Analysis of Chlorophyll and Stomata of the Leaves of Three Improved Varieties of Manihot esculenta Crantz

*Adeleke, Martina T. V[1]. and Nwogu, Ruth
[1]Department of Plant Science and Biotechnology, Rivers State University, Nkpolu, Port Harcourt, Nigeria.
*Corresponding Author: mtadeleke@yahoo.co.uk

Abstract
This study was carried out to investigate the physiological efficiency of the leaves of three improved cassava varieties. The varieties include UMUCAS 44, TME 419 and TMS 96/0523. Determination of the density and stomata distribution were performed using the North Replica method. Stomatal count on the lower epidermis varied significantly between leaflets and among the cassava varieties. The leaf tissue was extracted in 80% acetone and analyzed for chlorophyll content by measuring the absorbance at 663nm and 645nm for chlorophyll a and b respectively, and at 652nm for chlorophyll a+b. The result obtained from this study showed significant variations in leaf stomata count, and in leaf chlorophyll concentrations among the cassava cultivars. Chlorophyll b content for all three cultivars was lowest since the plants were not under any water-limiting stress. UMUCAS 44 had the highest stomatal count, and TMS 96/0523 was seen to have the highest chlorophyll content of the three varieties.
Keywords: Chlorophyll, Stomata, Cassava varieties, Photosynthesis, Cassava leaves.
DOI: 10.7176/JBAH/12-6-03
Publication date: March 31st 2022

Introduction

Photosynthesis is the primary process by which plants use light energy to drive the synthesis of organic compounds, and is pivotal for crop growth and productivity. The photosynthetic capacity of leaves depends on the characteristics and amounts of the components of the photosynthetic machinery, the production of which depends on the availability of water, light, temperature, nutrients, etc. (El-Sharkawy, 2012). However, yield potential in cassava cultivation is an outcome of several processes at all stages in the growth and development of the crop, and the primary goal of any yield assessment is usually to identify superior cultivars for a targeted region (Yan *et al*., 2001) since, the actual yield and the consistency of yield of any adapted cultivar is its ultimate index of adaptation (Duane, 2003).

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories after rice and maize in the tropics. Cassava can grow on poor soils, is easily propagated, requires little cultivation and can tolerate periodic and extended periods of drought (Hillocks *et al*., 2003). *Manihot esculenta* is a shrubby perennial species that produces storage roots (Hillocks *et al*., 2000). The roots form large starchy tubers, somewhat similar to sweet potato, with a dark brown fibrous covering and white flesh (MOFA, 2001). The leaves are deeply indented, palmate 3 - 7 lobed, attached to a slender stem by long petioles. Smaller leaves are produced near the top and have a higher level irradiance and larger leaves towards the interior and base where light levels are lower. Many cassava leaves can change their leaf angles and orientation in response to a change in light. Some do this to increase interception, while others do it to avoid high light. Photosynthetic tissue can be concentrated equally on both sides of a leaf (isobilateral) to maximize use of light absorbed from either side, or preferentially on one side (dorsiventral) as is common in species where leaves are predominantly horizontal (Fujihara, 2000).

Chloroplast density and location within leaves is also sensitive to light climate, and energy capture varies accordingly (Gallo and Sayre 2009). Alignment along vertical cell walls will reduce overall absorption of incident light. Under conditions where water is limiting, however, stomata conductance may be reduced, sacrificing photosynthesis in favour of slower transpiration.

In order to meet the ever- growing demands of people through increasing the yield potential and quality of the crop, more improved varieties of cassava have been released (McCarl, 2007), especially those that are bio-fortified with beta-carotene (Pro-Vitamin A). Bio-fortification of staple foods with micronutrients is regarded as a sustainable approach to reduce micronutrient malnutrition. Therefore, as a way of improving the level of production and quality of cassava, evaluation of the physiology of the crop clones are often needed to ensure that selections made have a reliable and a predictable performance in the farmer's field.

There is the need to work on the Pro Vitamin A cassava clones to understand their performance agronomically. The objective of this study therefore is to examine some physiological parameters of three improved cassava clones: TMS 96/0523, UMUCAS 44 and TME 419; namely, stomatal distribution and chlorophyll content of their leaves.

Materials and Methods

Experimental Site

The three Cassava varieties: TMS 96/0523, UMUCAS 44 and TME 419 used for this study were obtained from the Agricultural Development Program, Port Harcourt, and the site for the experiment was in the Rivers State University Agricultural farm. Rivers State University is located on latitude 4°79 N and longitude 6° 98 N. It is situated in the Niger Delta wetland of Southern Nigeria. The study site is characterized by climate with mean annual temperature of 25°C to 28°C and annual rainfall of over 3000mm. The relative humidity is very high with an annual mean of 85%, while the soil is usually sandy or sandy loam underlain by a layer of impervious pan.

Determination of Stomata Distribution

The determination of the density and stomata distribution were performed using the North Replica method (North, 1956). Impression of the leaf surface was produced with cellulose acetate film adhesive smeared on it. The leaves were left to dry, and the acetate film were peeled off and mounted on a slide for microscopic assessment. A light microscope with eyepiece lens 15x and an objective lens 40x was used, thus giving a magnification of 600x and a field view of 0.39mm^2. Five fields were viewed per slide replicated three times for each analyzed leaf. Stomata density (number of stomata per unit area) was calculated. Stomata distribution was estimated for three different leaflets (1, 4 and 7) on leaves situated on different parts of the plant.

Determination of Leaf Chlorophyll Content

To measure chlorophyll content, 2g of fresh leaf samples for each cassava clone was crushed in a clean mortar with pestle; 5ml of acetone (80%) was added and homogenized. The mixture was decanted through a filter paper into a 100ml volumetric flask and made up with 80 percent acetone. 5ml of the solution was transferred into 50ml volumetric flask with sterile pipette and made up with 80 percent acetone. The amount of chlorophyll in the leaf tissue extract was measured using a spectrophotometer to read the absorbance at wavelengths of 663nm and 645nm for chlorophyll a and b respectively, and at 652nm for chlorophyll a + b.
Determination of chlorophyll content of the leaves was calculated thus:
Chlorophyll a (mgg^{-1}) = [(12.7 x A_{663}) – (2.6 x A_{645})] ml acetone/g leaf tissue
Chlorophyll b (mgg^{-1}) = [(22.9 x A_{645}) – (4.68 x A_{663})] ml acetone/g leaf tissue
Total Chlorophyll = Chl a+b = 20.2(A_{645}) + 8.02(A_{663}) x [50/1000] x [100/5] x [½]

Results and Discussion

Leaf chlorophyll content

Of the three varieties of Cassava studied, UMUCAS 44 is the least in chlorophyll content for Chlorophyll a and b, as well as for total chlorophyll content (a+b). TME 419 is next to UMUCAS 44 in chlorophyll content (Fig. 1), and TMS 96/0523 has the highest values of the three varieties considered. Differences in leaf chlorophyll concentrations were significant among the three cassava varieties. Several studies have shown cultivar differences for leaf chlorophyll contents in cassava varieties (Ekanayake *et al.*, 1996, 1998; Pereira *et al*, 1986; Oka and Matsuda, 1983). Decreases in photosynthetic activity are paralleled by a reduction in leaf chlorophyll content (Ekanayake *et al.*, 1998), and leaf chlorophyll content is often indirectly associated with growth and yield of cassava (Ekanayake *et al.*, 1996; Oyetunji *et al.*, 1998).

The ratio of chlorophyll a to b is quite high in all three cassava cultivars studied. Johnston and Onwueme (1998) showed that chlorophyll a:b ratio was less in plants grown under shade than in plants exposed to full sunlight, and that compared with chlorophyll a, chlorophyll b increased significantly under low light. This suggests that chlorophyll b concentration may increase under stressful conditions compared to chlorophyll a.

Table 1. Leaf chlorophyll content of the Cassava Varieties

Cassava Variety	Chl a	Chl b	Chl a+b
UMUCAS 44	1.9846 ± 0.025	0.9280 ± 0.092	2.9051 ± 0.018
TME 419	2.1487 ± 0.060	0.9827 ± 0.026	3.1234 ± 0.041
TMS 96/0523	2.3244 ± 0.051	1.1099 ± 0.020	3.4254 ± 0.034

Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.12, No.6, 2022

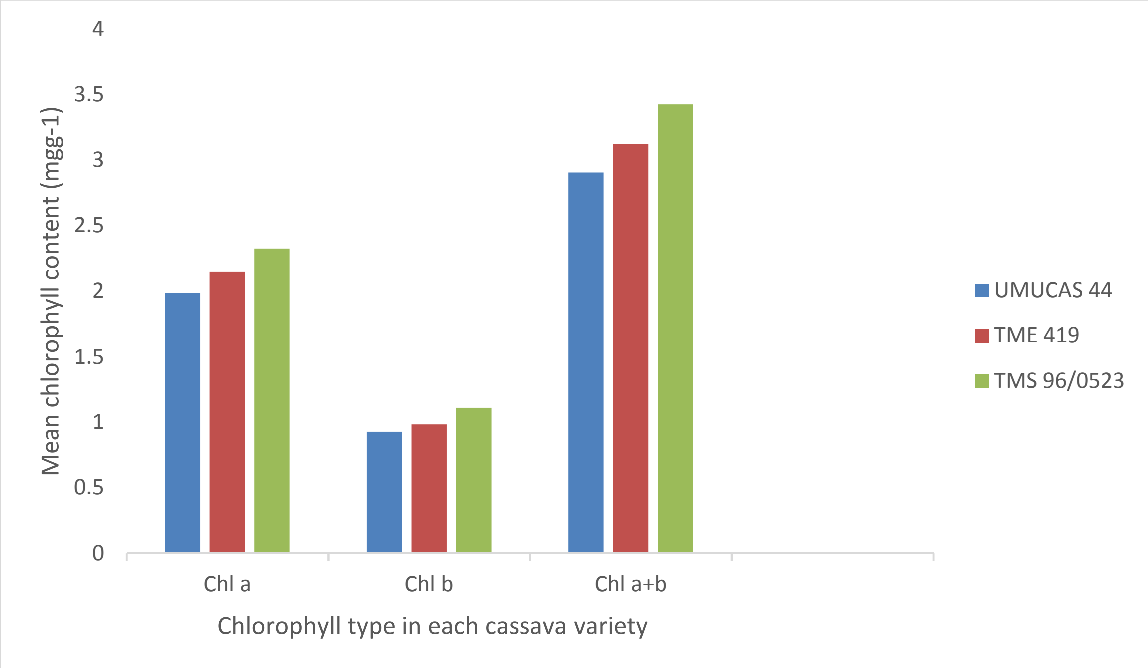

Figure 1. Chlorophyll content of the three cassava varieties

Of the three Cassava varieties studied, TMS 96/0523 could be assumed to be the most efficient photosynthetically because of its high chlorophyll content (especially chlorophyll a and total chlorophyll). Chlorophyll b is lowest in all three varieties, which may be due to the rain forest climate of the experimental site, and the period of sampling was almost at the peak of the rains, hence the cassava varieties were not under any kind of stress.

Stomata count of Cassava leaflets

The group chart for stomatal count (Fig. 2) show some variation in the stomatal counts among the leaflets. The middle leaflet (4) for UMUCAS 44 and TMS 96/0523 had significantly more stomata than the leaflets on both ends (I and 7). This agrees with the observation of Carlos et al. (2015), that there are differences in stomatal density, size and distribution depending on their position in the leaves of the different cassava genotypes.

Generally, UMUCAS 44 was observed to be the most abundant in stomatal count (Figs 2) with an average of 126.5 per mm^2 for all the leaflets examined, followed by TME 419 with 99.13, and TMS 96/0523 had 93.7. A high stomata count in any variety may not make for photosynthetic efficiency on the whole, especially when under any water-limiting condition. Stomatal density has been shown to increase with increasing temperature under CO_2 enrichment for five rose cultivars, increasing the potential for transpirational leaf cooling with warmer conditions (Pandey et al., 2007). Water was however not limiting in the experimental site and period of this study, therefore high stomata count would therefore not be necessary for transpirational leaf cooling. Hence UMUCAS 44 with the highest stomata count of the three varieties (Fig. 1) may be the most physiologically efficient, though it had the lowest chlorophyll content of the three (Fig. 2). The cassava variety TMS 96/0523 on the other hand had the highest values for chlorophyll content, and lowest for stomata count.

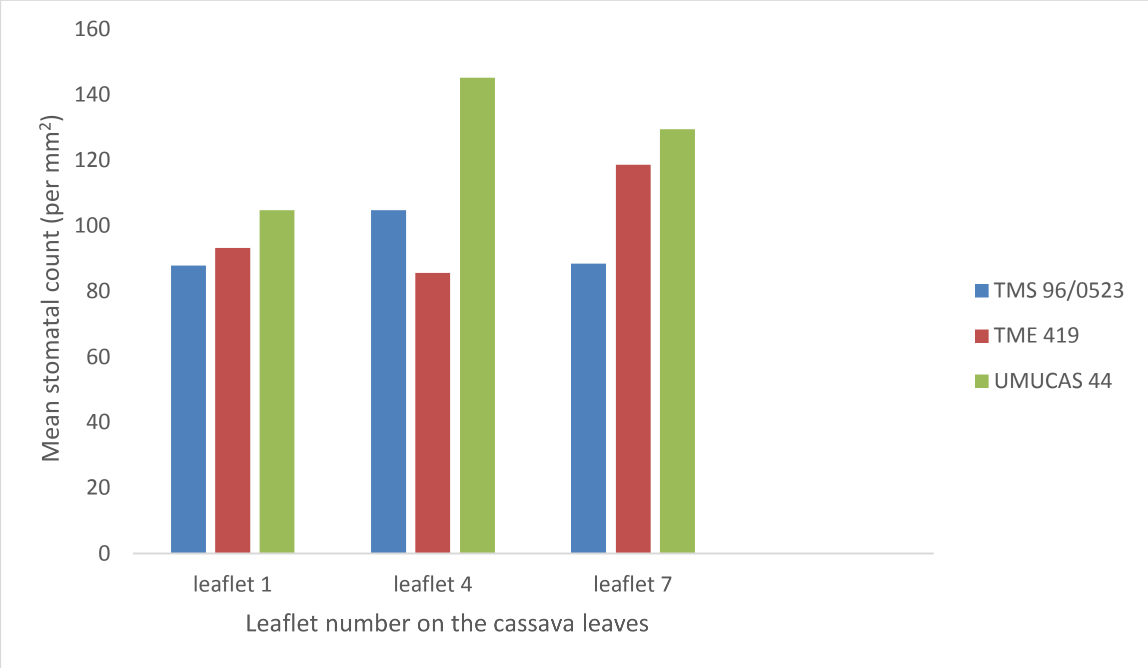

Figure 2. Stomata count on the abaxial surface of leaflets of the cassava varieties

The different genotypes of the same species can contain variations in the structure of the leaves, which may represent an excellent tool in the selection process of cassava plants (Carlos et al., 2015). Included in this is the chlorophyll content of the leaf tissue which is a good index of photosynthetic activity (Chowdhury and Kohri, 2003). This important pigment is also an index of plant growth and production of organic matter (Lahai et al., 2003). Two of the cassava varieties studied (TME 419 and 96/0523) were among the 40 new genotypes that were the results of a decade of genetic enhancement work at IITA in Ibadan, Nigeria in 2003. While UMUCAS 44 is among the second series of pro-vitamin A varieties released in the country in 2014. With the large number of options of genotypes that have superior dry root and forage yield, cultivators will have to grow and pick out their local best from the alternatives available, that is, the local niche match of the genotypes for the end use desired.

The plasticity in leaf anatomy in different genotypes of cassava cause differences in photosynthetic rate that affect CO_2 diffusion (Nassar et al., 2008). The leaves which are responsible for the synthesis of carbohydrates and root tuberization, present important features related to stomatal distribution and size (Carlos et al., 2015) and chlorophyll content (Lahai et al., 2003), depending on the genotype and their specific anatomy.

Despite the relatively lower chlorophyll content recorded in UMUCAS 44, its having the highest stomata count than the other two varieties makes up for its photosynthetic efficiency, as it has been reported to have a high yield record of 36t/ha (Dixon et al., 2008), unlike the other two which are less than 20t/ha.

In conclusion, the differences in stomata count in the three cassava varieties (UMUCAS 44 with the highest) is significantly higher than that in chlorophyll content (96/0523 with the highest), as more stomata makes for more photosynthetic efficiency when water is not a limiting factor (as was the case with this experimental site). This is further confirmed to be the case judging by the high yield of UMUCAS 44 (36t/ha) reported by Dixon et al. (2008), compared to those of TME 419 and 96/0523 which are less than 20t/ha.

Cassava leaf characteristics, chlorophyll content and stomata in particular, need to be further studied due to the plasticity in leaf anatomy in different varieties, which would cause differences in the photosynthetic rate, affecting CO_2 diffusion. Cassava is presented as a very rustic plant by its ability to adapt to different environments. There is some correlation in the Physiological properties of the leaf with respect to photosynthetic efficiency, and this breeders want to maximize.

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