

**Non-Destructive Determination of Photosynthetic Rates of Eight Varieties of  
Cassava (*Manihot Esculenta*, Crantz)**

A thesis presented to the

School of Nuclear and Allied Sciences

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## DECLARATION

This thesis is a result of research work undertaken by Ayesha Algade Amadu in the Department of Nuclear Agriculture and Radiation Processing of the School of Nuclear and Allied Sciences, University of Ghana, under the supervision of Prof. K. E. Danso and Dr. G. K. Owusu.

I hereby affirm that except for references which have been cited accordingly, this work is a result of my own research and that it has not been presented in part or whole for any other degree in this University or elsewhere.

Signed..... Date.....

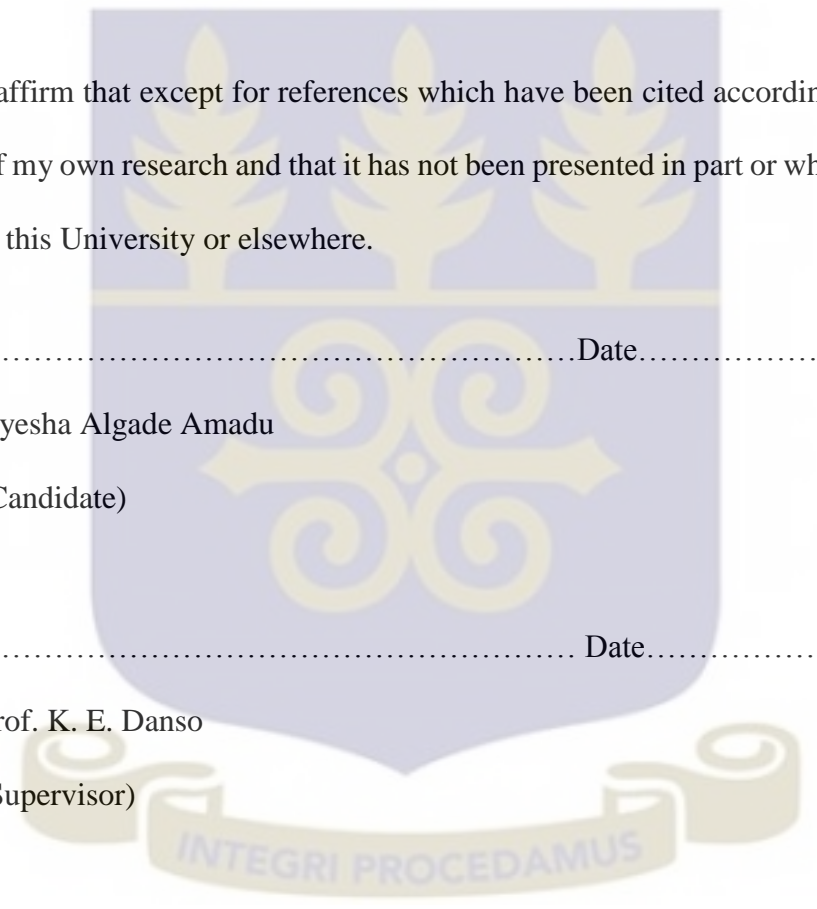
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## DEDICATION

This dissertation is dedicated to my parents, Hon Alhaji Amadu Seidu and Mrs. Meri Amadu for their love and support and also to Mr. Rauf Adam and my family and friends, your reward is with Allah almighty.



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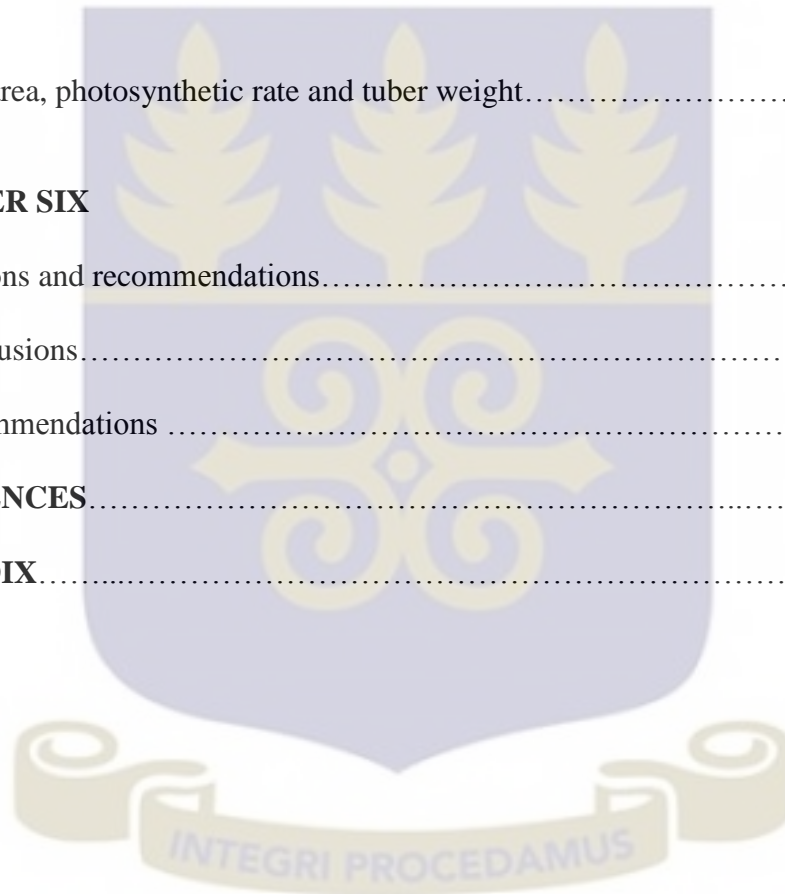
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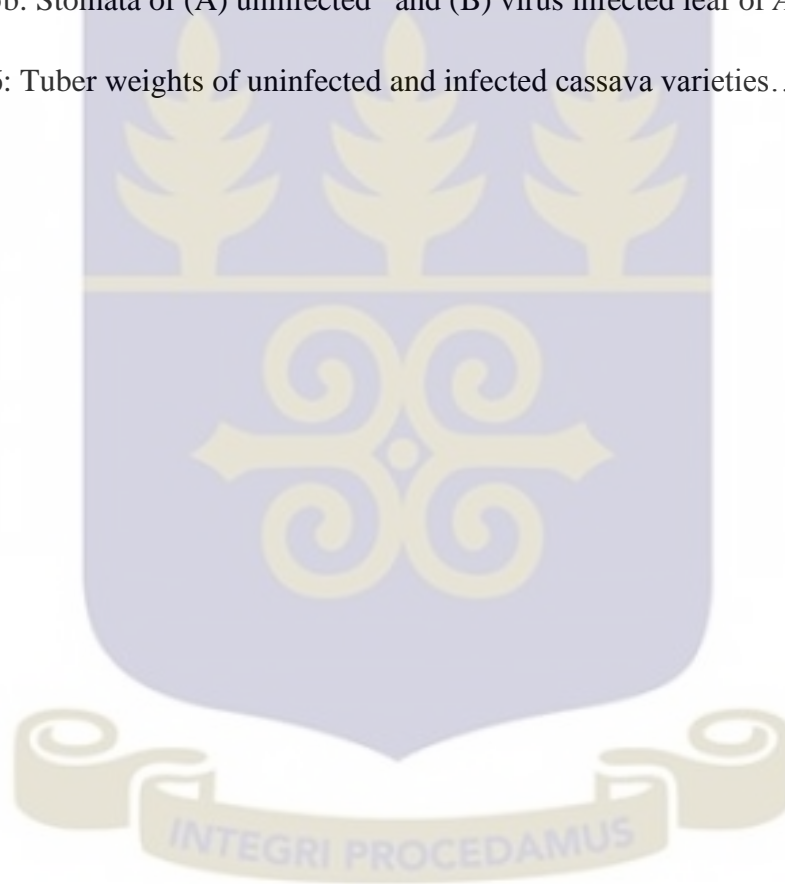
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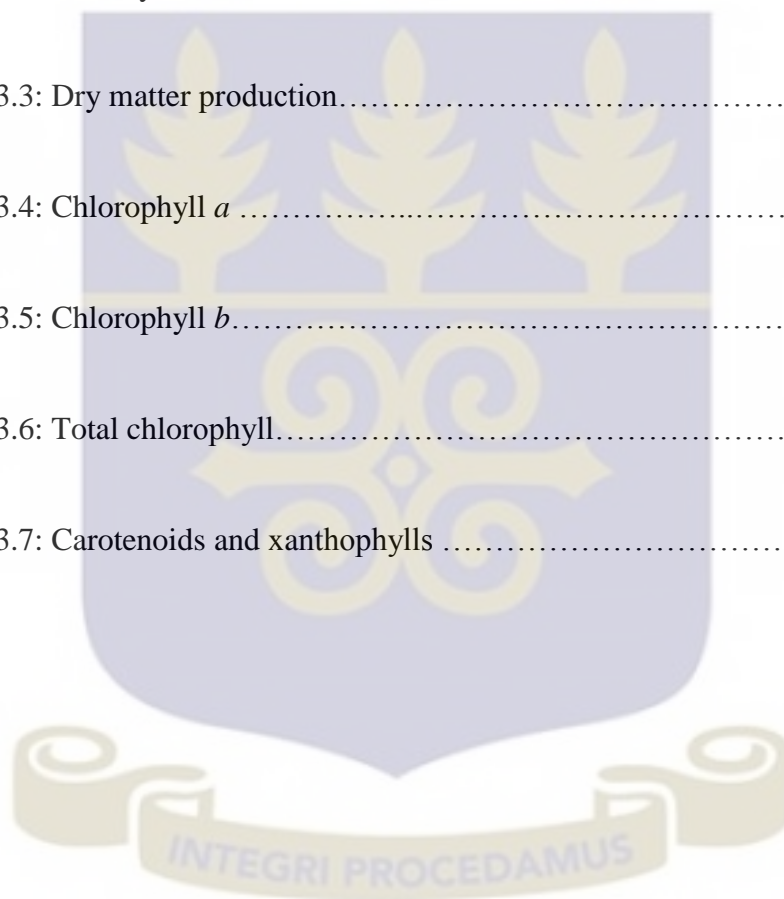
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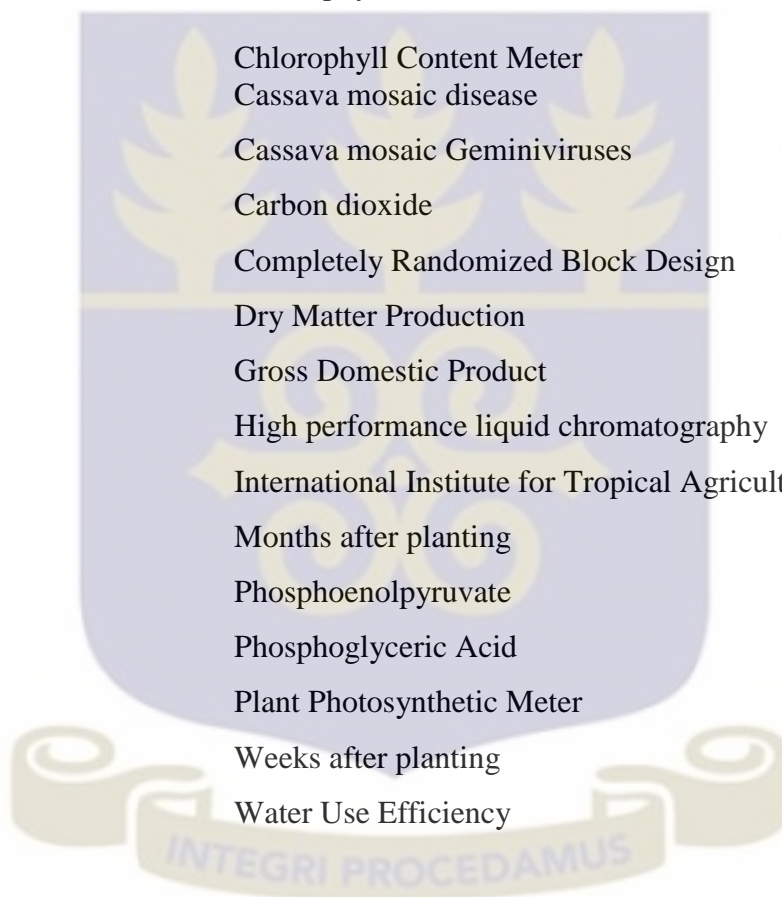
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## LIST OF ABBREVIATIONS

ACMD	African cassava mosaic disease
ACMV	African cassava mosaic virus
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CAM	Crassulacean Acid Metabolism
CCI	Chlorophyll content index
CCM	Chlorophyll Content Meter
CMD	Cassava mosaic disease
CMGs	Cassava mosaic Geminiviruses
CO <sub>2</sub>	Carbon dioxide
CRBD	Completely Randomized Block Design
DMP	Dry Matter Production
GDP	Gross Domestic Product
HPLC	High performance liquid chromatography
IITA	International Institute for Tropical Agriculture
MAP	Months after planting
PEP	Phosphoenolpyruvate
PGA	Phosphoglyceric Acid
PPM	Plant Photosynthetic Meter
WAP	Weeks after planting
WUE	Water Use Efficiency



## ABSTRACT

Cassava is an important food security crop in Ghana and in the wake of climate change there is the need for plant breeders to develop varieties with high water use efficiency as well as high photosynthetic rate in order to adapt to the changing climate. Thus, the photosynthetic rates of eight cassava (*Manihot esculenta* Crantz) varieties were non-destructively evaluated using photosynthesis meter miniPPM300, from June 2014 to May 2015, with the aim of selecting varieties with high photosynthetic rate for future breeding programmes. The mean photosynthetic rate varied depending on the varieties ranging from 40.5  $\mu\text{mol}/\text{m}^2\text{s}$  in *Bosom nsia* to 45.2  $\mu\text{mol}/\text{m}^2\text{s}$  in *Gbenze*. However, the presence of African cassava mosaic disease (ACMD) marginally reduced the photosynthetic rate to below 40  $\mu\text{mol}/\text{m}^2\text{s}$  in all the varieties. Similarly, the chlorophyll content index (CCI) as measured by chlorophyll meter and spectrophotometer also varied from one variety to another; it was low in *Nandom* (17.9 CCI) and high in *Gbenze* (39.93 CCI) using the chlorophyll meter and was also reduced by the presence of the virus. Although, the stomatal density varied between the varieties it was not influenced by virus infection. Furthermore, ACMD significantly decreased the leaf surface area from 5705.8mm<sup>2</sup> in uninfected plants to 1251.6mm<sup>2</sup> in infected plants, consequently reducing the number and weight of tubers produced 11 month after planting (MAP). Molecular Testing of the viruses using virus specific primers JSP001/JSP002, EAB555F/EAB555R, EACMV1e/EACMV2e at 6 MAP and 11MAP, showed that the mosaic symptoms were caused by African Cassava Mosaic virus disease. Cassava varieties with high

photosynthetic efficiency and low virus infection can be used in cassava improvement programmes in Ghana.



## CHAPTER ONE

### INTRODUCTION

Of the root and tuber crops cultivated in the tropics, Cassava (*Manihot esculenta*, Crantz) is the most economically important. It is the third most important staple food after rice and corn, providing a rich source of energy for over 800 million people living in the tropics (Angelucci, 2013). The tuberous roots are harvested and used either as food for human consumption or processed into starch for industrial use. Starch from the roots is used as raw material in the textile, paper and the pharmaceutical industries while the leaves with their rich source of vitamins, are also used as vegetable in many food preparations. Due to its industrial use, cassava has been transformed over the past 50 years from a domestic crop cultivated mainly for domestic consumption to an industrial cash crop (Nweke *et al.*, 2002).

According to Nassar (2002), cassava is believed to have originated from South America (Brazil) and was domesticated about 2000 to 4000 BC, but it is now cultivated widely in Africa (Nigeria, Democratic Republic of Congo, Ghana, Angola and Mozambique), Asia (Indonesia, Thailand, Viet Nam, India) and Central America (Brazil). Among the cassava-growing regions of the world, Africa accounts for more than 50 percent of the global production of 233.8 million metric tonnes (MT); Ghana is the sixth largest producer with an annual production of about 15,989,940MT (FAOSTAT, 2015). Cassava production in Ghana showed a moderate increase from 2008 (with an annual production of 11,351 mMT) up to 2012 (with annual production of 14,547 mMT) due to the introduction of high yielding and disease resistant varieties (Angelucci, 2013). Even with this increase, the

average production of cassava in Ghana is far below the estimated attainable yield of about 28,000,000 MT (MOFA, 2010).

Economically, cassava accounts for about 22% of Gross Domestic Product (GDP) in Ghana. In the rural and peri-urban areas of the country, the cultivation, production, and processing of cassava provides jobs for millions of Ghanaians, the majority of whom are women, thereby providing a backbone of the family's livelihood.

Taxonomically, cassava is a perennial dicotyledonous plant belonging to the family, Euphorbiaceae with great variation in its morphological features. It grows to a height of about 1m to 5m at maturity with several growth habits depending on the variety. The canopy shape and size as well as the shape and size of leaves also vary greatly from one genotype to the other and this may account for the variation in the yield of the crop. Additionally, the maturity period and yield of cassava have been reported to vary greatly among cultivars (IITA, 1990). However, the extent of these morphological variations on photosynthetic rate is not well documented especially among the varieties grown in Ghana. Also very little is known about the relationship between photosynthetic rate, time of maturity and yield of cassava varieties.

Generally plants are classified as C<sub>3</sub>, C<sub>4</sub>, or Crassulacean Acid Metabolism (CAM) depending on the first product of photosynthesis. According to Pessarakli (2002), about 95% of terrestrial plant species including cassava fix atmospheric carbon dioxide (CO<sub>2</sub>) by the C<sub>3</sub> pathway, while 1% and 4% fix CO<sub>2</sub> by the C<sub>4</sub> pathway and CAM pathway

respectively. For typical C<sub>3</sub> plants, the first stable product of carbon fixation is a three-carbon compound, 3-Phosphoglyceric Acid (3PGA).

In this photosynthetic pathway, atmospheric carbon dioxide is reduced to a three carbon sugar, 3-phosphoglyceric acid (3 PGA) in the Calvin Cycle by the chloroplast enzyme ribulose biphosphate carboxylase or oxygenase (Rubisco). During the process about 500 molecules of water are lost for every molecule of CO<sub>2</sub> fixed by photosynthesis, giving a transpiration ratio of 500 or water use efficiency (WUE) of 0.002 (Kang and Zhang 2004). The huge loss of water for fixation of one molecule of carbon dioxide makes the photosynthetic process inefficient especially under drought conditions where excessive loss of water may lead to withering of the plant. Secondly, photosynthetic activity of the pathway is reduced by photorespiration which leads to a loss of 50% of the carbon dioxide fixed due to competition from oxygenation of rubisco in the Calvin cycle (Häusler *et al.*, 2001).

Generally, plants possessing CAM have higher water-use efficiency than C<sub>3</sub> and C<sub>4</sub> plants. Black (1973) showed that the transpiration ratio for CAM plants ranged from 50 to 125kg H<sub>2</sub>Okg<sup>-1</sup> CO<sub>2</sub> compared to 250 to 350 kg H<sub>2</sub>O kg<sup>-1</sup> CO<sub>2</sub> for C<sub>4</sub> and 450 to 950 kg H<sub>2</sub>O kg<sup>-1</sup> CO<sub>2</sub> for C<sub>3</sub> plants (Han and Felker, 1996).

In C<sub>3</sub> plants about 97% of water uptake via roots is lost to transpiration, a high cost avoided by CAM plants (Raven and Edwards, 2001). Consequently, CAM plants have high photosynthetic efficiency and are thus adapted to ecologically dry habitats. The high photosynthetic efficiency in these plants is also attributed to closing of their stomata during

the day, possession of Kranz anatomy with cells which are rich in chloroplast in addition to mesophyll cells and the use of phosphoenolpyruvate (PEP) carboxylase enzyme which catalyzes the reaction instead of Rubisco (Keeley and Rundel, 2003). Thus, there is no photorespiration in this pathway, as there is no functional site in PEP carboxylase for oxygenation but rather a carbon dioxide concentrator had been added to the Calvin Cycle to ensure that it is always available for fixation.

Although cassava is a typical  $C_3$  plant and thus follows the  $C_3$  photosynthetic pathway (Ueno and Agarie, 1997), a recent study in Centro Internacional de Agricultura Tropical (CIAT) has shown that cassava can assimilate carbon dioxide at high rates under high humidity, temperature and high solar radiation and has also evolved drought tolerance mechanisms similar to  $C_4$  plants (El-Sharkawy, 2004). El-Sharkawy further reported that the leaves of cassava have high activities of  $C_4$  enzymes and PEP carboxylase but lack “Krantz” anatomy and this may explain why the crop has high photosynthetic efficiency (El-Sharkawy, 2006). Consequently, some studies have suggested that cassava and *Manihot* species are probably evolving biochemically towards the  $C_4$  photosynthetic pathway with  $C_3$ - $C_4$  intermediate behavior (El-Sharkawy, 2006).

Besides the effect of incident light and atmospheric  $CO_2$  on photosynthetic efficiency, the process is also reliant on a set of morphological, anatomical, physiological and biochemical features of the plant (Hanba *et al.*, 2002). Additionally, stomatal density, chlorophyll content as well as incidence of pests and diseases, especially on the leaves, have great influence on photosynthesis and subsequent yield of the plant (Salama *et al.*, 2011; Feng *et al.*, 2003; Dai *et al.*, 1995; Hewett, 1977). According to Hewett (1977), pests lower light

interception, reduce photosynthetic efficiency, and also alter normal distribution of assimilates within the plant. Gibson *et al.*, (1976) reported that any malformation of leaves will result in decreased light interception. For instance, leaf curling (known as "top-roll") in potato leaves caused by feeding of the aphid *Macrosiphum euphorbiae* showed that photosynthesis per unit leaf area was reduced by 50% in plants which had 'top-roll' compared with non-infested controls. They further stated that any damage which decreases the plants' leaf area also lowers light interception.

Thus, the effect of ACMV on photosynthesis and ultimate yield of cassava can be predicted based on extent of leaf surface area reduction by leaf curling (Fauquet and Fargette, 1990). The virus also affects the size and shape of leaves thereby destroying the photosynthetically active leaf surfaces of plants. Although ACMV causes yield losses of 20-90% (Agrios, 2005), and is prevalent in Ghana, its effect on photosynthetic rate and efficiency, chlorophyll content of leaves as well as tuber production (yield) in cassava has not been well documented.

Other factors which also have great influence on photosynthetic activity are chlorophyll content, stomatal size and stomatal density (Schlüter *et al.*, 2002; Pellet and El-Sharkawy, 1994; Fleischer, 1935). Although chlorophyll is a major pigment in the thylakoid disc of the stroma and together with accessory pigments traps solar energy for the photosynthetic process, the effect of ACMV on its content is not known. Similarly, the effect of ACMV on the stomatal density of cassava has also not been well documented. Thus, there is the need to assess the photosynthetic rates of different cassava varieties.

Moreover, both natural and human induced climate change and likely attendant long periods of drought are adversely affecting crop production globally. To mitigate climate change, there is the need to breed for resilient plants with high WUE as well as high photosynthetic rates for higher yield (FAO, 2011). Screening for photosynthetic rates will allow for the selection of cassava varieties with high photosynthetic efficiency to be incorporated into the breeding programmes.

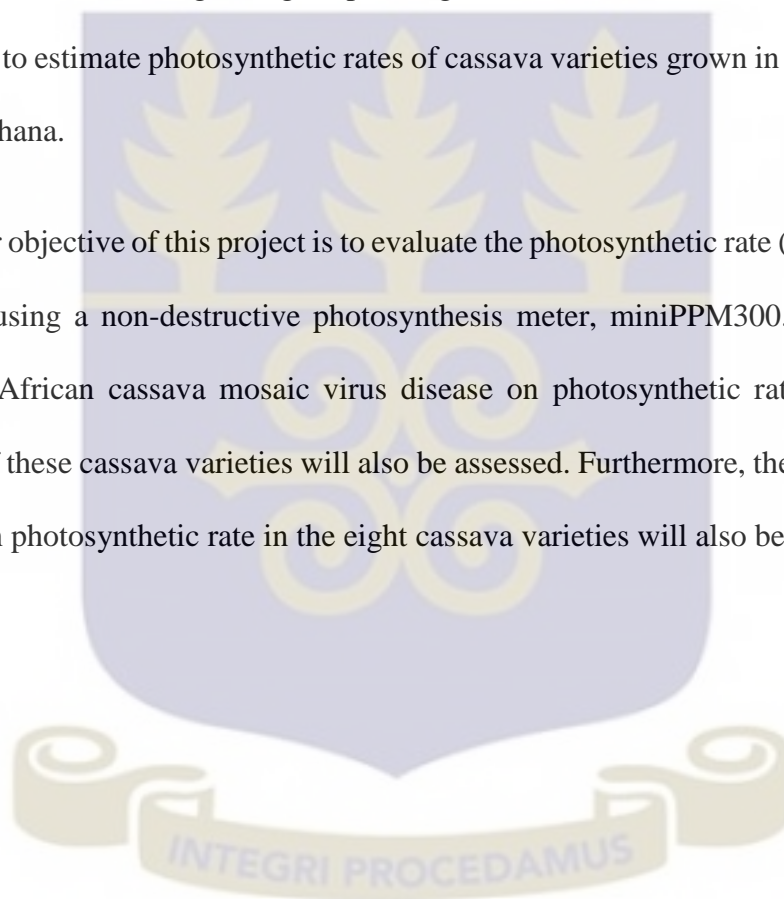
Several methods have been used to estimate photosynthetic rates in plants (Campas, *et al.*, 2008; Bassow and Bazzaz, 1998; Field *et al.*, 1989). These include both destructive and non-destructive methods such as the use of isotopes, calculations on dry matter accumulation and direct measurement of CO<sub>2</sub> and O<sub>2</sub> pressure changes in photosynthetic organisms. Recently, the use of photosynthesis meters has become popular (Bai and Kelly, 1999). Several photosynthesis meters have been developed for non-destructive measurement of photosynthesis. Each of these methods presents their advantages and limitations. Details of these methods are provided under section 2.7.1.

In this experiment, the mini Plant Photosynthesis meter (PPM300, EARS, Netherlands) was used to estimate photosynthetic rates in eight cassava varieties. The PPM300 has been in existence since the 1990's. The meter measures two fluorescence values that is, fluorescence yield (F) and maximum fluorescence yield (F<sub>m</sub>) and photosynthetic active radiation (PAR) incident on the leaf. The values of the fluorescence and PAR are then used to calculate the photosynthesis yield (Y) and photosynthesis rate (P) of the leaf. The dry matter production (DMP) can also be estimated from the photosynthesis yield. The

PPM300 automatically measures photosynthesis-light curves of plants, thereby providing information on optimum light level for growth and the corresponding photosynthesis.

According to El-Sharkawy, (2006) cassava has high inherent capacity to assimilate carbon in near optimum environment that correlates with both biological productivity and root yield across a wide range of germplasm grown in diverse environments. Thus, it is important to estimate photosynthetic rates of cassava varieties grown in a coastal savannah zone of Ghana.

The major objective of this project is to evaluate the photosynthetic rate (P) in eight cassava varieties using a non-destructive photosynthesis meter, miniPPM300. Additionally, the effect of African cassava mosaic virus disease on photosynthetic rate and chlorophyll content of these cassava varieties will also be assessed. Furthermore, the effect of stomatal density on photosynthetic rate in the eight cassava varieties will also be determined.



## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Origin, geographical distribution and economic importance of cassava

Cassava (*Manihot esculenta* Crantz) is an economically important crop principally grown in most tropical and subtropical countries for its starchy roots. It is estimated that about 800 million people in the tropics depend on this crop for their daily food (Nassar and Ortiz, 2010). Globally, Africa produces the largest cassava tubers annually of which 93% is used as food (Nweke *et al.*, 2002).

Although, genetically cassava has a chromosome number of  $2n=36$  and behaves like diploids at meiosis (Purseglove, 1968), natural triploids also occur among the *Manihot* species and are selected by farmers for cultivation. El-Sharkawy (2012) has also described cassava as amphi-diploid or sequential allopolyploids.

Many authors have proposed multiple centres of origin of cassava (Nassar, 2002 and Vavilov, 1951). However, according to Allem, (2002) the genus *M. esculenta* has its centre of origin in the southern edge of the Brazilian Amazon and was introduced into Africa, the Far East and the Caribbean by the early Portuguese traders in the 16<sup>th</sup> and 17<sup>th</sup> centuries. Although Nigeria is the world's largest producer of the crop (Nassar and Ortiz, 2010), Thailand is the highest exporter of cassava starch (FAO, 2013).



Figure 2.1: Geographical regions where cassava is grown in the world. Source: [www.google.com.gh](http://www.google.com.gh)

Globally, cassava is grown and utilized in several continents (Figure 2.1) with Nigeria producing the highest yield (54,000,000 MT) per annum followed by Thailand (29,848,000 MT), Indonesia (24,177,372 MT), and Democratic Republic of the Congo (16,000,000 MT) in that order. Ghana produces about 14,547,279 MT. [www.mapsofworld.com](http://www.mapsofworld.com)

## 2.2 Botanical description of cassava

Generally, cassava shows great variation in its morphometric traits. It grows to a height of 1 to 5m depending on the genotype, and show great variation in the number of lobes per leaf, leaf shape and size, tuber shape, colour of stem and petiole, time of maturity and size of tubers (IITA, 1990). The most important morphological feature which varies greatly is the branching habit. Most genotypes of the crop do not branch at all and for those that

branch, it occurs near the base, with the angle of branching determining whether the cultivar is an erect or spreading type. The leaves are spirally arranged with 2/5 phyllotaxis.

### **2.3 Propagation of cassava**

Cassava is propagated either by seed or by stem cuttings (comprising 3 to 4 nodes) which are prepared from lignified stakes. However, due to high heterozygosity as well as sterile seed production by some cultivars planting of cassava for domestic consumption or commercial plantation is usually through stem cuttings. Cassava thrives well in marginal sandy loamy soils. According to Cruz et al. (2003), the crop has evolved under conditions of low nitrogen availability. During the growth cycle of cassava, there are five distinct phases; leaf sprouting and root system development, canopy establishment and high carbohydrate translocation and dormancy (Lebot, 2009).

### **2.4 Economic importance of cassava**

Cassava produces edible tubers which are well known for its great diversity of uses. The tubers are important source of carbohydrates and are used for both human and animal consumption. They can be eaten either boiled or fried or can be precooked and frozen into croquette for preservation. Also, starch and its derivatives from the tubers are used by the paper, textile and pharmaceutical industries (CIAT, 2012). The leaves provide protein, minerals and vitamins and are thus used as vegetable in most food preparations (Awoyinka

*et al.*, 1995). In spite of its many uses in food preparations, cassava contains cyanogenic glucosides which may have toxic effects when consumed in large quantities. However, the rigorous processes during food preparation especially in fermentation and cooking make them safe for human consumption. The tubers are used for animal feed due to its rich carbohydrate content which provides high energy.

## **2.5 Cassava production in Ghana**

Ghana is ranked the sixth world producer of cassava and it has remained unchanged over the period of 2005-2010. In 2013, production increased up to 15,989,940 MT (FAOSTAT, 2015). The crop constitutes 22% of Ghana's agricultural Gross Domestic Product (GDP) and one of Ghana's main staple crops, with an annual production of about 10 MT in the last decade (Angelucci, 2013). Cassava production in Ghana showed a moderate increase between 2008 and 2010 with an annual production of 15 MT per hectare due to the introduction of high yielding and disease resistant varieties. In 2012 the total average production of cassava was about 868,550 MT (SRID, MOFA, 2013).

The crop can grow well in marginal soils with minimal rainfall of 50.8 mm per annum but an optimal rainfall of 400mm per annum have been found to be adequate for cassava growing when it is established (FAO, 2013). Under marginal conditions, the metabolic efficiency of cassava is high, thus it produces more energy per unit area than most cereal crops (Achinewhu and Owamanam, 2001). Additionally, cassava is highly tolerant to heat,

water stress and poor soils. These attributes make cassava a food security crop in regions severely affected by climate change.

## **2.6 Biotic factors affecting cassava production**

It has been estimated that diseases and pests of cassava can lead to yield losses of about 20-95% (Fargette *et al.*, 1988). The most important of these is mosaic virus diseases transmitted by the whitefly (*Bemisia tabaci*) which is very prevalent in Africa. Nine cassava mosaic diseases (CMD) have been reported to infect cassava worldwide (Fauquet, 2008) out of which seven have been reported in Sub-Saharan Africa. These diseases are caused by African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus (EACMCV), East African cassava mosaic Malawi virus (EACMMV), East African cassava mosaic Zanzibar virus (EACMZV), East African cassava mosaic Kenya virus (EACMKV) and South African cassava mosaic virus (SACMV) (Fauquet, 2008).

Of all the diverse begomoviruses causing mosaic disease (CMD) of cassava plants, only ACMV and EACMV have been identified in West Africa (Alabi *et al.*, 2008). The presence of ACMV in Ghana has been reported over several decades but EACMV was recently reported by Offei *et al.* (1999).

The symptoms of CMD are characteristic mosaic patterns on leaves, chlorotic yellow patches, reduced leaf area and size and these are observed at an early stage of leaf

development. Thus, mosaic diseases reduce the photosynthetic efficiency through the reduction of the leaf surface area as well as leaf greenness available for capture of sunlight. Although no resistant genotypes of cassava to ACMV have been found, some of the genotypes are able to recover from the diseases especially when environmental conditions are favourable for vigorous plant growth (Gibson and Otim-Nape, 1997).

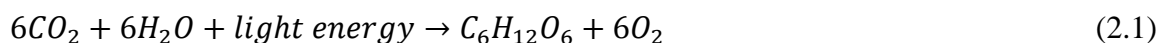
Sampol *et al.* (2003) found that virus infection led to a reduction in photosynthesis by about 50% and mesophyll conductance was the main factor contributing to this reduction, thus rubisco activity decreased in virus infected grape vine plants. Inhibition of primary light reaction by virus infection only had a minor effect on photosynthesis. Stomatal analysis also revealed that stomata closure was unaffected by the presence of the virus. It was concluded that a reduction in carboxylation and possibly in mesophyll conductance are the primarily mechanisms by which virus infection impairs photosynthesis in grape vine (*Vitis vinifera*)

Balachandran *et al.* (1994) studied photoacclimation in tobacco (*Nicotiana tabacum*) leaves infected with two strains of tobacco mosaic virus grown under different light and nitrogen nutrition regimes. The studies revealed that failure of expanding leaves to acclimate was due to the destruction of chloroplasts in yellow areas of the tissue, followed by severe reduction in ribulose-1,5-bisphosphate carboxylase or oxygenase levels, and corresponding reduction in photosynthesis on a leaf area basis.

## 2.7 Photosynthesis in Cassava

El-Sharkawy (2006), has reported that cassava varieties have high photosynthetic rates. However, photosynthetic rate in both improved and local cassava varieties in Ghana have not been given any serious scientific attention. It is therefore necessary to determine photosynthetic rates in cassava especially in the wake of climate change so that varieties with high photosynthetic efficiencies can be integrated into cassava breeding programmes in Ghana.

Photosynthesis is the most important biochemical process that can harvest light energy and convert it into chemical energy on which life on earth ultimately depends. The efficiency of photosynthesis is dependent on a set of anatomical, physiological and biochemical features of a plant. These features determine the adaptability of a plant to an ecological niche, classification of photosynthetic pathway and optimum carbohydrate production. Photosynthesis is light energy driven which synthesizes carbohydrates from carbon dioxide and water with the generation of oxygen. Land plants have been classified into different groups based on the first product of photosynthesis. The photosynthesis process is summarized by the equation below:



$$(\Delta G^{0'} = +2867 \text{ kJ/mol})$$

The light energy involved in photosynthesis is referred to as Photosynthetically Active Radiation (PAR) and it is defined as the number of moles of photons in the radiant energy.

It is in the spectral range of 400 to 700nm and it changes seasonally and also varies depending on the latitude and time of day. Although light is an essential prerequisite for plant life, too much light can cause photoinhibition and may lead to photooxidative destruction of the photosynthetic apparatus (Long *et al.*, 1994). The photosynthetic rate of a plant is measured in micromole per square meter per second ( $\mu\text{mol}/\text{m}^2\text{s}$ ).

Cassava is typically classified as a  $\text{C}_3$  plant and thus follows a  $\text{C}_3$  photosynthetic pathway (Angelov *et al.*, 1993; Ueno and Agarie, 1997). In this  $\text{C}_3$  photosynthetic pathway, carbon dioxide is captured by the ribulose-1,5-bisphosphate (Rubisco) to produce an unstable carbon-3 compound, 3-phosphoglyceraldehyde (3 PGA) in a reaction catalyzed by ribulose-1,5-bisphosphate carboxylase. The  $\text{C}_3$  photosynthetic pathway is inefficient principally due to photorespiration.

Typical  $\text{C}_3$  pathway is associated with photorespiration due to the dual functional site of ribulose-1,5-bisphosphate enzyme (Rokka, 2001) which makes it possible to accept either carbon dioxide or oxygen and thereby reduces the efficiency of  $\text{CO}_2$  assimilation and yield of  $\text{C}_3$ -crops. Photorespiration begins with the competitive inhibition of  $\text{CO}_2$  fixation by  $\text{O}_2$  at the active site of ribulose-1,5-bisphosphate carboxylase or oxygenase (Rubisco) and this can lead to a loss of about 50% of the  $\text{CO}_2$  fixed in ambient air.

However,  $\text{C}_4$  plants, have overcome photorespiration by having a  $\text{CO}_2$  concentrating mechanism, through which atmospheric  $\text{CO}_2$  is bound to a  $\text{C}_4$ -carbon compound (oxaloacetate) which is transported from the mesophyll cells into bundle-sheath cells. The bound carbon is then released and enters the Calvin cycle. Thus,  $\text{C}_4$  plants release  $\text{CO}_2$  at

higher rates in a localized area of Rubisco than C<sub>3</sub> plants thereby avoiding photorespiration (Häusler *et al.*, 2001).

The efficiency of C<sub>4</sub> is also enhanced by their possession of Kranz anatomy (Lundgren *et al.*, 2014 and Häusler *et al.*, 2001) In 'Kranz' anatomy, mesophyll cells are surrounded by thin cell walls and bundle-sheath cells surrounded by thick cell walls. Both the mesophyll and bundle sheath cells are rich in chloroplast and are interconnected by plasmodesmata.

Häusler *et al.*, (2001) stated that the anatomical split-up into different cell types is also supplemented by a spatial separation of the prefixation of atmospheric CO<sub>2</sub> in the mesophyll cells followed by the release of CO<sub>2</sub> and its refixation through the C<sub>3</sub> cycle (Calvin cycle) in the bundle-sheath cells. Thus, when grown under their respective optimum conditions, C<sub>4</sub> plants are more productive than C<sub>3</sub> plants. Brown, (1999) attributed the higher dry matter production in C<sub>4</sub> plants to higher water and nitrogen use efficiencies compared to C<sub>3</sub> plants.

Moreover, it has been observed that cassava has a distinctive chlorenchymatous vascular bundle sheath located below a single layer of palisade cells. Unlike C<sub>3</sub>-C<sub>4</sub> intermediates and C<sub>4</sub> species, the bundle sheaths of cassava are not surrounded by mesophyll cells. The high density of chlorenchymatous cells in cassava may be the reason behind its high photosynthetic efficiency and transport of photosynthates in the leaf (Edward, 1990).

Edward, (1990) stated that physiological and biochemical photosynthetic characters of cassava also indicate that the crop is a C<sub>3</sub> plant and not a C<sub>4</sub> plant. A study conducted on CO<sub>2</sub> compensation points of ten cassava cultivars ranged between 55 and 62 µl, which was

typical for C<sub>3</sub> plants including castor bean, a member of the same family. Edward, (1990) further indicated that the initial products of photosynthesis in cassava are C<sub>3</sub>-like and the activities of several key C<sub>4</sub> enzymes in cassava are low and similar to those of C<sub>3</sub> plants. Furthermore data on the rates of photosynthesis per unit of leaf area and the photosynthetic response of cassava to CO<sub>2</sub> is also consistent with C<sub>3</sub> photosynthesis (Edward, 1990).

El-Sharkawy and Cock (1990) have reported that cassava leaves have low photorespiration, low CO<sub>2</sub> compensation point, and a high PEP-carboxylase activity, but cassava does not have the typical 'C<sub>4</sub>-Kranz' anatomy. All these arguments suggest that cassava is not a C<sub>4</sub> plant.

However, recent studies by El-Sharkawy (2004, 2005) suggest that cassava and other *Manihot* species are probably evolving biochemically towards the C<sub>4</sub> photosynthetic pathway with C<sub>3</sub>.C<sub>4</sub> intermediate behaviour, perhaps due to the changing climate. This assertion is attributed to higher assimilation of carbon dioxide at high rates under high humidity, temperature and high solar radiation by the crop (El-Sharkawy, 2004). Additionally, it has been reported that the crop has evolved drought tolerance mechanisms similar to C<sub>4</sub> plants and also cassava leaves have high activities of C<sub>4</sub> enzyme, PEP carboxylase (El-Sharkawy, 2006). Besides the effect of the anatomy of the leaf on photosynthesis, other abiotic and biotic factors play very significant role in photosynthesis.

### 2.7.1 Measurement of photosynthetic rate

Several methods have been employed to measure photosynthetic rate in field grown plants. These include biochemical, physiological and hand-held photosynthesis meters. Some of the traditional methods used for the measurement of photosynthesis in plants are destructive.

Measuring dry matter accumulation in plants as a means of determining photosynthetic efficiency is an example of destructive method of measuring photosynthesis. This method was described by Hodson *et al.*, (2005). It involves cutting plant parts or whole plants (from the point of germination to the time it is cut). The plant is dried and weighed and the value is used to estimate cumulative photosynthetic activity in the plant. The dry weight gives an estimate of the photosynthetic capacity of the plant, due to the fact that photosynthesis produces the bulk of dry matter (Millan-Almaraz *et al.*, 2009). Since this is a destructive method, it cannot be used for determination of photosynthesis in the same plant over a period of time.

The isotopic method based on the use of carbon isotopes  $^{11}\text{C}$ ,  $^{12}\text{C}$  and  $^{14}\text{C}$  have been used to estimate net photosynthetic rate in plants (Irvine, 1967). Chen, (2006) measured photosynthetic rates of plantlets cultured *in vitro* using this method.

Other non-destructive methods involve the use of a portable devices *in situ*. Portable photosynthesis meters and chlorophyll content meters (CCMs) have been used for several years by agronomists (Chapman and Barretto 1997, Dwyer *et al.*, 1994, Tenga *et al.*, 1989). They are non-destructive to leaf tissue, rapid and easy to use. Using these hand-held

portable meters does not require hazardous compounds or specially trained personnel. Additionally, data generated can be downloaded for rapid analysis (Cate and Perkins, 2003).

The use of photosynthesis meters to non-destructively determine or estimate photosynthetic rates has become a common practice due to their ease of use, accuracy and low labour cost (Bai and Kelly, 1999). Many authors have reported the use of these meters to accurately measure photosynthetic rate of algae and higher plants like asparagus, evening primrose and grapevine (Koyama and Takemoto, 2014; Sampol *et al.*, 2003; Bai and Kelly, 1999). The LI-COR Biosciences photosynthesis meter (LI-6000/LI-6200/LI-6400/LI-6400XT, USA) is a typical example of these meters and was designed by William Biggs in 1971. The Li-COR concurrently measures fluorescence and gas exchange, temperature, light intensity, CO<sub>2</sub> and H<sub>2</sub>O concentration in the same intact leaf. Pridacha *et al.*, (2012) used the LI-COR to measure the photosynthetic rate of different birch species in Karelia, Russia.

The CID Bio-Science meter (CI-340, Washington) is another useful hand-held digital photosynthesis meter that measures not only photosynthesis but also transpiration, stomatal conductance, photosynthetic active radiation (PAR) and internal CO<sub>2</sub>. The meter was used by Lee *et al.*, (2012) to measure photosynthesis in *Quercus variabilis* (Chinese cork oak).

The miniPPM300 employed in this study, besides its portability and affordability is also useful in screening damaged crops in greenhouses. The photosynthesis yield of such crops is usually below 40%. Thus the meter can be used to easily detect stress. It is also very

useful in plant selection and breeding especially in seedlings and cuttings. In Canada the PPM has been used for fifteen years to select spruce seedlings on the basis of their winter hardiness. Seedlings are subjected to very low, freezing temperatures during the night and measured thereafter. Those with the highest photosynthesis yield are then selected for further breeding. Herbicide efficacy can also be monitored using this photosynthesis meter. High doses of herbicides cause damage to crops and pollute the environment. A minimum lethal herbicide dose method (MLHD) has been developed by the manufacturers of this meter. The success of the herbicide treatment cannot be verified visually, thus the meter is used to measure the weed's photosynthesis yield after two days which should be 20% or less. The MLHD method has been introduced in China and there has been about 50 to 70% reduction in herbicide use and a 7% increase in maize yield ([www.ears.nl/ppm](http://www.ears.nl/ppm)).

### **2.7.2 Chlorophyll content and photosynthesis**

Chlorophyll is the principal light receptor in the leaf of a plant. It consists of chlorophylls *a* (Chl*a*) and chlorophylls *b* (Chl*b*) and together with other antenna pigment are the most important pigments for light receptors. These pigments absorb solar radiation, and through resonance transfer, emit the light energy to the reaction center pigments, which release electrons and set in motion the photochemical process. Thus, from physiological perspective, leaf chlorophyll content is therefore a significant parameter in photosynthesis that needs to be investigated (Richardson *et al.*, 2002). Curran *et al.* (1990) and Filella *et al.* (1995) have independently reported that low concentrations of chlorophyll can directly limit photosynthetic potential and hence primary production.

Both abiotic and biotic stresses have influence on pigment content of leaves. Ayanru and Sharman, (1981) reported that concentrations of chlorophyll *a* and chlorophyll *b*, as well as leaf surface area, leaf dry weight, and petiole dry weight of ACMV infected leaves were reduced significantly compared with healthy leaves. It has been reported that reduction in cassava yield is due to diminished chlorophyll content and reduced leaf surface area caused by cassava mosaic disease (Ayanru and Shrman, 1981).

### **2.7.3 Effect of stomata on photosynthesis**

Another important anatomical structure of the leaf which significantly affects photosynthesis is the stomata. The dual role of stomata involving the diffusion of carbon dioxide into the plant and exit of excess water makes it play a critical role in photosynthesis. Koyama and Takemoto (2014) have reported that in *Oenothera biennis* (common evening-primrose), the net photosynthetic rate under natural sunlight intensity increased from sunrise, reached a maximum at mid-morning, and then showed midday depression. This observation correspond to opening of stomata under light conditions during the day thereby driving gaseous exchange for photosynthesis to occur. Koyama and Takemoto (2014) suggested that, midday suppression of photosynthesis is caused by stomatal limitation and other factors such as photoinhibition, photorespiration and reduced Rubisco activation under high temperature. *Oenothera biennis* has been described as a C<sub>3</sub> plant by Evans *et al.* (2005) thus, midday suppression of photosynthesis could also occur in cassava.

Tanaka *et al.* (2013) investigated the effect of alteration of stomatal density on leaf photosynthetic capacity in *Arabidopsis thaliana*. They modulated stomatal density by

overexpressing or silencing stomagen, which is a positive regulator of stomatal development and observed a 30% increase in leaf photosynthetic capacity and plant growth in transgenic plants compared to wild-type plants. Thus, they concluded that increased stomatal density enhanced leaf photosynthetic capacity by modulating gas diffusion.

#### **2.7.4 Tuber production in cassava**

The production of storage tubers is the main driving force for the large scale cultivation of cassava in the tropics. The tubers are thus the predominant sinks that store the carbohydrates which are utilized in the food and the pharmaceutical industries (CIAT, 2012). Tuber production in cassava is varietal or genotype-dependent as the number and weight of tubers varies among the genotypes. Studies have indicated that cassava can be both sink and source limited, thus, achieving an optimal sink-source relationship is a means of reaching high yield (El-Sharkawy and Cock, 1990). In the study, it was proposed that the use of photosynthetic rate in breeding parental materials can lead to high storage-root since sink strength and photosynthetic rates are related. Tuber production is directly influenced by leaf structure and number. According to Karim (2004), leaf is a primary source of photoassimilates in cassava and it is an important factor governing tuber production. Islam *et al.*, (2007) have reported that leaf production, size or area, and weight directly affect the growth of storage root in cassava. Continuous leaf shed is often accompanied by emergence of new leaf in cassava and it is very important for tuber development in cassava.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Experimental site

The research was carried out at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Kwabenya, Accra. The field trials were conducted on the experimental fields of the Nuclear Agriculture Center, located in the coastal savannah zone, 05°40' N, 0° 13' W. The area has an annual precipitation of less than 1000 mm (Morris *et al.*, 1999).

#### 3.2 Planting material

The cassava varieties used in the study were obtained from a farmer's field at Samsam, a village near Amasaman in the Ga West District in the Greater Accra Region. The eight cassava varieties were *Ankrah* (Ank), *Yeveshevi* (Yev), *Atomo* (Ato), *Gbenze* (Gbe), *Afisiafi* (Afi), *Nandom* (Nan), *Agriculture* (Agr), and *Bosom nsia* (Bos). These varieties vary in their maturity period and cooking quality. *Bosom nsia* as the vernacular name suggests, is an early maturing variety, producing tubers after six months of planting; however, for higher yield, the tubers are usually harvested after nine months of planting. *Gbenze* matures nine months after planting while the remaining varieties (*Afisiafi*, *Ankrah*, *Nandom*, *Atomo*, *Yeveshevi* and *Agriculture*) mature twelve months after planting (MAP). Most of the varieties are local varieties or farmers' materials. These varieties were chosen for the study because of their commercial value and good cooking qualities. All the

varieties are good for making *fufu*, a typical traditional Ghanaian food except *Afisiafi* which is good for making *gari* and cassava dough.

### **3.3 Planting and Experimental Design**

#### **3.3.1 Land preparation and cultural practices**

Weeding (with a hoe and cutlass) was done before planting and throughout the study period in order to rid the field of weeds that may otherwise compete with the cassava plants. Planting was done during the wet season (May, 2014), thus the soil was moist. The field was kept under rain-fed conditions and no weedicides or pesticides were used throughout the experimental period as weedicides block photosynthesis pathways.

#### **3.3.2 Experimental design**

The experimental field was laid out in a Randomized Complete Block Design (RCBD) with four replications. Each replicate or block measured 10m x14.5m with eight plots per block. Each plot represented a cassava variety.

#### **3.3.3 Planting**

Stem cuttings consisting of four nodes were prepared from the stakes and used as planting material. The cuttings were planted on a field measuring 35m x 25m. Thirty cuttings were planted per plot at a distance of 1m between rows and 0.5m within rows. The stem cuttings

were planted slanted at 45°. The African cassava mosaic virus (ACMV) was allowed to spread naturally on the field.

### **3.4 Symptom score for cassava mosaic disease on eight cassava varieties**

Cassava mosaic disease (CMD) symptom severity was determined using visual observation followed by scoring using a five point scale as described by Fauquet and Fargette (1990) as follows:

0 – No symptoms,

1 – Faint mosaic symptoms on leaves

2 – Yellowish mosaic pattern and malformation of leaves,

3 – Severe mosaic distortions and reduced leaf size,

4 – Severe mosaic, severe distortion, and up to 50% leaf size reduction,

5 – Leaf size reduced to tendrils, 50-80% size reduction.

Symptom severity score was done at one, two, three, four, five and six months after planting.

### 3.5 Detection of ACMV and EACMV by Polymerase Chain Reaction (PCR)

After the symptomatic score, the specific viruses were determined using the polymerase chain reaction.

#### 3.5.1. Extraction of genomic DNA

Genomic DNA was extracted from young cassava leaf tissues according to the method described by Dellaporta *et al.*, (1983). Leaves of infected plants were harvested and 2g were placed in 2.0ml microfuge tubes and labeled. Each tube containing the leaf discs was dipped into a flask containing liquid nitrogen and ground with a plastic micro pestle into a fine powder and eight hundred (800  $\mu$ l) of 2% CTAB and 0.5 $\mu$ l of 0.1% mercaptoethanol were added to each powdered sample and then placed in a water bath at 65°C for 30 minutes with intermittent swirling. The mixture was then cooled at room temperature and 800 $\mu$ l of chloroform: isoamylalcohol (24:1) was added to each and mixed by gently shaking the tubes. The mixture was centrifuged at 14000 rpm for 15min after which the supernatant was transferred into clean 1.5ml Eppendorf tubes and labelled correspondingly. The chloroform:isoamylalcohol step was repeated, the supernatant was transferred into a clean 1.5ml microfuge tube and again centrifuged at 14000rpm for 15min. Four hundred (400 $\mu$ l) of ice cold isopropanol was added to precipitate nucleic acids by gently swirling the mixture, which was kept on ice for 30min and centrifuged at 14000rpm for 5min. The isopropanol was decanted and the nucleic acid pellet was washed with 500 $\mu$ l of washing buffer and centrifuged again at 6000rpm for 4min. The buffer was decanted and the pellets were washed in 400  $\mu$ l of 80% ethanol then centrifuged again at 6000rpm for 4min. The

ethanol was then decanted and the pellet was dried at 37°C. The final DNA product was then suspended in 50 µl TE buffer and centrifuged at high speed for 30sec to remove all insoluble materials. To confirm the DNA extraction was successful, 10 µl of the genomic DNA plus 2 µl bromophenol blue was run on 1% agarose gel prepared from agarose (Sigma, St Louis, USA), 10µl ethidium bromide and 1xTAE buffer. The same buffer preparation was used to run the gel in a 200 ml tank at a constant voltage of 90 for 45minutes. After running, the gel was viewed under a high performance ultraviolet transilluminator (UVP, Cambridge, UK) and images were captured with the aid of a UVP Life Sciences Software (Doc-It LS Image Acquisition).

### **3.5.2 DNA amplification**

Two (2) µl of the extracted DNA from each cassava variety was amplified using virus-specific primers, JSP001/JSP002, EAB555F/EAB555R and EACMV1e/EACMV2e (Appendix 2). The amplification reaction was performed in a 20 µl reaction mixture consisting of 2µl 10x PCR buffer, 0.1µl dNTPs, 1.0µl MgCl<sub>2</sub>, 0.2µl JSP001 (forward primer), 0.2µl JSP002 (reverse primer), 14.3µl water, 0.2µl Tag DNA polymerase and 2µl of genomic DNA. The PCR reaction was performed in a 96-well Thermal Cycler (Eppendorf AG, Hamburg). The PCR program used for the amplification involved an initial denaturation step at 94°C for 2 minute followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 52°C for 1 minute and elongation at 72°C for 1 minute followed by a final elongation step at 72°C for 10 minute.

### 3.5.3 Agarose gel electrophoresis

Gel electrophoresis was done using a protocol by Sambrook *et al.*, (1989). Two percent (2%) agarose was prepared by weighing 3g (Sigma, USA) into 150ml of 1xTAE buffer in a conical flask and boiled in a microwave oven for about 5minutes and allowed to cool to about 55°C. Ten (10) µl of ethidium bromide solution was added to the agarose and poured into a horizontal gel electrophoresis tray fitted with a 50-tooth comb and allowed to solidify. After solidification, the comb was removed and the electrophoresis tank was filled with 1xTAE buffer. Ten (10) µl of PCR product was mixed with 2µl of bromophenol blue (Sigma, USA) and loaded into the wells. The gel was run at a constant voltage of 90V for one hour and then visualized under a high performance ultraviolet transilluminator (UVP, Cambridge, UK) and photographed with UVP Life Science Software (Doc – It LS Image Acquisition) and a digital camera (Samsung WB250F).

### 3.6 Measurement of photosynthetic rate in eight cassava varieties.

The photosynthetic rates of eight cassava varieties were determined using the mini Plant Photosynthesis Meter (miniPPM-300, Netherlands). The photosynthetic rate is measured using visually uninfected (healthy) and infected intact leaves of five plants per plot. Three healthy and infected leaves were randomly selected from mid portion of each plant and their photosynthetic rates were measured using the central lobe. Upon pressing the “measure” button on the meter, light is emitted (for a few seconds) and this was directed unto the central lobe by gently holding it upright and two fluorescence; F (fluorescence

yield) and  $F_m$  (maximum fluorescence) and photosynthetic active radiation (PAR) were read on the LED screen. To ensure accuracy, measurements were taken three times on each lobe and the means were calculated. These values were then used to calculate the photosynthesis yield (Y) and photosynthesis rate (P) using the following equations:

$$Y = 1 - F/F_m \quad (3.1)$$

Where F is fluorescence yield and  $F_m$  is maximum fluorescence. The photosynthetic rate (P) was then calculated using the equation below:

$$P = Y * PAR \text{ (mmol/m}^2\text{s)} \quad (3.2)$$

Where PAR is photosynthetic active radiation.

The calculated photosynthetic rate was then used to calculate the dry matter production (DMP) using the equation below:

$$DMP = P/100 \text{ (gram/m}^2\text{hr)} \quad (3.3)$$

Where P is the photosynthetic rate.

### **3.7 Measurement of chlorophyll content of uninfected and infected leaves of cassava varieties**

Two methods were used for the measurement of the chlorophyll content in intact infected and uninfected leaves. The first method involves non-destructive measurement of chlorophyll content using a portable chlorophyll meter and a destructive method involving the use of spectrophotometer.

#### **3.7.1 Measurement of chlorophyll content using Chlorophyll Content Meter**

Chlorophyll content of the cassava leaves were determined using Opti-Science (CCM 200 Plus, Tyngsboro, MA, USA) chlorophyll meter. To measure the chlorophyll content, the central lobe of intact leaves of healthy and infected leaves were placed in the chamber of the meter and pressed to close. The chlorophyll content is read on LED screen. Three randomly selected intact leaves of both infected and uninfected leaves from the mid portion of a plant were used and five plants per plot were sampled for the study. The meter was calibrated after each measurement to ensure accuracy. The CCM-200 Plus uses transmittance to estimate the chlorophyll content in leaf tissue. Two wavelengths (940 and 665nm) are used for absorbance determinations. One wavelength falls within the chlorophyll absorbance range while the other serves to compensate for mechanical differences due to tissue thickness. The meter measures the transmittance of both wavelengths and calculates chlorophyll content index (CCI) value that is proportional to the amount of chlorophyll in the leaf sample (Cate and Perkins, 2003).

### 3.7.2 Measurement of chlorophyll content using Spectrophotometer

The amount of chlorophyll in leaves of eight cassava varieties was also determined using the spectrophotometric method described by Arnon, (1949) to ensure accuracy of results. To allow for ease of comparison, the same infected and uninfected leaf lobes used for chlorophyll content measurement with the chlorophyll meter were used. Since this is a destructive method, the chlorophyll content was measured once at 6 months after planting.

The spectrophotometer (Jenway, 6305 UK) was used to estimate chlorophyll *a* and *b* content as well as carotenoids in infected and uninfected leaves. Fresh leaves were plucked and placed in zip lock bags and labelled. The leaves were then placed in a cooler with ice cubes to avoid wilting. The leaves were weighed using an analytical weighing balance (Mettler Toledo, USA) and then chopped into small pieces, care being taken to avoid major veins, tough and fibrous tissues. The leaf samples were then ground with a mortar and pestle and 0.05g of the powder was weighed. The powder was transferred into a 25ml extraction bottle, and 5ml of 80% acetone [80:20 (v: v)] was added and the homogenate was filtered through a 1mm filter paper. The residue was discarded and the extract was collected into a 5ml test-tube.

To measure the chlorophyll content, a clean 4ml quartz cuvette was filled with two-thirds of 80% acetone and placed in the spectrophotometer and the absorbance was read at a wavelength of 663nm (this served as a blank). The blank was then removed and saved for the next measurement. A second cuvette was similarly filled with two-thirds full of the leaf extract after gentle swirling, the absorbance read as previously described at 663nm and

645nm. The procedure was repeated and the absorbance was read at 470nm. The chlorophyll content was estimated using Arnon's (1949) equation which converts absorbance to mg Chl g<sup>-1</sup> of leaf tissue as shown below:

$$Chl_a (mg g^{-1}) = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \text{ ml acetone/mg leaf tissue} \quad (3.4)$$

$$Chl_b (mg g^{-1}) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \text{ ml acetone/mg leaf tissue} \quad (3.5)$$

The total chlorophyll content was estimated using the formula below:

$$Total\ Chl = Chl_a + Chl_b \quad (3.6)$$

Carotenoids and xanthophylls were also estimated using the equation below:

$$C_{x+c} = 1000A_{470} - 1.90Chl_a - 63.14Chl_b / 214 \quad (3.7)$$

Where x + c = xanthophyll and carotenes

### 3.8 Measurement of morphological features

#### 3.8.1. Measurement of plant height

Plant height was measured from soil surface to shoot tip using a measuring tape at 2, 3, 4, 5 and 6 MAP. Plants with several branches were measured by recording the average height of all the branches.

### **3.8.2. Measurement of leaf area**

The leaf area of the eight cassava varieties was measured using a graphical method six months after planting. Six leaves each of infected and uninfected cassava plants were detached and placed on a graph paper. The outline of the leaves was traced using a pencil and the leaf area was estimated from the graph paper by counting the number of squares.

### **3.9 Determination of stomatal size and density**

The stomatal densities of eight varieties were determined using a digital microscope (Leica, Germany). Two replicates of both CMD-infected and uninfected leaves of the eight varieties were harvested and wiped clean. The cleaned leaves were brushed with nail varnish, air-dried on a laboratory bench for five minutes and then peeled off using a cellotape. The peeled varnish was placed on a slide and the stomatal impressions made on the dried nail varnish were viewed under the microscope. The number of stomata was determined by counting the number of stomata within a  $100\text{mm}^2$  of a graticule placed in the eye piece of the microscope. Also, the stomata size was measured by reading the calibrations on the graticule. The shape of the leaves of each variety was described using a study guide by CIAT, (1983).

### **3.10 Determination of tuber weight**

Tuber weight of non-infected and infected eight cassava varieties was determined by counting the number of tubers per plant and weighing the fresh tubers. Using four replicates and five plants per replicate, making a total of twenty plants of each variety were harvested 11 months after planting and the tubers were weighed using a scale (Camry FD50-150, China).

### **3.11 Measurement of microclimatic factors of the study area.**

Microclimatic factors namely; relative humidity, temperature, wind speed and light intensity of the study area were measured using Extech, (45170-EU-EN V1.3, USA) 4-in-1 meter. The vane or airflow sensor of the meter was placed towards the direction of the wind to measure the wind speed while the light sensor was directed towards light in order to measure light intensity. The measurement of these microclimatic factors were done monthly throughout the experimental period.

### **3.12 Statistical Analysis**

Comparison between varieties were done using data from four replicates, however, those involving comparison between ACMV infected and uninfected plants were done using data from three replicates. Statistical analysis was done using Statgraphics (Centurion version

16.1.11). Data collected on photosynthesis, chlorophyll and plant morphometry were subjected to analysis of variance (ANOVA). Correlation analysis was done where applicable. Significant ( $p \leq 0.05$ ) means were separated using Duncan's multiple range test (DMRT).



## CHAPTER FOUR

### RESULTS

#### 4.1 Symptom severity of CMD on cassava varieties

Visual observation of leaves of the eight cassava varieties showed symptoms of the presence of cassava mosaic disease which were expressed as chlorotic yellowish spots, wrinkle surfaces and reduction in leaf size (Figure 4.1B-D). Symptom severity was scored using a five point scale (from 0 to 5) described by Fauquet and Fargette (1990). Scoring for the symptoms was done at one, two, three, four, five and six months after planting

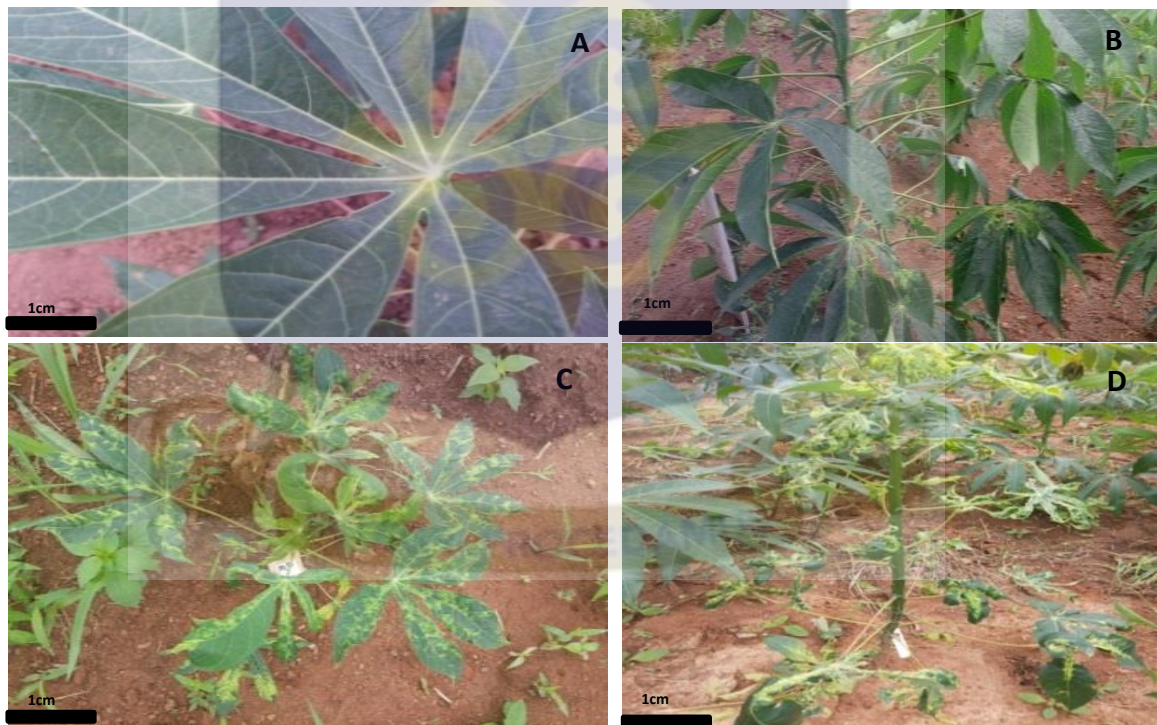


Figure 4.1: Cassava plants showing symptoms mosaic virus disease. (A) Uninfected leaf (healthy), (B) and (C) ACMV infected leaf with yellowish chlorotic patches (D) ACMV infected leaf with wrinkled or distorted or reduced leaf surface.

Symptoms of the mosaic virus disease were observed one month after planting in all the varieties except *Agric* and *Afisiafi*, which started showing symptoms two months after planting indicating that the virus was either transmitted from the stem cuttings to the developing shoots or transmission from white flies (*Bemisia tabaci*) (Table 4.1). *Bosomnsia* had the highest percentage of plants infected with the ACMV virus (12.9%) one month after planting. Monthly percentage of infection almost remained the same except at the 6 month where more than 23% of the plants were ACMV infected. Throughout the study period, *Ankrah* had the least percentage infection suggesting that it is tolerant to the virus, although in the second month after planting it had the highest percentage (15%) of ACMV infected plants. However, it visually showed recovery from the mosaic disease at the third and fourth months after planting. Similarly, *Atomo* and *Afisiafi* also showed visual recovery from the mosaic infection at the fourth and fifth months after planting. The variety with the highest number of ACMV infected plants was *Nandom* with 98% infection at the end of the study period.

The percentage of plants ACMV infected per variety ranged from 29.0 - 98.0. Of all the varieties, *Nandom* had the highest percentage (98.0) of ACMV infected plants (Table 4.1), although it had a low mean virus score of 1.9 (Table 4.1, Appendix 1).

Table 4.1 Percentage of plants infected by mosaic virus disease per month

MAP	Afisiafi	Agric	Ankrah	Atomo	Bosom nsia	Gbenze	Nandom	Yeveshevi
1	0	0	7	3	11	8	2	2
2	6	2	18	2	11	6	5	4
3	4	4	0	3	12	9	4	8
4	1	3	0	0	11	8	4	10
5	0	10	1	0	13	6	9	8
6	55	39	8	50	20	17	90	6
NIP	66	58	34	58	78	54	114	38
TNP	114	111	117	95	85	110	116	98
% Infection	58	52	29	61	92	49	98	49

MAP = Months after planting; NIP = Number of ACMV infected Plants; TNP = Total Number of Plants.

The mean virus score throughout the study period was low in *Afisiafi* (1.2) and high in *Gbenze* (2.8) (Table 4.1, Appendix 1). Two of the varieties namely; *Afisiafi* and *Atomo* had virus scores below 1.5 and thus may be classified as highly mosaic tolerant varieties. These varieties also showed recovery from the disease (Table 4.1, Appendix 1) as there was no score for the virus 4 and 5 months after planting. Of the remaining varieties *Ankrah*, *Nandom* and *Yeveshevi* had virus scores ranging from 1.5 – 2.0, suggesting that they are moderately tolerant to the mosaic disease while *Agric*, *Bosom nsia* and *Gbenze* had virus scores higher than 2.0 and thus may be highly susceptible to the disease.

#### **4.2 Determination of specific viruses using Polymerase Chain Reaction**

For determination of the specific viruses causing the mosaic disease, three primers were used to amplify the viruses in the genomic DNA of the cassava varieties using the polymerase chain reaction (Appendix 2). Of the three primer pairs used, only JSP001/JSP002 was able to amplify ACMV in two cassava varieties, *Bosom nsia* and *Yeveshevi* (Figure 4.2) even though all the remaining varieties showed visual symptoms of the disease.



Figure 4.2: Gel electrophoresis of PCR amplification of Cassava DNA using the primer JSP001/JSP002, Lane M = 1 kb DNA ladder; 1 - 40 represent DNA of forty cassava leaves randomly selected from the field for ACMV detection; Lane 1, 2, 11 & 12 = *Ankrah*; Lane 3, 4, 5, 13, 21 & 40 = *Bosom nsia*; Lane 6, 7, 8, 14, 22 & 23 = *Yeveshevi*; Lane 9, 10, 15 & 16 = *Atomo*; Lane 17, 18, 19, 20, 24 & 25 = *Gbenze*; Lane 26, 27, 31, 32 & 33 = *Afisiafi*; Lane 28, 29, 30, 34 = *Agric*; Lane 35 – 39 = *Nandom*.



### 4.3 Photosynthetic rate of eight cassava varieties

Photosynthetic rates of uninfected and ACMV infected cassava varieties were measured from September 2014 to May 2015 using a portable photosynthetic meter (miniPPM-300, Netherlands) and the results are presented in Figure 4.3a&b. Generally, the photosynthetic rate in all the uninfected varieties followed the same pattern throughout the study period (Figure 4.3a). It decreased from September 2014 (3 MAP) in all the varieties to the lowest value in November 2014 (5 MAP) and thereafter increased again in the December and finally declined in May 2015 (11 MAP). For uninfected plants, the photosynthetic rates ranged from 58  $\mu\text{mol}/\text{m}^2\text{s}$  in *Atomo* in September to 31.5  $\mu\text{mol}/\text{m}^2\text{s}$  in *Agric* in May. Although *Atomo* had the highest photosynthetic rate (58  $\mu\text{mol}/\text{m}^2\text{s}$ ) in September 2014, *Gbenze* had the highest mean photosynthetic rate (45.2  $\mu\text{mol}/\text{m}^2\text{s}$ ) at the end of the study period, followed by *Nandom* and *Yeveshevi* with 44.6  $\mu\text{mol}/\text{m}^2\text{s}$  each. *Ankrah* and *Atomo* had similar mean photosynthetic values of 43.8 and 43.9  $\mu\text{mol}/\text{m}^2\text{s}$  respectively, while the least mean photosynthetic rate (41.2  $\mu\text{mol}/\text{m}^2\text{s}$ ) was observed in *Afisiafi*. There were no significant differences ( $p \geq 0.05$ ) in mean photosynthetic rates among the varieties (Appendix 4).

Figure 4.3b shows the photosynthetic rates of ACMV infected plants which were measured concurrently with the uninfected plants for all the eight varieties while Figure 4.3c shows a comparison of infected and uninfected plants at the end of the study period.

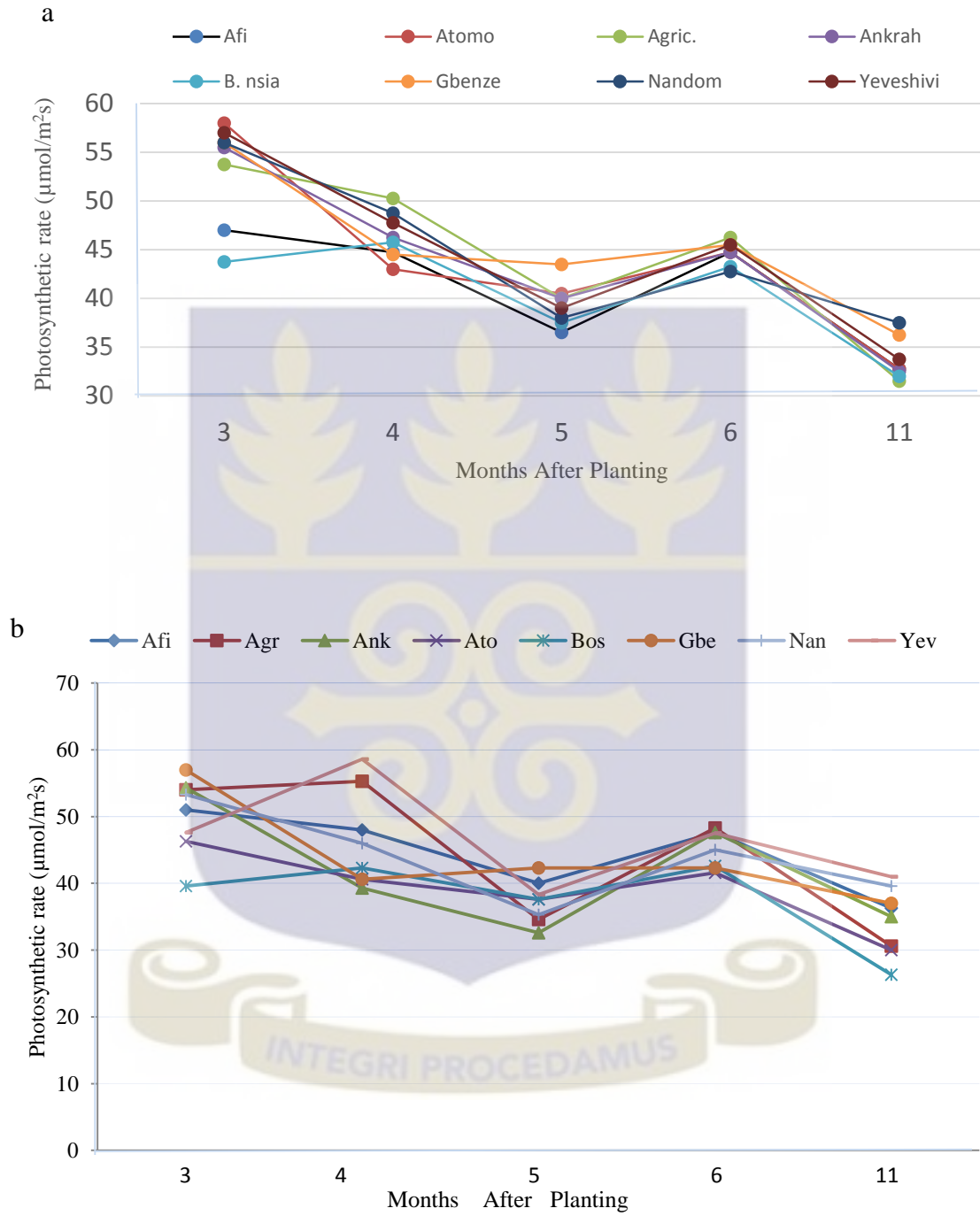


Figure 4.3a and b: Monthly photosynthetic rate of uninfected (a) and ACMV infected (b) cassava varieties from September 2014 to May 2015 as measured by portable photosynthetic meter (miniPPM-300, Netherlands).

The photosynthetic rates of ACMV infected plants of the eight varieties followed a similar trend to that of uninfected plants with the exception of *Agric* and *Yeveshevi* which increased in photosynthetic rates between September and October (month 3 and 4) (Figure 4.3b). *Bosom nsia* had the lowest photosynthetic rate 3 MAP September ( $39.6 \mu\text{mol}/\text{m}^2\text{s}$ ) while *Gbenze* had the highest photosynthetic rate ( $57 \mu\text{mol}/\text{m}^2\text{s}$ ). Healthy plants of *Bosom nsia* also had the lowest photosynthetic rate 3 MAP. In October, *Atomo* and *Ankrah* had the lowest photosynthetic rates ( $40.6 \mu\text{mol}/\text{m}^2\text{s}$ ) and *Yeveshevi* had the highest ( $58.6 \mu\text{mol}/\text{m}^2\text{s}$ ). There was a general decline in photosynthetic rate in all the varieties between October and November (Month 4 and 5), except *Gbenze* whose photosynthetic rate increased from  $40.6 \mu\text{mol}/\text{m}^2\text{s}$  to  $42.3 \mu\text{mol}/\text{m}^2\text{s}$ . Between November (5 MAP) and December (6 MAP), the photosynthetic rates of all the infected varieties increased, with *Agric* having the highest ( $48.3 \mu\text{mol}/\text{m}^2\text{s}$ ) and *Atomo* having the lowest photosynthetic rate of  $41.6 \mu\text{mol}/\text{m}^2\text{s}$ . At the end of the study period, all the varieties exhibited low photosynthetic rates suggesting that aging was having effect on the photosynthetic process. *Bosom nsia* showed the greatest decline in photosynthetic rate of  $26 \mu\text{mol}/\text{m}^2\text{s}$  which was the lowest throughout the study period.



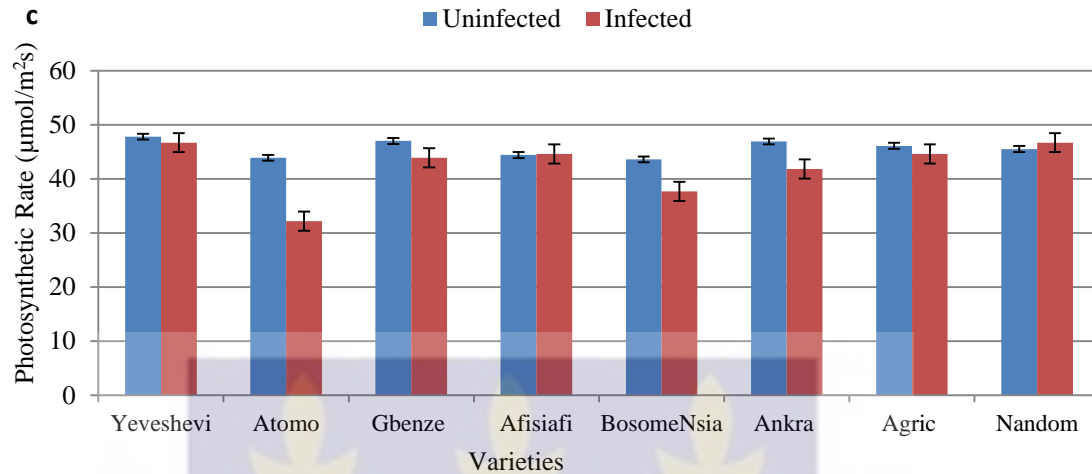


Figure 4.3c Mean photosynthetic rates of ACMV infected and uninfected cassava varieties from September 2014 to May 2015 across 3 replicates.

With the exception of *Nandom* and *Afisiafi*, the mean photosynthetic rate in all the varieties was reduced by the presence of the virus disease (Fig. 4.3c). With the exception of *Nandom* and *Afisiafi*, the photosynthetic rates of uninfected plants were comparatively higher than that of ACMV infected plants (Figure 4.3c). However, the differences were not statistically significant.

Figure 4.5a-c show the relationship between ACMV score and monthly photosynthetic rate of both uninfected and ACMV infected plants of *Afisiafi*, *Agric*. and *Ankrah*. Generally, as the ACMV infection increased, photosynthetic rate also increased and vice versa and this observation is seen in all the remaining varieties. In all the varieties, ACMV score decreased drastically to a minimum five months after planting and the photosynthetic rate also correspondingly decreased; in *Afisiafi* and *Ankrah*, the ACMV score decreased to zero suggesting that these varieties were able to recover from ACMV infection.

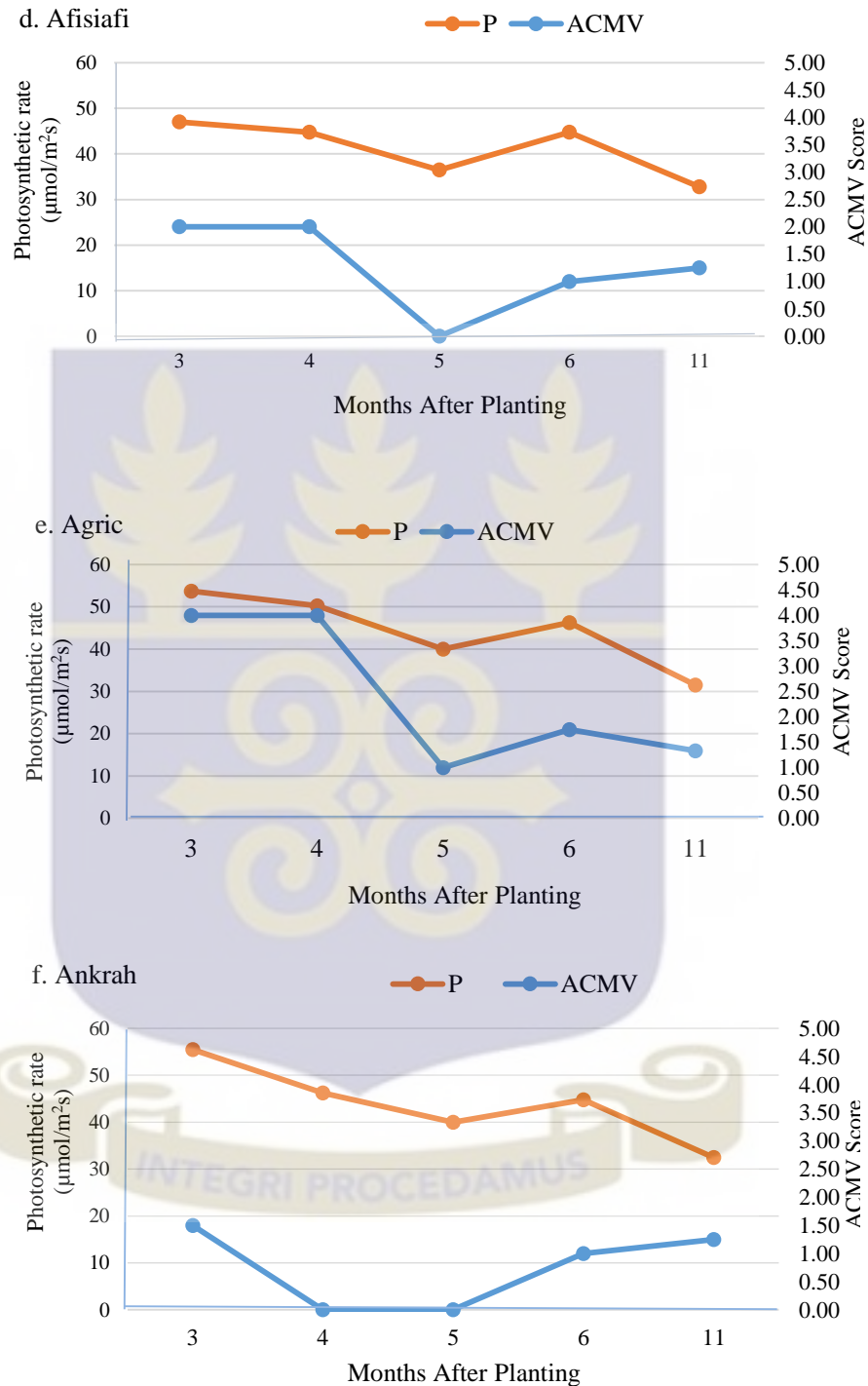


Figure 4.3d-f: Relationship between ACMV score and monthly photosynthetic rate of both uninfected and ACMV infected plants of (d) *Afisiafi*, (e) *Agric* and (f) *Ankrah*. P = Photosynthetic rate; ACMV = ACMV score

#### 4.4 Chlorophyll content of eight cassava varieties

##### 4.4.1 Determination by Chlorophyll Content Meter (CCM 200, USA)

The Chlorophyll Content Index (CCI) of uninfected and ACMV infected plants of the eight cassava varieties was also measured using the Chlorophyll Content Meter (CCM 200, Tyngsboro, MA, USA) and Spectrophotometer (Jenway6305, UK). Using the Chlorophyll Content Meter, the chlorophyll content of healthy plants ranged from 17.9 to 39.93 CCI with *Gbenze* having the highest mean chlorophyll content (39.93 CCI) while *Nandom* had the lowest (17.90 CCI). *Gbenze* was significantly different from all the other varieties (Table 4.4a, Appendix 5). With the exception of *Nandom* and *Agric*, the chlorophyll content of all the varieties was generally higher than 20 CCI.

Table 4.4a Chlorophyll Content Index (CCI) of eight cassava varieties

Variety	CCI
Afisiafi	24.38±1.23 <sup>c</sup>
Agriculture	19.93±1.25 <sup>cd</sup>
Ankrah	21.40±1.69 <sup>cd</sup>
Atomo	34.23±2.19 <sup>b</sup>
Bosom nsia	36.90±1.76 <sup>ab</sup>
Gbenze	39.93±1.75 <sup>a</sup>
Nandom	17.90±1.92 <sup>d</sup>
Yeveshevi	25.28±1.63 <sup>c</sup>

Values in a column followed by different letters are significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range test

Generally, chlorophyll content index (CCI) of all the varieties was reduced by the presence of CMD (Figure 4.4a) except for *Nandom* and *Yeveshevi* where the ACMV infected plants had higher chlorophyll content than the uninfected plants.

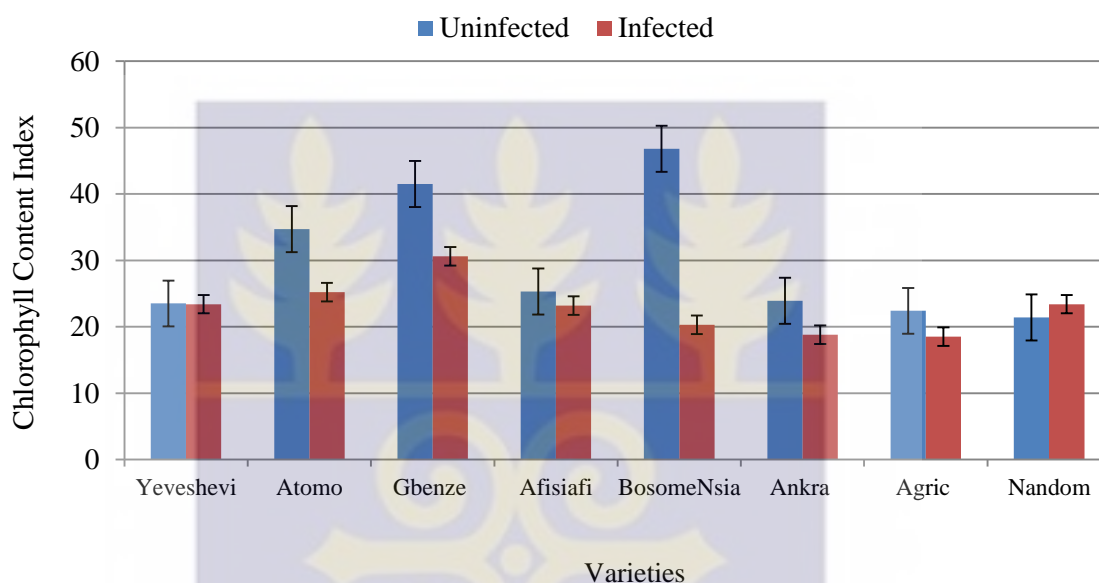


Figure 4.4a Chlorophyll content of ACMV infected and uninfected plants of eight cassava varieties as measured by the chlorophyll meter CCM200.

In *Bosome nsia*, the chlorophyll content of the uninfected plant was 48 CCI and this was higher than the ACMV infected plant (20 CCI). Similarly, the chlorophyll content of uninfected *Gbenze* (42 CCI) and *Atomo* (35 CCI) was also higher than the ACMV infected (30 and 25 respectively). Of the remaining varieties namely *Ankra*, *Afisiafi* and *Agric*, the chlorophyll content of the uninfected plants were marginally (1 – 4 CCI) higher than the ACMV infected plants.

#### 4.4.2 Determination by Spectrophotometer (Jenway 6305, UK)

Additionally, the spectrophotometer was also used to determine the total chlorophyll concentration, chlorophyll *a* and *b*, xanthophyll and carotenoid content of healthy and ACMV infected plants using Arnon's equation (see sections 3.5.2). The results obtained for the total chlorophyll concentration of healthy plants showed a trend similar to results obtained using the chlorophyll meter. *Gbenze* had significantly the highest ( $P \leq 0.05$ ) total chlorophyll concentration ( $3.39 \text{ mg.g}^{-1}$ ), chlorophyll *a* and *b* ( $2.26$  and  $1.09 \text{ mg.g}^{-1}$  respectively), xanthophyll and carotenoid ( $7.42 \text{ mg.g}^{-1}$ ) compared to the remaining varieties while *Nandom* had the lowest values of the same parameters ( $1.53$ ,  $1.08$ ,  $0.45$ , and  $2.91 \text{ mg.g}^{-1}$ ) respectively (Table 4.4b). Significant differences ( $p \leq 0.000$ ) were observed among the varieties for total chlorophyll, chlorophyll *a* and *b*, xanthophyll and carotenoids (Appendix 6 - 9). Total chlorophyll, chlorophyll *a* and *b*, xanthophyll and carotenoids of *Gbenze* differed significantly from the remaining varieties. There was no significance difference in total chlorophyll concentration between *Bosom nsia*, *Atomo* and *Yeveshevi*. The total chlorophyll content in all the varieties ranged between  $1.53 \text{ mg.g}^{-1}$  and  $3.40 \text{ mg.g}^{-1}$  (Table 4.4b).

Figures 4.4b and 4.4c show chlorophyll *a* and *b* concentrations in both ACMV infected and uninfected plants of the eight varieties using the spectrophotometer. Again, the mosaic disease reduced chlorophyll *a* and *b* concentrations of the ACMV infected plants. ACMV infected plants of *Ankrah* had about 7% reduction in chlorophyll *a* concentration compared with uninfected plants. *Gbenze* and *Atomo* had about 23% reduction each. In *Bosom nsia* and *Afisiafi* however, the ACMV infected plants had higher chlorophyll content than

uninfected plants. The chlorophyll *a* content of uninfected plants ranged from 1.3 mg.g<sup>-1</sup> in *Nandom* and *Ankrah* to 2.5 mg.g<sup>-1</sup> in *Gbenze*, while for the ACMV infected plants it ranged from 0.84 mg.g<sup>-1</sup> to 2.02. A similar trend was observed in chlorophyll *b* concentration.

Chlorophyll *b* concentration of uninfected plants ranged from 0.55 mg.g<sup>-1</sup> in *Nandom* to 1.3 mg.g<sup>-1</sup> in *Gbenze*. For the ACMV infected plants *Bosom nsia* had the highest chlorophyll content. Chlorophyll *b* concentration of infected *Afisiafi* plants did not differ in uninfected as they both had a concentration of 0.61 mg.g<sup>-1</sup>. Again, ACMV infected plants of *Bosom nsia* had higher chlorophyll *b* concentrations (1.07 mg.g<sup>-1</sup>) compared with uninfected plants (0.67 mg.g<sup>-1</sup>) (Figure 4.4c).

Table 4.4b Mean total chlorophyll, chlorophyll *a*, chlorophyll *b*, xanthophyll and carotenoid (Cx+c) concentration of eight cassava varieties.

Cassava variety	Total Chlorophyll (mg.g <sup>-1</sup> )	Chlorophyll <i>a</i> (mg.g <sup>-1</sup> )	Chlorophyll <i>b</i> (mg.g <sup>-1</sup> )	Cx+c (mg.g <sup>-1</sup> )
Afisiafi	2.12±0.14 <sup>bc</sup>	1.50±0.53 <sup>b</sup>	0.61±0.03 <sup>cd</sup>	4.42±0.25 <sup>cd</sup>
Agric	1.99±0.81 <sup>bc</sup>	1.39±0.06 <sup>bcd</sup>	0.61±0.02 <sup>cd</sup>	3.88±0.16 <sup>de</sup>
Ankrah	1.79±0.19 <sup>c</sup>	1.24±0.13 <sup>cd</sup>	0.55±0.63 <sup>d</sup>	3.93±0.49 <sup>de</sup>
Atomo	2.49±0.23 <sup>b</sup>	1.68±0.14 <sup>b</sup>	0.81±0.09 <sup>bc</sup>	5.32±0.51 <sup>bc</sup>
Bosom nsia	2.64±0.28 <sup>b</sup>	1.77±0.15 <sup>b</sup>	0.87±0.13 <sup>b</sup>	6.16±0.40 <sup>b</sup>
Gbenze	3.39±0.28 <sup>a</sup>	2.26±0.17 <sup>a</sup>	1.09±0.09 <sup>a</sup>	7.42±0.61 <sup>a</sup>
Nandom	1.53±0.20 <sup>c</sup>	1.08±0.14 <sup>d</sup>	0.45±0.06 <sup>d</sup>	2.91 ±0.48 <sup>e</sup>
Yeveshevi	2.62±0.10 <sup>b</sup>	1.80±0.53 <sup>b</sup>	0.82±0.04 <sup>bc</sup>	5.32±0.29 <sup>bc</sup>

Values in a column followed by different letters are significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range test f

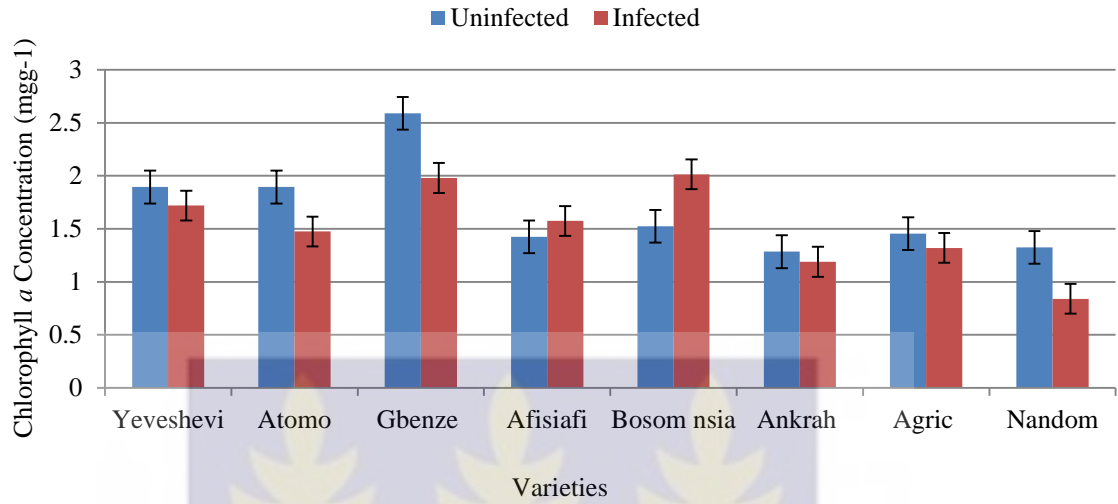


Figure 4.4b: Effect of ACMV on Chlorophyll *a* concentration (mg.g<sup>-1</sup>) of eight cassava varieties

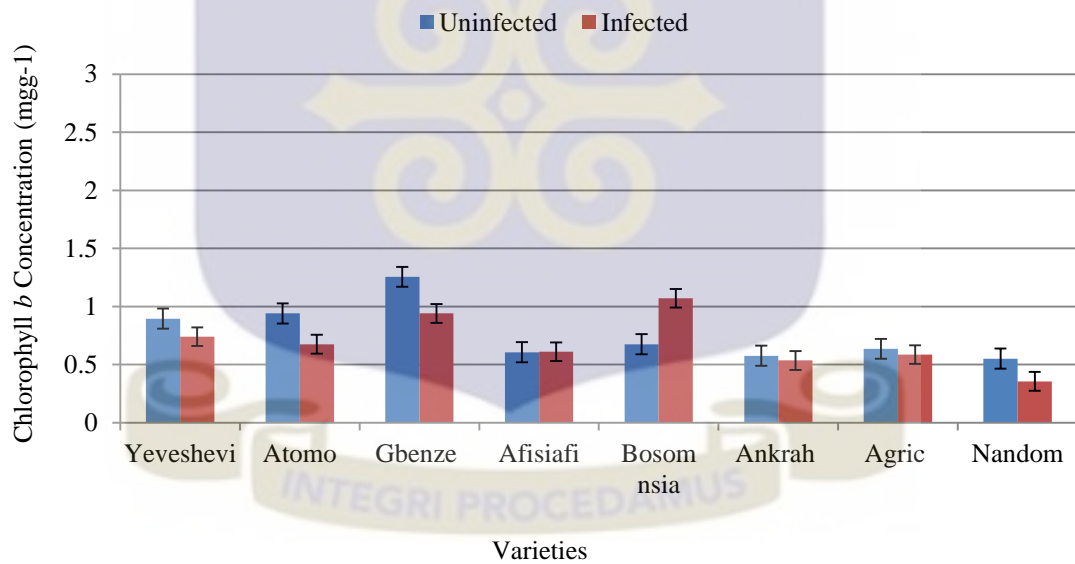


Figure 4.4c: Effect of ACMV on Chlorophyll *b* concentration (mg.g<sup>-1</sup>) of eight cassava varieties

#### 4.5 Plant height, leaf area, stomatal density and size of eight cassava varieties

The leaf area, stomata density and stomata size as well as height of plants were measured in order to determine their effect on photosynthetic rate of the eight varieties. Of all the varieties studied, *Ankrah* grew tallest reaching a height of 127.35cm which was significantly taller ( $p \leq 0.05$ ) than all the remaining varieties (Table 4.5a, Appendix 11). Although the height of *Bosom nsia* (109.73cm), *Gbenze* (111.40cm) and *Afisiafi* (103.15cm) varied morphologically, statistical analysis did not show any significant difference ( $p \geq 0.05$ ) between these varieties (Table 4.5a). Among all the varieties, *Yeveshevi* was the shortest (87.6 cm), however, its height was not significantly different from *Nandom* (89.40cm), *Agric.* (98.95) and *Afisiafi* (103.15).

Morphologically, the shape of the leaf lobes varied among the eight varieties. The leaf lobes were either, lanceolate (*Yeveshevi*, *Atomo*, *Agric* and *Ankrah*) or arched (*Gbenze*) or elliptic (*Afisiafi*, and *Nandom*) or obovate-lanceolate (*Bosom nsia*) (Figure 4.5a). The presence of ACMV infection reduced the leaf area due to distortions compared to the healthy leaves. Due to variation in shape, the leaf area varied significantly ( $p \leq 0.001$ ) among the varieties (Appendix 10) among the varieties. *Gbenze* with arched leaves had the broadest leaves among all the varieties with a significant leaf area of 5705.8 mm<sup>2</sup>, which is twice larger than the leaf area of *Afisiafi* (2041.0 mm<sup>2</sup>) and *Agric* (2397.3 mm<sup>2</sup>) (Table 4.5a).

However, the presence of ACMV infection reduced the leaf area to 3105.7 mm<sup>2</sup> in *Gbenze* and 1715.3 mm<sup>2</sup> in *Afisiafi*. Similarly, *Yeveshevi* with arched shaped lobes had leaf area of

4934.20mm<sup>2</sup> which was reduced to 3816.7 mm<sup>2</sup> by ACMV infection. The leaf areas recorded for healthy leaves of *Nandom*, *Ankrah* and *Bosom nsia* were below 2000mm<sup>2</sup> and these were further reduced as a result of ACMV infection.

Table 4.5a: Plant height, leaf area and stomata density and size of ACMV infected and uninfected leaves of cassava varieties.

Varieties	Plant Height (cm)	Stomata Density (mm <sup>2</sup> )	Stomata Size (µm)
Afisiafi	103.15±23.66 <sup>ab</sup>	7.75±0.25 <sup>b</sup>	0.80±0.00 <sup>d</sup>
Agric	98.95±11.64 <sup>b</sup>	7.75±0.25 <sup>b</sup>	1.00±0.00 <sup>a</sup>
Ankrah	127.35±16.94 <sup>a</sup>	7.25±0.48 <sup>ab</sup>	0.87±0.25 <sup>bcd</sup>
Atomo	94.53±12.56 <sup>b</sup>	7.25±0.25 <sup>ab</sup>	0.90±0.40 <sup>bc</sup>
Bosom nsia	109.73±10.16 <sup>ab</sup>	6.25±0.25 <sup>c</sup>	0.95±0.29 <sup>ab</sup>
Gbenze	111.40±10.56 <sup>ab</sup>	9.25±0.25 <sup>a</sup>	0.95±0.29 <sup>ab</sup>
Nandom	89.40±7.87 <sup>b</sup>	6.50±0.28 <sup>c</sup>	0.82±0.25 <sup>cd</sup>
Yeveshevi	87.63±21.75 <sup>b</sup>	7.25±0.63 <sup>ab</sup>	0.87±0.25 <sup>bcd</sup>

Values in a column followed by different letters are significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range test



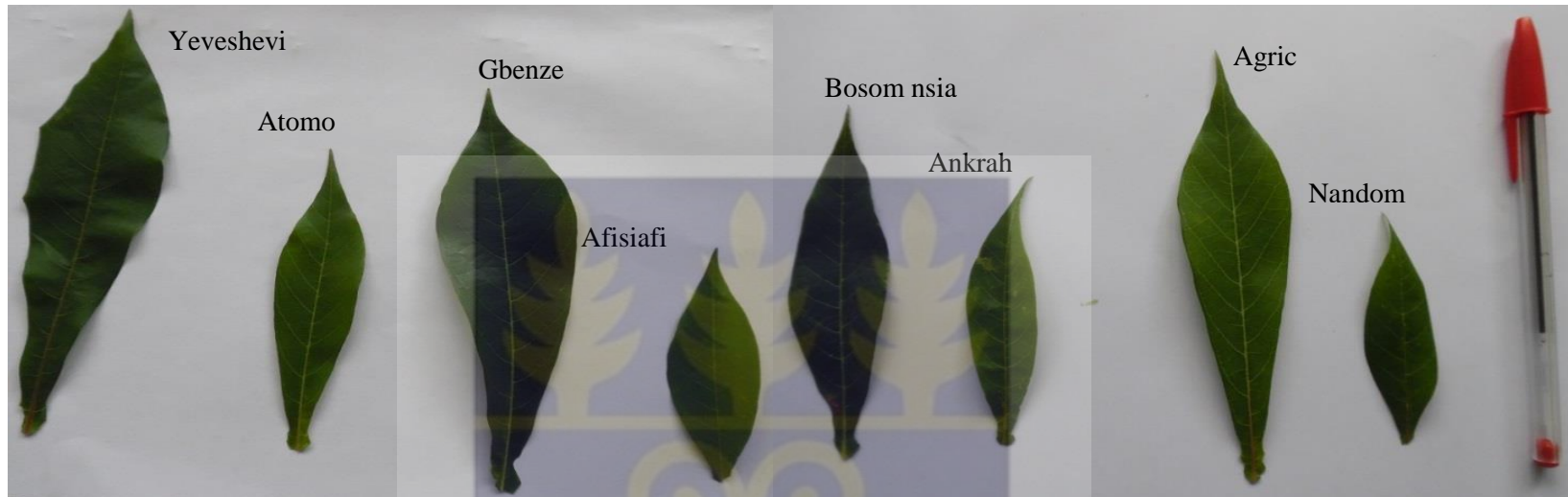


Figure 4.5a Different shapes of leaf lobes of cassava varieties

Yeveshevi = Lanceolate; Atomo = Lanceolate; Gbenze = Arched; Afisiafi = Elliptic; Bosom nsia = Obovate-lanceolate; Ankrah = Lanceolate; Agric = Lanceolate; Nandom = Elliptic.



Table 4.5b: Leaf area of ACMV infected and uninfected leaves of eight cassava varieties.

Varieties	Leaf Area (mm <sup>2</sup> )	
	Uninfected	Infected
Afisiafi	2041.0±53.90	1715.3±33.20 <sup>d</sup>
Agric	2397.3±38.90	2754.6±52.50 <sup>d</sup>
Ankrah	1181.3±34.43	1087.7±45.63 <sup>f</sup>
Atomo	3590.6±35.90	3644.8±25.35 <sup>c</sup>
Bosom nsia	1686.6±41.40	1503.6±41.10 <sup>e</sup>
Gbenze	5705.8±18.20	3105.7±16.42 <sup>a</sup>
Nandom	1659.3±16.08	1251.6±22.38 <sup>f</sup>
Yeveshevi	4472.5±44.57	3816.7±47.81 <sup>b</sup>

Microscopic examination of the lower leaf surfaces showed the presence of kidney-shaped guard cells with stomata (Figure 4.5a). However, the stomata density as well as the size varied significantly among the varieties ( $p \leq 0.001$ ) (Table 4.5b; Appendix 12 and 13). The presence of the mosaic disease also reduced the stomata density and size in *Gbenze*, *Ankrah*, *Agric* and *Nandom*. Additionally, it packed the stomata tightly together compared to the uninfected plants as the leaf surface area reduced due to the virus (Figure 4.5a). For the healthy plants, the stomata density was highest in *Gbenze* (9.25mm<sup>2</sup>) compared to the remaining accessions while *Bosom nsia* had the lowest density of 6.25mm<sup>2</sup>. *Agric* and *Afisiafi* had the same stomata densities of 7.75mm<sup>2</sup> each.

The presence of ACMV reduced the stomatal densities of *Gbenze* from 9.3 to 7mm<sup>2</sup>, *Ankrah* (7.3 to 6.5 mm<sup>2</sup>), *Agric* (7.7 to 4.7 mm<sup>2</sup>) and *Nandom* (6.7 to 6.3mm<sup>2</sup>) but had no effect on *Yeveshevi*, *Atomo*, *Afisiafi* and *Bosom nsia*. The stomatal size was

correspondingly reduced by the presence of the virus. The stomatal sizes of *Gbenze* and *Agric* were reduced but virus had no reduction effect on the remaining varieties. Contrarily, *Yeveshevi*, *Atomo*, *Afisiafi*, *Ankrah* and *Nandom* had higher stomata sizes in ACMV infected plants. *Agric* and *Gbenze* had the largest stomata sizes (1.00 and 0.95  $\mu\text{m}$  respectively).

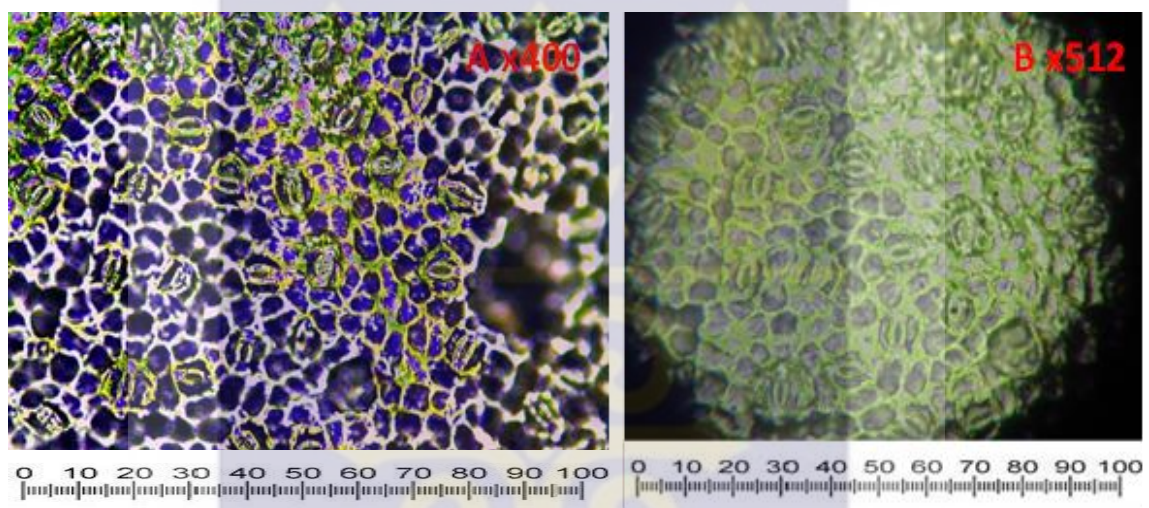


Figure 4.5b Stomata of (A) uninfected and (B) virus infected leaf of *Atomo*.

#### 4.6 Effect of photosynthetic rate and ACMV on fresh weight of leaves and tubers and number of tubers

Plants were harvested 11MAP by uprooting the stakes. Table 4.6 shows fresh weight of leaves and tubers as well as the number of tubers of the eight cassava varieties. For uninfected plants, the tuber weights ranged from 1.28 kg to 2.9 kg with *Yeveshevi* having the highest weight (2.93 kg), followed by *Atomo* (2.57 kg) and *Gbenze* (2.25 kg) in that order while *Ankrah* had the lowest tuber weight of 1.28 kg.

Tuber weight in uninfected *Afisiafi*, *Agric.*, *Ankrah*, *Atomo*, *Bosom nsia* and *Gbenze* did not show any significant ( $P>0.05$ ) variation among the varieties (Table 4.6, Appendix 15 & 16). However, the weights of these varieties were lower than *Yeveshevi* which had the highest mean fresh tuber weight (Table 4.6). The presence of mosaic virus disease reduced the fresh tuber weight in all the varieties except *Yeveshevi*, *Afisiafi* and *Agric* (Figure 4.6) where the ACMV-infected had higher tuber weights than the uninfected. The weights of the ACMV infected plants ranged from 2.8 kg to 1kg with *Agric* having the highest weight. *Bosom nsia*, *Ankrah* and *Nandom* had the lowest yields of 1kg each.

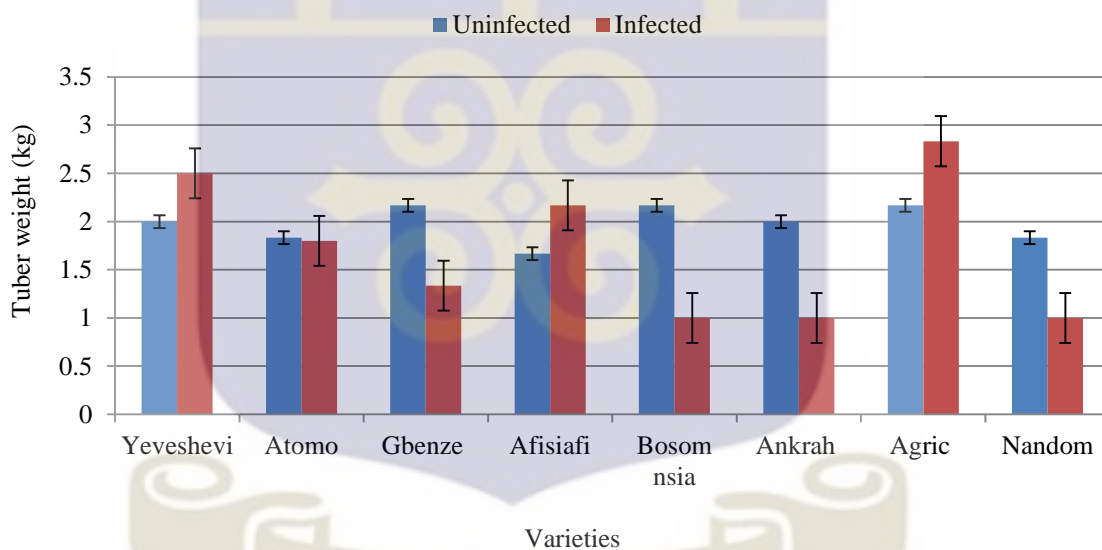


Figure 4.6: Tuber weights of uninfected and infected cassava varieties

The tuber weight was also influenced by photosynthetic rate. There was a strong correlation between tuber weight and photosynthetic rate ( $p\leq 0.01$ ) (Appendix 3). Similarly, there were significant differences in fresh leaf weight of the eight varieties (Appendix 14). Fresh

leaves weighed between 2.46g and 0.71g. Gbenze had the highest fresh leaf weight (2.46 g), followed by *Yeveshevi* (2.29 g) and *Atomo* (2.16) while Nandom had the lowest (0.71).

Table 4.6: Mean fresh leaf weight, fresh tuber weight and number of eight uninfected cassava varieties.

Variety	Fresh Leaf Weight (g)	Fresh Tuber Weight (kg)	Number of Tubers
Afisiafi	0.80±0.22 <sup>c</sup>	1.70±1.08 <sup>ab</sup>	6.00±1.58
Agric	1.39±0.89 <sup>bc</sup>	2.13±0.85 <sup>ab</sup>	7.25±0.75
Ankrah	0.89±0.63 <sup>c</sup>	1.28±0.39 <sup>b</sup>	5.50±0.50
Atomo	2.16±0.47 <sup>ab</sup>	2.57±1.43 <sup>ab</sup>	5.00±1.22
Bosom nsia	0.84±0.32 <sup>c</sup>	1.50±0.27 <sup>ab</sup>	4.75±0.63
Gbenze	2.46±0.98 <sup>a</sup>	2.25±0.21 <sup>ab</sup>	4.75±0.25
Nandom	0.71±0.17 <sup>c</sup>	1.35±0.21 <sup>b</sup>	6.50±1.19
Yeveshevi	2.29±0.62 <sup>ab</sup>	2.93±1.56 <sup>a</sup>	6.00±0.00

Values in a column followed by different letters are significantly different ( $p \leq 0.05$ ) according to Duncan's Multiple Range test

The number of tubers produced by the varieties also varied from one variety to another. Of all the varieties, *Afisiafi* produced the highest mean number (7.25) of tubers while *Bosom nsia* and *Gbenze* produced the lowest number of tubers (4.75). However, the numbers of tubers produced did not differ significantly among the varieties. Correlation analysis showed a strong positive relationship between fresh leaf weight and fresh tuber weight ( $p \leq 0.001$ ) (Appendix 3).

#### 4.7 Climatic conditions and photosynthesis

Mean temperatures throughout the study period ranged between 29.7 to 47.1°C. There was a decline in mean temperature between September and October 2014 from 47.1°C to 31.4°C (Table 4.7). The decline in temperature between September and October is comparatively, more than 10°C difference while temperature difference between October to December was marginal.

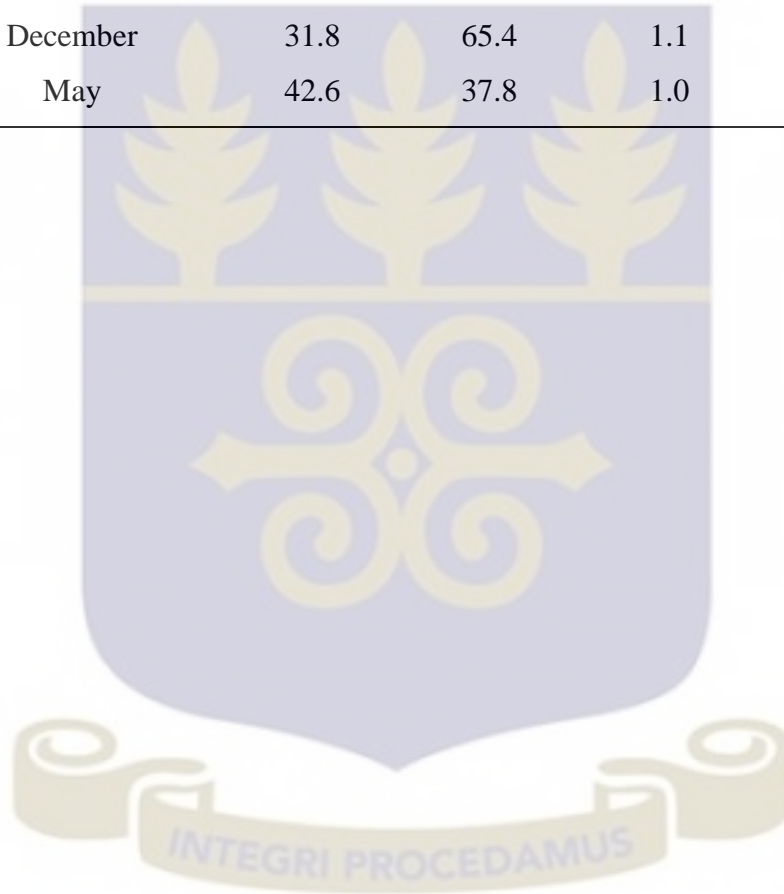
Similarly, the relative humidity also changed from one month to the other during the study period. It was high (70.1%) in November but low in October (50.3%). Average wind speed ranged between 1ms<sup>-1</sup> and 2.4ms<sup>-1</sup>.

The light intensity also varied and it ranged from 7495 lux and 17800 lux. It was high in September but very low in October. The light intensity significantly decreased between September (17800lux) to October (7590lux) but increased again from October to November.

Wind speed rather increased from 1.2 to 2.4 m/s from September to October. There was a continuous decline in wind velocity from October till May (2.4 – 1m/s).

Table 4.7 Mean temperature, relative humidity, wind speed and light intensity on the field during the study

Month	Temp (°C)	R.H (%)	Wind (m/s)	Light intensity (lux)
September	47.1	62.6	1.2	17800
October	31.4	50.3	2.4	7590
November	31.0	70.1	1.4	15890
December	31.8	65.4	1.1	12400
May	42.6	37.8	1.0	17370



## CHAPTER FIVE

### DISCUSSION

#### 5.1 ACMD symptom severity and disease incidence

Six of the eight cassava varieties expressed symptoms of African cassava mosaic disease (ACMD) one month after planting; the exceptions being *Afisiafi* and *Agric*. Symptom severity score and disease incidence infection rate varied among the varieties. Symptom severity was very high in *Gbenze* and *Bosom nsia* (2.8 and 2.5 respectively) but was very low in *Afisiafi* and *Atomo* (1.2 and 1.4, respectively), suggesting that the susceptibility of the cassava varieties to ACMD varied; while some appeared to be tolerant, others appeared to be highly susceptible. Also, there was no patterned relationship between symptom severity (disease score) and infection rate. *Afisiafi* and *Atomo* which had symptom severity score of less than 1.5 could be classified as highly ACMD tolerant varieties while *Ankrah* and *Nandom* with severity score higher than 1.5 but less than 2.0 could be classified as moderately tolerant varieties.

To date, no cassava variety in Africa has been found to be resistant to ACMD and most research efforts globally are aimed at developing cassava varieties resistant to the disease (Alabi *et al.*, 2011). Historically, *Afisiafi* and *Ankrah* were introduced as CMVD-resistant varieties into cassava breeding programmes in Ghana (Owusu and Donkor, 2012). However, due to high disease pressure in the cassava growing areas as well as other environmental factors, these varieties have lost their resistance to the disease.

## 5.2 Detection of ACMV by PCR

Using the polymerase chain reaction one primer pair JSP001/JSP002 out of three confirmed the presence of African Cassava Mosaic Virus (ACMV) in two of the eight cassava varieties, *Bosom nsia* and *Yeveshevi*, although the remaining varieties clearly showed the symptoms of the virus on the leaves and the primers had previously been used successfully to screen for ACMV in many cassava varieties at the Plant Biotechnology laboratory of BNARI (Elegba, 2013; Appiah, 2010). The failure of the remaining primers to amplify the virus in several attempts was not investigated further due to time constraint, but might be due to the problem of freezing and de-freezing of the primers in storage due to the intermittent power supply to the laboratory at the time of carrying out the research.

## 5.3 Photosynthetic rate

The photosynthetic rate of the varieties generally declined with the age of the plants and varied from one variety to the other. The mean photosynthetic rate for all the varieties was higher than  $40\mu\text{mol}/\text{m}^2\text{s}$ ; this was marginally reduced by the presence of ACMV except in *Atomo* where the effect of the disease was clearly visible. Additionally, there was a positive correlation between the photosynthetic rate and virus infection: as the ACMV symptom score increased, the photosynthetic rate also increased and vice versa.

Abiotic factors such as temperature, relative humidity and light intensity might have contributed to the observed trends between photosynthesis and virus severity as has been

reported by Yamori *et al.* (2013) and El-Sharkawy, (1990). Generally, wind speed had an effect on photosynthesis in this study. It was observed that when wind speed increased from  $1.2 \text{ ms}^{-1}$  to  $2.4 \text{ ms}^{-1}$  from September to October (3<sup>rd</sup> and 4<sup>th</sup> months), photosynthetic rates of all the varieties decreased. There was also a reduction in temperature ( $47.1 \text{ }^{\circ}\text{C}$  to  $31.4 \text{ }^{\circ}\text{C}$ ), relative humidity (62.6% to 50.3%) and light intensity which corresponded with the reduction in photosynthesis.

Several factors including both biotic (diseases and pests) and abiotic stress (light intensity and temperature) have been shown to influence photosynthetic rates in plants. Effect of leaf age and its position on photosynthesis has been reported by Henning *et al.* (1979) in peanuts (*Arachis hypogaea*). They reported that the youngest fully expanded leaves had the highest photosynthetic rate compared with the oldest leaves, suggesting that photosynthesis decreased with the age of the plant. Lin and Ehleringer (1982) also reported a decline in maximum net photosynthetic rate of *Carica papaya* (pawpaw) with time. The results of all these experiments indicate that photosynthetic rate decreases with age of the leaf.

In this study, the photosynthetic rate measured in all the cassava varieties ranged between 40 to  $50 \mu\text{mol/m}^2\text{s}$ . El-Sharkawy (2002) has also reported that maximum net photosynthetic rates in cassava grown under field conditions in Columbia were between 40 and  $50 \mu\text{mol/m}^2\text{s}$  of  $\text{CO}_2$ , an observation similar to the present report. According to El-Sharkawy *et al.* (1992), the high photosynthetic rates may explain in part, the high productivity observed in cassava field trials in Colombia.

The presence of ACMV marginally reduced the photosynthetic rate to 30 - 42 $\mu\text{mol}/\text{m}^2\text{s}$ . Diseases and pests of cassava can lead to yield losses of about 20-95% (Fargette *et al.*, 1988). Nine cassava mosaic begomovirus (CMB) species have so far been reported to infect cassava worldwide (Fauquet, 2008) out of which seven are found in sub-Saharan Africa (SSA). Notable among these viruses is ACMV transmitted by *Bemisia tabaci*, which is the most limiting biotic factor affecting cassava production in Africa (Thresh *et al.*, 1994), causing extremely high yield losses.

In this study, ACMV was detected by PCR in two of the cassava varieties (*Bosom nsia* and *Yeveshevi*) and the virus could have been present in the other varieties since they showed conspicuous leaf symptoms which partly accounted for the marginal decrease in photosynthetic rate observed in infected plants. Although, the direct effect of the disease on photosynthesis in cassava is least documented, it may be due to its effect on reduction of leaf surface area caused by chlorosis and leaf distortions.

Physiological and histological examinations have revealed that mosaic-infected leaves have either short or undifferentiated palisade cells from those of the spongy mesophyll tissues (Chant and Beck, 1959). Bates and Chant, (1970) have reported that leaves of plants affected by CMD have marked reductions and distortions of the chloroplasts, increased respiration and peroxidase activity, and decreased total carbohydrate and rates of photosynthesis. Furthermore, changes in peroxidase isoenzyme components occur in mosaic-infected plants (Bates and Chant, 1970; Chant *et al.*, 1971). All these effects may have contributed to the marginal decrease in photosynthetic rate of infected plants. According to Fauquet and Fargette (1990), vector distribution, virus concentration and

susceptibility of leaf to virus inoculation are all related to leaf age which eventually cause reduction in photosynthesis.

Ambavaram *et al.* (2014) reported that photosynthesis and related carbon metabolism are primarily affected by stress. Stress caused by abiotic factors such as unfavourable climatic conditions and biotic factors like diseases and pests can lead to reduction in photosynthetic efficiency and subsequent low yields. The reduction in photosynthetic rate by ACMVD may also be attributed to the reduction of the chlorophyll content by the disease. Chlorophyll and its associated accessory light receptor pigments (xanthophyll and carotenoids) as determined by both the chlorophyll meter and the spectrophotometer was decreased by the presence of the virus except in *Nandom* and *Yeveshevi* varieties. In photosynthesis, chlorophyll is the principal light receptor pigment and its reduction may adversely affect photosynthetic rate. Filella *et al.* (1995) have argued that solar radiation absorbed is a function of concentration of photosynthetic pigments and thus low concentration of the pigment can limit photosynthesis. It has been suggested that cassava mosaic disease-induced yield loss can be attributed to reduced chlorophyll content and reduction in leaflet size (Ayanru and Sharman, 1981).

#### **5.4 Photosynthesis and ACMVD on tuber production**

The present study showed that photosynthetic rate had a positive significant correlation with fresh tuber weight ( $p = 0.0087$ ). Although in this study, of all the eight varieties studied, *Gbenze* had the highest mean photosynthetic rate ( $45.2\mu\text{mol}/\text{m}^2\text{s}$ ), largest leaf area

(5931.2mm<sup>2</sup>) and highest fresh leaf weight (2.46g), it did not have the highest tuber weight, thus suggesting that other factors may be influencing tuber production. In cassava, vegetative growth and reproductive phases are simultaneously developed, hence photoassimilates are partitioned between growth of shoots and roots (El-Sharkawy, 2004), thus there is competition for photoassimilates (Gray, 2002). This may have accounted for the low tuber production. El-Sharkawy (2004) suggests that photoassimilates were perhaps being used to provide energy for vegetative growth rather than root storage, as also observed in this study.

Tuber weight was also reduced by the presence of ACMVD in some of the varieties (*Gbenze*, *Bosom nsia*, *Ankrah* and *Nandom*) while in the remaining varieties, the disease did not reduce tuber weight. The reduction in tuber weight could be due to the adverse effect of the disease on photosynthetic rate and chlorophyll content. It could also depend on the maturity period of the cassava varieties. The cassava varieties used in this study varied in their maturity periods. For example, *Bosom nsia* (as the vernacular name suggests) matures 6 months after planting, *Gbenze* (nine months) while the remaining varieties (*Afisiafi*, *Ankrah*, *Nandom*, *Atomo*, *Yeveshevi* and *Agric.*) mature 12 months after planting. It is therefore probable that differences in maturation time were influencing tuber yield. However, *Bosom nsia* which matures after six months rather had lower tuber weight than *Yeveshevi* and *Atomo* which mature twelve months after planting. Thus, differences in tuber weight cannot be explained away by differences in maturity time. Interestingly, tuber weight of infected plants of *Yeveshevi*, *Afisiafi* and *Agric* were higher than that of non-infected plants. Sweet cassava varieties are known to be susceptible to rodent attack.

Although cultural practices, especially weeding, were observed throughout the experiment, some of the varieties were badly affected by rodents between 9 MAP and 11 MAP. This must have led to the lower tuber weight observed in *Yeveshevi*, *Afisiafi* and *Agric*.

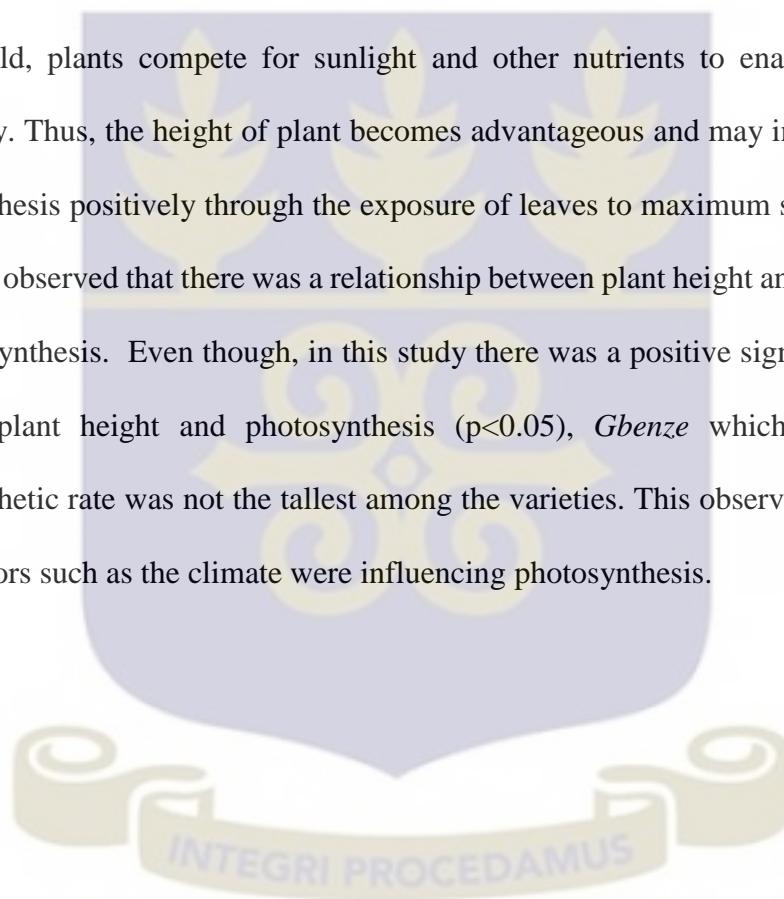
### **5.5 Leaf area, photosynthetic rate and tuber weight**

Tuber weight increased correspondingly with leaf area, indicating a significant ( $p < 0.01$ ) positive correlation between leaf area and fresh tuber weight, thereby explaining the differences in tuber weight observed. For example, *Ankrah*, *Bosom nsia* and *Nandom*, had small leaf surface areas (below  $2000\text{mm}^2$ ), and had very low tuber weights. Physiologically, the leaf is the seat of photosynthesis as it contains chlorophyll for light reception in the plant. Mariscal *et al.* (2002) observed that a reduction in cassava leaf area resulted in reduced tuber production. Similarly, Islam *et al.* (2007) have reported that leaf production, area and weight directly affect tuber production. Jůzl and Štefl (2002) have reported a positive correlation between potato yield and leaf area index.

Besides leaf area, the stomatal density is another important factor that affects photosynthesis and hence tuber production. The stomata constitute an essential anatomical feature which allows for the diffusion of carbon dioxide into the leaf and loss of excess water. Thus, it plays a significant role in carbon fixation in the Calvin cycle. In this study, the stomatal density significantly varied ( $P < 0.001$ ) among the varieties. There was a positive but non-significant correlation between the stomatal density and photosynthesis in this study. Schlüter *et al.* (2002) have reported that the relationship between stomatal

density and photosynthesis is species dependent. While Kundu and Tigerstedt (1998) reported a positive correlation between stomatal density and photosynthesis in neem (*Azadirachta indica*), Heichel (1971) observed a negative correlation between stomatal density and photosynthesis in maize plants. These findings suggest that the relationship between stomatal density, leaf area and photosynthesis is species dependent.

In the field, plants compete for sunlight and other nutrients to enable them to grow vigorously. Thus, the height of plant becomes advantageous and may indirectly influence photosynthesis positively through the exposure of leaves to maximum sunlight. Rijkers *et al.* (2000) observed that there was a relationship between plant height and light availability on photosynthesis. Even though, in this study there was a positive significant correlation between plant height and photosynthesis ( $p < 0.05$ ), *Gbenze* which had the highest photosynthetic rate was not the tallest among the varieties. This observation suggests that other factors such as the climate were influencing photosynthesis.



## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

In spite of the significant role of cassava as a food security crop in Ghana, its production per hectare is low, a total annual production of approximately 15,989,940 MT in 2013 (FAOSTAT, 2015). Most research on the crop which aimed at its improvement has centered mainly on overall yield and breeding for resistance to pests and diseases, with little effort geared towards studying other factors such as photosynthetic rate and efficiency and its effect on tuber production.

This research was therefore aimed at studying the photosynthetic rates of eight varieties of cassava and the major conclusions of the study were:

- All the cassava varieties studied were affected by cassava mosaic virus disease which was expressed mainly as chlorotic patterns and patches on the leaves, wrinkled and distorted leaves and reduced leaf surface area. Molecular analysis using Polymerase Chain Reaction (PCR) confirmed that the virus present was ACMV.
- Photosynthetic rates varied among the eight varieties, however, the variation was not statistically significant. It was high in *Gbenze* ( $45.25\mu\text{mol}/\text{m}^2$ ) and low in *Bosom nsia* ( $40.5\mu\text{mol}/\text{m}^2$ ).

- Chlorophyll content varied among the varieties. *Gbenze* had the highest chlorophyll content (39.93 CCI) and *Nandom* had the lowest (17.90 CCI).
- ACMVD incidence reduced the rate of photosynthesis, leaf area and chlorophyll content in all the varieties.
- *Gbenze* and *Yeveshevi* can be incorporated into future breeding programs due to their high photosynthetic rates, high chlorophyll content and good yield despite their susceptibility to the African Cassava Mosaic Virus.

## 6.2 Recommendations

The present study has shown variation in photosynthetic rate in cassava. It was also observed that biotic factors such as diseases also affected photosynthesis. However, due to time constraint, some observations which need further investigation could not be done. The following studies are therefore recommended for further investigations.

- i. More primers should be used under improved testing conditions in the Polymerase Chain Reaction to detect the viruses that affected the plants leading to the reduction in the photosynthetic rate. Such studies will also show whether the infections are mixed or not.
- ii. Measurement of photosynthetic rate should begin one month after planting in order to determine its efficiency throughout the plant propagation cycle

- iii. Other factors such as carbon dioxide concentration, transpiration relative humidity, canopy size on photosynthesis may be investigated.



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**APPENDIX 1****Presence and severity of ACMV on eight cassava varieties**

Varieties	No. of Infected		Mean ACMD score
	plants	Infection (%)	
Afisiafi	58	59.6	1.2
Agric	58	52.3	2.3
Ankra	34	29.1	1.8
Atomo	58	61.1	1.4
Bosome nsia	78	91.8	2.5
Gbenze	54	49.1	2.8
Nandom	114	98.3	1.9
Yeveshevi	38	38.9	2.0

**APPENDIX 2****Details of specific primer used for the detection of ACMV**

Primer	Sequence (5' – 3')	Virus species
JSP001/F	ATGTCGAAGCGACCAGGAGAT	ACMV
JSP002/R	TGTTTATTAATTGCCAATACT	ACMV
EAB555/F	TACATCGGCCTTTGAGTCGCATGG	CMBs
EAB555/R	CTTATTAACGCCTATATAAACACC	CMBs
EACMV1e	GTTTCGGCTATCACCTTCTAGAACA	EACMV
EACMV2e	CAAGGCTTACATTGAAAAGGGA	EACMV

APPENDIX 3

Pearson's correlations analysis between Traits of 8 varieties of Cassava (*Manihot esculenta* Crantz.).

	CCI	Chla	Chlb	Cx+c	PH	LA	FLW	TNo.	P	SG	SD	SS	T.Chl	TW
CCI														
Chla	<b>0.0979<sup>ns</sup></b> <u>0.5939</u>													
Chlb	<b>0.1176<sup>ns</sup></b> <u>0.5214</u>	<b>0.9977***</b> <u>0.0000</u>												
Cx+c	<b>0.1213<sup>ns</sup></b> <u>0.5085</u>	<b>0.9967***</b> <u>0.0000</u>	<b>0.9989***</b> <u>0.0000</u>											
PH	<b>0.2784<sup>ns</sup></b> <u>0.1228</u>	<b>0.1835<sup>ns</sup></b> <u>0.3148</u>	<b>0.1722</b> <u>0.3459</u>	<b>0.1802</b> <u>0.3237</u>										
LA	<b>0.4659**</b> <u>0.0072</u>	<b>0.1034<sup>ns</sup></b> <u>0.5734</u>	<b>0.1173</b> <u>0.5227</u>	<b>0.1065</b> <u>0.5619</u>	<b>-0.1084</b> <u>0.5547</u>									
FLW	<b>0.3977*</b> <u>0.0242</u>	<b>-0.0123<sup>ns</sup></b> <u>0.9469</u>	<b>-0.0003</b> <u>0.9985</u>	<b>-0.0110</b> <u>0.9525</u>	<b>0.6861***</b> <u>0.8084</u>	<b>0.3841*</b> <u>0.0000</u>								
T No.	<b>0.1143<sup>ns</sup></b> <u>0.5334</u>	<b>0.2250<sup>ns</sup></b> <u>0.2156</u>	<b>0.2066</b> <u>0.2566</u>	<b>0.1914</b> <u>0.2940</u>	<b>0.2561</b> <u>0.1571</u>	<b>0.2827</b> <u>0.1170</u>	<b>0.2504*</b> <u>0.1668</u>							
P	<b>0.0152<sup>ns</sup></b> <u>0.9344</u>	<b>0.1950<sup>ns</sup></b> <u>0.2848</u>	<b>0.1878</b> <u>0.3034</u>	<b>0.1858</b> <u>0.3086</u>	<b>0.3634*</b> <u>0.0409</u>	<b>0.3841*</b> <u>0.0300</u>	<b>0.2690</b> <u>0.1366</u>	<b>0.5202**</b> <u>0.0023</u>						
SG	<b>0.5375**</b> <u>0.0015</u>	<b>0.6064***</b> <u>0.0002</u>	<b>0.6102***</b> <u>0.0002</u>	<b>0.6062***</b> <u>0.0002</u>	<b>0.3803*</b> <u>0.0318</u>	<b>0.4530**</b> <u>0.0092</u>	<b>0.4763**</b> <u>0.0059</u>	<b>0.5032**</b> <u>0.0033</u>	<b>0.4056</b> <u>0.0213</u>					
SD	<b>0.0397<sup>ns</sup></b> <u>0.8291</u>	<b>0.9790***</b> <u>0.0000</u>	<b>0.9656***</b> <u>0.0000</u>	<b>0.9650</b> <u>0.0000</u>	<b>0.1945</b> <u>0.2860</u>	<b>0.0225</b> <u>0.9027</u>	<b>-0.0644</b> <u>0.7264</u>	<b>0.2714</b> <u>0.1330</u>	<b>0.1983</b> <u>0.2767</u>	<b>0.5650***</b> <u>0.0008</u>				
SS	<b>0.0143<sup>ns</sup></b> <u>0.9383</u>	<b>0.9632***</b> <u>0.0000</u>	<b>0.9498***</b> <u>0.0000</u>	<b>0.9445***</b> <u>0.0000</u>	<b>0.1712</b> <u>0.3488</u>	<b>0.0059</b> <u>0.9746</u>	<b>-0.0766</b> <u>0.6768</u>	<b>0.2772</b> <u>0.1245</u>	<b>0.1833</b> <u>0.3154</u>	<b>0.5234**</b> <u>0.0021</u>	<b>0.9815***</b> <u>0.0000</u>			
T.Chl	<b>0.1045<sup>ns</sup></b> <u>0.5692</u>	<b>0.9998***</b> <u>0.0000</u>	<b>0.9989***</b> <u>0.0000</u>	<b>0.9980***</b> <u>0.0000</u>	<b>0.1805</b> <u>0.3228</u>	<b>0.1074</b> <u>0.5586</u>	<b>-0.0088</b> <u>0.9618</u>	<b>0.2190</b> <u>0.2285</u>	<b>0.1929</b> <u>0.2901</u>	<b>0.6079***</b> <u>0.0002</u>	<b>0.9754***</b> <u>0.0000</u>	<b>0.9590***</b> <u>0.0000</u>		
TW	<b>0.2573<sup>ns</sup></b> <u>0.1552</u>	<b>0.3989*</b> <u>0.0237</u>	<b>0.3877*</b> <u>0.0283</u>	<b>0.3717*</b> <u>0.0362</u>	<b>0.1860</b> <u>0.3080</u>	<b>0.4760**</b> <u>0.0059</u>	<b>0.5765***</b> <u>0.0006</u>	<b>0.7320***</b> <u>0.0000</u>	<b>0.4562**</b> <u>0.0087</u>	<b>0.7399***</b> <u>0.0000</u>	<b>0.4064*</b> <u>0.0210</u>	<b>0.4121*</b> <u>0.0191</u>	<b>0.3946*</b> <u>0.0254</u>	

CCI = Chlorophyll Content Index    Chla= Chlorophyll a    Chlb = Chlorophyll b    Cx+c = xanthophyll and carotenoid    PH = Plant Height  
 LA = Leaf Area    FLW = Fresh Leaf weight    T.No = Number of Tubers    P = Photosynthetic rate    SG = Stem Girth    SD = Stomatal Density  
 SS = Stomatal Size    T.Chl = Total Chlorophyll    TW = Tuber weight  
 Below each correlation coefficient (**bolded**) is P-value (underlined). \*, \*\*, \*\*\* = significant at P ≤ 0.05, 0.01, 0.001 respectively  
 ns = not significant at P ≤ 0.0

**APPENDIX 4****ANOVA table for photosynthetic rates ( $\mu\text{mol}/\text{m}^2\text{s}$ ) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	83.609	7	11.944	1.177	0.352
Within Groups	243.590	24	10.150		
Total	327.199	31			

**APPENDIX 5****ANOVA table for chlorophyll content index (CCI) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	1957.730	7	279.676	24.014	0.000
Within Groups	279.518	24	11.647		
Total	2237.247	31			

**APPENDIX 6****ANOVA table for total chlorophyll (a+b) ( $\text{mg}\cdot\text{g}^{-1}$ ) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	9.628	7	1.375	8.430	0.000
Within Groups	3.916	24	0.163		
Total	13.544	31			

**APPENDIX 7****ANOVA table for chlorophyll a (mg.g<sup>-1</sup>) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	3.908	7	0.558	8.551	0.000
Within Groups	1.567	24	0.65		
Total	5.475	31			

**APPENDIX 8****ANOVA table for Chlorophyll b (mg.g<sup>-1</sup>) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	1.219	7	0.174	7.722	0.000
Within Groups	0.541	24	0.023		
Total	1.760	31			

**APPENDIX 9****ANOVA table for xanthophyll and Carotenoid (Cx+c) (mg.g<sup>-1</sup>) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	57.890	7	8.270	11.286	0.000
Within Groups	17.586	24	0.733		
Total	75.476	31			

**APPENDIX 10****ANOVA table for leaf area (mm<sup>2</sup>) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	7.032E7	7	1.005E7	457.200	0.000
Within Groups	527300.690	24	21970.862		
Total	7.084E7	31			

**APPENDIX 11****ANOVA table for plant height (cm) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	4871.480	7	695.926	2.946	0.022
Within Groups	5669.112	24	236.213		
Total	10540.592	31			

**APPENDIX 12****ANOVA table for Stomatal density (mm<sup>2</sup>) of eight cassava varieties**

<b>Source</b>	<b>Sum of</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
	<b>Squares</b>				
Between Groups	23.469	7	3.353	6.569	0.000
Within Groups	12.250	24	0.510		
Total	35.719	31			

**APPENDIX 13****ANOVA table for Stomatal size ( $\mu\text{m}$ ) of eight cassava varieties**

<b>Source</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
Between Groups	0.127	7	0.018	6.977	0.000
Within Groups	0.062	24	0.003		
Total	0.190	31			

**APPENDIX 14****ANOVA table for fresh Leaf Weight (g) of eight cassava varieties**

<b>Source</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
Between Groups	15.605	7	2.229	6.569	0.000
Within Groups	8.916	24	0.372		
Total	24.521	31			

**APPENDIX 15****ANOVA table for fresh Tuber Weight (kg) of eight cassava varieties**

<b>Source</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
Between Groups	10.165	7	1.452	1.721	0.151
Within Groups	20.250	24	0.844		
Total	30.415	31			

**APPENDIX 16****ANOVA table for Number of Tuber of eight cassava varieties**

<b>Source</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
Between Groups	22.219	7	3.174	0.949	0.489
Within Groups	80.250	24	3.344		
Total	102.469	31			

