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 Caribbean 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, phytchemistry, 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioscorea alata L.</td>
<td>Water yam</td>
</tr>
<tr>
<td>Dioscorea bulbifera L.</td>
<td>Aerial yam</td>
</tr>
<tr>
<td>Dioscorea cayenensis Lam.</td>
<td>Yellow yam</td>
</tr>
<tr>
<td>Dioscorea dumetorum (Kunth) Pax</td>
<td>Bitter yam</td>
</tr>
<tr>
<td>Dioscorea esculenta (Lour.) Burkill.</td>
<td>Chinese yam</td>
</tr>
<tr>
<td>Dioscorea rotundata Poir.</td>
<td>White yam</td>
</tr>
</tbody>
</table>

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.

Table 2. Stomatal anatomy in some species of Dioscorea.

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf surface</th>
<th>Stomatal complex types</th>
<th>Frequency of stomatal complex type (%)</th>
<th>Stomatal size (µm)</th>
<th>Stomatal density (mm$^{-2}$)</th>
<th>Stomatal index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioscorea alata</td>
<td>Abaxial</td>
<td>Paracytic</td>
<td>50.00</td>
<td>0.74±0.01</td>
<td>10.57±2.06 (3-20)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Tetracytic</td>
<td>32.00</td>
<td>18.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anisocytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioscorea bulbifera</td>
<td>Abaxial</td>
<td>Paracytic</td>
<td>87.60</td>
<td>1.64±0.02</td>
<td>27.83±4.23 (12-55)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Anisocytic</td>
<td>12.40</td>
<td>0.82±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioscorea cayenensis</td>
<td>Abaxial</td>
<td>Anisocytic</td>
<td>100.00</td>
<td>1.47±0.04</td>
<td>17.75±4.46 (7.42)</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Tetracytic</td>
<td></td>
<td>8.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioscorea dumetorum</td>
<td>Abaxial</td>
<td>Tetracytic</td>
<td>91.05</td>
<td>1.79±0.03</td>
<td>10.85±2.29 (4-20)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Paracytic</td>
<td></td>
<td>8.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diacytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioscorea esculenta</td>
<td>Abaxial</td>
<td>Paracytic</td>
<td>100.00</td>
<td>1.38±0.02</td>
<td>21.25±1.52 (15-28)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioscorea rotundata</td>
<td>Abaxial</td>
<td>Paracytic</td>
<td>100.00</td>
<td>1.38±0.02</td>
<td>21.25±1.52 (15-28)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Diacytic</td>
<td></td>
<td>10.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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
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 \(\mu m\) as "small" and those with guard cells measuring more than 38 \(\mu m\) 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-Seteolu, 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. dumetorum
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


Adegbite, A. K. (1995). Leaf epidermal studies in three of
Nigerian species of Aspilia (Helianthae – Asteraceae) and two hybrids, Nig. J. Bot. 18, 25-33.


