year round making it an important food security crop for West tropical African countries [5].

Dioscorea spp. contains thiocynate, which has the potential for protection against sickle cell anemia [6]. It prevents colon cancer risk by preventing toxic compounds in the food from sticking to the colon mucosa and also reduces constipation. The tuber milk contains allatoin that hastens healing when applied externally on ulcer, boil and abscissions. The decoction is used to stimulate appetite and to relieve bronchial irritation and cough. Yam is eaten during pregnancy, there are sapogenins known as diosgenin at the rhizome. Diosgenin help to regulate menstrual flow, relieves cramping and pain during pregnancy.

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Micromorphological features have been useful in taxonomic study. Micromorphology have been used in leaf morphology of *Saponaria* (Caryophyllaceae) [7], *Gomphrena* and *Achyranthes* (Amaranthaceae) [8, 9]. Seed coat micromorphology have been studied in Aizoaceae [10] and Molluginaceae [11]. This work would be useful in understanding the taxonomic and phylogenetic relationship within the species and genera.

2. Materials and Methods

Plants materials were collected from the experimental farm of National Root Crops Research Institute (NCRCI), Umudike, Nigeria and were deposited in the departmental herbaria. Samples were soaked in Jeffrey' solution (equal parts of 10 % chromic acid and concentrated Nitric acid) and placed for 15 – 60 minutes in water bath depending on the thickness of the leaf. An area of about 1 cm² was removed from a central position of the matured leaf [12]. The adaxial and abaxial epidermis was peeled using a forcep and dissecting needles. The samples were washed in water, stained in safranin. The membranes were put in 50% ethyl alcohol for 5 minutes. The epidermis were then dehydrated and mounted in Canada balsam. The epidermal characters of the cell, stomata, and trichome were studied. Quantitative features such as length of the epidermal cell, size of stomata and stomatal index have been determined as follows:

$$SI = (S \times 100 / E + S)$$

Where SI is the stomatal index percent, S is the number of stomata/field of study, and E is the number of epidermal cells/field of study.

All observations and photomicrographs of epidermal peels were taken from the slide preparation using a Leitz Wetzler Ortholux fitted with Vivatar-335 Camera.

3. Result

The descriptive terminologies used by Metcalfe and Chalk (1983) have been followed in this work. A summary of the localities of the plants investigated are shown in Table (1). All the leaves of the plants studied are hypostomatic. The stomatal types were anisocytic in Agbocha, Dorban, He-abalo, Dente, Obiaturugo and Atalibe. Alosi and Nwanyieri were anomocytic. The stomatal length and width were more in Dorban, He – abalo, Obiaturugo, Dente, Agbaocha, Adaka and Ataibe but low in Alosi, Nwanyieri and Ame (Table 2). Stomatal index were higher in Agbocha (47.37%), Alosi (36.84%), Adaka (36.84%), Dente (36%), Nwanyieri (44.64%) and but were low in Obiaturugo (20 %), Dorban (20 %), Atalibe (23 %), He-abalo (31.25 %) and Ame (32.43 %) (Table 2).

Micromorphological features of the cultivars investigated showed that the abaxial and adaxial surfaces have ordinary epidermal cells with polygonal sinuous anticlinal epidermal cell walls (Plate 1-6). The epidermal cells of the leaves of Ame were paracytic and anisocytic (Table 2). Length of the epidermal cells varied from 3.67 ± 0.58 µm in the adaxial surface of Atalibe to 61.67 ± 1.53 µm in the adaxial surface of He-abalo. Long pointed and needle like trichomes were present in the adaxial surfaces of Alosi and Nwanyieri (Plate 2b and 3d)

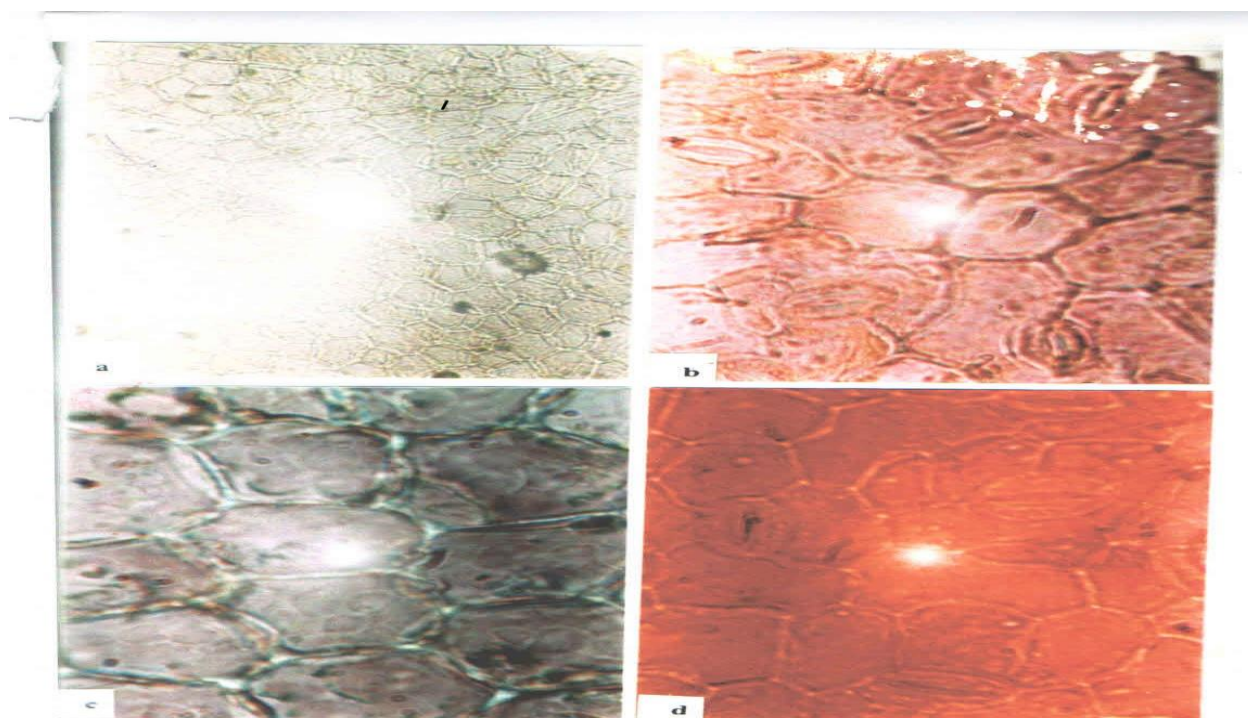


Plate 1: (a) Agbaocha (UE): Showing straight wall type arrangement of leaf epidermal cell wall (b) Agbaocha (LE): Showing curved shape wall arrangement and also the presence of stomata. (c) Adaka (UE): Showing curved shape wall arrangement of leaf epidermal cell wall. (d) Adaka (LE): Showing curved shape wall arrangement and also the presence of stomata.

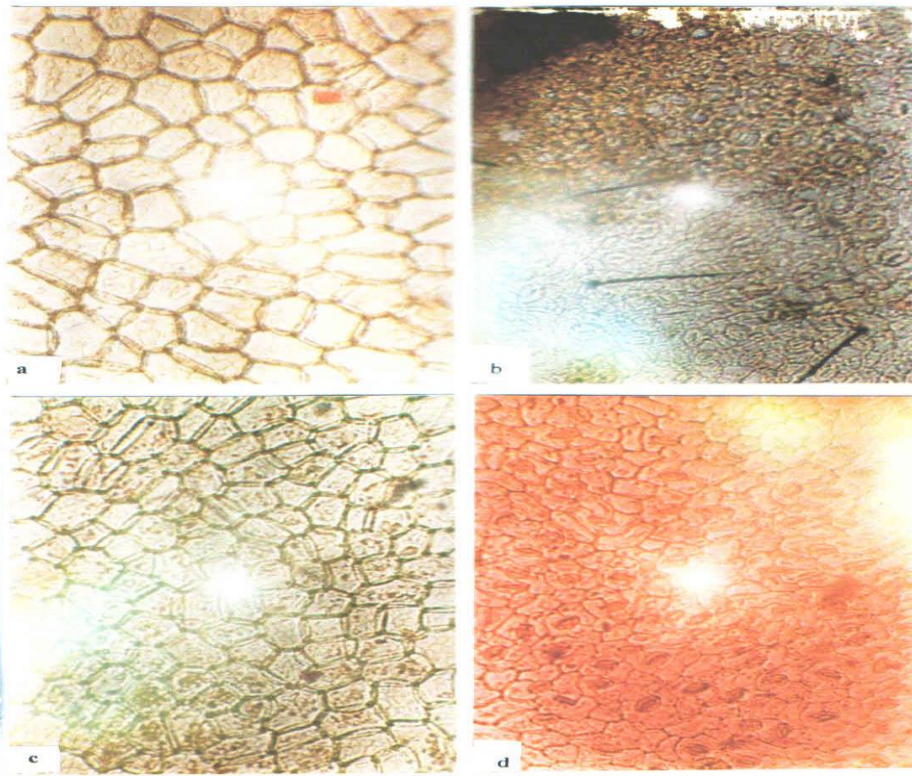


Plate 2: (a) Alosi (UE): Showing curved shape wall arrangement of leaf epidermal cell wall
 (b) Alosi (LE): Showing curved shape wall arrangement with presence of stomata trichomes which appeared as a nail-like structure.
 (c) Nwanyieri (UE): Showing straight arrangement of leaf epidermal cell wall.
 (d) Nwanyieri (LE): Showing curved arrangement and also the presence of stomata.

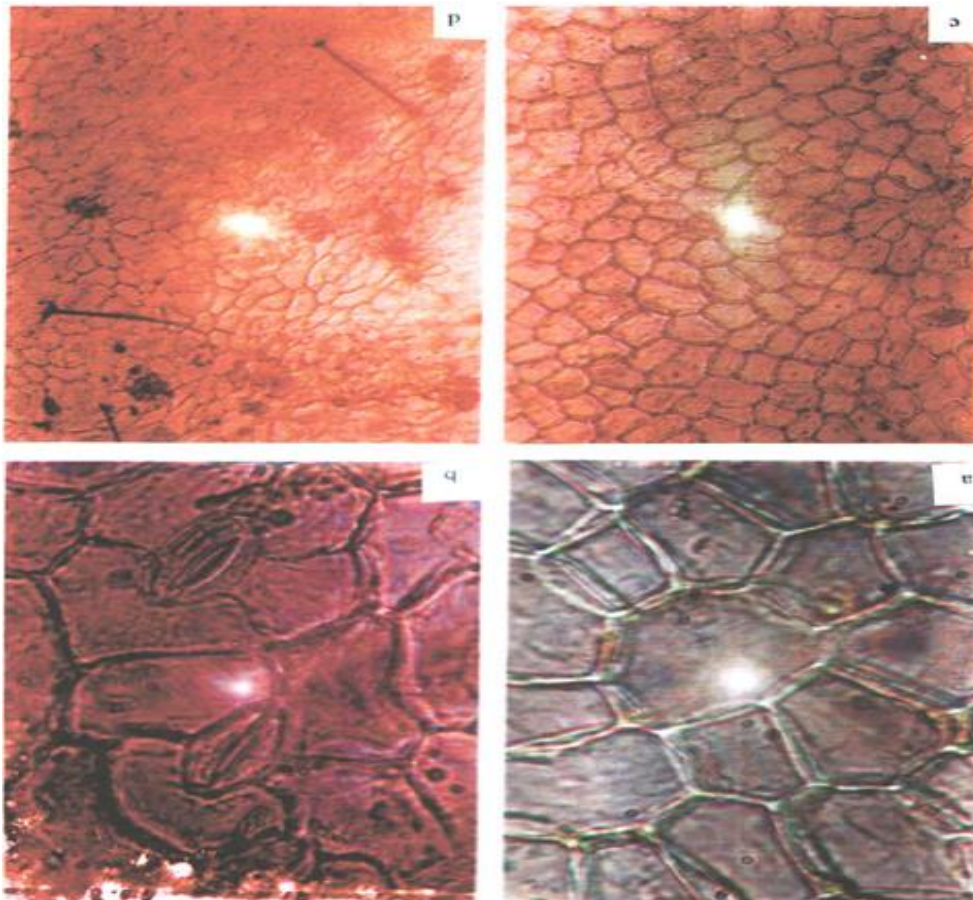


Plate-3. (a) Dorban (UE): Showing a straight arrangement of leaf epidermal cell wall
 (b) Dorban (LE): Showing curved arrangement and presence of stomata
 (c) Nwanyieri (UE): Showing curved arrangement of leaf epidermal cell wall.
 (d) Nwanyieri (LE): Showing curved arrangement and presence of stomata with trichomes which appeared as a nail-like structure.

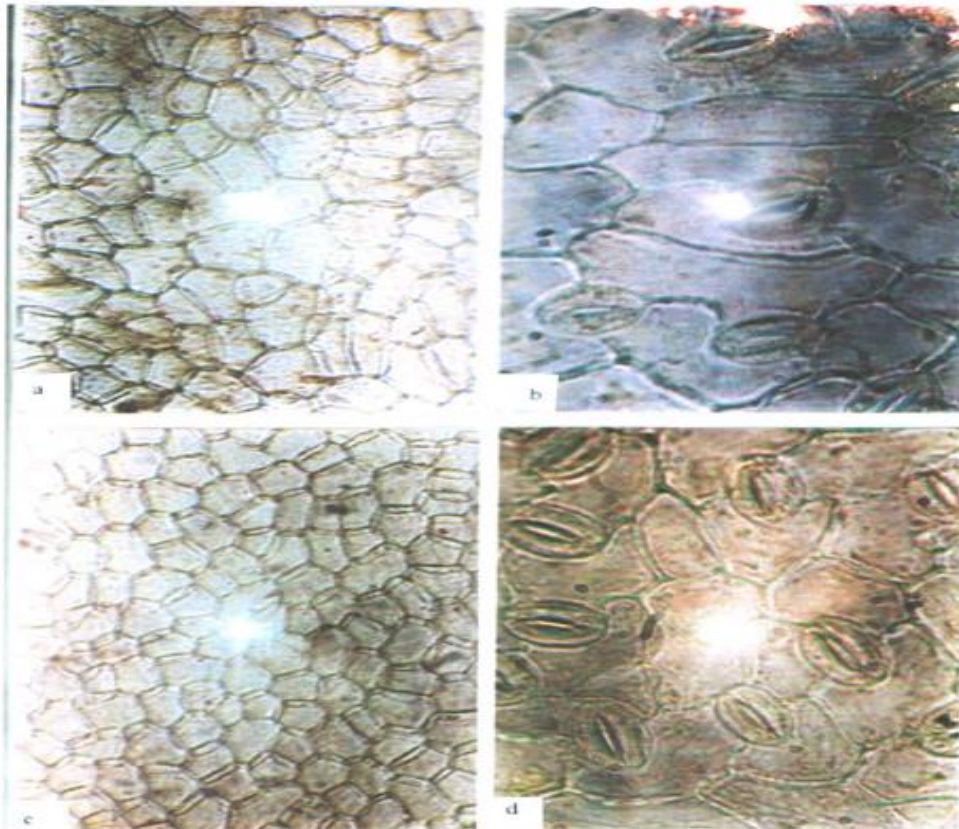


Plate 4: (a) He-abalo (UE): Showing a curved arrangement of leaf epidermal cell wall
 (b) He-abalo (LE): Showing curved arrangement and presence of stomata
 (c) Dente (UE): Showing curved arrangement of leaf epidermal cell wall.
 (d) Dente (LE): Showing curved arrangement and presence of stomata.

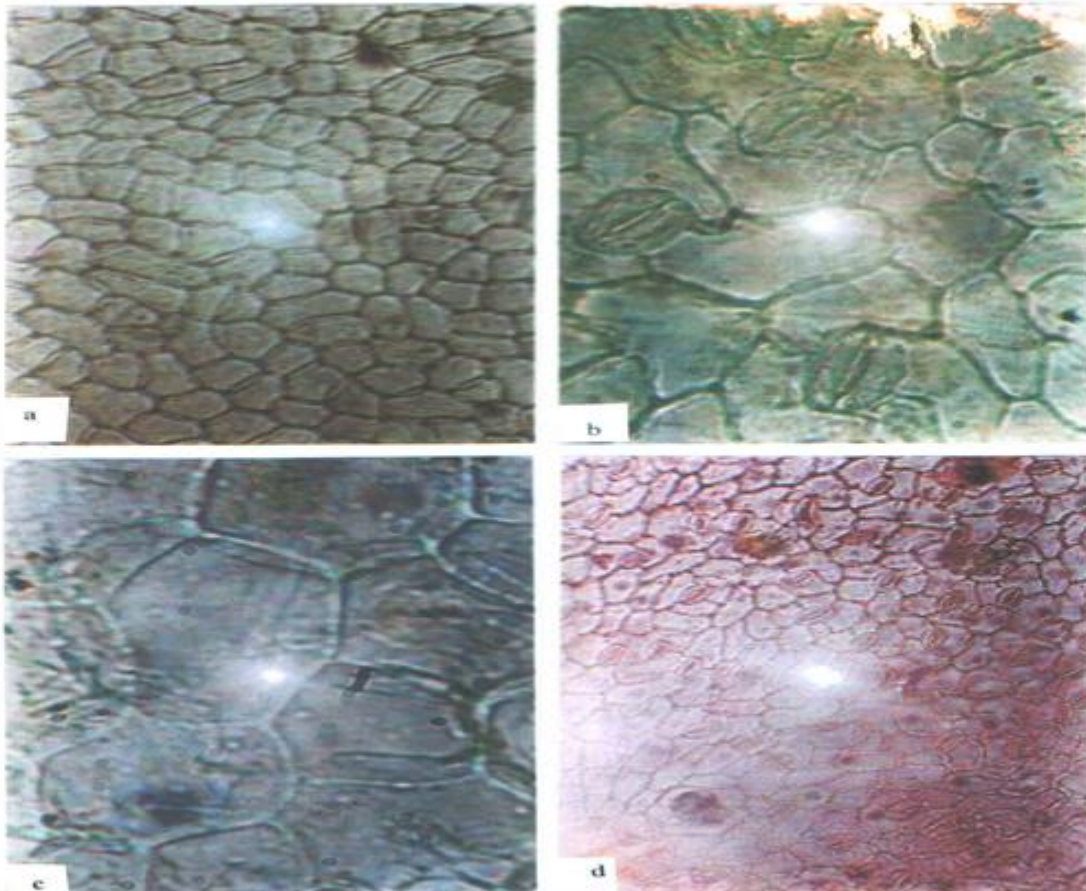


Plate 5: (a) Oblaturugo (UE): Showing a curved arrangement of leaf epidermal cell wall
 (b) Obiaturugo (LE): Showing curved arrangement and presence of stomata
 (c) Ame (UE): Showing curved arrangement of leaf epidermal cell wall.
 (D) Ame (LE): Showing curved arrangement and presence of stomata.

UP=Upper epidermis (abaxial surface)
 LE=Lower epidermis (adaxial surface)

Table-1. List of the locality of the land races investigated

Cultivar	Locality
A gbaocha	Abakiliki, Nigeria
Adaka	Achala, Anambra, Nigeria
Aloshi	Benue, Nigeria
Nwanyieri	Ndoki, Abia, Nigeria
Dorban	Benue, Nigeria
He-abalo	Togo
Dente	Ghana
Obiaturugo	Umudike, Abia, Nigeria
Ame	Benue/Nasarawa, Nigeria
Atalibe	Togo

Table-2. Stomatal characteristics of the land races investigated

Cultivar	Type of Stomata	Stomatal length	Stomatal width	Stomatal index (%)	Distribution of stomata
Agbaocha	Anisocytic	21.1±1.05	14.2±1	47.37	Hypostomatic
Adaka	Anisocytic	21.3±0.80	14.2±0.95	36.84	Hypostomatic
Aloshi	Anomocytic	8.1±0.9	5.3±0.9	45.45	Hypostomatic
Nwanyieri	Anomocytic	6.2±1.1	4.3±0.96	44.64	Hypostomatic
Dorban	Anisocytic	30.1±0.95	19.3±1.1	20	Hypostomatic
He-abalo	Anisocytic	24.1±1.1	14.1±1.2	31.25	Hypostomatic
Dente	Anisocytic	22.2±0.95	16.3±1.1	36	Hypostomatic
Obiaturugo	Anisocytic	24.1±1	16.4±1.2	20	Hypostomatic
Ame	Paracytic & Anisocytic	8.2±0.95	4.3±0.95	32.43	Hypostomatic
Atalibe	Anisocytic	21.5±0.83	12.3±1.1	23	Hypostomatic

Stomatal length and width in μm (mean \pm standard deviation)

Table-3. Epidermal characteristics of the land races investigated

Cultivar	Side	Shape of epidermal cell	Length of epidermal cell (μm)	Type of trichome
Agbaocha	Abaxial	Polygonal	10.3±0.58	
	Abaxial	Polygonal to sinuous	40.3±0.58	
Adaka	Abaxial	Polygonal to sinuous	39.6±1.53	
	Abaxial	Polygonal to sinuous	35±0.58	
Aloshi	Abaxial	Polygonal	18±1	
	Abaxial	Polygonal to sinuous	4.67±0.58	Long pointed
Nwanyieri	Abaxial	Polygonal	12±1	
	Abaxial	Polygonal to sinuous	10.3±0.58	Long pointed
Dorban	Abaxial	Polygonal	46.3±1.52	
	Abaxial	Polygonal to sinuous	41.6±0.58	
He-abalo	Abaxial	Polygonal	21±1	
	Abaxial	Polygonal to sinuous	61.67±1.53	
Dente	Abaxial	Polygonal	16±1	
	Abaxial	Polygonal to sinuous	40.3±1.53	
Obiaturugo	Abaxial	Polygonal	15±1	
	Abaxial	Polygonal to sinuous	39.7±1.53	
Ame	Abaxial	Polygonal	49±3.6	
	Abaxial	Polygonal to sinuous	3.67±0.58	
Atalibe	Abaxial	Polygonal	15.3±0.58	
	Abaxial	Polygonal to sinuous	23.67±1.58	

Epidermal length and width in μm (mean \pm standard deviation)

4. Discussion

This study depicts the micromorphological characters of ten cultivars of *D. rotundata* namely; Agbaocha, Adaka, Aloshi, Nwanyieri, Dorban, He-abalo, Dente, Obiaturugo, Ame and Atalibe. All the leaves were found to be hypostomatic. In anisocytic stomata, the subsidiary cells of the guard cells pair with the three surrounding subsidiary cells and are of unequal size. Anomocytic stomata are also called irregular celled type of stomata surrounded by limited number of cells which cannot be distinguished from other epidermal cells. Subsidiary cells are absent. Paracytic stomata are stomatal complex having one or more subsidiary cells parallel to each guard cell.

Anomocytic and anisocytic stomata have been found in *D. bulbifera*. Anomocytic stomata have been reported in *Dioscorea hispida* [13], *D. cayenensis*, *D. dumetorum* and *D. atala* [14]. The presence of anomocytic, anisocytic and paracytic stomata may be used as a distinguishing feature for the identification and separation of *D. rotundata* from other *Dioscorea* species. The presence of trichomes in Aloshi and Nwanyieri can be used to separate the two

cultivars from the other land races investigated. Presence of trichomes with multicellular head is an indication of medicinal lodgement of bioactive compounds and principles in plant and may correlate with its therapeutic potential [15]. Leaf epidermal micromorphological studies have been carried out on several species and genera of plants which include; *Boerhavia* L. (Nyctaginaceae) [16], *Cercis* (Fabaceae) [17], *Portulaca* [18], *Pistacia* (Anacardiaceae) [19], *Peucedanum* (Umbelliferae) [20], *Melastoma* L. (Melastomataceae) [21], *Myrceugenia rufa* (Myrtaceae) [22], *Elaeagnus angustifolia* L. (Elaeagnaceae) [23].

Combination of diagnostic features such as quantitative and microscopic parameters including stomatal trichomes and epidermal cell types, sizes and shapes have been employed for taxonomic distinction and recognition of different angiosperm families. Hence, foliar micromorphological description of plants are relevant in taxonomic examination and delineation of plants and can complement other taxonomic characters which could be useful in the preparation of monograph for its identification, therefore generating important tool for the collection and preservation of different plant species. In summary, the macromorphological characters identified in these ten land races of *Dioscorea rotundata* L. from West Africa can be useful for intra specific separation of this *Dioscorea rotundata*

References

- [1] Dehl, L., 1981. "Smallholder farming systems with yam in the Southern Guinea Savannah of Nigeria Eschborn GTZ (Gesellschaft fur Technische Zusammenarbeit)."
- [2] Hahn, S. K., Osiru, D. S. O., Akoroda, M. O., and Otoo, J. A., 1987. "Yam production and its future prospects." *Outlook on Agriculture*, vol. 16, pp. 105-110.
- [3] Okwor, G. C., 1993. *The production, economic and socio-cultural importance of yam in Africa. Keynote address at the inauguration and first seminar of the first of the African Yam Network (AYN) 26-28 Oct., 1993*. Cotonu: Republic of Benin. p. 21.
- [4] Burkill, I. H., 1960. "The organography and the evolution of Dioscoreaceae, the family of the yams." *Journal of the Linnean Society of London Botany*, vol. 56, pp. 319-412.
- [5] IITA, 2009. *International institute of tropical agriculture yam (Dioscorea species)*. Ibadan Nigeria.
- [6] Agbai, O., 1989. "Anti-sickling effect of dietary thiocyanate in prophylactic control of sickle cell anemia." *Journal of the National Medical Association*, vol. 78, pp. 1053-1056.
- [7] Atasslar, E., 2004. "Morphological and anatomical investigations on the saponaria kotschy boiss. (Caryophyllaceae)." *Turkish Journal of Botany*, vol. 28, pp. 193-199.
- [8] Frank -de- Carvalho, S. M., Gomes, R. D. A., Silva, P. F. T., and Bao, S. N., 2010. "Leaf surface of Gomphrena spp (Amaranthaceae) from Cerrado biome." *Biocell*, vol. 34, pp. 23-35.
- [9] Agarwal, T. and Yadav, A. S., 2007. "Micromorphology of the leaf epidermis of Achyranthes aspera L." *Journal of Phytological Research*, vol. 20, pp. 61-66.
- [10] Hassan, N. M. S., Meve, U., and Liede-Schumann, S., 2005. "Seed coat morphology of Aizoaceae - Sesuvioideae, Gisekiaceae and Molluginaceae and its systematic significance." *Botanical Journal of the Linnean Society*, vol. 148, pp. 189-206.
- [11] Sivarajan, V. V. and Gopinathan, M., 1985. "Seed coat micromorphology of Caryophyllates: observations on some Molluginaceae." *Proceedings: Sciences*, vol. 94, pp. 51-57.
- [12] Olowokudejo, J. D., 1990. "Comparative morphology of the epidermis in the genus Annona (Annonaceae) in West Africa." *Phytomorphology*, vol. 40, pp. 407-422.
- [13] Salmah, T., Nashriyah, M., Abdul-Ghani, Y., and Shamsul, B. A., 2013. "Anatomical study of petiole, leaf, tuber, root and flower of Dioscorea hispida Dennst (Dioscoreaceae) by using optical microscope, SEM and TEM." *J. Agrobiotechnol*, vol. 4, pp. 32-41.
- [14] Aina, O. D. and Atumeyi, S., 2011. "Foliar epidermal anatomy of four species of Dioscorea." *Adv. Applied Sc. Research*, vol. 2, pp. 21-24.
- [15] Sonibare, M. A. and Adeniran, A. A., 2014. "Comarative micromorphological study of wild and micro propagated Dioscorea bulbifera Linn." *Asian Pac J. Trop Biomed*, vol. 4, pp. 176-183.
- [16] Edeoga, H. O. and Ikem, C. I., 2001. "Comparative morphology of the leaf epidermis in three species of Boerhavia L. Nyctaginaceae." *Journal of Economic and Taxonomic Botany*, vol. 19, pp. 197-205.
- [17] Zou, P., Liao, J., and Zhang, D., 2008. "Leaf epidermal micromorphology of Cercis (Fabaceae : Caesalpinoideae)." *Botanical Journal of the Linnean Society*, vol. 158, pp. 539-547.
- [18] Srivastava, A., Joshi, A. G., and Raole, V. M., 2013. "Leaf epidermal micromorphology of Portulaca L. species found in Vadodara, Gujarat, India 2013." *Journal of Botany*, pp. 1-5.
- [19] Belhadj, S., Denidj, A., Aigouy, T., Gers, C., Gauquelin, T., and Mevy, J. P., 2007. "Comparative morphology of leaf epidermis in eight populations of Atlas Pistachio (Pistacia atlantica Desf.) Anacardiaceae." *Microsc. Res. Tech.*, vol. 70, pp. 837- 846.
- [20] Zhou, J., Wang, W., Gong, X., and Liu, Z., 2014. "Leaf epidermal morphology in Peucedanum L. (Umbelliferae) from China." *Acta Botanica Gallica*, vol. 161, pp. 21-31.
- [21] Haron, N. W., Anuar, N., and Veeramohan, R., 2015. "The taxonomic significance of leaf micromorphology in the genus Melastoma L. (Melastomataceae)." *Sains Malaysiana*, vol. 44, pp. 645-650.
- [22] Retamales, H. A., Cabello, A., Serra, M. T., and Scharaschkin, T., 2015. "Leaf micromorphology and anatomy of Myrceugenia rufa (Myrtaceae). An endemic coastal shrub of north -central Chile." *Gayana Bot*, vol. 72, pp. 76-83.

- [23] Li, M., Liu, Y., Liu, M., and Liu, D., 2015. "Comparative studies on leaf epidermal micromorphology and mesophyll structure of *Elaeagnus angustifolia* L." *Science in Cold and Arid Regions*, vol. 7, pp. 229-237.