



Leaf anatomical investigations of four indigenous vegetables in south west Nigeria

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Abstract

Anatomical investigations of four indigenous vegetables in South West Nigeria was conducted, the vegetables are *Celosia argentea* L.; *Crassocephalum rubens*, (Juss.) S. Moore; *Senecio biafrae* (Olive. and Hern) C. Jeffrey and *Solanum americanum* Mill. The anatomical materials were prepared according to standard methods and observations were made with compound light microscope. Anatomical observations made from this study revealed diagnostic characters that can be used for the identification of each of these vegetables. *Celosia argentea* can be delimited from the other vegetables studied by the presence of straight anticlinal wall (on the adaxial surface), anomocytic stomata and low stomata index. *Crassocephalum rubens* has relatively the smallest epidermal cell and the largest stomata size with the absence of crystals. *Senecio biafrae* and *Solanum americanum* have the highest percentage of stomata index on the adaxial surface and have prismatic crystals. Conclusively, the results show leaf anatomical characters which can be employed for the identification of the vegetable species.

Keywords: abaxial, adaxial, crystal, prismatic, stomata

Introduction

Vegetables are valuable sources of nutrition and they supply most of the required vitamins, essential minerals, fibers, carbohydrate and protein that the body needs (Schippers, 2000) [8]. *Celosia argentea* L. belongs to the family *Amaranthaceae*. It is one of the four species of *Celosia* occurring in Nigeria. *Celosia argentea*, called sokoyokoto in Yoruba dialect is an erect annual herb with alternate leaves. The stems are angular, pinkish at the base but green at the top. *Crassocephalum rubens* (Juss.) S. Moore and *Senecio biafrae* (Olive. and Hern) C. Jeffrey belong to the family *Asteraceae*. There are nine species in the genus *Crassocephalum* in Nigeria. *Crassocephalum rubens* called Ebole is an erect annual herb with alternate-opposite leaves. There are four species of *Senecio* in Nigeria. *Senecio biafrae* is an annual herb with compound leaves that are alternate to opposite. *Solanum americanum* Mill is called American nightshade and it belongs to the family *Solanaceae*. There are seventeen species of *Solanum* in Nigeria but *Solanum americanum* called odu in Yoruba dialect is a leafy vegetable. It is an herbaceous flowering plant with alternate leaves.

The four vegetables used for this study are under leafy traditional or indigenous vegetables that over the years have contributed to household food security. These vegetables have their ethno-botanical importance in the area of study. The present study is aimed at investigation the phylogenetic relationship between the vegetables using morphological and leaf anatomical parameters of the vegetables.

Materials and Methods

Plant materials were collected from various locations around Obafemi Awolowo University, Ile-Ife (latitude 07 30° and longitude 04 40°) in South- West Nigeria.

Sizeable portions of the matured leaves of the species were cut from the median part of well expanded leaves. For epidermal peels preparations, Metcalfe (1960) [6] scrape method was used. Epidermal peels of both adaxial and abaxial surfaces were made by placing the desired epidermal surface face down on a glass slide; scraping off with a sand paper and a sharp razor blade all tissues above the desired epidermis and wash the scraped off part with water until the epidermis is reached. The epidermal peels were stained in 1% aqueous Safranin O solution for 3-5 minutes, rinsed thoroughly with water to remove excess stain and then mounted in 25% glycerol for microscopic examination. All processed materials were preserved in 50% ethyl alcohol until when required.

All microscopic measurements were taken with the aid of an ocular micrometer inserted in the eyepiece of the microscope. These measurements were later multiplied by ocular constant with respect to the power under which they were taken. Observations were made under a light microscope of both abaxial and adaxial surfaces. The guard cell, stoma, trichomes and epidermis were carefully studied. Photomicrographs of the epidermal surfaces were taken on different observations made on both surfaces of the sections of the four species with Amscope MT microscope camera version 3.001 attached to a light microscope. The guard cell area was calculated by measuring the length and the width of ten guard cells per species (this was done for both the abaxial and adaxial surfaces). The length and breadth of the guard cells were measured using the ocular micrometer at the magnification X400.

The Stomata index (I) was calculated by expressing the number of stomata (S) per unit area as a percentage of the sum of total number of epidermal cells (E) and stomata of the same unit area. This was done for both surfaces of the leaves using

the formula:

$$I = S / (E + S) \times 100 \quad (1)$$

Where,

I - Stomata Index

S - Number of stomata per unit area

E - Number of epidermal cells in the same unit area

The mean, standard deviation and standard error were calculated.

Results

Table 1: Species Studied and their Geographical locations

Names of Species	Sites of Collection	Coordinates
<i>Celosia argentea</i>	Farm along Road 7 gate Area, O.A.U. Ile-Ife	N 07 ⁰ 30.789, E 004 ⁰ 32.923, 251m
	Old Bukateria O.A.U. Ile-Ife	N 07 ⁰ 31.299, E 004 ⁰ 31.207, 298m
<i>Crassocephalum rubens</i>	Along the stream to Conference Centre, O.A.U. Ile-Ife.	N 07 ⁰ 31.420, E 004 ⁰ 31.836, 269m
	Along Road 7, Olonade street, Ile-Ife.	N 07 ⁰ 31.145, E 004 ⁰ 31.643, 275m
<i>Senecio biafrae</i>	Botanical Garden. O.A.U. Ile-Ife	N 07 ⁰ 31.248, E 004 ⁰ 31.593, 280m
	Along the stream to Conference Centre, O.A.U. Ile-Ife.	N 07 ⁰ 31.420, E 004 ⁰ 31.836, 269m
<i>Solanum americanum</i>	Parks and Garden, O.A.U. Ile-Ife.	N 07 ⁰ 31.404, E 004 ⁰ 31.823, 263m
	Old Bukateria O.A.U. Ile-Ife	N 07 ⁰ 31.299, E 004 ⁰ 31.207, 298m
<i>Solanum americanum</i>	Farm along Road 7 gate Area, O.A.U. Ile-Ife.	N 07 ⁰ 30.789, E 004 ⁰ 32.923, 251m
	Behind Conference Centre, O.A.U. Ile-Ife.	N 07 ⁰ 31.421, E 004 ⁰ 31.835, 271m
	Botanical Garden. O.A.U. Ile-Ife.	N 07 ⁰ 31.248, E 004 ⁰ 31.593, 280m
	Farm along Road 7 gate Area, O.A.U. Ile-Ife	N 07 ⁰ 30.789, E 004 ⁰ 32.923, 251m

Anatomical Descriptions

Celosia argentea Linn.

Epidermal cells are polygonal in shape with straight anticlinal wall on the adaxial surface and wavy anticlinal wall on the abaxial surface. On the adaxial surface, the epidermal cells are 7.49- 14.2 µm long and 3.21 – 7.49µm width and they are 5.35 – 16.00µm long and 3.57 – 15.35µm wide on the abaxial surface (Fig 1)

Stomata are anomocytic, elliptic in shape (Fig 1), stomata size for the adaxial surface is 0.53 – 62.8µm² and 1.60–3.28µm² on the abaxial, stomata index for the adaxial surface is 8.27– 12.00 % and 7.80 – 13.00% on the abaxial surface. Trichomes are sparse on epidermal.

Crassocephalum rubens (Juss.) S. Moore

Epidermal cells are irregular in shape on the adaxial surface; the epidermal cells are 0.35 – 4.99µm long and 1.07 – 2.14 µm wide with sinous anticlinal walls on both surfaces. On the abaxial surface, the epidermal cells are 1.78- 3.21 µm long and 0.71 – 2.14µm wide (Fig 2).

Stomata are mainly paracytic and elliptic in shape with small protrusions at the plar ends in some stomata. Stomata size for the adaxial surface is 0.01 – 0.53µm² and 0.21–0.53µm² on the abaxial, stomata index for the adaxial surface is 11.90 - 38.50 % and 11.10 – 15.40% on the abaxial surface. Trichomes are present on both surfaces with a greater distribution on the abaxial surface. The trichomes length ranges between 7.85 – 22.80µm and the width between 2.14 – 2.14µm on the

adaxial. On the abaxial surface it is 7.85 – 22.80µm long and the width is between 2.14 – 2.14µm on the abaxial (Table 3).

Senecio biafrae (Olive and Hern) C. Jeffrey

Epidermal cells are polygonal in shape, on the adaxial surface, the epidermal cells are 1.78- 4.21µm long and 0.35 – 1.07µm wide with wavy anticlinal walls. On the abaxial surface, they are 3.57 – 7.85µm long and 2.14 – 6.06µm wide with wavy anticlinal wall (Fig 3)

Stomata are mainly paracytic, elliptic in shape. Stomata size for the adaxial surface is 6.42 – 28.56µm² and 1.42 - 4.74µm² on the abaxial, stomata index for the adaxial surface is 9.80– 43.86% and 11.00 – 30.60% on the abaxial surface (Table 4). The trichomes length ranges between 16.00 – 48.10µm and the width between 2.49 – 30.30µm on the adaxial.

Solanum americanum Mill.

Epidermal cells are polygonal in shape on both surfaces. On the adaxial surface, the epidermal cells are 4.64- 14.90µm long and 6.06 – 13.56µm wide with wavy anticlinal walls. On the abaxial surface, they are 2.85 – 14.90µm long and 0.35 – 25.30µm wide with sinous anticlinal walls (Fig 4)

Stomata are paracytic, elliptic in shape. Stomata size for the adaxial surface is 1.24 – 4.06µm² and 1.14 - 5.42µm² on the abaxial, stomata index for the adaxial surface is 16.50 – 32.80% and 20.00 – 42.41% on the abaxial surface (Table 5). Trichomes are present on the abaxial, On the abaxial surface it is 25.70– 72.10µm long and 3.57 – 17.85µm wide.

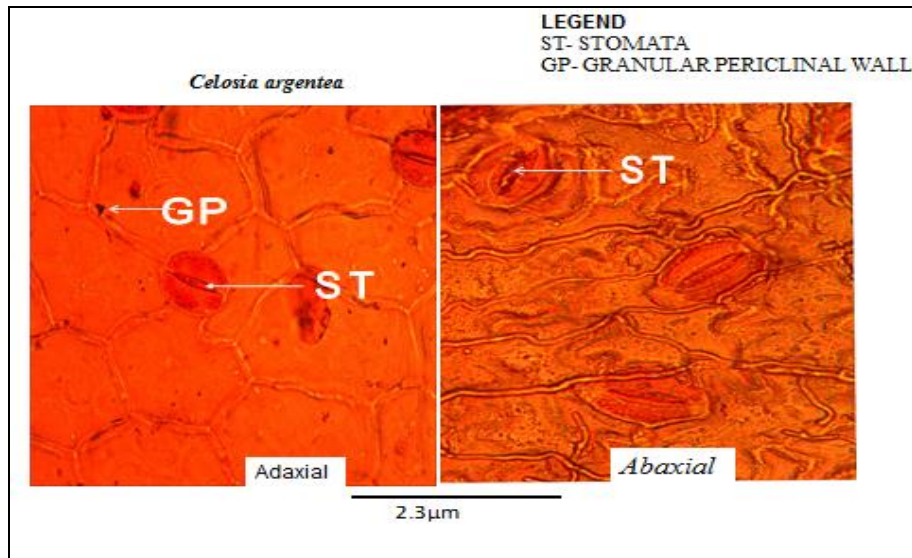


Fig 1

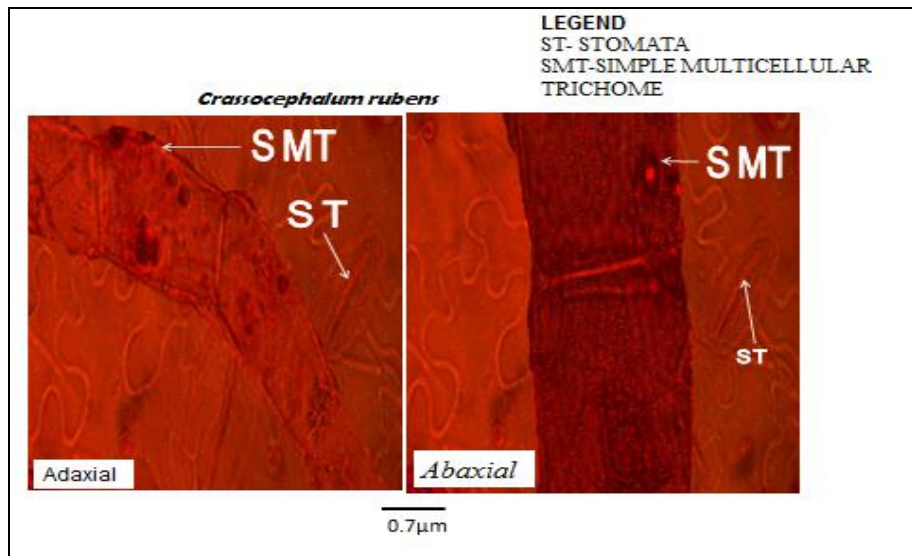


Fig 2

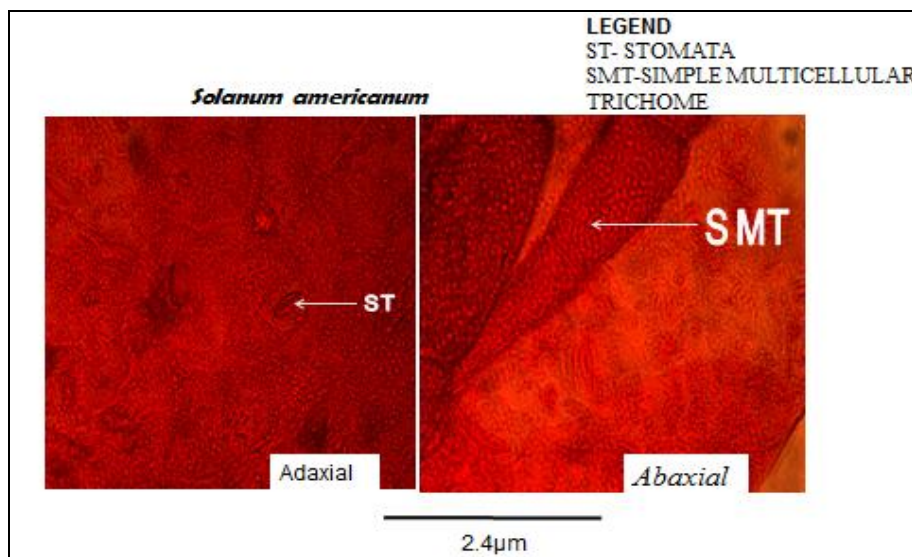


Fig 3

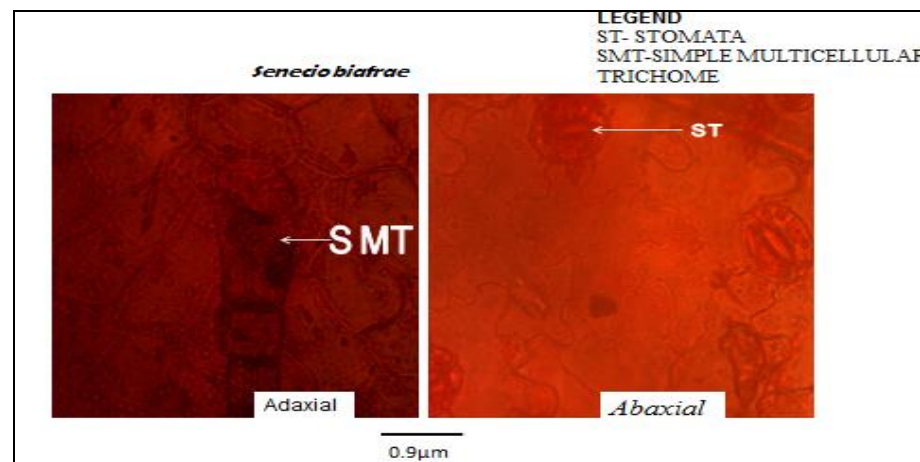


Fig 4

Table 2: Quantitative Parameters of the Adaxial Epidermal Structures of the Vegetables studied

Variables	<i>Celosia argentea</i>					<i>Crassocephalum rubens</i>					<i>Senecio biafrae</i>					<i>Solanum americanum</i>				
	Min Value	Max Value	Mean	S.E	S.D	Min Value	Max Value	Mean	S.E	S.D	Min Value	Max Value	Mean	S.E	S.D	Min Value	Max Value	Mean	S.E	S.D
length of epidermal cells (µm)	7.49	14.20	9.81	0.03	0.01	0.35	4.99	3.28	0.03	4.37	1.78	4.21	2.78	4.51	14.28	4.64	14.90	9.38	0.09	2.92
width of epidermal cells (µm)	3.21	7.49	7.06	2.47	0.07	1.07	2.14	1.60	1.07	0.03	0.35	1.07	0.53	5.02	1.50	6.06	13.56	8.81	0.05	0.16
Stomata index (%)	8.27	12.00	9.93	0.01	0.05	11.90	38.50	17.70	0.05	0.18	9.80	43.60	19.80	0.05	0.15	1.24	4.06	2.29	0.05	0.18
stomata size (µm ²)	0.53	62.80	4.26	0.03	0.09	0.01	0.53	0.40	42.50	0.07	6.42	28.56	18.58	3.30	0.01	16.50	32.80	25.41	9.03	0.16
length of trichomes (µm)	-	-	-	-	-	7.85	22.80	15.32	0.01	0.01	16.00	48.10	32.13	0.05	0.07	-	-	-	-	-
width of trichomes (µm)	-	-	-	-	-	2.14	2.14	2.14	0.01	0.01	2.49	30.30	16.40	0.04	0.05	-	-	-	-	-

Table 3: Quantitative Parameters of the Abaxial Epidermal Structures of the Vegetables studied

Variables	<i>Celosia argentea</i>					<i>Crassocephalum rubens</i>					<i>Senecio biafrae</i>					<i>Solanum americanum</i>				
	Min Value	Max Value	Mean	S.E	S.D	Min Value	Max Value	Mean	S.E	S.D	Min Value	Max Value	Mean	S.E	S.D	Min Value	Max Value	Mean	S.E	S.D
length of epidermal cells (µm)	5.35	16.00	9.31	0.77	0.02	1.78	3.21	1.35	0.01	3.60	3.57	7.85	6.24	0.56	1.78	2.85	14.90	9.03	0.55	1.75
width of epidermal cells (µm)	3.57	15.35	9.56	0.99	0.02	0.71	2.14	1.53	0.05	1.74	2.14	6.06	4.60	1.76	0.56	0.35	25.30	5.74	0.01	0.05
stomata index (%)	7.80	13.30	10.15	0.33	0.01	11.10	15.40	14.50	0.05	0.01	11.00	30.60	17.70	0.03	0.11	1.14	5.42	3.78	0.03	0.01
stomata size (µm ²)	1.60	3.28	2.43	0.03	0.15	0.21	0.53	0.40	0.01	1.90	1.42	4.74	2.28	6.90	2.19	20.00	42.40	31.00	0.05	0.15
length of trichomes (µm)	-	-	-	-	-	7.85	22.80	15.32	0.01	0.01	-	-	-	-	-	25.70	72.10	48.90	0.09	0.03
width of trichomes (µm)	-	-	-	-	-	2.14	2.14	2.14	0.01	0.01	-	-	-	-	-	3.57	17.85	10.71	0.09	2.80

Table 4: Summary of foliar epidermal features of the adaxial and abaxial surfaces of the species of vegetables studied

Character	Epidermal features of the Adaxial surface				Epidermal features of the Abaxial surface			
	<i>Celosia argentea</i>	<i>Crassocephalum rubens</i>	<i>Senecio biafrae</i>	<i>Solanum americanum</i>	<i>Celosia argentea</i>	<i>Crassocephalum rubens</i>	<i>Senecio biafrae</i>	<i>Solanum americanum</i>
Epidermal cell shape	Polygonal	Irregular	Polygonal	Irregular	Polygonal	Irregular	Polygonal	Irregular
Anticlinal wall pattern	Straight	Sinuous	Wavy	Wavy	Wavy	Sinuous	Wavy	Wavy
Stomata shape	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Stomata type	Anomocytic	Paracytic	Paracytic	Paracytic	Anomocytic	Paracytic	Paracytic	Paracytic
Epidermal size range	14.13-28.56µm	0.21-2.49µm	0.17-1.28µm	8.81-54.2µm	5.35-38.3µm	0.53-1.60µm	2.57-13.35µm	2.03-33.2µm
Stomata size	0.53-6.28µm ²	0.01-0.53µm ²	6.42-28.80µm ²	1.24-4.06µm ²	1.60-3.28µm ²	0.21-0.53µm ²	1.42-4.74µm ²	1.14-5.42µm ²

Discussion

The use of anatomical characters for the identification of plant species has been an established process in the history of plant systematics. Prominent among workers in this line of study are Chandra P., Khare R. C. (1980) ^[1] and Illoh and Inyang (1998) ^[5]. The four vegetables studied showed consistent anatomical characters when examined. Epidermal cells are polygonal in shape with straight anticlinal wall on the adaxial surface and wavy anticlinal wall on the abaxial surface in *Celosia argentea* whereas they are irregular in shape with sinuous anticlinal wall on both surfaces in *Crassocephalum rubens*. The epidermal cells are polygonal in shape with sinuous anticlinal wall on both surfaces of *Senecio biafrae* but irregular in shape with sinuous anticlinal wall on the surfaces of *Solanum americanum*. The stomata are anomocytic in *Celosia argentea* and paracytic in *Crassocephalum rubens*, *Senecio biafrae* and *Solanum americanum*. The stomata are densely distributed on the abaxial and adaxial surfaces of *Celosia argentea*, *Senecio biafrae* and *Solanum americanum* when compared to the abaxial and adaxial surfaces of *Crassocephalum rubens*. In all the species, stomata are present on both adaxial and abaxial surfaces, thus the leaf is said to be amphistomatic, and also the guard cell shape is elliptic in the species. Presence of simple multicellular trichomes are present made them not too hard for edible vegetables.

Anatomical observation made from this study reveals diagnostic characters that can be used for the identification of each of these vegetables. The presence of straight anticlinal wall, anomocytic stomata and low stomata index in *Celosia argentea* delimit this vegetable from the other vegetables studied in this work. *Crassocephalum rubens* has relatively the smallest epidermal cell and the largest stomata size with the absence of crystals. *Senecio biafrae* and *Solanum americanum* have the highest percentage of stomata index on the adaxial surface. The findings in this study is in consonant with the reports of Illoh, H. C. (1995) ^[4] and Illoh, H.C., & Inyang U.E., (1998) ^[5].

Conclusion

The anatomical characters of *Celosia argentea* made it unique and differentiate it as a member of the family Amaranthaceae. *Crassocephalum rubens* and *Senecio biafrae* though belonging to the same family Asteraceae can be distinguished by the stomata size and index. *Solanum americanum* can also be distinguished by having the highest stomata index.

Conclusively, the results of this study show the leaf anatomical characters which can be employed for the identification of the vegetable species and also contributed to the knowledge on anatomical studies of the vegetables

References

1. Chandra P, Khare RC. Leaf epidermis in some cultivated *Nephrolepis* New Botanis. 1980; 7:145-160.
2. Illoh HC. Taxonomic evaluation of mango (*Mangifera indica* L.) varieties in Nigeria. Ph.D Thesis submitted to Botany Department, University of Ife, Ile-Ife, Nigeria, 1986.

3. Illoh HC, Olorode O. Numerical taxonomic studies of mango (*Mangifera indica* L) varieties in Nigeria. Kluwer Academic Publishers, 1991, 197-205.
4. Illoh HC. Foliar epidermis and petiole anatomy of four species of *Celosia* L. in Nigeria, *Feddes Repertorium*. 1995; 106(1-2):15-23.
5. Illoh HC, Inyang UE. Foliar epidermis and petiole anatomy in some Nigerian *Solanum* Linn. Species in the sub-genus *Leptostemonum* (Bitt) Dun. *Glimpses in Plant Research*. 1998; 12:73s-86
6. Metcalfe CR. Anatomy of the Monocotyledons I – Gramineae. 1st Edn., Oxford University Press, London, 1960.
7. Olorode O. Taxonomy of West African Plants. Longman Ltd, London, 1984, 8-72.
8. Schippers RR. African Indigenous Vegetables. An Overview of the Cultivated Species. Natural Resources Institute, Chatham, UK, 2000.