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Full Length Research Paper

Agro-physiological responses of okra genotypes cultivated under water deficit conditions

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Water is a scarce resource, and Okra (*Abelmoschus esculentus*) is grown in wet and dry seasons. For its dry season cultivation, alternative water sources are used for watering, but these sources are not sustainable in Burkina Faso. The aim of this study was to determine water deficit effect on okra's behavior. Five genotypes of okra were subjected to three water regimes: (i) T1, watering at 100% of soil field capacity (SFC); (ii) T2, watering at 50% SFC; and (iii) T3, watering at 25% SFC. Results showed that water restrictions at 50% SFC and 25% SFC caused a reduction in growth's parameters. This reduction was very pronounced under watering at 25% SFC. In addition, the restrictive water supply at 25% SFC significantly reduced the number of capsules and the number of seeds. Results also revealed a large inter-genotypes variation on agro-physiological parameters under effects of water stress. The genotype G259 had a better tolerance under water regime at 25% SFC while, under watering at 50% SFC, genotypes O2 and L2 have been the least sensitive for capsules and seed yield. For irrigated okra, it would be better to bring water to plants at 50% SFC, if the soil is sandy-loamy.

Key words: *Abelmoschus esculentus*, ecotype, water regime, effect, yield.

INTRODUCTION

Water is the main environmental factor limiting agricultural production, especially in the least-watered regions. Thus, in the context of recurrent climatic changes, the irregular nature and uneven distribution of rains are factors that seriously compromise agricultural production. According to Niang (2009), agriculture is negatively affected by climate change, especially in developing countries (Nelson et al., 2009). This causes selective pressure on plant genetic resources, which

could be a source of genetic erosion. According to Trinchant et al. (2004), about 20 million hectares of land are lost worldwide each year due to water and salt stress. Irrigated agriculture is from a distance the largest water use in the world, accounting for 69% of global removals (FAO, 2002).

In Burkina Faso, agriculture is essentially rain-fed and production remains dependent on weather conditions. Drought is one of the main environmental constraints that

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Table 1. Characteristics of the studied genotypes.

| Genotype | Main characteristics | | Climate zones of origin | Average annual rainfall of climatic zones |
|----------|-----------------------|--------------------------------|-------------------------|---|
| | Cycle | Morphology | | |
| KKO5 | Late 80-100 days | Very tall plant and long fruit | Sudanese | > 900 mm |
| L2 | Precocious 40-60 days | Short fruit | | |
| G259 | Precocious 40-60 days | Short fruit | Sudano-Sahelian | 600 - 900 mm |
| B2 | Late 80-100 days | Very tall plant and long fruit | Sahelian | < 600 mm |
| O2 | Precocious 40-60 days | Short fruit | | |

Data source: Ouédraogo (2016) and Züllich et al. (2012)

causes the most damage to agricultural production globally and in Burkina Faso in particular. Indeed, Burkina Faso's agricultural sector sustains the brunt of drastic effects of climate variability, particularly of rainfall and temperatures. Studies on agromorphological characterization of okra [*Abelmoschus esculentus* (L.) Moench] genetic resources in Burkina Faso have shown the traditional character of cultivated varieties (Ouédraogo, 2016). In addition, other studies on the genetic and molecular diversity of okra accessions have identified genotypes of interest that could help boost okra production in Burkina Faso. Other studies have already discussed water stress in okra in Burkina Faso, but these studies have focused on development stages the most vulnerable to water deficit (Nana et al., 2009a, b). However, ignorance of their adaptation to water stress limits their appropriate use especially in areas subject to periodic water deficits.

The success of agricultural production is also based on the possibility to use plant material that has an adaptive potential for water deficit conditions. Indeed, the availability of soil water is one of the factors limiting the growth of plants (Reynolds et al., 2004; Otieno et al., 2005). Water stress is reflected in the plant by a series of changes that affect morphological, physiological and biochemical characters, when the water needs of plant exceed the available quantities (Mefti et al., 2001). As a result, the physiological mechanism adopted in case of water stress can be an effective tool for differentiating between varieties (Radhouane, 2013). Indeed, tolerance to different stresses depends on species, varieties and even genotypes (Ullah et al., 2008). Understanding the response of okra accessions to water stress and identification of water deficit tolerance characters would strengthen the process of varietal selection of okra in Burkina Faso. It is in this perspective that this study was initiated with the general objective of identifying genotypes presenting the characters of tolerance in water deficit conditions. Specifically, it is about:

(i) Evaluating physiological and agronomic characteristics of Okra genotypes cultivated under water deficit

conditions;

(ii) Identifying the best water treatments for the production of okra in cultivation in irrigation.

MATERIALS AND METHODS

Plant material used

The plant material used for this study was represented by five genotypes of okra, collected in three climatic zones of Burkina Faso, from a participatory selection (Ouédraogo, 2016). They were selected on the basis of their agromorphological performance and consumer preferences. Characteristics and provenance of the five genotypes are presented in Table 1.

Characteristics of the culture substrate

The substrate used was soil taken at 20 cm deep and submitted to National Office of Soils for granulometric and physicochemical analyzes. Analytical results (Table 2) show that textural class was sandy-loamy (62.75% sand, 23.52% silt and 13.30% clay). In addition, this soil had fairly good chemical characteristics because it contained the necessary nutrients (organic matter, total carbon, assimilable phosphorus, assimilable potassium, Ca²⁺, Mg²⁺, etc.) to allow a good growth of plants under a sufficient water supply. The choice of this type of substrate was guided by its saturation rate, its ability to good soil water retention capacity and its pH, okra preferring slightly acidic to neutral soils (Hamon and Charrier, 1997). In addition, a soil of similar texture was used by Nana (2010) because the okra's roots manage to collect easily the water it contains.

Determination of the soil capacity of the soil

The soil field capacity will be determined with a PVC pipe 2 cm in diameter and 18 cm high approximately, with one of its ends closed with a nylon mesh screen having mesh smaller than 2 mm. The pipe will then be filled with soil up to 2 cm from the edge. The dry weight (X) of the earth will be determined and thereafter saturated with water. After draining for 48 h, the wet weight of the earth (Y) is determined. The soil field capacity (SFC) is calculated by the following formula:

$$SFC = \frac{Y - X}{X} \text{ in g and H}_2\text{O/g of dry earth}$$

Table 2. Physico-chemical characteristics of the cultivation substrate.

| Characteristics | | Quantity | |
|---|------------|--|-------|
| Texture | Sandy-loam | Clay (%) | 13.30 |
| | | Silt (%) | 23.52 |
| | | Sand (%) | 62.75 |
| Organic matter and carbon | | Total organic matter (%) | 1.23 |
| | | Total carbon (%) | 0.72 |
| Nitrogen | | Total nitrogen (%) | 0.06 |
| | | C/N (%) | 12 |
| Phosphorus | | Total phosphorus (ppm) | 786 |
| | | Assimilable phosphorus (ppm) | 16.09 |
| Potassium | | Total potassium (ppm) | 864 |
| | | Assimilable potassium (ppm) | 49.67 |
| Chemical factors of soil fertility for 100g | | Ca ²⁺ (meq) | 1.42 |
| | | Mg ²⁺ (meq) | 0.42 |
| | | K ⁺ (meq) | 0.17 |
| | | Na ⁺ (meq) | 0.02 |
| | | Sum of bases (Ca ²⁺ . Mg ²⁺ . K ⁺ . Na ⁺) (meq) | 2.03 |
| | | Cation exchange capacity (CEC) (meq) | 3.40 |
| | | Saturation rate (S/T) (%) | 60 |
| Ground reaction | | pH (H ₂ O) | 6.12 |

Experimental device and treatments

Each genotype was subjected to three water regimes in a greenhouse using a split-plot design and 5 replications. Plants were grown in pots arranged in three randomized blocks. Each pot received 17 kg of homogenized dry soil as a growing substrate. Seedlings were sown on 5th August, 2017 at three seeds per pot, and thinned to one per pot two weeks after sowing (19 August, 2017). Plants were subjected to three water regimes beginning three weeks after sowing (vegetative stage); combining two factors: three-levels of water regime versus soil field capacity (100% SFC, 50% SFC and 25% SFC) and one-level watering frequency (every three days). Plants in pots receiving 100% SFC (control) were watered every three days with 3 L of water, while plants in treatments T1 and T2 were watered at 50% or 25% SFC with 1.5 or 0.75 L per pot, respectively. A sample of three plants (pots) of each genotype was selected for physiological evaluations.

Temperature and relative humidity inside the greenhouse

Given the influence of environmental factors, especially temperature and relative humidity on the effects of water treatments on plants, these two parameters were recorded daily from the induction of water stress at different times of day (7 am, 12 pm and 5 pm) using a thermohygrometer. Thus, during the induction period of water stress, temperature and relative humidity in the greenhouse oscillated respectively between 25.45 and 33.43°C and between 70 and 84.33%. The maximum temperature was recorded

at 12 o'clock when the relative humidity was lower (Figure 1). On the other hand, humidity was higher at 7 o'clock when the temperature was relatively low.

Evaluation of growth parameters

Height of plants

The height of plants was measured at the vegetative stage, at the fructification and at the end of the cycle; respectively on 44th day after sowing (DAS), 65th DAS and 104th DAS using a measuring tape.

Number of leaves, length and width of leaves

Foliar performances of plants cultivated under the three hydric regimes were evaluated at the vegetative stage (44th DAS) and at the fruiting stage (65th DAS). These performances were the number of leaves per plant (NLea/P), leaves length (LLe) and leaves width (LWi).

- (i) The number of leaves per plant (NLea/P) was determined *in situ* by counting leaves of each plant.
- (ii) The length and width of the leaves (LLe and LWi) expressed in centimeters, were determined by in-situ measurements, using a graduated ruler.

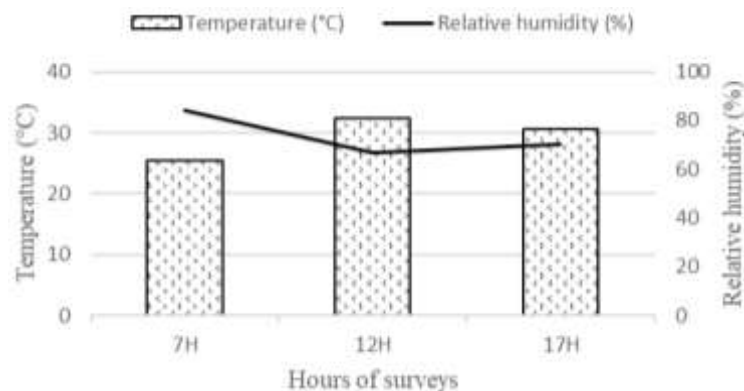


Figure 1. Evolution of temperature and relative humidity inside the greenhouse during the water stress induction.

Yield estimation

Yield evaluation consisted of the measurement of quantitative aspects such as capsule yield; seed yield and its components. The studied parameters were the following:

- (i) Number of capsules per plant (N_{Cap/P});
- (ii) Number of seeds per capsule (N_{Se/Cap});
- (iii) Weight of seeds per capsule (P_{Se/Cap}) in grams;
- iv) Weight of 100 seeds (W_{100Se}) in grams.

The number was determined by counting and weighing was carried out using a precision balance of 0.01 g.

Data analysis

Collected data were subjected to an analysis of variance (ANOVA) using the software XLSTAT 7.5.8. The Fisher test at the 5% threshold was used for averaging discrimination. Graphics were made using the Excel 2016 spreadsheet.

RESULTS

Influence of water treatments on growth parameters

Height of plants

The height of plants subjected to watering 100% SFC (T1); 50% SFC (T2) and 25% SFC (T3) was measured at the vegetative and fruiting stages and at harvest.

Results showed that at the vegetative stage, the decrease in water supply at 50 and 25% of the soil field capacity does not have a negative effect on the height of plants (Figure 2a). At this stage of development, plants of genotypes L2, G259 and KKO5 watered at 50% SFC, presented better performance in terms of height (Figure 2a).

At the fruiting stage and at harvest, plants of all five genotypes watered at 25% of the soil field capacity were the lowest (Figures 2b and 2c). Reducing the amount of

water supplied to plants impacts negatively on their growth and normal development, compared to the non-limiting water regime (Son, 2010). However, the height of plants of genotypes O2, L2 and KKO5 watering at 50% SFC, was higher than that of plants of T1 and T3 treatments. Statistical analysis reveals a very highly significant difference between T2 and T3 water regimes ($P < 0.0001$) and between T1 and T2 treatments ($P < 0.0001$) for plants height to fruiting (Table 3); on the other hand, there is no significant difference between T1 and T2 water treatments for plant height at this stage of development. In addition, the comparison of plants height at the end of the life cycle shows the same trend as at the fruiting stage. Indeed, T2 and T3 water treatments had significantly different effects ($P = 0.000$) on height; as well as T1 and T3 treatments ($P = 0.002$).

Comparison of plants height based on the genotype factor (Table 4) showed that genotype KKO5 was distinguishable from genotypes B2, G259 and L2, but this depended on the stage of development of the plants. Indeed, at the vegetative stage there was a highly significant difference between plants height of KKO5 and that of B2 ($P = 0.003$); and between the height of KKO5 and that of G259 ($P = 0.003$). In addition, plants height of KKO5 and L2 were significantly different ($P = 0.028$) at the vegetative stage, very significantly different at the fruiting stage ($P = 0.004$) and at the end of the life cycle ($P = 0.007$). A significant difference was also revealed between plants height of KKO5 and plants height of B2 at the end of the life cycle ($P = 0.032$).

Foliar performance of plants

The number of leaves per plant (N_{Lea/P}), the length and width of the leaves (L_{Le} and L_{Wi}) have varied according to water treatments (Table 5) and genotypes (Table 6). In the vegetative stage, the best performance of leaves was observed at the control treatment (T1). However, the

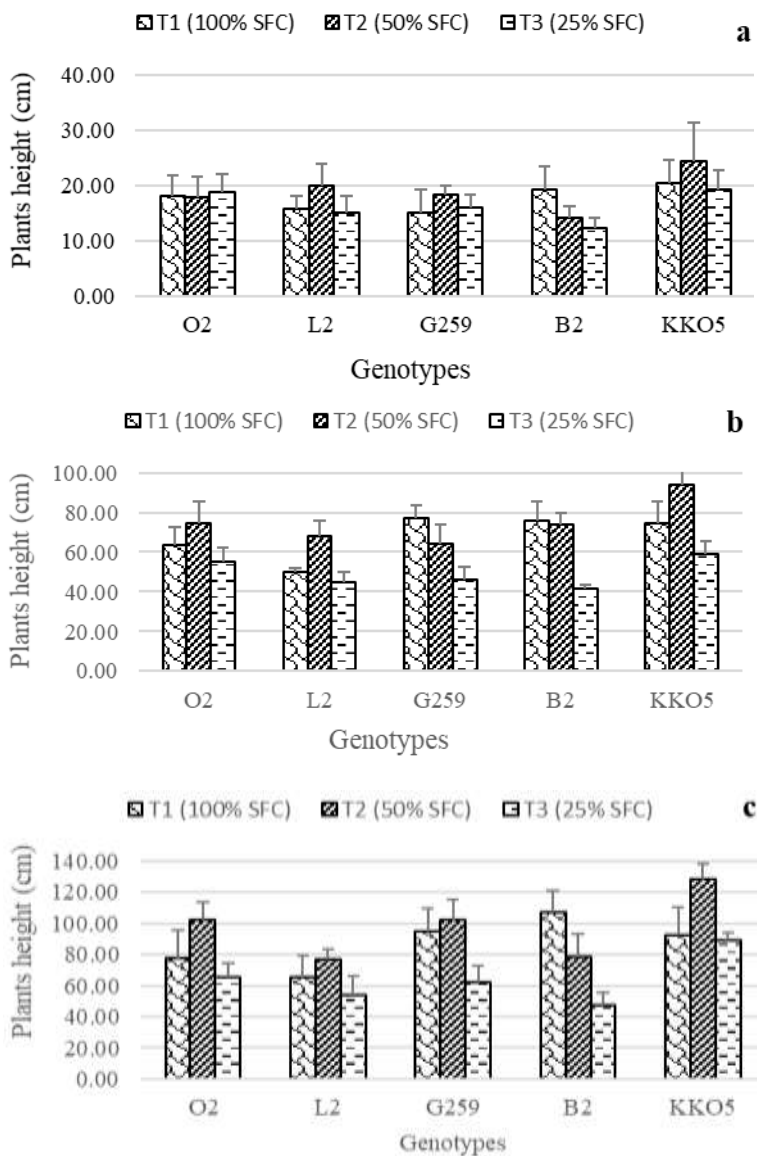


Figure 2. Variation of plants height according to water regimes and genotypes. a: vegetative stage; b: fruiting stage; c: end of the life cycle. Error bars represent standard deviations.

Table 3. Multiple comparisons of plants height according to water treatments using Fischer’s test at the 5% threshold.

| Water treatment | Vegetative stage | | | Fruiting stage | | | End of life cycle | | |
|-----------------|------------------|-------|-------|----------------|----------|-------|-------------------|-------|-------|
| | Diff. | Pr. | Sign. | Diff. | Pr. | Sign. | Diff. | P | Sign. |
| T2 ~ T3 | 3.088 | 0.057 | ns | 25.831 | < 0.0001 | *** | 34.033 | 0.000 | ** |
| T2 ~ T1 | 0.147 | 0.926 | ns | 7.022 | 0.101 | ns | 7.067 | 0.379 | ns |
| T1 ~ T3 | 2.941 | 0.069 | ns | 18.809 | < 0.0001 | *** | 26.967 | 0.002 | ** |

Diff. = difference, Sign. = Significant, Pr = probability, ns: = no significant; ** = highly significant at the 5% level; *** = very highly significant at the 5% level

effect of water stress was more pronounced on NLea/P, LLe and LWi, with low values when watering was done at

25% SFC (Table 5a and 5b). Analysis of variance revealed no significant difference between water

Table 4. Multiple comparisons of plants height with respect to the genotype's variable according to Fischer test at the 5% threshold.

| Genotype | Vegetative stage | | | Fruiting stage | | | End of life cycle | | |
|-------------|------------------|-------|-------|----------------|-------|-------|-------------------|-------|-------|
| | Diff. | Pr. | Sign. | Diff. | Pr. | Sign. | Diff. | Pr. | Sign. |
| KKO5 ~ B2 | 6.093 | 0.003 | ** | 11.981 | 0.094 | ns | 25.722 | 0.032 | * |
| KKO5 ~ G259 | 5.967 | 0.003 | ** | 13.163 | 0.067 | ns | 17.056 | 0.149 | ns |
| KKO5 ~ L2 | 4.335 | 0.028 | * | 21.381 | 0.004 | ** | 32.778 | 0.007 | ** |
| KKO5 ~ O2 | 2.993 | 0.123 | ns | 11.144 | 0.118 | ns | 21.389 | 0.072 | ns |
| O2 ~ B2 | 3.100 | 0.110 | ns | 0.837 | 0.905 | ns | 4.333 | 0.710 | ns |
| O2 ~ G259 | 2.974 | 0.125 | ns | 2.019 | 0.774 | ns | 4.333 | 0.710 | ns |
| O2 ~ L2 | 1.343 | 0.483 | ns | 10.237 | 0.150 | ns | 11.389 | 0.331 | ns |
| L2 ~ B2 | 1.757 | 0.360 | ns | 9.400 | 0.186 | ns | 7.056 | 0.546 | ns |
| L2 ~ G259 | 1.631 | 0.395 | ns | 8.219 | 0.246 | ns | 15.722 | 0.182 | ns |
| G259 ~ B2 | 0.126 | 0.947 | ns | 1.181 | 0.866 | ns | 8.667 | 0.459 | ns |

Diff. = difference, Sign. = Significant, Pr = probability, ns: = no significant; ** = highly significant at the 5% level; *** = very highly significant at the 5% level

Table 5. Effect of water treatment on leaf parameters at the vegetative stage and fruiting stage.

| Level of water factor | NLea/P | LLe (cm) | LWi (cm) |
|-------------------------------------|------------------------------|-----------------------------|-----------------------------|
| Vegetative stage^a | | | |
| T1 | 5.578 ± 0.960 ^a | 8.858 ± 1.75 ^a | 8.251 ± 2.490 ^a |
| T2 | 5.133 ± 0.407 ^a | 6.648 ± 1.018 ^{ab} | 6.714 ± 1.072 ^a |
| T3 | 5.111 ± 0.288 ^a | 6.482 ± 0.784 ^b | 2.839 ± 0.758 ^b |
| Pr. > Diff (T1 ~ T3) | 0.957 | 0.038 | < 0.0001 |
| Pr. > Diff (T1 ~ T2) | 0.258 | 0.053 | 0.159 |
| Pr. > Diff (T2 ~ T3) | 0.281 | 0.882 | 0.001 |
| Fruiting stage^b | | | |
| T1 | 10.622 ± 0.377 ^a | 12.220 ± 1.730 ^a | 14.431 ± 1.379 ^a |
| T2 | 10.044 ± 0.377 ^{ab} | 14.307 ± 1.730 ^a | 16.089 ± 1.950 ^a |
| T3 | 9.800 ± 0.267 ^b | 12.053 ± 1.223 ^a | 10.173 ± 1.971 ^b |
| Pr. > Diff (T2 ~ T1) | 0.520 | 0.200 | 0.400 |
| Pr. > Diff (T2 ~ T3) | 0.133 | 0.235 | 0.004 |
| Pr. > Diff (T3 ~ T1) | 0.035 | 0.924 | 0.035 |

NLea/P: number of leaves per plant; LLe: Length of the leaves; LWi: Width of the leaves; cm: Centimeter; T1: water treatment 100% of soil field capacity; T2: Water treatment 50% of soil field capacity; T3: water treatment 25% of soil field capacity. Values with the same letters on a column means that they are not significantly different between treatments from statistical view point.

treatments for the number of leaves per plant (NLea/P). On the other hand, for the length of the leaves (LLe), there was a significant difference between treatments T1 (control) and T3. In addition, water stress negatively affected width of the leaves. The reduction was of 65.59% under water regime at 25% SFC compared to 18.62% under water regime at 50% SFC. Fisher's test reveals a very highly significant water regime effect between T1 and T3 ($P < 0.0001$) for leaf width (Table 5a). At the fruiting stage, the same trend was observed for the three leaf parameters. However, analysis of variance did

not reveal any significant difference between water treatments for number of leaves and leaf length. The width of the leaves of plants subjected to water treatment T2 was very significantly different ($P = 0.004$) from that of leaves of plants to treatment T3 (Table 5b). It also appears that the width of leaves of treatment T1 is significantly different ($P = 0.035$) from that of leaves of treatment T3.

Inter-genotypes comparison shows that at the vegetative stage, genotype KKO5 had a relatively larger number of leaves with relatively longer and wider leaves

Table 6. Effect of genotypes on foliar performance at vegetative stage and fruiting stage.

| Level of genotype factor | NLea/P | LLe (cm) | LWi (cm) |
|-------------------------------------|------------------------------|-----------------------------|-----------------------------|
| Vegetative stage^a | | | |
| KKO5 | 6.148 ± 0.497 ^a | 9.189 ± 1.438 ^a | 7.391 ± 1.760 ^a |
| B2 | 5.296 ± 0.497 ^{ab} | 5.857 ± 1.123 ^b | 4.769 ± 1.680 ^a |
| O2 | 5.074 ± 0.351 ^b | 6.222 ± 1.017 ^b | 5.172 ± 1.244 ^a |
| L2 | 5.074 ± 0.369 ^b | 7.246 ± 1.133 ^{ab} | 6.001 ± 1.760 ^a |
| G259 | 4.778 ± 0.435 ^b | 8.131 ± 1.220 ^{ab} | 6.341 ± 1.760 ^a |
| Pr. > Diff (KKO5 ~ G259) | 0.009 | 0.446 | 0.975 |
| Pr. > Diff (KKO5 ~ L2) | 0.037 | 0.184 | 0.932 |
| Pr. > Diff (KKO5~ O2) | 0.037 | 0.026 | 0.716 |
| Pr. > Diff (KKO5~ B2) | 0.094 | 0.046 | 0.575 |
| Fruiting stage^b | | | |
| B2 | 11.222± 0.407 ^a | 13.252 ± 2.330 ^a | 13.635 ± 2.789 ^a |
| KKO5 | 10.556 ± 0.397 ^{ab} | 12.356 ± 2.152 ^a | 13.501 ± 2.291 ^a |
| G259 | 10.037 ± 0.411 ^{bc} | 13.689 ± 2.228 ^a | 14.662 ± 2.846 ^a |
| O2 | 9.630 ± 0.287 ^c | 12.689 ± 1.648 ^a | 13.363 ± 2.436 ^a |
| L2 | 9.333 ± 0.425 ^c | 12.315 ± 2.243 ^a | 12.662 ± 2.012 ^a |
| Pr. > Diff (B2 ~ L2) | < 0.0001 | 0.690 | 0.734 |
| Pr. > Diff (B2 ~ O2) | 0.000 | 0.810 | 0.924 |
| Pr. > Diff (B2~ G259) | 0.006 | 0.852 | 0.720 |
| Pr. > Diff (KKO5~ L2) | 0.005 | 0.986 | 0.770 |
| Pr. > Diff (KKO5~ O2) | 0.028 | 0.887 | 0.961 |

NLea/P: Number of leaves per plant; LLe: leaves Length ; LWi: Leaves Width; cm: Centimeter. Values with the same letters on a column mean that there are not significantly different between genotypes form statistical view point.

(Table 6a). On the other hand, genotype G259 had the low number of leaves per plant; and genotype B2 had shorter leaves. Analysis of variance shows a significant difference between genotypes KKO5 and L2 ($P = 0.037$); between KKO5 and O2 ($P = 0.037$) and highly significant ($P = 0.009$) between genotype KKO5 and genotype G259 for number of leaves per plant (NLea/P). At the fruiting stage, genotypes B2 and KKO5 had more leaves per plant, but the low number of leaves per plant was observed at genotypes O2 and L2 (Table 5b). Analysis of variance revealed a significant difference between KKO5 and O2 ($P = 0.028$); highly significant between B2 and O2 ($P = 0.000$), between B2 and G259 ($P = 0.006$), between KKO5 and L2 ($P = 0.005$); and highly significant between B2 and L2 ($P < 0.0001$) for NLea/P. At the fruiting stage, for the length and width of the leaf, no significant difference was observed between genotypes.

Effect of water deficit on yields

Number of capsules per plant (NCap/P)

The number of capsules per plant (NCap/P) ranged from 4.20 to 6.00 in treatment T1 (100% SFC), with the most capsules recorded in genotype G259 (6 capsules on

average). In plants subjected to watering 50% SFC, the number of capsules per plant ranged from 4.00 to 6.32. However, in plants subject to 25% SFC, the number of capsules per plant ranged from 3.33 to 5.00. The effect of water stress induced by watering 25% SFC, resulted in a decrease in the number of capsules per plant of four genotypes (Figure 3) compared to the control water regime (100% SFC). This reduction was 47% for each of genotypes L2 and KKO5; 17 and 5% respectively for genotypes O2 and G259. However, under water regime of 50% SFC, three genotypes (B2; L2 and KKO5) recorded a decrease in the number of capsules per plant of the order of 4, 5 and 33% respectively. In general, genotype G259 produced fewer capsules per plant, but the number of capsules per plant was less influenced by deficient water regimes (Figure 3). Analysis of variance shows that this genotype G259 is significantly different from genotypes O2 ($P = 0.017$) and L2 ($P = 0.024$).

Number of seeds per capsule and its components

Plants watered at 25% SFC had a reduced number of seed per capsule regardless of genotypes (Figure 4).

This reduction was approximately 30% for O2, L2 and G259; and about 60% each for both B2 and KKO5.

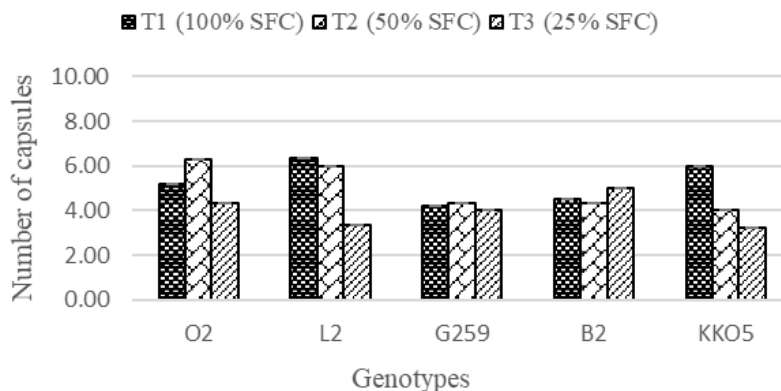


Figure 3. Variation of capsules number per plant depending on water treatments and genotypes.

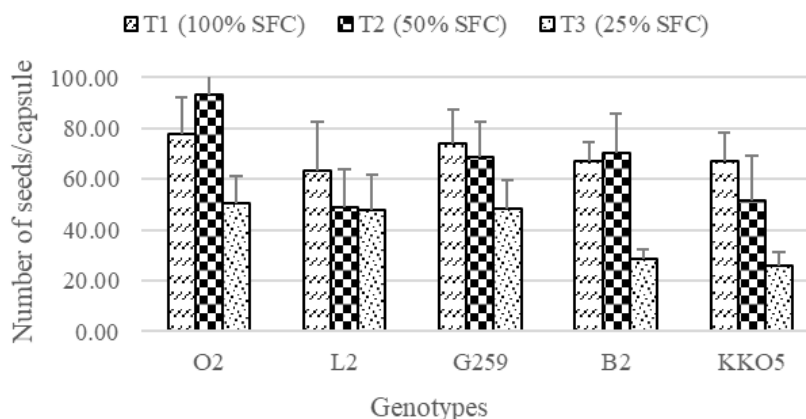


Figure 4. Variation of the number of seeds per capsule depending on water treatments and genotypes. Error bars represent standard deviations.

Regardless of water regime, KKO5 had the lowest number of seeds per capsule compared to O2 which had the highest number of seeds. Analysis of variance reveals a significant difference ($P = 0.03$) between these two genotypes (O2 and KKO5).

For seed yield components, water regime T3 (25% SFC) decreased seed weight per capsule (WSe/Cap) and weight of 100 seeds (W100Se) of all genotypes (Figures 5 and 6). On the other hand, with T2 water treatment (50% SFC), four genotypes (O2, G259, B2 and KKO5) had reduced WSe/Cap and W100Se compared to plants receiving the control treatment (100% SFC). In addition, genotype effect showed that the WSe/Cap and W100Se of genotypes L2 and KKO5 were lower than those of other genotypes. However, genotype effect was not significant for these two parameters.

Results show that capsule and seed yields were significantly influenced by water regimes (Table 7). Water stress imposition by decreasing the amount of water supplied to plants caused a significant reduction in the

number of capsules per plant (NCap/P), number of seeds per capsule (NSe/Cap), weight of seeds per capsule (WSe/Cap) and the 100-seeds weight (W100Se) in treatments 50 and 25% SFC compared to the control treatment (100% SFC). This reduction was much more accentuated in the 25% SFC treatment for which, the difference with the control treatment (100% SFC) was very highly significant. Our results are consistent with those of Son et al. (2011) who had found that in sesame (*Sesamum indicum*), water stress induced by reducing water supply to 80, 60 and 40% SFC caused a reduction in capsules production and weight.

DISCUSSION

The present results showed a highly significant effect of water regime on plants height at fruiting, but also at the end of the life cycle. Indeed, the stress induced by the water supply at 25% SFC caused a reduction in the

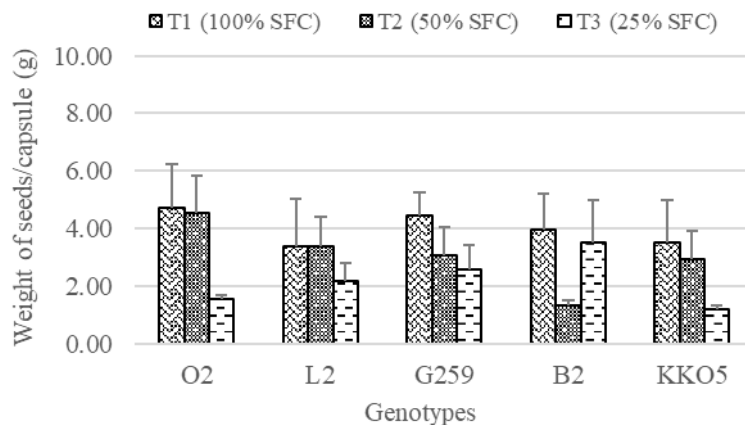


Figure 5. Variation of seeds weight per capsule depending on water treatments and genotypes. Error bars represent standard deviations.

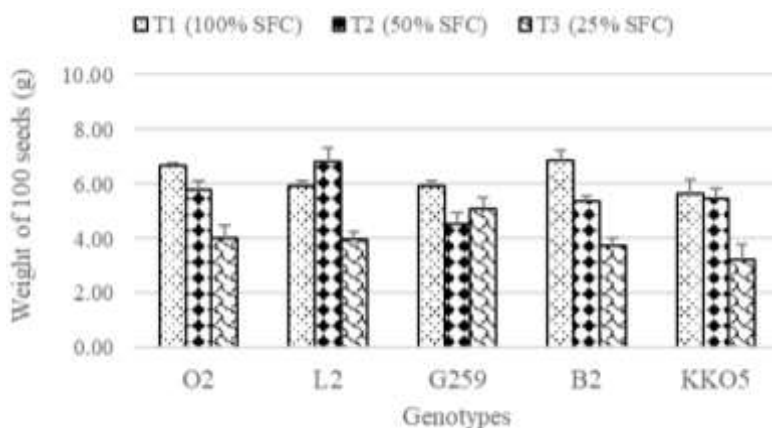


Figure 6. Variation of the weight of 100 seeds according to water treatments and genotypes. Error bars represent standard deviations.

Table 7. Water treatments effect on yield and its components.

| Level of water factor | NCap/P | NSe/Cap | WSe/Cap (g) | W100Se (g) |
|-----------------------|----------------------------|-----------------------------|----------------------------|----------------------------|
| T1 | 5.246 ± 0.221 ^a | 69.933 ± 5.482 ^a | 4.115 ± 0.275 ^a | 6.199 ± 0.166 ^a |
| T2 | 4.996 ± 0.312 ^a | 66.533 ± 5.753 ^a | 3.561 ± 0.388 ^a | 5.590 ± 0.235 ^b |
| T3 | 3.972 ± 0.315 ^b | 40.200 ± 5.015 ^b | 1.770 ± 0.397 ^b | 4.013 ± 0.240 ^c |
| Pr. > Diff (T1 ~ T3) | 0.000 | 0.000 | < 0.0001 | < 0.0001 |
| Pr. > Diff (T1 ~ T2) | 0.427 | 0.663 | 0.161 | 0.013 |
| Pr. > Diff (T2 ~ T3) | 0.002 | 0.002 | < 0.0001 | < 0.0001 |

NCap/p: Number of capsules per plant; NSe/Cap: number of seeds per capsule; WSe/Cap: weight of seeds per capsule; W100Se: Weight of 100 seeds; g: gram. Values with the same letters on a column mean that there are not significantly different between treatments from statistical view point.

height of plants by 28% at fruiting and 30% at the end of the life cycle. Our results corroborate those of Son (2010) who observed a reduction in the height of sesame plants (*S. indicum*) when they were watered to less than

50% SFC. On the other hand, at the vegetative stage, no significant difference between water regimes was observed. These results may be justified by the fact that at the vegetative phase, measurements were made at 23

days after the beginning of the imposition of deficit water regimes, that is, 8 deficient water supplies; while at fruiting and at the end of the life cycle, measurements were made respectively at 44 and 83 days after the onset of stress, respectively 15 and 28 deficient water supplies. This assumes that water deficit has gradually increased over time. In addition, water requirements of juvenile plants are lower compared to their needs at reproductive stage during which metabolic reactions requiring water are higher. Based on our results, water treatment T3 (25% SFC) does not seem to favor the height growth of okra genotypes after their vegetative stage. This depressive effect of water stress on growth follows the loss of turgor of cells. Water stress induced by water supply at 50% SFC or 25% SFC did not have a significantly reducing effect on the number of leaves per plant at the vegetative stage as well as with fruiting in okra genotypes studied. However, Meftah (2012) found a decrease in number of leaves in cowpea grown under water stress. Gorai et al. (2010) have noticed a significant decrease in the number of leaves on a *Medicago sativa* crop irrigated at 40% SFC compared to controls. These results may be due to severe water stress suffered by plants. The number of leaves is a genetic trait (Lorgeou and Martin, 2005); therefore, it is not influenced by water regime. Under restrictive water regime conditions, the yield and its components were negatively impacted compared to the control water regime. Indeed, our results showed that the number of capsules per plant (NCap/P), the number of seeds per capsule (NGr/Cap), the seed weight per capsule (WSe/Cap) and the weight of 100 seeds (W100Se) were significantly reduced under water conditions at 25% SFC. Ohashi et al. (2009) found a significant decrease in seed yield on a soybean crop under water stress. According to Farooq et al. (2009), the reduction of seeds replenishment is attributable to a decrease in the distribution of photo assimilates between different reserve organs. Any water stress negatively affects key physiological phenomena such as photosynthesis and translocation of photo assimilates, which are directly related to fruits and seeds formation. Indeed, the transport of photo assimilates across the phloem plays a key role in crop productivity and yield (Hopkins, 2014). The significant decrease in the yield of capsules and its different components noted in the treatment 25% SFC could be explained by the concomitant reduction in plant height at the fruiting stage and at the end of the cycle, but also by the decrease in the number of capsules, leaves and leaves dimensions at the time of fruiting.

Inter-genotypes comparison showed a reduction in the number of capsules per plant by 47% for each of genotypes L2 and KKO5, 17 and 5% respectively for genotypes O2 and G259 under restrictive water regimes 25% SFC. On the other hand, under water regime 50% SFC, these are genotypes B2, L2, and KKO5 recorded a decrease in the number of capsules per plant of the order

of 3, 5 and 33% respectively. In general, genotypes B2 and G259 were tolerant of water restriction while genotype KKO5 was the most sensitive to water deficit for capsule yield. In addition, genotypes O2 and L2 were less sensitive to water supply 50% SFC. This diversity in sensitivity to water deficit can be attributed to climatic zones where these genotypes are from. Indeed, genotype KKO5 comes from the Sudan's worst-weathered climate zone (> 900 mm rainfall/year) while genotypes G259 and B2 come from respectively the medium-watered areas (600 - 900 mm/year) and less watered (<600 mm/year). These last two genotypes seem already to be adapted to medium or low water regimes. In addition, independently of water regime, genotype G259 produces fewer capsules per plant compared to other genotypes. This result could be justified by an influence of genetic factors rather than by the water factor which is environmental. Processes involved in crop yield development are influenced by both genetic factors that are intrinsic to the plant and environmental factors (Radhouane et al., 2014).

Conclusion

From the results obtained, it appears that the restriction of water supply to 25% SFC negatively affects not only the height of plants at the fruiting stage and at the end of the life cycle, the length and the width of the leaf but also, the yield in capsules, seeds yield and its components. In case of irrigated cultivation, restrictive water supply can be applied at the vegetative stage of okra, but these restrictive contributions should be avoided during the reproductive stage, especially during flowering-fruiting until full maturity. With regard to agronomic parameters, under the water deficit induced by water supply at 25% SFC, genotype KKO5 was the most sensitive; however, genotype that has been less sensitive was G259. In addition, genotypes O2, L2 and B2 showed some tolerance to water restriction at 50% SFC for capsules and seeds yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Taxonomic significance of the vegetative anatomy of members of genera *Colocasia* (L.) Schott and *Xanthosoma* (L.) Schott in the family Araceae

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Anatomical attributes are important for taxonomic studies of plants. This study investigated foliar and petiole anatomy of some members of the genera *Colocasia* and *Xanthosoma*. Similar and diagnostic characters critical for the taxonomy of the two genera were identified. The similar characters include, polygonal epidermal cell shape, straight adaxial anticlinal wall pattern, brachyparacytic stomata type, elliptic shaped stomata and unmodified raphide type. The presence of papillae on the adaxial surfaces of the members of genus *Colocasia* but not in the *Xanthosoma* taxa; lamellar collenchyma type in *Xanthosoma mafaffa* (Red), and unicellular non-glandular trichomes in *Xanthosoma mafaffa* (White) were recorded as diagnostic characters.

Key words: Brachyparacytic stomata, collenchyma, druses, foliar anatomy, petiole anatomy, papillae, raphides, trichome.

INTRODUCTION

Anatomical methods have been found to be very useful in many taxonomic investigations. Several authors have employed leaf, petiole, and even wood anatomical characters that are not influenced by the environment in solving taxonomic problems among groups of plants (Adedeji and Illoh, 2004; Adedeji et al., 2007; Thakuri and Patil, 2011; Akinloye et al., 2012; Oladipo and Oyaniran, 2013; Akinnubi et al., 2014; Osuji and Nwala, 2015; Mudasiru et al., 2016; Rodriguez et al., 2016; Arogundade and Adedeji, 2017). The Araceae is a large family of plants, found in the New World and Old World tropics, and north temperate regions (Vargas et al., 2004;

Mora et al., 2006). Members of the family are known as Aroids (Bown, 2000). Although research works have been carried out on the Araceae to settle some taxonomic positions of genera, more research is needed to resolve persistent confusions reported among lower ranks in the family (Green and Oguzor, 2009). According to Keating (2003), leaf and petiole anatomy of the Araceae have a high potential use as character states in resolving taxonomic problems in the family.

Colocasia (L.) Schott, tribe Colocasieae, and *Xanthosoma* (L.) Schott, tribe Caladieae, are two genera in the family Araceae. They grow in different ecological

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zones of the world (Croat, 1990). There are 3,750 species of aroids from 114 genera according to Petruzzello (2018), though Boyce and Croat (2018) reported 3,645 species from 144 genera. Members of these two genera, *Colocasia* and *Xanthosoma*, are important sources of food and medicine, especially in rural and poor communities where food security is posing a major challenge (Matemilola and Elegbede, 2017).

An interesting feature or character of aroids is their toxicity. Their cells possess calcium oxalate crystals in form of raphides and druses (Franceschi and Nakata, 2005; Arogundade and Adedeji, 2017). Therefore, Araceae members are enlisted as poisonous plants (Mulligan and Munro, 1990). Nevertheless people had devised methods for eliminating the poison to make these edible (Okiy, 1960; Ibe and Iwueke, 1984; Amanze, 2009). The corms and leaves of members of genera *Colocasia* and *Xanthosoma* have been part of delicacies around the world (Cable, 1984; Okeke, 1992, Amanze, 2009).

Morphological descriptions of these two genera have been studied (Purseglove, 1972; Burkill, 1985; Gill, 1988; Mayo et al., 1997; Ngoka, 1997; Bown, 2000), resolving some descriptive problems between the two genera. A ready distinction between *Xanthosoma* and *Colocasia* as ascertained by Burkill (1985), lies in the junction of the leaf lamina with the petiole. The leaf is attached to the petiole on the leaf margin in *Xanthosoma*, while in *Colocasia*, the attachment is towards the centre. That is, unlike the leaves of *Colocasia*, those of *Xanthosoma* are usually not *peltate*—the upper v-notch extends in to the point of attachment of the leaf petiole to the blade.

The genus *Colocasia* include six species of tuberous perennials from tropical Asia, grown there as a staple starchy food. Common names include Elephant-ear, Taro, Cocoyam, Dasheen, and Eddoe. *Colocasia esculentum* has between 28 and 42 chromosomes per cell that behave erratically during cell division. Thus, new permutations, resulting in new varieties are being formed without recourse to cross-pollination and seed production (Onwueme, 1978). Although cultivated plants rarely bloom, they are cultivated all the year round in subtropical and tropical areas. Taro is closely related to *Xanthosoma* and *Caladium*, which are commonly grown as ornamentals (Bown, 2000).

The genus *Xanthosoma* is a group of fleshy herbaceous stem-less plant with leaves arising from the crown of a central corm usually surrounded by a mass of cormels. A number of varieties exist based on yield, the colour of the flesh or skin, size of corm, storage quality, palatability and so on. The young leaves are eaten as a green vegetable (Kay, 1987). Mayo et al. (1997) reported chromosome number $2n = 22, 26, 39$ and 52 for the genus.

This work is intended to shed more light on taxonomy of genera *Colocasia* and *Xanthosoma* which has been reported to be confusing (Green and Oguzor, 2009) using

their leaf and petiole anatomical characters.

MATERIALS AND METHODS

Five taxa from the genera *Colocasia* and *Xanthosoma* were included in this work. The species and varieties are: *C. esculentum* var. *esculentum* (L.) Schott, *C. esculentum* var. *antiquorum* (L.) Schott, the red and white varieties of *X. mafaffa* Schott and *X. saggitifolium* Schott. The taxa were collected from different locations in the South Western part of Nigeria and were authenticated at Forestry Herbarium Ibadan (FHI), Oyo State and IFE Herbarium at Obafemi Awolowo University, Osun State, Nigeria. Voucher specimen was deposited at the IFE herbarium (Table 1).

Epidermal studies

The well expanded median portion of the leaf of each of the taxa was scrapped following the standard method described by Metcalfe (1960) and as adopted by Arogundade and Adedeji (2016). Epidermal peels from both the adaxial and abaxial surfaces were obtained and afterwards stained with Safranin O. The peels were later mounted in dilute glycerine in readiness for microscopic examination. Features observed on the epidermal peels included the epidermal cell shape, the anticlinal wall patterns, stomata shape and size. The stomata area was calculated by multiplying the length and breadth of 50 stomata from at least five different plant accessions. Stomata indices of the adaxial and abaxial surfaces were calculated using the formula:

$$\text{Stomata Index (I)} = \frac{S}{S + E} \times 100$$

Where S = Number of stomata and E = Number of ordinary epidermal cells plus the subsidiary cells in the same unit area.

Photomicrographs of the epidermis were taken for both the adaxial and the abaxial surfaces.

Petiole anatomy

A Reichert sliding microtome manufactured at Vienna, Austria NR. 367 019 was employed in cutting the transverse section of the petiole of the taxa at the proximal, median and distal regions. The sections were made at a thickness of 8 – 15 μm . For staining, Safranin O and Alcian blue stains were used. After which differentiation and dehydration were carried out on the sections by passing them through varying percentages of ethanol (50%, 70%, 80%, 90% and absolute). The sections were later mounted in 25% glycerine and observed under the microscope Olympus XSZ-107BN binocular biological microscope manufactured by Zenith Laboratories, California. Photomicrographs of the different sections were taken.

All microscopic measurements were taken with the aid of an ocular micrometer inserted into the eyepiece of a microscope. They were later multiplied by the ocular constant with respect to the power under which they were taken in order to convert them to micrometer.

RESULTS

Tables 2 and 3 show the summary of the important

Table 1. Voucher specimen numbers of the taxa at IFE herbarium.

| Species/varieties | Voucher specimen numbers |
|---|--------------------------|
| <i>C. esculentum</i> var. <i>esculentum</i> (L.) Schott | IFE - 17761 |
| <i>C. esculentum</i> var. <i>antiquorum</i> (L.) Schott | IFE - 17762 |
| <i>X. mafaffa</i> Schott (Red variety) | IFE - 17763 |
| <i>X. mafaffa</i> Schott (White variety) | IFE - 17764 |
| <i>X. saggitifolium</i> Schott | IFE - 17765 |

Table 2. Summary of important qualitative foliar epidermal characteristics of the adaxial surfaces of the taxa studied.

| Species | Epidermal cell shape | Anticlinal wall pattern | Stomata shape | Stomata type | Cell inclusions | Other features |
|---|----------------------|-------------------------|--------------------|-----------------------------|-----------------|----------------------|
| <i>C. esculentum</i> var. <i>antiquorum</i> | Polygonal | Straight | Elliptic | Brachyparacytic | Druses | Papillae |
| <i>C. esculentum</i> var. <i>esculentum</i> | Polygonal | Straight | Elliptic | Brachyparacytic, anisocytic | Nil | Papillae |
| <i>X. mafaffa</i> (Red) | Polygonal | Straight | Elliptic, circular | Brachyparacytic, anomocytic | Nil | Cuticular striations |
| <i>X. mafaffa</i> (White) | Polygonal | Straight | Elliptic | Brachyparacytic, anomocytic | Nil | Nil |
| <i>X. saggitifolium</i> | Polygonal | Straight | Elliptic | Brachyparacytic, anomocytic | Nil | Nil |

Table 3. Summary of important qualitative foliar epidermal features of the abaxial surfaces of the taxa studied.

| Species | Epidermal cell shape | Anticlinal wall pattern | Stomata shape | Stomata type | Other features |
|---|----------------------|-------------------------|--------------------|-----------------------------|---------------------------------------|
| <i>C. esculentum</i> var. <i>antiquorum</i> | Polygonal | Straight | Elliptic, circular | Brachyparacytic, Anomocytic | Druses; apillae; Cuticular striations |
| <i>C. esculentum</i> var. <i>esculentum</i> | Polygonal | Straight | Elliptic, circular | Brachyparacytic, Anomocytic | Papillae |
| <i>X. mafaffa</i> (Red) | Polygonal | Straight | Elliptic, circular | Brachyparacytic | Papillae; cuticular striations |
| <i>X. mafaffa</i> (White) | Polygonal | Straight | Elliptic, circular | Brachyparacytic Anomocytic | Papillae; cuticular striations |
| <i>X. saggitifolium</i> | Polygonal | Straight to wavy | Elliptic, circular | Brachyparacytic Anomocytic | Papillae; cuticular striations |

qualitative foliar epidermal features of the adaxial and abaxial surfaces respectively while Table 4 shows the summary of the quantitative foliar anatomical features.

Adaxial epidermal characteristics

In *C. esculentum* var. *antiquorum*, epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 213.12 and 532.8 μm^2 (mean = 348.68

μm^2). Brachyparacytic stomata types, elliptic in shape, were observed (Plate 1A and B). Stomata size ranges between 116.55 and 179.82 μm^2 (mean = 141.59 μm^2) and stomata index ranged between 4.47 - 8.89% (mean = 6.87%). Druses and papillae were present.

In *C. esculentum* var. *esculentum*, epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 336.6 and 761.6 μm^2 (mean = 536.79 μm^2). Brachyparacytic, occasionally anisocytic stomata types, elliptic in shape, were observed (Plate 1F).

Table 4. Summary of the quantitative foliar anatomical parameters.

| Species | Range and mean | Epidermal adaxial area (μm^2) | Epidermal abaxial area (μm^2) | Stomata area Adaxial (μm^2) | Stomata Area Abaxial (μm^2) | Stomata Index Adaxial (%) | Stomata Index Abaxial (%) |
|---|----------------|--|--|--|--|---------------------------|---------------------------|
| <i>C. esculentum</i> var. <i>antiquorum</i> | Range | 213.12-532.8 | 139.86-439.56 | 116.55-179.82 | 149.85-266.4 | 4.47-8.89 | 8.15-14.78 |
| | Mean | 348.68 \pm 8.54 | 261.21 \pm 9.50 | 141.59 \pm 2.07 | 173.36 \pm 3.09 | 6.87 \pm 0.23 | 10.51 \pm 0.28 |
| <i>C. esculentum</i> var. <i>esculentum</i> | Range | 336.6-761.6 | 219.78-799.2 | 142.8-214.2 | 159.84-233.1 | 2.70-7.59 | 9.42-13.13 |
| | Mean | 536.79 \pm 13.77 | 419.98 \pm 16.85 | 175.24 \pm 2.38 | 192.87 \pm 2.84 | 4.75 \pm 0.27 | 11.31 \pm 0.18 |
| <i>X. mafaffa</i> (Red) | Range | 214.2-578.0 | 166.6-442.0 | 183.6-299.2 | 183.6-272.0 | 4.33-8.33 | 11.98-15.60 |
| | Mean | 391.20 \pm 11.74 | 311.85 \pm 7.79 | 242.49 \pm 3.98 | 215.02 \pm 3.49 | 6.56 \pm 0.18 | 13.37 \pm 0.20 |
| <i>X. mafaffa</i> (White) | Range | 261.8-652.8 | 214.2-652.8 | 183.6-285.6 | 183.6-244.8 | 4.41-7.32 | 8.78-12.65 |
| | Mean | 445.81 \pm 11.75 | 428.13 \pm 14.77 | 216.38 \pm 3.63 | 212.16 \pm 2.61 | 5.66 \pm 0.17 | 10.78 \pm 0.18 |
| <i>X. saggitifolium</i> | Range | 309.4-1142.4 | 261.8-867.0 | 261.8-397.8 | 238.0-367.2 | 3.16-8.51 | 11.11-16.79 |
| | Mean | 745.62 \pm 20.03 | 564.60 \pm 18.37 | 312.32 \pm 3.63 | 305.66 \pm 4.35 | 5.67 \pm 0.25 | 13.67 \pm 0.25 |

Stomata size ranges between 142.8 and 214.2 μm^2 (mean = 175.24 μm^2) and stomata index ranges between 2.70 and 7.59% (mean = 4.75%). Papillae were present.

In *X. mafaffa* (Red variety), epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 214.2 and 578.0 μm^2 (mean = 391.20 μm^2). Brachyparacytic, occasionally anomocytic stomata types, elliptic, occasionally circular in shape were observed (Plate 1I and J). Stomata size ranges between 183.6 and 299.2 μm^2 (mean = 242.49 μm^2) and stomata index ranges between 4.33 - 8.33% (mean = 6.56%). Cuticular striations radiating from the guard cells were present.

In *X. mafaffa* (White Variety), epidermal cells were polygonal with straight anticlinal wall. They also varied in size, shape and arrangement. Epidermal cell area ranges between 261.8 and 652.8 μm^2 (mean = 455.81 μm^2). Brachyparacytic,

occasionally anomocytic stomata types, elliptic in shape were observed (Plate 1M). Stomata size ranges between 183.6 - 285.6 μm^2 (mean = 216.38 μm^2) and stomata index ranges between 4.41 and 7.32% (mean = 5.66%).

In *X. saggitifolium*, epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 309.4 and 1142.4 μm^2 (mean = 745.62 μm^2). Brachyparacytic stomata types, elliptic in shape, were observed (Plate 1O). Stomata size ranges between 261.8 and 397.8 μm^2 (mean = 312.32 μm^2) and stomata index ranges between 3.16 - 8.51% (mean = 5.67%).

Abaxial epidermal characteristics

In *C. esculentum* var. *antiquorum*, epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal

cell area ranges between 139.86 and 439.56 μm^2 (mean = 261.21 μm^2). Brachyparacytic, occasionally anomocytic stomata types as well as two stomata sharing the same subsidiary cells, were observed. Stomata were elliptic in shape though occasionally circular (Plate 1C, D and E). Stomata size ranges between 149.85 - 266.4 μm^2 (mean = 173.36 μm^2) and stomata index ranges between 8.15 and 14.78% (mean = 10.51%). Papillae and druses are present.

In *C. esculentum* var. *esculentum* epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 219.78 and 799.2 μm^2 (mean = 419.98 μm^2). Brachyparacytic, occasionally anomocytic stomata types, elliptic in shape, though occasionally circular were observed (Plate 1G and H). Stomata size ranges between 159.84 and 233.1 μm^2 (mean = 192.87 μm^2) and stomata index ranges between 9.42 and 13.13% (mean =

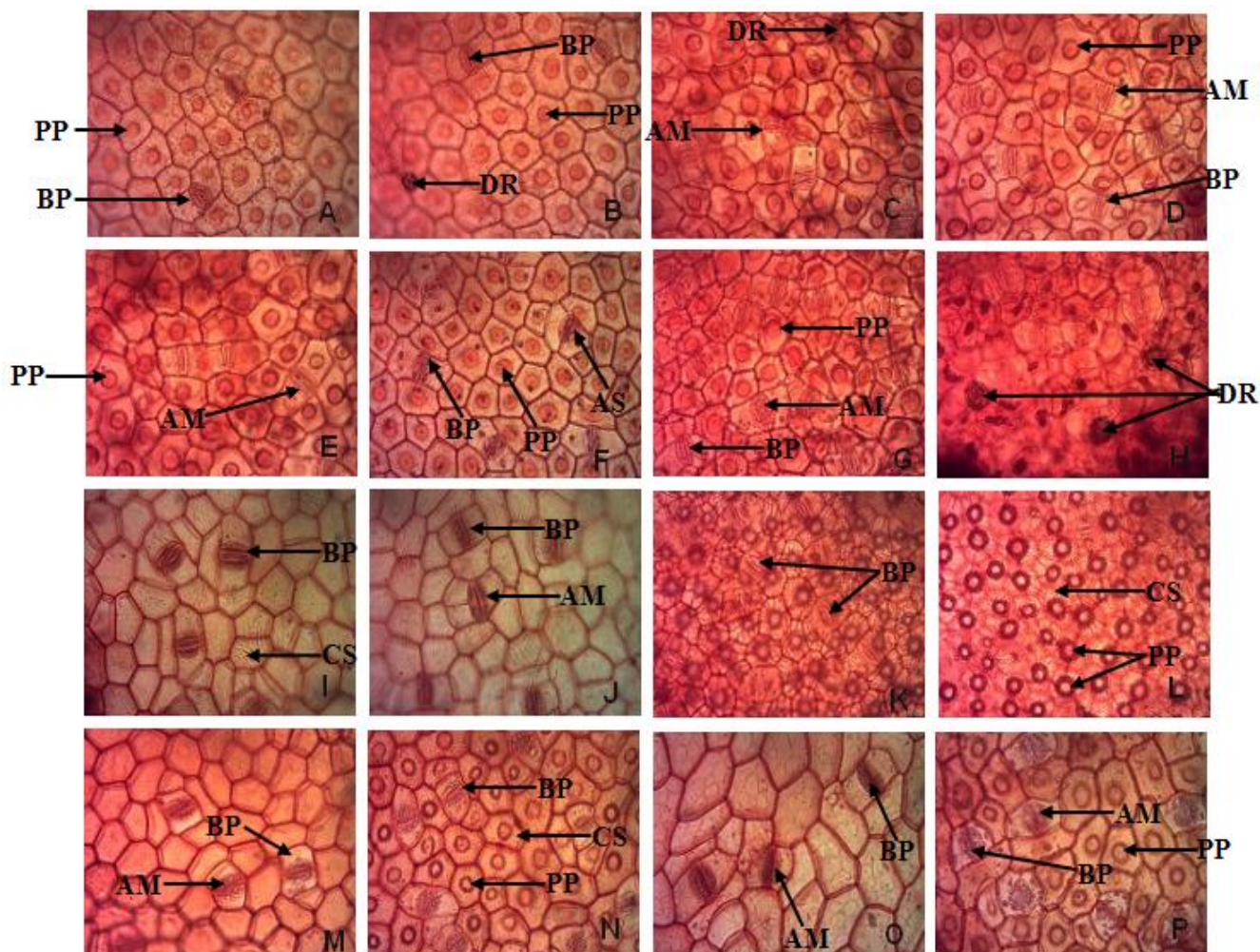


Plate 1. Leaf epidermal surfaces of taxa of *Colocasia* and *Xanthosoma*. A and B. Adaxial epidermis of lamina of *C. esculentum* var. *antiquorum*; C, D and E. Abaxial epidermis of lamina of *C. esculentum* var. *antiquorum*; F. Adaxial epidermis of lamina of *C. esculentum* var. *esculentum*; G and H. Abaxial epidermis of lamina of *C. esculentum* var. *esculentum*; I and J. Adaxial epidermis of lamina of *X. mafaffa* (Red Variety). K and L; Abaxial epidermis of lamina of *X. mafaffa* (Red Variety); M. Adaxial epidermis of lamina of *X. mafaffa* (White Variety); N. Abaxial epidermis of lamina of *X. mafaffa* (White Variety); O. Adaxial epidermis of lamina of *X. saggitifolium*; P. Abaxial epidermis of lamina of *X. saggitifolium*.

11.31%). Papillae were present.

In *X. mafaffa* (Red variety) epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 166.6 and 442.0 μm^2 (mean = 311.85 μm^2). Brachyparacytic stomata types, elliptic in shape though occasionally circular, were observed (Plate 1K and L). Stomata size ranges between 183.6 and 272.0 μm^2 (mean = 215.02 μm^2) and stomata index ranges between 11.98 and 15.60% (mean = 13.37%). Papillae with cuticular striations radiating from the guard cells were present.

In *X. mafaffa* (White variety) epidermal cells were polygonal with straight anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 214.2 and 652.8 μm^2 (mean = 428.13

μm^2). Brachyparacytic, occasionally anomocytic stomata types, elliptic in shape, though occasionally circular were observed (Plate 1N). Stomata size ranges between 183.6 and 244.8 μm^2 (mean = 212.16 μm^2) and stomata index ranges between 8.78 and 12.65%; (mean = 10.78%). Papillae with cuticular striations radiating from the guard cells were present.

In *X. saggitifolium* epidermal cells are polygonal to irregular with straight to wavy anticlinal wall. They varied in size, shape and arrangement. Epidermal cell area ranges between 261.8 and 867.0 μm^2 (mean = 564.6 μm^2). Brachyparacytic, occasionally anomocytic stomata types, elliptic in shape were observed (Plate 1P). Stomata size ranges between 238.0 and 367.2 μm^2 (mean = 305.66 μm^2) and stomata index ranges between 11.11 and 16.79% (mean = 13.67%). Papillae with

Table 5. Summary of the proximal, median and distal regions of the petiole anatomy of the taxa studied.

| Species | Characters | Adaxial outline | Abaxial outline | Layers of parenchyma cells | Type of collenchyma cells | Raphides (+/-) | Druses (+/-) | Tannins (+/-) | Starch grains (+/-) |
|--|------------|-------------------------|-----------------|----------------------------|---------------------------|----------------|--------------|---------------|----------------------|
| <i>C. esculentum</i> var. <i>antiquorum</i> | Proximal | Convex | Round | 1-4 | Lacunar | + | - | - | - |
| | Median | Concave | Round | 1-3 | Lacunar | + | + | - | + |
| | Distal | Concave | Round | 1-4 | Lacunar | + | + | - | - |
| <i>C. esculentum</i> var. <i>esculentum</i> | Proximal | Convex | Round | 1-2 | Lacunar | + | - | - | - |
| | Median | Flat to slightly convex | Round | 1-2 | Lacunar | + | + | - | - |
| | Distal | Concave | Round | 1-2 | Lacunar | + | + | + | - |
| <i>X. maffafa</i> (Red) | Proximal | Flat to slightly convex | Round | 1-3 | Lamellar | - | - | + | + |
| | Median | Convex | Round | 2-4 | Lamellar | + | + | - | + |
| | Distal | Concave | Round | 2-4 | Lamellar | - | + | - | + |
| <i>X. maffafa</i> (White) | Proximal | Convex | Round | 1-2 | Lacunar | + | + | - | + |
| | Median | Convex | Round | 1-2 | Lacunar | + | + | - | + |
| | Distal | Concave | Round | 1-3 | Lacunar | - | + | - | Unicellular trichome |
| <i>X. sagittifolium</i> | Proximal | Convex | Round | 1-6 | Lacunar | + | - | - | - |
| | Median | Convex | Round | 1-6 | Lacunar | + | + | - | + |
| | Distal | Convex | Round | 4-6 | Lacunar | + | + | - | + |

+, Present; -, Absent.

cuticular striations radiating from the guard cells were present.

Petiole anatomy

The summary of the proximal, median and distal regions of the petiole anatomy of the taxa studied is as shown on Table 5.

C. esculentum var. *antiquorum*

The outline of the adaxial surface of the proximal region was convex while that of the median and distal regions was concave. The outline is consistently round on the abaxial side of the petiole at the three regions. The epidermis was uniseriate and it was a single layer. One to four layers of parenchyma cells were encountered at the proximal and distal regions of the petiole, while one to three layers were identified at the median region. Lacunar collenchyma cells were encountered as discontinuous bundles on both the adaxial and abaxial sides of the petiole in all the three regions except in the distal region where it was not present on the adaxial side. Air spaces of varying diameter were present through the three

regions of the petiole. Collateral vascular bundles were scattered throughout the ground tissue in the three regions with the xylem being consistently surrounded by one or two layers of xylem parenchyma. Unmodified and spindle-shaped raphides were present in the three regions of the petiole, starch grains were encountered in the median region while druses were found in both median and distal regions (Plates 2 to 4).

C. esculentum var. *esculentum*

The outline of the adaxial surface of the proximal region was convex, flat to slightly convex at the median region and concave at the distal region. The outline was consistently round on the abaxial side of the petiole at the three regions. The epidermis was uniseriate and it was a single layer. One to two layers of parenchyma cells were encountered at the three regions of the petiole. Lacunar collenchyma cells were encountered as discontinuous bundles on both the adaxial and abaxial sides of the petiole in all the three regions except in the distal region where it was not present on the adaxial side. Air spaces of varying diameter are present through the three regions of the petiole. Collateral vascular bundles are scattered throughout the ground tissue in the three regions with the

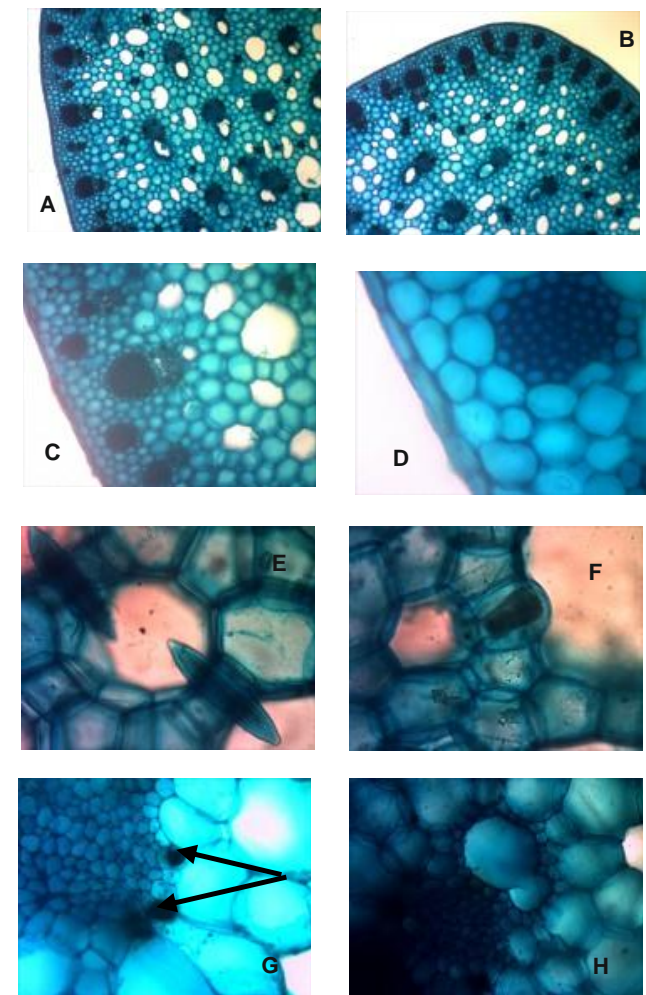


Plate 2. Transverse section of petiole and cell inclusions in the proximal region of *C. esculentum* var. *antiquorum*. A. Abaxial outline ($\times 40$); B. Adaxial outline ($\times 40$); C and D. Petiole transects ($\times 100$ and $\times 400$ respectively); E. Unmodified raphide ($\times 400$); F. Spindle-shaped raphide ($\times 400$); G. Tannins ($\times 400$, Arrowed); H. Vascular bundle ($\times 400$).

xylem being consistently surrounded by a layer of xylem parenchyma. Spindle-shaped raphides were present in the three regions of the petiole, bifurcate raphides were observed only in the proximal region, unmodified raphide types and tannins were also observed in the distal region only while druses were present in both the median and distal regions (Plates 5 to 7).

***Xanthosoma mafaffa* (Red)**

The outline of the adaxial surface of the proximal region was flat to slightly convex, convex at the median region and concave at the distal region. The outline was consistently round on the abaxial side of the petiole at the three regions. The epidermis was uniseriate and it was a

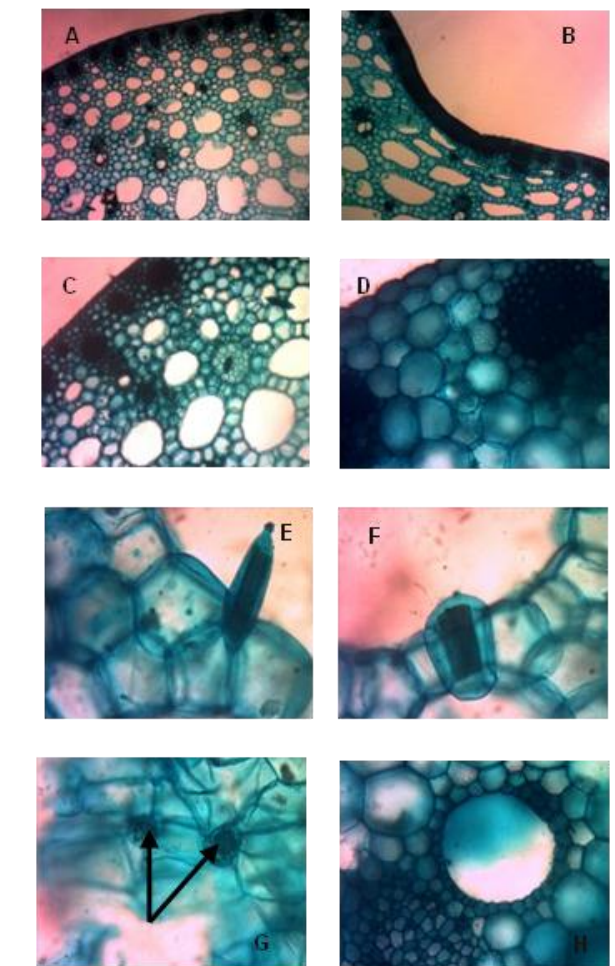


Plate 3. Transverse section of petiole and cell inclusions in the median region of *C. esculentum* var. *antiquorum*. A. Abaxial outline ($\times 400$); B. Adaxial outline ($\times 400$); C and D. Petiole transects ($\times 100$ and $\times 400$ respectively); E. Unmodified raphide ($\times 400$); G. Druses ($\times 400$, Arrowed); H. Vascular bundle ($\times 400$).

single layer. Two to three layers of parenchyma cells were encountered at the proximal region of the petiole, while two to four layers were identified at the median and distal regions. Lamella collenchyma cells were encountered as discontinuous bundles on both the adaxial and abaxial sides of the petiole in all the three regions except in the distal region where it was absent on the adaxial side. Air spaces of varying diameter were present through the three regions of the petiole. Collateral vascular bundles were scattered throughout the ground tissue in the three regions with the xylem being consistently surrounded by one layer of xylem parenchyma. Unmodified raphides were encountered only in the median region of the petiole, starch grains were present in all the three regions, tannins were present only at the proximal region while druses were encountered at the median and distal regions (Plates 8 to

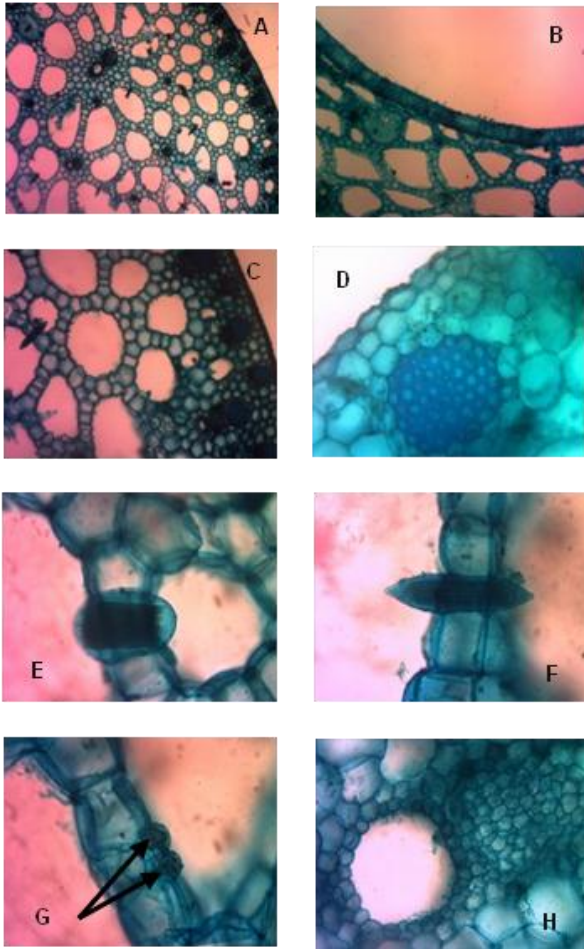


Plate 4. Transverse section of petiole and cell inclusions in the distal region of *C. esculentum* var. *antiquorum*. A. Abaxial outline ($\times 400$); B. Adaxial outline ($\times 400$); C and D. Petiole transects ($\times 100$ & $\times 400$ respectively); E. Unmodified raphide ($\times 400$); F. Spindle-shaped raphide ($\times 400$); G. Druses ($\times 400$, Arrowed); H. Vascular bundle ($\times 400$).

10)

X. mafaffa (White)

The outlines of the adaxial surface of the proximal and median regions were convex while that of the distal region was concave. The outline was consistently round on the abaxial side of the petiole at the three regions. The epidermis was uniseriate and it was a single layer. One to two layers of parenchyma cells were encountered at the proximal and median regions of the petiole, while one to three layers were identified at the distal region. Lacunar collenchyma cells were encountered as discontinuous bundles on both the adaxial and abaxial sides of the petiole in all the three regions except in the distal region where it was absent on the adaxial side. Air spaces of varying diameter are present through the three regions of

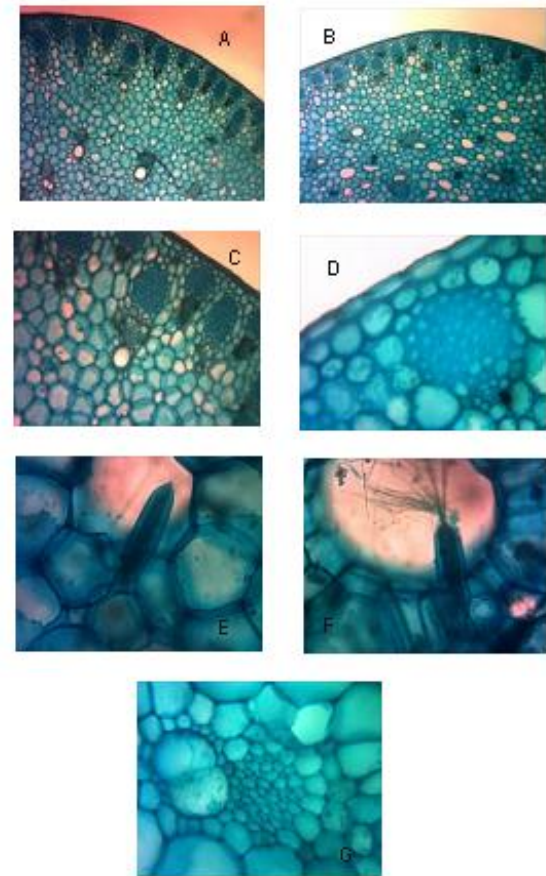


Plate 5. Transverse section of petiole and cell inclusions in the proximal region of *C. esculentum* var. *esculentum*. A. Abaxial outline ($\times 40$); B. Adaxial outline ($\times 40$). C and D. Petiole transects ($\times 100$ and $\times 400$ respectively). E. Spindle-shaped ($\times 400$). F. Bifurcated raphides ($\times 400$). G. Vascular bundle ($\times 400$)

the petiole. Collateral vascular bundles were scattered throughout the ground tissue in the three regions with the xylem being consistently surrounded by one layer of xylem parenchyma. Unmodified raphides were encountered in the proximal and median regions, druses and starch grains were present in the three regions of the petiole. Trichome-like (eglandular, uniseriate trichomes) structures were identified in the distal region of the petiole (Plates 11 to 13).

Xanthosoma saggitifolium

The outline of the adaxial surface of the three regions was convex while that of the abaxial side is consistently round. The epidermis was uniseriate and it was a single layer. One to six layers of parenchyma cells were encountered at the proximal and median regions of the petiole, while four to six layers were identified at the distal region. Lacunar collenchyma cells were encountered as

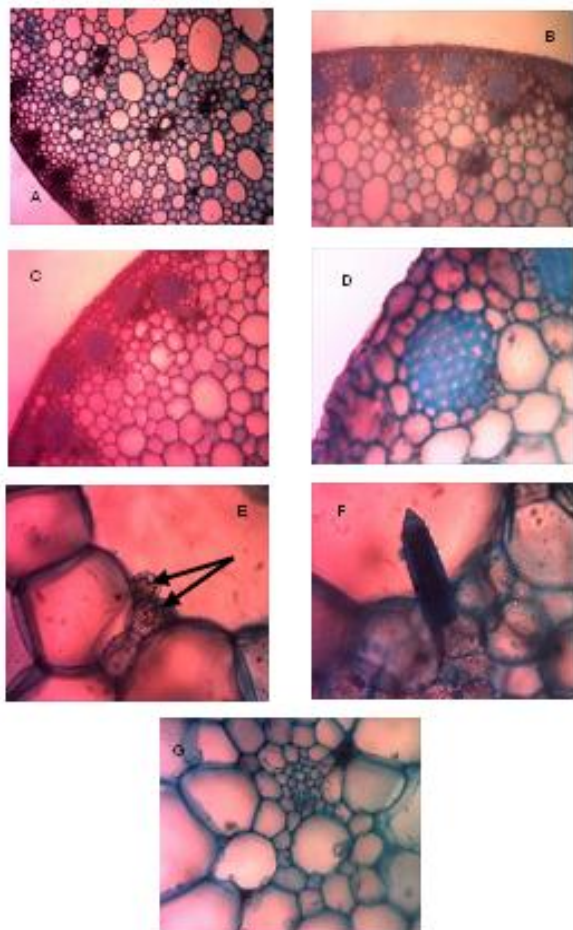


Plate 6. Transverse section of petiole and cell inclusions in the median region of *C. esculentum* var. *esculentum*.. A. Abaxial outline (×400); B. Adaxial outline (×400); C and D. Petiole transect (×100 & ×400 respectively); E. Druses (×400, Arrowed); F. Spindle-shaped raphide (×400); G. Vascular bundle (×400).

discontinuous bundles on both the adaxial and abaxial sides of the petiole in the three regions. Air spaces of varying diameter were present through the three regions of the petiole. Collateral vascular bundles were scattered throughout the ground tissue in the three regions with the xylem being consistently surrounded by one layer of xylem parenchyma. Spindle-shaped raphides were encountered in all the three regions, while druses and starch grains were encountered in the median and distal regions (Plates 14 to 16).

DISCUSSION

Anatomical features have been found very useful in the classification of plant species and so the use of anatomical features in separating or delimiting species is germane. This is because most anatomical features are

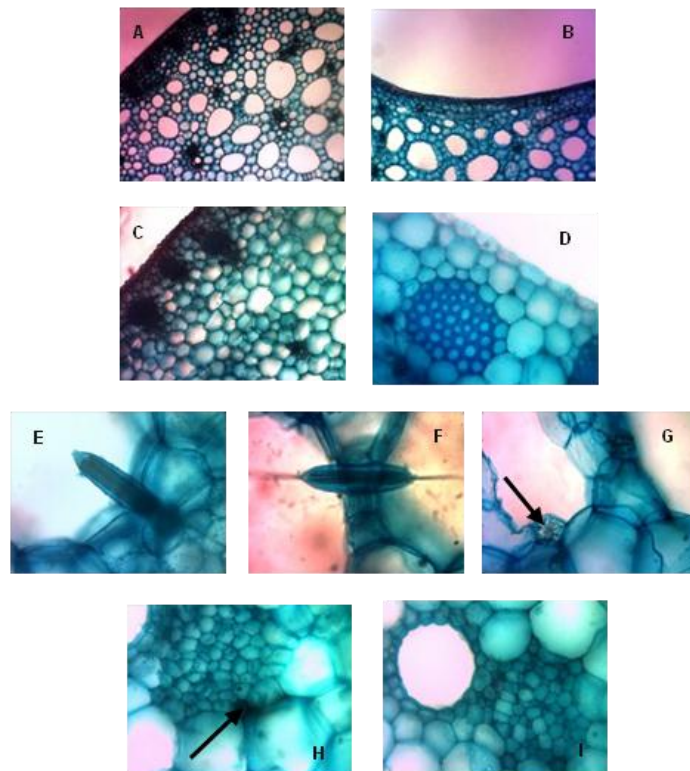


Plate 7. Transverse section of petiole and cell inclusions in the distal region of *C. esculentum* var. *esculentum*. A. Abaxial outline (×400); B. Adaxial outline (×400); C & D. Petiole transect (×100 & ×400 respectively); E. Spindle-shaped raphide (×400); F. Bifurcated raphide (×400); G. Druses (×400, Arrowed); H. Tannins (×400, Arrowed); I. Vascular bundle (×400).

not affected or altered by the environmental factors. Anatomical research works that have been done on some species of Araceae, which include the works of Green and Oguzor (2009) on four selected species from genera *Xanthosoma*, *Dieffenbachia* and *Colocasia* in the South Eastern part of Nigeria; Ina and Eka (2013) on leaf surface comparison of some species in the genera *Alocasia*, *Colocasia* and *Remuatia* in Indonesia; Osuji and Nwala (2015) on some cultivars of *Xanthosoma* and *Colocasia* also in the South Eastern part of Nigeria, were limited to the epidermal studies of the selected species or cultivars only. This study provided some more detailed information on the epidermal surfaces of more taxa as well as the details of the transverse sections of the petioles in the three petiole regions, proximal, median and distal regions.

Similar and diagnostic characters which were useful tools in the taxonomy of the two genera were identified in this study. The epidermal cell shape on the adaxial and abaxial surfaces of the two varieties of *Colocasia* studied; *C. esculentum* var. *antiquorum* and *C. esculentum* var. *esculentum* as well as that of the three taxa in the genus *Xanthosoma*; *X. mafaffa* (Red), *X. mafaffa* (White) and *X.*

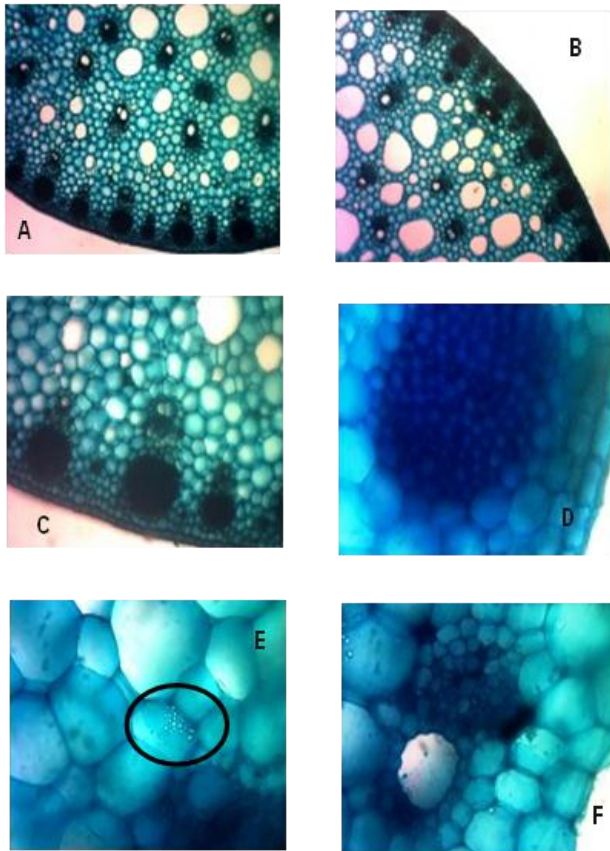


Plate 8. Transverse section of petiole and cell inclusions in the proximal region of *X. maffafa* Red variety. A. Abaxial outline (x40); B. Adaxial outline (x40); C & D. Petiole transects (x100 & x400 respectively); E. Starch grains (x400, Circled); F. Vascular bundle.

sagittifolium is polygonal. This was a common character to the two genera. Anticlinal wall pattern on the adaxial surface was straight in all the taxa of *Colocasias* and *Xanthosoma* studied. On the abaxial surface however, though the wall pattern was straight in all the taxa, it was straight to wavy in *X. sagittifolium*. This separated *X. sagittifolium* from the two varieties of *X. maffafa*. Adedeji et al. (2007) also employed anticlinal wall pattern in the separation of some species in the family Solanaceae. Generally, there were more stomata on the abaxial surfaces than on the adaxial surfaces of all the taxa studied. The presence of brachyparacytic stomata complex type in all the taxa agrees with the findings of Osuji and Nwala (2015) who reported the presence of paracytic and brachyparacytic stomata types in the cultivars of *Xanthosoma* and *Colocasias* that they studied. However, additional stomata complex types were encountered on one or both surfaces of some of the taxa studied. On the adaxial surface, anisocytic stomata were found in *C. esculentum* var. *esculentum* which separated it from the other variety, *C. esculentum* var. *antiquorum*.

On the abaxial surface, anomocytic stomata were the

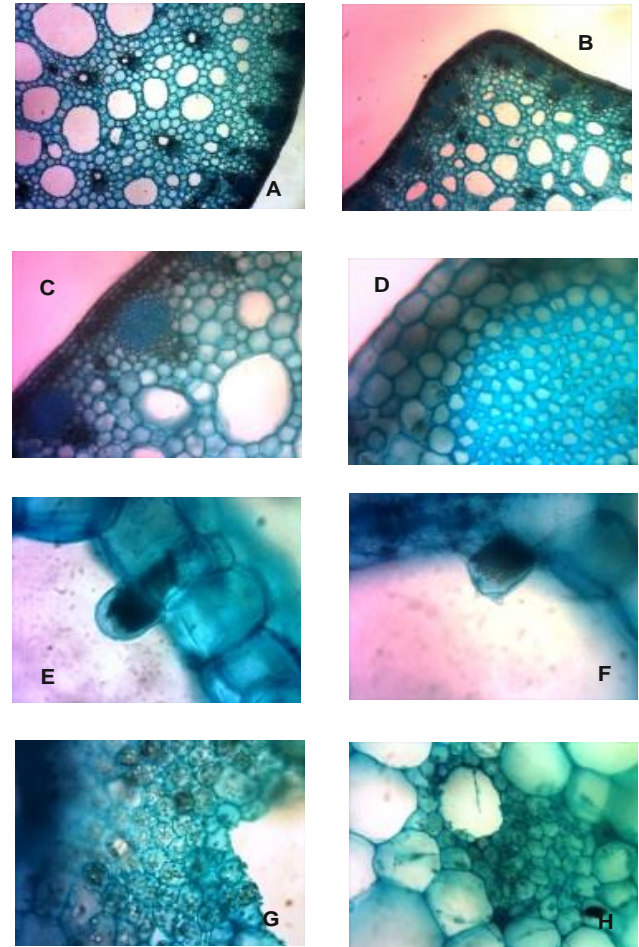


Plate 9. Transverse section of petiole and cell inclusions in the median region of *X. maffafa* Red variety. A. Abaxial outline (x40). B. Adaxial outline (x40). C and D. Petiole transects (x100 & x400 respectively). E & F. Unmodified raphides (x400). G. Druses and starch grains (x400). H. Vascular bundle (x400).

additional stomata encountered in all the taxa but not in the red variety of *X. maffafa*. According to Hetherington and Woodward (2003), stomata types are genetically determined and so cannot be influenced by the environment.

Stomata size is also quite diagnostic (Thair and Rajput, 2009). The largest stomata sizes were encountered in *X. sagittifolium* while the smallest sizes were encountered in *C. esculentum* var. *antiquorum*. Elliptic shaped stomata were common to all the taxa studied; notwithstanding, some of the taxa have additional circular shaped stomata especially on their abaxial surfaces. Stomata index was generally higher on the abaxial surface than on the adaxial surface in all the taxa studied. Druses of calcium oxalate crystals were found on the adaxial and abaxial epidermal surfaces of *C. esculentum* var. *antiquorum* only. This distinguished it from *C. esculentum* var.

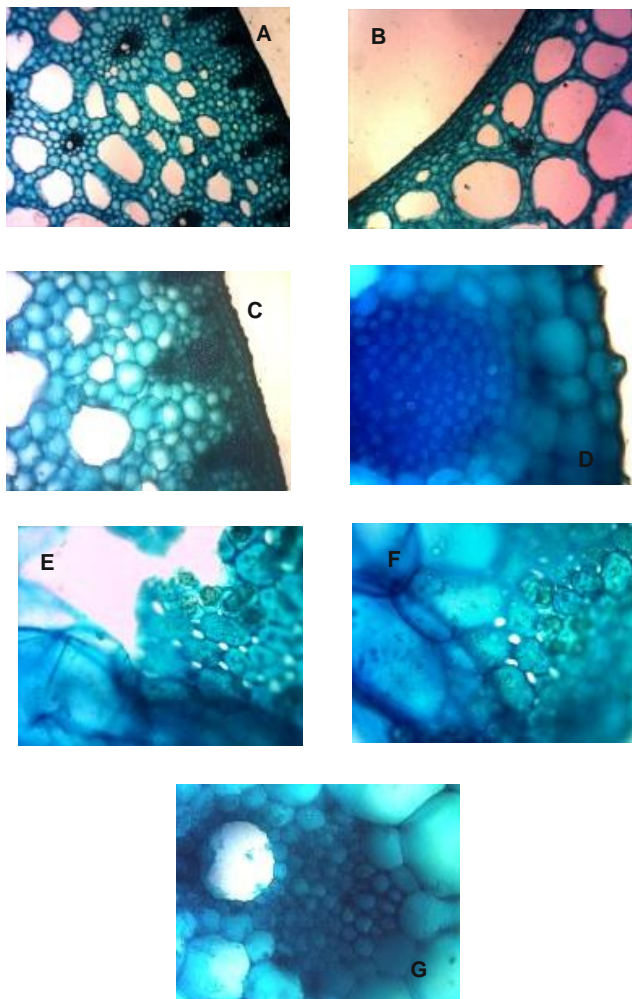


Plate 10. Transverse section of petiole and cell inclusions in the distal region of *X. maffafa* Red variety. A. Abaxial outline (x40). B. Adaxial outline (x40); C & D. Petiole transects (x100 &x400 respectively);E:Druses (x400); F. Starch grains (x400); G. Vascular bundle (x400).

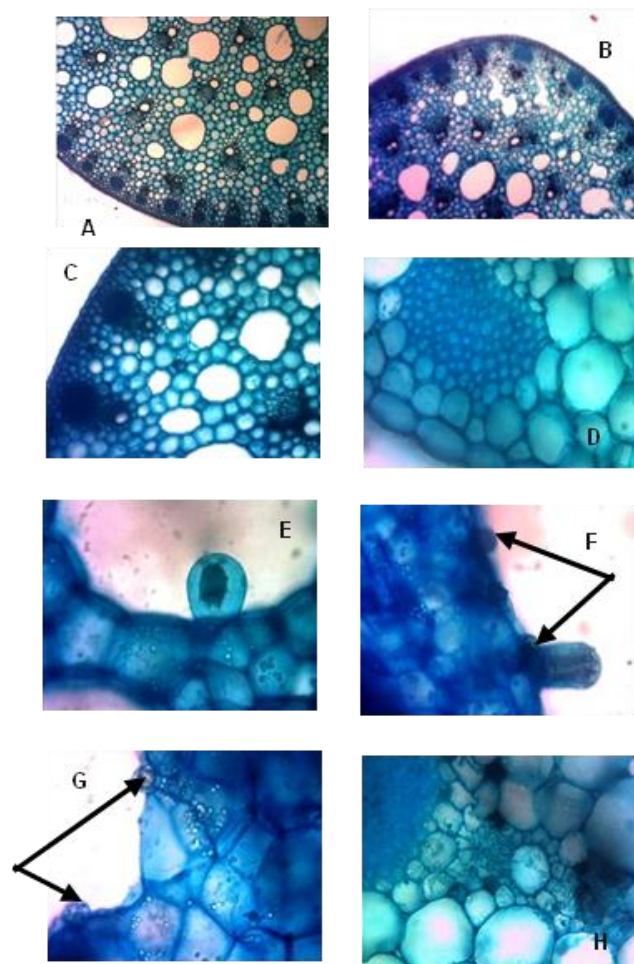


Plate 11. Transverse section of petiole and cell inclusions in the proximal region of *X. maffafa* White variety. A. Abaxial outline (x40). B. Adaxial outline (x40). C & D. Petiole transects (x100 &x400 respectively). E. Unmodifiedraphide (x400). F. Unmodified raphide and druses (x400, Arrowed). G. Druses (Arrowed) and starch grains (x400). H. Vascular bundle (x400).

esculentum and the members of the genus *X.* in this study. Adedeji and Illoh (2004) also reported a separation among some species of *Hibiscus* based on the presence of druses.

The presence of globular or spherical papillae in some of the taxa studied is of diagnostic value. Papillae were encountered on the adaxial surfaces of the two varieties of *Colocasia* studied, that is, *C. esculentum* var. *antiquorum* and *C. esculentum* var. *esculentum* but not on those of the three taxa of *Xanthosoma*. This can be employed in the delimitation of the members of the genus *Colocasia* from the members of the genus *Xanthosoma*. On the abaxial surface, however, they were encountered in all the species and varieties of the two genera. They were of variable shapes and sizes. The subsidiary cells in most of these taxa have no papillae. Osuji and Nwala

(2015) also reported the presence of papillae on the abaxial surfaces of the *X. maffafa* accessions but did not report it on the two surfaces of *Colocasia*. Osuji (2006) separated two species in the genus *Musa* based on the presence or absence of papillae.

Ridges or folds of the cuticle form ornamentalations on them; these ornamentalations largely consist of striae, hence, striated cuticle (Metcalf and Chalk, 1979).

According to Solereder (1908), these striations are very useful for specific diagnosis and are not always developed in the same way on the two surfaces of the leaf, they are also taxonomically stable. Adedeji and Illoh (2004) used cuticular striations to separate some species of *Hibiscus*. In this study, striated cuticle was found on the adaxial surface of the red variety of *X. maffafa* as well

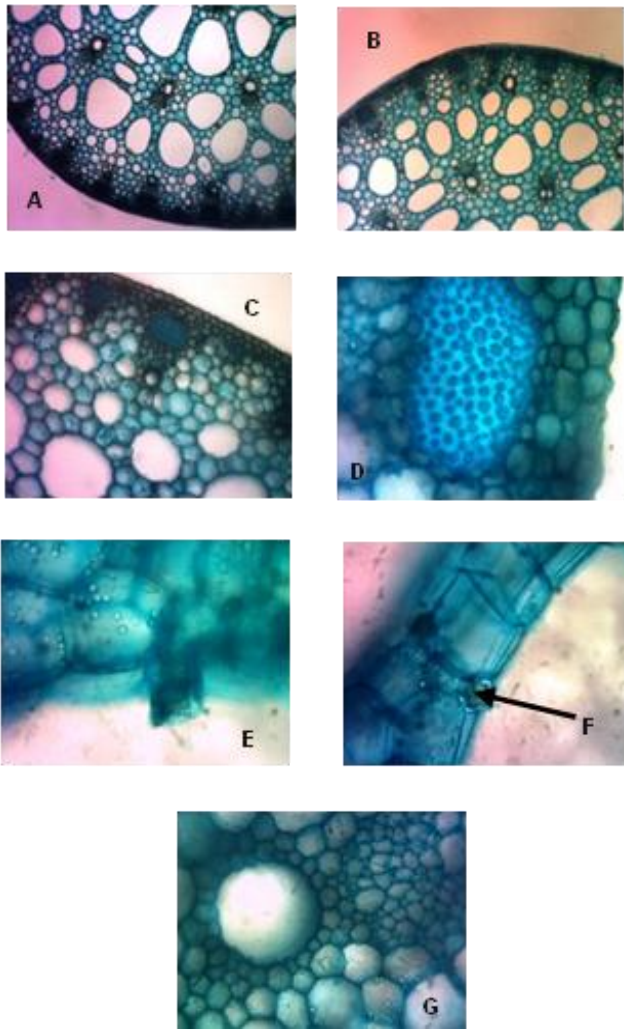


Plate 12. Transverse section of petiole and cell inclusions in the median region of *X. maffafa* White variety. A. Abaxial outline (x40); B. Adaxial outline (x40); C & D. Petiole transects (x100 & x400 respectively); E. Unmodified raphide and starch grains (x400); F. Druses (x400, Arrowed); G. Vascular bundle (x400).

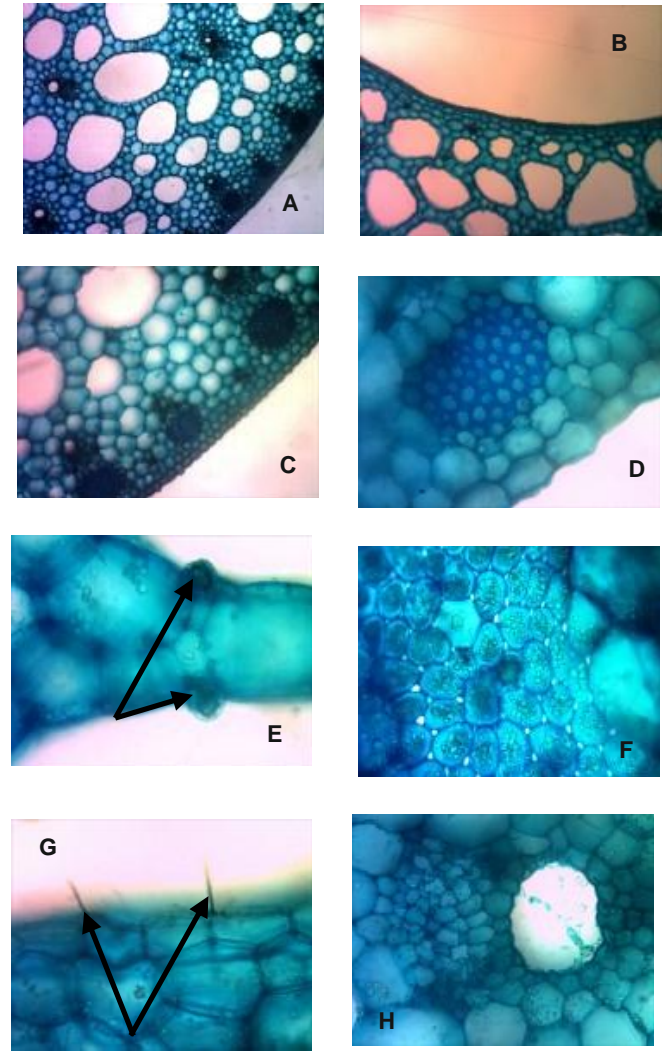


Plate 13. Transverse section of petiole and cell inclusions in the distal region of *X. maffafa* White variety. A. Abaxial outline (x40); B. Adaxial outline (x40); C and D. Petiole transects (x100 & x400 respectively). E. Druses (x400, Arrowed); F. Starch grains (x400); G. Trichome-like structures (x400, Arrowed); H. Vascular bundle (x400).

as on the abaxial surfaces of all the taxa of the genus *Xanthosoma* studied, that is, the red and white varieties of *X. maffafa* and *X. saggitifolium*. However, the striation pattern on the abaxial surface of the red variety of *X. maffafa* revealed a unique feature as it formed a continuous network with the papillae in such a way that it kept the stomata and epidermal cells out of focus. This is a delimiting factor among the members of the genus *Xanthosoma*. Also, no cuticular striations were observed in the two varieties of *C. esculentum*.

The results of the anatomy of the petiole have provided a wide range of characters that were equally of diagnostic value in delimiting the Araceae species studied. Thakuri and Patil (2011) have separated some species of the family Euphorbiaceae using petiole anatomy. Concave

adaxial petiole outline in the median region of *C. esculentum* var. *antiquorum* differentiated it from *C. esculentum* var. *esculentum* and the three members of genus *Xanthosoma* in this study with convex or flat to slightly convex outline. In the distal region, however, convex adaxial petiole outline separated *X. saggitifolium* from all the other taxa where concave adaxial petiole outline was observed. The cells of the epidermis in the petiole of all the taxa studied were of one layer and uniseriate. Parenchyma cells of varying number of layers from one to six were present in the proximal, median and distal regions of the petiole of the taxa studied.

All the taxa studied have the collenchyma cells as discontinuous bundles but the types and location of

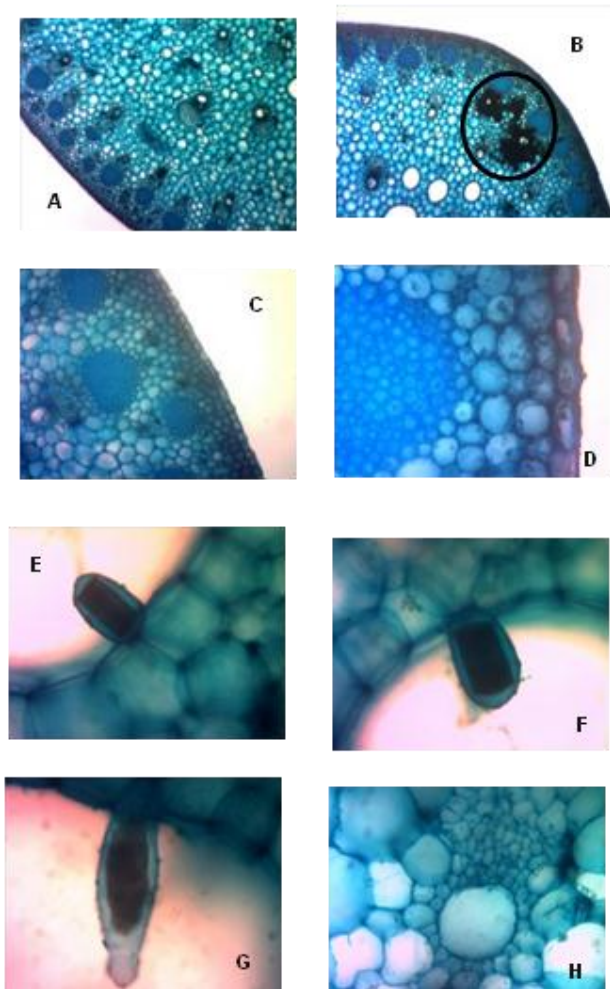


Plate 14. Transverse section of petiole and cell inclusions in the proximal region of *X. saggitifolium*. A. Abaxial outline ($\times 40$); B. Adaxial outline with Tannins (Circled) ($\times 40$); C and D. Petiole transects ($\times 100$ & $\times 400$ respectively). E, F and G. Spindle-shaped raphides ($\times 400$); H. Vascular bundle ($\times 400$).

collenchyma cells can be employed in separating them. Lacunar collenchyma cells were encountered in the two varieties of *Colocasia*; *C. esculentum* var. *antiquorum* and *C. esculentum* var. *esculentum*, as well as *X. mafaffa* (White) and *X. saggitifolium* whereas the collenchyma cells were the lamellar type in *Xanthosoma mafaffa* (Red) which separated it from the white variety and the other taxa. The collenchyma cells were observed on both the adaxial and abaxial surfaces of the proximal and median regions of all the taxa in this study. The variation observed was in the distal region where it occurred on both surfaces of *X. saggitifolium* and only the adaxial surfaces of the other four taxa. Collateral vascular bundles scattered in the ground tissues were found in the three regions of all the taxa studied and all the xylem cells were surrounded by xylem parenchyma. Vascular bundles are known to be scattered in the ground tissues

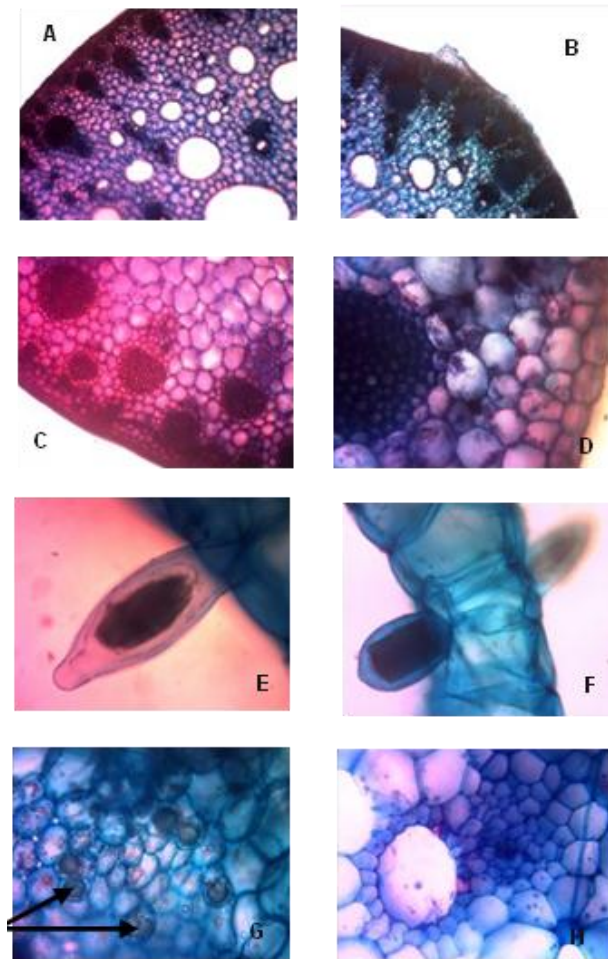


Plate 15. Transverse section of petiole and cell inclusions in the median region of *X. saggitifolium*. A. Abaxial outline ($\times 40$); B. Adaxial outline ($\times 40$); C & D. Petiole transects ($\times 100$ & $\times 400$ respectively); E & F. Spindle-shaped raphides ($\times 400$); G. Starch grains and druses (Arrowed) ($\times 400$); H. Vascular bundle ($\times 400$).

of Monocots, which is one of their major characteristics (Fahn, 1974).

Raphides, druses, tannins and starch grains were observed in petiole of all the taxa. The presence of raphides and druses which are calcium oxalate crystals have been well established among the members of the family Araceae (Middendorf, 1982; Mayo et al., 1997; Arogundade and Adedeji, 2016). From the work of Keating (2004), eight types of raphides have been established. They are unmodified, styloids, wide cells (a form of the unmodified raphide crystal), elongated cells, tubular cells, articulated tubes, spindle-shaped, and bifurcate raphides. The unmodified raphide type was observed in all the taxa studied. The spindle-shaped type was observed only in the two varieties of *Colocasia* and *X. saggitifolium* while the bifurcates were observed only in *C. esculentum* var. *esculentum*. This can also be

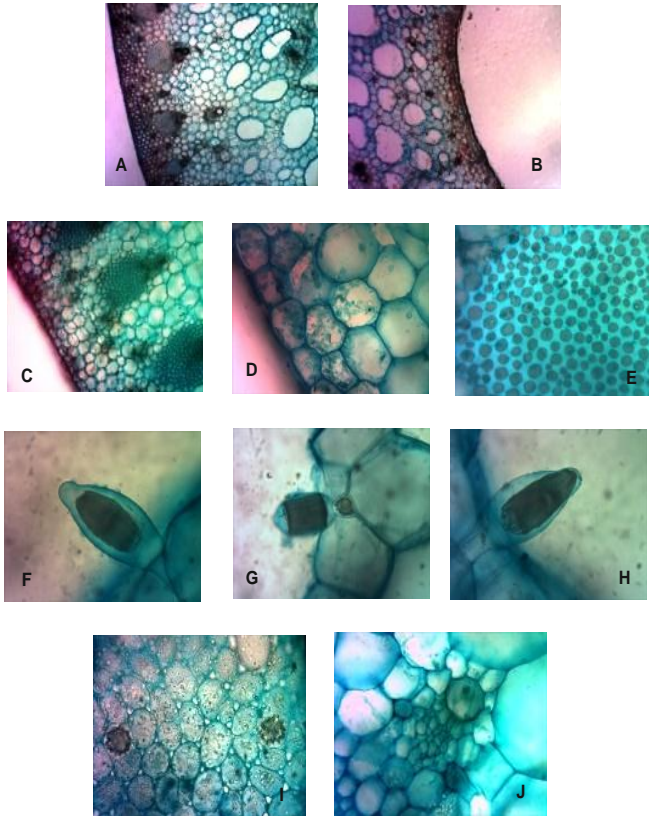


Plate 16. Transverse section of petiole and cell inclusions in the distal region of *X. saggitifolium*. A. Abaxial outline ($\times 40$); B. Adaxial outline ($\times 40$); C & D. Petiole transects ($\times 100$ & $\times 400$ respectively); E. Lacunar collenchyma type ($\times 400$); F, G and H. Spindle-shaped raphides ($\times 400$); I. Druses ($\times 400$, Arrowed); J. Vascular bundle ($\times 400$).

employed in delimiting the taxa in this study. Tannins were observed in *C. esculentum* var. *esculentum* and *X. mafaffa* (Red). They were not encountered in the other taxa. Where present, they were always found close to the vascular bundles.

Aroids, plants in the Araceae family are generally known to be glabrous. Trichomes are highly unusual in their leaves and stems (Solereder and Meyer, 1928; Mayo et al., 1997; Bown, 2000). Interestingly, in this study, some trichome-like structures were found on the adaxial side in the distal region of the petiole of *X. mafaffa* (White). They were more or less unicellular, non-glandular trichomes. Solereder and Meyer (1928) also reported the presence of unicellular hairs in *X. pubescens*. This obviously separated *X. mafaffa* (White) from its Red variety and the other taxa in this study.

Conclusion

In conclusion, the members of genera *Colocasia* and *Xanthosoma* can be separated based on their anatomical

features although many of the observed characters are common to them affirming their familial classification. Some other characters cut across the two genera. The unifying features observed include polygonal epidermal cell shape, straight adaxial anticlinal wall pattern, brachyparacytic stomata, elliptic shaped stomata and unmodified raphide type. Diagnostic features that can be employed in separating the two genera are the presence of papillae on the adaxial surfaces of the two varieties of *Colocasia* which were not observed on the adaxial surfaces of the *Xanthosoma* taxa. Also cuticular striations observed only in the members of genus *Xanthosoma* but not in the members of genus *Colocasia*. Worthy of note is the presence of unicellular non-glandular trichomes observed only in *X. mafaffa* (White).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutrient and antinutrient constituents in seeds of *Sphenostylis stenocarpa* (Hochst. Ex A. Rich.) Harms

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Nutrient and antinutrient constituents in seeds of *Sphenostylis stenocarpa* were determined. The proximate composition revealed that *S. stenocarpa* seed contains 12.44% moisture, 18.55% protein, 2.41% lipid, 2.93% ash, 1.95% fibre, 74.16% carbohydrate and 392.50 kcal caloric value. The antinutrients analysis showed that the seed contains 15.25 mg/100 g hydrocyanide, 144.14 mg/100 g tannins, 1.01 mg/100 g phytate and 61.60 mg/100 g oxalate. It may be concluded that the seeds of *S. stenocarpa* contribute to nutrient intake by the consuming populations in Nigeria and contain some pharmacological evidence for the treatment of stomach aches and acute drunkenness can serve as an antimalarial, antidiabetic, fertility agent, anti-cancer, anti-ulcer and cardioprotective agent.

Key words: Nutrient, antinutrient, *Sphenostylis stenocarpa*.

INTRODUCTION

The African yam bean (*Sphenostylis stenocarpa* Hochst. ex. A. Rich) is a leguminous crop belonging to the family Fabaceae, sub-family papilionoidae, tribe Phaseoleae, sub-tribe Phaseolionae and genus *Sphenostylis* (Okigbo, 1973; Allen and Allen, 1987). It is a minor crop cultivated together with cassava and yam. Being a minor crop shows it is highly not exploited (Klu et al., 2001). It is cultivated in West Africa, specifically Cameroon, Cote d'Ivoire, Ghana, Nigeria and Togo (Porter, 1992). In Nigeria, it is seen in local places in the southern part of Nigeria, where it is cultivated by poor farmers as a security crop. It has risk of disappearing due to the high payment on the major legumes enumerated earlier and others like Soya bean.

The economic importance of African yam bean is immense. High population, high prices of staple food

items, policy restrictions on importation of food worsen food security in developing nations with predominantly protein deficiency and malnutrition (Weaver, 1994; FAO, 1994, 2008). To fill the high gap in the balanced food provision for developing nations' increasing population, lesser-known crops are given attention, those that play great roles in the economy of peasant farmers (Ezeagu et al., 2002). These crops include African yam beans *S. stenocarpa* (Hochst. ex A. Rich.) Harms and pigeon pea (*Cajanus cajan* L. Mill Sp.). They are planted to be consumed at home and for sale in Nigeria (Saka et al., 2004) irrespective of their abilities to give enough require nutrients. Plants like these are called under-exploited, under-utilized, orphan or abandoned (Jaenicke et al., 2009). The nutritious seeds are sweet, and many places of Nigeria people prefer it to other leguminous seeds.

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Besides its palatable leaves and pods, the tubers can be cooked as vegetable (Rice et al., 1986).

Grain legumes constitute the main source of protein in the diets of the average Nigerian home. The most important ones are cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogea*) and lima bean (*Phaseolus lunatus*). However, there are other pulses that could help meet dietary needs but are cultivated only in localized areas and used less (Klu et al., 2001). These underexploited legumes include African yam bean (*S. stenocarpa*), Bambara groundnut (*Vigna subterranea*) and pigeon pea (*C. cajan*).

The current unpredictability in the supply of food globally and the increased demand expected demands searching for other food sources that everyone can access. A lot of researchers like Fetuga et al. (1973), Aletor and Aladetimi (1989) and Fowomola and Akindahunsi (2007) have investigated the nutritional potentials of plant seeds less known as other food sources. Developing nations' desire to advance in achieving the Millennium Development Goals (MDGs), mostly to eradicate severe destitution and famine specifically necessitates doing great study in certain less used native crops and tree plants.

Antinutrients are found at some level in almost all foods for a variety of reasons. However, their levels are reduced in modern crops, probably as an outcome of the process of domestication. Nevertheless, the large fraction of modern diets that come from a few crops, particularly cereals, has raised concerns about the effects of the antinutrients in these crops on human health (Cordian, 1999). The possibility now exists to eliminate antinutrients entirely using genetic engineering; but since these compounds may also have beneficial effects (such as polyphenols which reduce the risk of cancer, heart disease or diabetes), such genetic modifications could make the foods more nutritious but not improve people's health (Welch and Graham, 2004).

Many traditional methods of food preparation such as fermentation, cooking and malting increase the nutritive quality of plant foods though reducing certain antinutrients such as phytic acid, polyphenols and oxalic acid (Hotz and Gibson, 2007). Such processing methods are widely-used in societies where cereals and legumes form a major part of the diet (Chavan and Kadam, 1989; Phillips, 1993). Other antinutrients found in crops include saponins and hydrocyanide. These anti-nutrients when subjected to cooking are denatured thus making them to be non toxic.

Unlike animals, plants lack teeth, claws and legs that would enable to get out of trouble. Many plants stay on in a place and they depend on chemical defences to drive enemies not wanted. For this reason, plants store a great number of chemicals that are harmful to bacteria, fungi, insects, herbivores, and man. Luckily these different chemicals contain a lot of compounds useful to man; vitamins, nutrients, antioxidants, anticarcinogens,

and a lot of other compounds that are important medically (Novak and Haslberger, 2000).

A lot of plants such as food plants have certain levels of natural plant pollutants. The levels of pollutants in plant tissues are measured by a chemist to analyze their safety for animal feed and drug. The impact of natural plant pollutants seen at reduced levels in a lot of drugs and foods and drugs consumed by us, on man and animals, is due to laboratory tests that use very high amounts of toxin than usually seen in food and drug. Each edible plant species has its own nutrient content apart from its phytochemicals that are useful pharmacologically. These nutrients needed for the physiological functions of man body. Such nutrients and biochemicals such as carbohydrates, fats and proteins satisfy man's needs for life and energy (Hoffman et al., 1998; Dingman, 2002).

Due to the transformations all over Africa, wild plants are at risk of going into extinction and might affect the nutrition of the local people (Herzog et al., 1994). Numerous scientific data indicate that the consumption of grains is associated with a lower risk of several chronic diseases, such as cancers and cardiovascular diseases. The preventive effect is often associated to naturally occurring antioxidant components, such as anthocyanins, flavonoids, and other phytochemicals that are predominantly present in the seed coat (Bomser et al., 1996; Wang and Mazza, 2002).

All legumes contain phytate (also known as phytic acid). Phytate works in the gastro-intestinal tract to tightly bind minerals such as zinc, copper, iron, magnesium and calcium. It has a particularly strong affinity for zinc, a mineral that supports wound healing, protein synthesis, reproductive health, nerve functions, and brain development. It is believed that people living in developing countries are shorter than those in developed countries because of zinc deficiency caused by eating too many legumes. There is also evidence that mental development can be negatively impacted by a diet high in phytate (Minton, 2009). In most legumes such as other varieties of beans, soaking is enough to break down most of the phytate content. However, soybean requires that the enzymes be released during the fermentation process to reduce its phytate content to the point where it becomes fit for consumption.

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. One common example is phytic acid, which forms insoluble complexes with calcium, zinc, iron and copper (Cheryan, 1980). Proteins can also be antinutrients, such as trypsin inhibitors and lactins found in legumes (Gilani et al., 2005). These enzyme inhibitors interfere with digestion. Another particularly wide-spread form of anti-nutrient is flavonoids, which are a group of polyphenolic compounds that include tannins (Beecher, 2003). These compounds chelate metals such as iron and zinc and reduce the absorption of these nutrients, but they also inhibit digestive enzymes and may also precipitate proteins.

Table 1. Proximate composition (%) of *S. stenocarpa*.

| Nutrient | Sample composition % |
|----------------------|----------------------|
| Moisture contents | 12.44 |
| Crude protein | 18.55 |
| Crude lipid | 2.41 |
| Ash | 2.93 |
| Crude fibre | 1.95 |
| Carbohydrate | 74.16 |
| Caloric value (Kcal) | 392.50 |

However, polyphenols such as tannins have anticancer properties, so foods such as green tea that contain large amounts of these compounds might be good for the health of some people despite their antinutrient properties (Chung et al., 1998). Locally, very little is known about the economic and nutritional value of *S. stenocarpa*, this studies aim to add to the existing information the nutritional and antinutritional value of the said seed crop.

MATERIALS AND METHODS

Sources and collection of seeds

The seeds of African yam bean *S. stenocarpa* (Hochst. ex.A. Rich) Harms were collected from local farmers in Use Offot, and Nsukara Offot in Uyo Local Government Area of Akwa Ibom State. The seeds were identified by a taxonomist in the Department of Biological Sciences, Akwa Ibom State University. The seeds were extracted from the dried pods. Observation showed that two sizes of seeds and two colours were present, a small size (SS) and a large size (LS), brown and white. The weight of each seed was also determined. Premature and infected seeds were discarded, and selected seeds were taken to the laboratory for analysis. Seeds from both sources (Use Offot and Nsukara Offot) were pooled together and the brown colour seeds were used for the studies.

Proximate analysis

Analysis of the nutrient content of *S. stenocarpa* was carried out using the method of AOAC (1984).

Analysis of anti-nutrients

The method of AOAC (1984) was employed to determine the level of Hydrocyanide, Oxalate, phytate and tannins.

RESULTS

The results of the proximate analysis of *S. stenocarpa* seeds are presented in Table 1. The results show that the seed contains (12.44%) moisture, (18.55%) protein, (2.41%) lipid, (2.93%) Ash, (1.95%) fibre, (74.16%) carbohydrate and (392.50 kcal) caloric value. Table 2

shows the antinutrients content of *S. stenocarpa* seeds. The results indicate that the seed contains hydrocyanide (15.256 mg/100 g), tannins (144.144 mg/100 g), phytate (1.006 mg/100 g) and oxalate (961.60 mg/100 g).

DISCUSSION

The results of the proximate composition of the seed of *S. stenocarpa* as shown in Table 1 show that the moisture contents (12.44%) was within the range obtained for red kidney bean (12.39 ±4.60) (Sasanam et al., 2011) but was more than 4.85 as in gourd seed (9.13 ±8.0%) (Ogunbenie, 2006), pumpkin seed (5.02%) (Aisegbu, 1987), pumpkin seed (5.5%) (Fagbemi and Oshodi, 2005) and shelled lima bean (4.42%) (Oyenuga, 1968). This observed variation might have resulted from genetic geographic, climate and season variations.

The protein content value (18.55%) was comparable to that of *Azelia africana* seed (16:52 ± 0.79%) (Ogunlale et al., 2011) and low in comparison to other palatable leguminous seed flours like pigeon peas, cowpeas and soybeans (Olaofe et al., 1994), certain lima bean varieties (Oshodi, 1993), some cowpea varieties (Aletor and Aladetimi, 1989) and related less used legumes (*Caesalpinia pulcherima*) (Olaofe et al., 2004). Its protein content makes it nutritionally a good source of plant protein; this validates its use in diet as a plant protein which can supplement animal protein thereby alleviating kwashiorkor and marasmus. Its palatability however, depends on handling when prepared as meal.

The value for the lipids content (2.41%) was similar to the fruit pulp of *Spondias mombin* (2.0 ± 0.05) (Adepoju, 2009) and were low when compared with 43.2% for calabash kernel (Olaofe et al., 2009) and 23.5% for soybean seed (Paul and Southgate, 1985). The seed of *S. stenocarpa* recorded ash content of 2.93%. This value was lower than (3.00 to 3.8%) in certain varieties of cowpea (Aletor and Aladetimi, 1989) and (3.1 ± 3.6%) for various lima bean flours (Oshodi and Adeladun, 1993), but comparable favourably with values (2.62 ± 0.025%) reported for mango seed (Fowomola, 2010). This suggests that the seeds of this plant might be mineral sources the body needs to develop well. The crude fibre

Table 2. Antinutrients contents (mg/100 g) of *S. stenocarpa* seeds.

| Antinutrient content | Sample composition (mg/100 g) |
|----------------------|-------------------------------|
| Hydrocyanide | 15.256 |
| Tannins | 144.144 |
| Phytate | 1.006 |
| Oxalate | 61.60 |

value 1.95% was low in comparison to the 2.8 and 4.28% for gourd seeds (Ogungbenle, 2006) and soybean (Temple et al., 1991). The low fibre composition can reduce the taking in of bile (Marfo et al., 1990). The content of carbohydrate (74.16%) was high as compared with values reported for *Pachira glabra* and *Azelia africana* seeds (52.32 ± 0.8) and (45.92 ± 0.72) respectively (Ogunlade et al., 2011). This shows that *S. Stenocarpa* seed is a good source of carbohydrate with protein and the gross energy value qualify it as a good energy source.

Food analysts are greatly concerned about oxalate due to its adverse effect on the availability of minerals. Food having high level of oxalate can result in kidney stones as its high levels tantamount to increased absorption of calcium in the kidneys (Chai and Liebman, 2004). The levels of oxalate in *S. stenocarpa* seeds were higher than 0.23 to 1.10 g/100 g (Bello et al., 2008) and 0.4% (Amoo and Agunbiade, 2010) found in certain Nigerian fruits and full seed flour of *Pterygota macrocarpa*. Oxalate builds complex if taken by animals and also great amount of oxalate diets can increase the risk of absorbing renal calcium (Osagie and Eka, 1988). Also, dietary oxalate can form complex with calcium, magnesium and iron resulting in insoluble oxalate salts and oxalate stone.

Tannins are responsible for the formation of insoluble complexes with proteins leading to low digestibility of food proteins. Tannin values got (61.60) were lower than 13.3% cashew nut, 19.1% fluted pumpkin (Fagbemi et al., 2005) and 7.0% hulled seed of *P. macrocarpa* (Amoo and Agunbiade, 2010). Tannins are pleasant compounds that contain groups of phenols. They mingle with salivary proteins and glycoproteins in the mouth and make the tissues bitter. The bitterness makes tannin valueable medically for the prevention of diarrhea and dysentery and to control haemorrhage (Jones, 1965). Also, tannins save plants from dryness, rot, destruction from animals and pathogens. Their polymerization forms insoluble protective barrier which stops attack of microbes (Stumpf and Conn, 1981). Thus they can be used on wounds as defence coating. Bichel and Bach (1968) said that continued absorption of tannin has symptoms like gastritis, irritation and edema of the intestine. Glick and Joshyn (1970) said that intake of 0.5% of tannic acid reduced the retaining of nitrogen and led to 5% death in rats. Bressani et al. (1983) reported that tannins show their adverse impacts by building protein tannin

complexes via multiple hydrogen binding between their hydroxyl groups and carboxyl groups of protein peptide bounds of proteolytic enzymes in the gastrointestinal tract.

Phytate reduce the growth of chicks given phytate-casein diet by forming complex with zinc making the later not available. Phytate value got (1.006 mg/100 g) was low. Omosaiye and Cheryan (1979) noted that, phytate formed complex with protein via the activity of cations, normally calcium, zinc or magnesium acting as a bridge between the negatively charged protein carboxyl groups and former. The content of the hydrocyanide was 15.256 mg/100 g. Chen et al. (1934) reported that the lowest harmful dose of hydrogen cyanide taken by mouth was between 0.5 and 3.5 mg/kg body weight. The hydrogen cyanide signs are peripheral numbness, light-headedness, mental confusion, stupor, cyanosis and convulsion (Halstrom and Moller, 1945).

The outputs of plant normally have higher levels of antinutritional features than those of animals. The values of the antinutritional factors that are slightly high might be because the seeds are not processed. The availability of some of these antinutrients can be decreased by different processing methods (Elegbede, 1998). Farag (2001) and Agunbiade and Olanlokun (2006) showed that soaking and boiling, autoclaving for 30 min and irradiation up to 20 KGY and roasting and boiling terribly decreased the antinutritional factors seen in mango seed kernels, leading to improvement in their features nutritionally. It is clear in this work that *S. stenocarpa* had high levels of antinutrients apart from phytate. All the antinutritional parameters levels were lower than what can make up the health risk or wrong intake of other nutrients in high amount.

Conclusion

The seed of *S. sternocarpa* can be seen as a potential source of useful items for food and drugs formulation. Further research work is ongoing to confirm some of the ethno-pharmacological claims on *S. stenocarpa*.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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