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# Evaluation of Chlorophyll Contents of Wild Type and a 'Variant' *Telfairia occidentalis* (Hooker Fil.) and Soil Properties of Their Locations on a Farm at Ikot Ekpene, Akwa Ibom State, Nigeria

**A. O. Egbe[1], E. E. O. Ndibukke[1], O. S. Bello[2] and A. A. J. Mofunanya[3*]**

*[1]Department of Biological Sciences, Cross River University of Technology, Calabar, P.M.B 1123, Cross River State, Nigeria.*
*[2]Department of Soil Sciences, University of Calabar, Calabar, P.M.B 1115, Cross River State, Nigeria.*
*[3]Department of Botany, University of Calabar, Calabar, P.M.B 1115, Cross River State, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors AOE and AAJM wrote the first draft of the manuscript and chlorophyll estimation. Author EEON handled the statistical analysis. Author OSB handled the soil analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To measure the chlorophyll contents of wild type and 'variant' *Telfairia occidentalis* and determine the soil properties of their locations on a farm at Ikot Ekpene, Akwa Ibom State, Nigeria.
**Study Design:** Soil and leaves were randomly sampled at three locations; IKI, IKII and IKIII.
**Methodology:** Leaves were randomly sampled at three locations; IKI, IKII and IKIII for determination of chlorophyll a, b and a+b contents of the wild type and 'variant'. Chlorophyll contents were determined spectrophotometrically. Soil samples were also collected at two depths 0-15 cm and 15-30 cm at the root base of the plants to make a composite from the three locations in four replicates each. The soil samples were air dried and passed through a 2 mm sieve before

**Corresponding author: E-mail: amofunanya@yahoo.com;*

analysis for the various nutrients. Particle size was determined by hydrometer method. Organic C, P, soil pH, total N, exchangeable bases, effective cation exchange capacity (ECEC), K, Na, Ca and Mg were determined.

**Results:** Results of t-test for paired comparison of leaf chlorophyll contents showed a highly significant (P<0.025) difference between the wild type and 'variant'. Mean values for chlorophyll a for the wild type ranged from 66.08±1.16 to 79.35±1.02 mg/g FW for the three locations, while the 'variant ' had a ranged in values from 19.28 ± 0.02 to 32.40 ± 1.45 mg/g FW. Chlorophyll b for wild type ranged from 73.46 ± 2.16 to 87.76 ±1.28 mg/g FW compared to mean values for 'variant' which ranged from 22.91 ± 0.18 to 42.03 ± 1.50 mg/g FW. Mean values for a+b for the wild type ranged from 140.26 ± 2.12 to 167.11±1.34 mg/g FW, while the 'variant' had a ranged in values from 42.25±0.35 to 74.43±1.44 mg/g FW. Results of soil analysis revealed low values for Ca, Mg, K and N of 1.2-4.0, 0.4-1.8, 0.08-0.11 cmol/kg and 0.08-0.15% respectively.

**Conclusion:** The low values of the major mineral nutrients; N, Ca, Mg and K suggest that the chlorosis of the leaves of the 'variant' *T. occidentalis* may be attributed to mineral nutrients deficiencies. However, viral infection can also cause chlorosis. Whether there was any underlying genetic causes could not be established.

*Keywords: Chlorophyll contents; Telfairia occidentalis; 'variant'; wild type.*

## 1. INTRODUCTION

*Telfairia occidentalis* Hooker Fil. (family Cucurbitaceae) commonly called fluted pumpkin is native to West Africa [1,2]. It is a dioecious, perennial, drought tolerant, climbing plant, which attaches to its support by means of coiled often branched tendrils [1,3].

The plant derives its common name fluted pumpkin from the fact that females produce fluted fruits [4]. *Telfairia occidentalis* is an important economic crop in West Africa. Among many other benefits, it is a major cheap source of protein in West African sub-region where majority of the people are poor and can hardly afford animal protein. The main use of *Telfairia occident talis* is as a leaf and seed vegetable and from it consumers derive vitamins and minerals necessary for healthy life [5]. In Nigeria, it is used in traditional medicine for the treatment of some ailments [4,6] and the seeds are considered rich in oil (about 45%), and suitable for making soap [3,1].

Chlorophyll formation is influenced by light, nutritional and genetic factors [7]. In the dark plants do not produce chlorophyll. The leaves of plants grown in full sun often produce leaves that are lighter green in colour than the leaves of the same plant grown under low light intensity [8]. When plants are deficient in an essential element in most cases nitrogen (N), magnesium (Mg), or potassium (K), the amount of chlorophyll produced may be low and the plant produces yellow or yellowish green leaves, a condition named as chlorosis. Each individual plant also has a different inherent ability to produce chlorophyll. On a farm site at Ikot Ekpene, Akwa Ibom State, Nigeria, some *T. occidentalis* plants with chlorotic leaves were noticed (Plate 1) among the normal green leaf coloured plants.

**Plate 1. Wild type and 'Variant' *Telfairia occidentalis* plants growing side by side**

In Nigeria *T. occidentalis* is a highly cherished vegetable used in the preparation of soups. Its market value is dependent on how fresh, tender and green the leaves are. The chlorosis is a problem in this regard as it reduces its market value. The study was carried out to understand the possible cause of the problem and possibly proffer solution.

This study was carried out to find explanation for the occurrence of a few plants with yellow leaves

among the normal green plants at different locations on the farm. The objective was to determine the amount of chlorophyll a, b as well total chlorophyll (a + b) in the wild type and 'variant' *T. occidentalis* plants and also to determine the nutritional status of the soil at locations where the 'variant' occurred.

## 2. MATERIALS AND METHODS

### 2.1 Estimation of Chlorophyll

Leaves of the wild type and 'variant' *Telfairia occidentalis* plants were randomly sampled in four replicates at three locations, IK I, IK II and IK III. Chlorophyll was extracted by grinding 2 g fresh leaves sampled from both wild type and variant *T. occidentalis* from each of the three locations in 10 ml of 80% aqueous acetone using porcelain mortar and pestle. The homogenate was decanted into test tubes and centrifuged at 4500 rpm (Model 0406 – 2) for 3 minutes. After centrifugation the supernatant was decanted and used for determination of chlorophyll content using a cuvette a blank was prepared with 2 ml of 80% aqueous acetone. This was used to set the spectrophotometer to 100% transmittance zero absorbance at 663 nm for chlorophyll a and 643 nm for chlorophyll b [9]. The blank was removed and then to 3 ml of 80% aqueous acetone was added 0.1 ml of the chlorophyll extract: The cuvette containing the chlorophyll extract was inserted into the sample holder. The chlorophyll content was estimated using the formula:

$$\text{Chl a} = (11.6A663 - 1.3\ A643)\ Vx^{-1}$$
$$\text{Chl b} = (19.6\ A643 - 4.7\ A663)\ Vx^{-1}$$
$$\text{Chl a + b} = (\text{mg/g FW})$$

Where, A663 and A643 are the absorbance at 663 and 643 nm.

A = Absorbance
V = Volume (ml) of 80% acetone
$Vx^{-1}$= Fresh weight of samples used
mg = Milligram
g = Gram
FW = Fresh weight

### 2.2 Soil Analysis

Composite soil samples were collected from the *Telfairia occidentalis* farm at Ikot Ekpene, Akwa Ibom State, Nigeria at two depths of 0-15 cm and 15-30 cm. Samples were collected at the root base of the 'variant' plant and wild type at these depths in three locations in four replicates each. The samples were taken to the laboratory, air dried and passed through a 2 mm sieve before analysis for the various nutrients. Particle size analysis was carried out by hydrometer method [10], organic carbon by [11], available phosphorus by Bray and Kurtz [12] and soil pH in soil: water solution ratio of 1:1 in distilled water using a pH meter. Total Nitrogen was determined by Kjeldahl digestion, the exchangeable bases were extracted with neutral I N $NH_4OAC$. Potassium and sodium in the extract were determined by flame photometry, while calcium and magnesium were determined by titration.

### 2.3 Data Analysis

The experiment was laid out in a Completely Randomized Design. Data obtained for chlorophyll content was analysed by t-test for paired comparison at ($P< 0.025$).

## 3. RESULTS AND DISCUSSION

### 3.1 Chlorophyll Contents of Wild Type and 'Variant' of *Telfairia occidentalis*

This study was carried out to investigate the probable causes of chlorosis observed on the leaves of *Telfairia occidentalis* on a farm at Ikot Ekpene, in Akwa Ibom State, Nigeria. One common reason for chlorosis is poor nutrition.

Table 1 shows the estimated chlorophyll in milligrams per gram fresh weight in leaves of the wild type and 'variant' *Telfairia occidentalis* sampled at the three locations IKI, IKII and IKIII, on the farm. Results of t-test for paired comparison of chlorophyll contents revealed a highly significant difference ($P < 0.025$) between the wild type and 'variant' in chlorophyll a, b and total chlorophyll (a+b) contents at the three locations. Mean values for chlorophyll a for the wild type ranged from 66.80 ± 1.16 to 79.35 ± 1.02 mg/g FW for the three locations, while for the 'variant' the values ranged from 19.28 ± 0.02 to 32.40 ± 1.45 mg/g FW. Chlorophyll b values for wild type ranged from 73.46 ± 2.16 to 87. 76 ± 1.28 mg/g FW, compared to mean values for 'variant' which ranged from 22.91 ± 0.18 to 42.03 ± 1.50 mg/g FW. Mean values for total chlorophyll (a+b) for the wild type ranged from 140.26 ± 2.12 to 167. 11 mg/g FW, while those for the 'variant' ranged from 42.25 to 74.43 mg/g FW.

Results of the t-test for paired comparison of chlorophyll contents showed that there were significant (P<0.025) differences in chlorophyll a, b and (a+b) between wild type and 'variant' *T. occidentalis*.

### 3.2 Physicochemical Properties of Soil Samples

Table 2 shows the physicochemical properties of the soil sampled from the three locations; IKI, IKII and IKIII. The pH at the three locations ranged from 5.0 to 5.8 with an average of 5.2, indicating strongly acidic soil [13], organic carbon ranged from 1.04% to 1.79% with a mean of 1.38% indicating low organic carbon content [13,14].

Total nitrogen ranged between 0.08% and 0.15% with a mean of 0.11% showing low total nitrogen in all the locations, IKI, IKII and IKIII. The soil at all locations had very high available P with a range of 27.75 to 57.50 mg/kg [14,15]. Exchangeable Ca, Mg, K and Na, had low values 1.2 – 4.0, 0.4 -1.8, 0.08 – 0.11 and 0.08 – 0.09 centimole per kilogram respectively. Effective cation exchange capacity (ECEC) ranged from 4.53 to 7.00 which are low values [14,15].

One common reason for chlorosis is poor nutrition. Deficiencies in Ca, K, and especially in N and Mg which are constituents of chlorophyll [16,17,18,19] all cause reduction in leaf chlorophyll content referred to as chlorosis [20,21]. The low values of total N, available Ca, Mg and K in Table 2 may therefore be responsible for the chlorosis of the leaves of the 'variant' *Telfairia occidentalis* observed on the farm. The Al content of the soil is not likely to be in soluble form since the soil pH was greater than 4.7 [22].

Though the values in Table 2 show that the P content of the soil was high at the three locations, it cannot be utilized by the plants because under acidic condition P is fixed. The wild type and 'variant' grew side by side (Plate I) at the three locations, yet the leaves of the wild type remained normal green, whereas those of the 'variant' were chlorotic. Viral infections of plants can cause chlorosis. Mutation involving X chromosomes (lyonization) may cause variation in leaf colour, this has been suggested in some plants. The chlorosis of the 'variant' may therefore be attributed to mineral nutrient deficiencies, viral infection or have genetic basis. Attempts made to further the study by investigating whether the chlorosis of the 'variant' had genetic bases or not was hindered by some problems. When the yellow leafed plants were noticed on the farm, all the plants had reached a stage of maturity that made it difficult to obtain young roots for mitotic studies. The farm was observed until the plants flowered, but none of the 'variant' plants flowered. So there was no fruit from which to obtain seeds for follow up studies. Also, it was not known whether all the plants on the farm were raised from seeds of the same or different provinces to give clue of what may have predisposed the leaves of the 'variant' to chlorosis. The search for another such occurrence is ongoing, with the hope that an attempt at viral and genetic studies can still be made.

It is suggested that government should establish bio-conservation centres where uncommon plants that need to be studied and preserved for posterity are kept. Government should employ the appropriate expertise as seed producers where seeds of high yielding pure breeding plants can be obtained. The commercial production of seeds of high yielding plants can be a source of revenue to government. *Telfairia occidentalis* is grown in Nigeria mainly for its leaves which are used for making soup. Chlorosis reduces the market value of the leaves leading to economic loss to the farmer. Further investigation of this problem will throw light on why chlorosis of some *Telfairia occidentalis* plants might occur on a farm, and provide remedy to avoid such losses to the farmers.

**Table 1. Chlorophyll contents of Wild Type and 'Variant' of *Telfairia occidentalis***

| Site | mg/g FW | | | | | |
|---|---|---|---|---|---|---|
| | Chlorophyll a | | Chlorophyll b | | Chlorophyll (a + b) | |
| | Wild type | 'Variant' | Wild type | 'Variant' | Wild type | 'Variant' |
| IK I | 79.35 ± 1.02 | 32.40±1.45 | 87.76±1.28 | 42.03±1.50 | 167.11±1.34 | 74.43 ±1.44 |
| IK II | 66.80±1.16 | 19.28±0.02 | 73.46 ±2.16 | 22.91±0.18 | 140.26 ±2.12 | 42.25 ±0.35 |
| IK III | 76.23 ±1.01 | 30.21±0.76 | 81.17 ±0.27 | 36.81±1.27 | 157.40 ±0.77 | 67.02 ± 0.86 |

*Means ±standard deviations of four replicates*

**Table 2. Physicochemical properties of soil samples**

| Description/depth | pH | % | % | Mg/kg | Cmol/kg | | | Cmol/kg | | Cmol/kg | | % | % | % | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Org. C | T.N | Avail. P | Ca | Mg | K | Na | $Al^{+++}$ | $H^{+}$ | ECEC | BS | Clay | Silt | Sand |
| IKI Top soil | 5.0 | 1.77 | 0.15 | 44.00 | 2.4 | 0.6 | 0.10 | 0.07 | 0.84 | 1.12 | 5.13 | 62.0 | 15.0 | 13.7 | 71.3 |
| IKI Sub soil | 5.0 | 1.73 | 0.14 | 42.12 | 2.2 | 0.4 | 0.09 | 0.06 | 0.96 | 1.20 | 4.91 | 56.0 | 14.0 | 12.7 | 73.3 |
| IKI control top | 5.8 | 1.79 | 0.15 | 57.50 | 4.0 | 1.6 | 0.12 | 0.09 | 0.04 | 1.00 | 6.85 | 85.0 | 11.0 | 13.7 | 75.3 |
| IKI Control sub | 5.0 | 1.51 | 0.13 | 54.87 | 3.4 | 1.8 | 0.10 | 0.06 | 0.44 | 1.20 | 7.00 | 76.0 | 14.0 | 12.7 | 73.3 |
| IKII Top soil | 5.1 | 1.57 | 0.13 | 47.50 | 2.4 | 0.8 | 0.09 | 0.06 | 0.24 | 1.08 | 4.67 | 72.0 | 13.0 | 10.7 | 76.3 |
| IKII sub soil | 5.0 | 1.12 | 0.09 | 35.75 | 1.2 | 0.6 | 0.08 | 0.05 | 1.16 | 1.44 | 4.53 | 43.0 | 18.0 | 10.7 | 71.3 |
| IKII control top | 5.1 | 1.51 | 0.12 | 42.25 | 2.0 | 0.4 | 0.09 | 0.06 | 1.20 | 1.32 | 5.07 | 50.0 | 17.0 | 10.7 | 72.3 |
| IKII control sub | 5.1 | 1.24 | 0.10 | 27.75 | 1.4 | 0.6 | 0.08 | 0.06 | 1.32 | 1.24 | 4.70 | 46.0 | 18.0 | 9.7 | 72.3 |
| IKIII Top soil | 5.1 | 1.04 | 0.08 | 34.00 | 2.8 | 0.6 | 0.10 | 0.07 | 0.72 | 0.88 | 5.17 | 69.0 | 14.0 | 10.7 | 75.3 |
| IKIII Sub soil | 5.1 | 1.12 | 0.08 | 27.75 | 1.8 | 0.4 | 0.09 | 0.08 | 1.32 | 1.04 | 4.73 | 50.0 | 22.0 | 8.7 | 69.3 |
| IK III Control top | 5.6 | 1.14 | 0.09 | 41.75 | 2.6 | 1.0 | 0.11 | 0.08 | 0.04 | 0.84 | 4,67 | 81.0 | 10.0 | 6.7 | 83.3 |
| IK III Control sub | 5.5 | 1.06 | 0.08 | 37.12 | 3.0 | 1.4 | 0.11 | 0.08 | 0.04 | 1.08 | 5.67 | 81.0 | 11.0 | 9.7 | 79.3 |

## 4. CONCLUSION

Results of this study showed a significantly higher chlorophyll a, b and a+b contents in the wild type than the 'variant'. Also soil analysis revealed low values of the major mineral nutrients; N, Ca, Mg and K. Plant virus infections are also known to cause leaf chlorosis. Lyonization has also be reported as a cause of chlorosis in some plants. The chlorosis of the 'variant' may therefore, be attributed to either mineral nutrients deficiencies, viral infection or have genetic basis.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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