MAKERERE UNIVERSITY

COLLEGE OF NATURAL SCIENCES

SCHOOL OF BIOSCIENCES

DEPARTMENT OF PLANT SCIENCES MICROBIOLOGY AND BIOTECHNOLOGY

EFFECT OF *Bemisia tabaci* (WHITEFLY) ON PHOTOSYNTHETIC CAPACITY AND SECONDARY METABOLITE COMPOSITION OF SELECTED CASSAVA VARIETIES GROWN IN UGANDA

BY

MWEINE PEREZ

18/U/9420/PS

SUPERVISOR: DR. SENKU E. JAMILU

A RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF PLANT SCIENCES MICROBIOLOGY, AND BIOTECHNOLOGY, COLLEGE OF NATURAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWAED OF DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY OF MAKERERE UNIVERSITY

MARCH 2021

DECLARATION

I **Perez Mweine** declare that this report is my original compilation and that none of its sections is plagiarized. I further declare that this report has never been submitted to any university or higher institute of learning for any award of any degree.

Signed: _____ Date: 06/04/2022

This report has been submitted after my approval as supervisor:

Dr. Ssenku E. Jamilu (PhD)

Signature: _____ Date: 22/04/2022

Contents

ABSTRACT

Cassava is the largest source of functional food and nutrition in many countries. It is considered a staple food in many countries as well a cash crop as a source of income for farmers who play a role in improving their own welfare. This study was focused on understanding the effect of the whitefly on the photosynthetic machinery which would later aid in accessing the performance of both tolerant and susceptible varieties through additional study of production of secondary metabolites. This knowledge will be used by breeders to effectively perform a breeding program to obtain improved cultivars. Four varieties NASE 3, NASE 13, NASE 14, and Mkumba were used in this study and samples analyzed at 6months from planting. Samples taken showed variation in the chlorophyll contents ($p<0.05$) with NASE 13 having a significantly higher chlorophyll content retained after infestation compared to other varieties. Cynogenic potential was too measured across all the varieties and there was a strong significant difference ($p<0.01$) between the varieties with NASE 13 have the least significant levels which I considered a better adaptation than Mkumba which had the highest significance of 0.59ppm. The less significant levels proved the palatability of infested plants even when mixed with the controls or the non infested. However, NASE 13 was highly significant with production of other phenolic acid derivatives as these were the responses to whitefly infestation which placed it as a better variety compared to the others. This study, therefore presents comprehensive evaluation of selected varieties and attained results that show NASE 13 as a better variety from which to breed for tolerance and resistance against the whitefly.

CHAPTER ONE

1.0 INTRODUCTION

1. 1 BACKGROUND TO THE STUDY

Cassava (*Manihot esculenta* Crantz), in the tropics, is one of the most grown food crops as source of calories to humans and domesticated animals, as feeds. It is the largest source of functional food and nutrition in many countries(Pratama et al., 2021). Although cassava has long been a staple crop for most of the countries in Africa, it is increasingly considered as a cash crop (Moreno-Cadena et al., 2021). In most regions, cassava has become a strategic crop commodity used as a source of income for farmers who play a role in improving their own welfare and other farmers(Analianasari et al., 2020). In some African communities, both the root and leaf systems are used as a source of food and these have exceedingly high nutritive value as described in nutrient composition by (Egesi et al., 2007).

Most notable is cassava inherent high capacity to assimilate carbon in near optimum environments that correlates with both biological productivity and root yield across a wide range of germplasm grown in diverse environments. Under prolonged water shortages in seasonally dry and semiarid zones, the crop, once established, tolerates stress and produces reasonably well compared to other food crops. Because of its inherent tolerance to various edapho-climatic stresses(Mabrouk A. El-Sharkawy, 2012), the crop has recently been expanding into more marginal lands, particularly in sub-Saharan Africa, where other staple food crops are failing to produce reasonably because of the increasing degradation of these marginal African ecosystems. This has placed cassava at a high exposure to different pests and most of these affect the leaf system which is the photosynthetic machinery of the plants, this leads to high loss in crop yield. Of the invertebrate pests that attack cassava in East Africa, the whitefly, *Bemisia tabaci* is among the most challenging to control. In Uganda, limited whitefly management options are available to smallholder farmers, and pesticides are not readily accessible(Katono et al., 2021). While pest control techniques may prove substantial remedy to kill pests, *Bemisia tabaci* eggs and the older

nymphal stages are highly immune to these pesticides(Act, 2019), and this means soon the white fly will again emerge and cause similar effect to reduce yields.

Whiteflies can damage plants directly or indirectly. Direct damage is caused through their feeding on the leaf undersides, which removes plant sap and stunts plant growth, especially in young plants. Infested leaves have numerous chlorotic spots. These spots grow together forming different sized yellow areas. In severe cases, only the veins remain green. Some leaves appear completely brown and dried. This is because sap removed goes along with various leaf nutrients and leading to severe photosynthetic pigment degradation and subsequently may lead to leaf abscission and death. Indirect whitefly damage is caused by the large amounts of sticky honeydew secreted during feeding. Honeydew may cover plants and support the growth of sooty mold, which reduces the plant's ability to use light for photosynthesis. In addition to direct and indirect damage, whiteflies may carry and transmit viral diseases that can severely damage susceptible plants (Polston et al., 2014).

The effects of the whitefly can presciently be expected to increase and lead to more losses if the ongoing research does not look to the ways to mitigate and obviate them. In Uganda, research to identify resistant varieties has been ongoing and this shall look to improve the susceptible varieties to the whitefly. Understanding the effect of the whitefly on the photosynthetic machinery will aid in accessing the performance of both the resistant and susceptible varieties as knowledge needed to effectively perform a breeding program to obtain improved cultivars.

1.2 Statement of the problem

Despite the vast importance of cassava (*Manihot esculenta* Crantz), for small scale holder farmers in Africa, and considered a staple food for more than a billion people in 105 countries(Chetty et al., 2012), yield per unit land acre have not increased over the past 55 years. Despite significant efforts in breeding and agronomy, cassava productivity in sub-Saharan Africa has declined at a rate of 0.024 t ha−1 year−1 between 2004 and 2014. This requires the understanding of limitations to photosynthesis within existing germplasm(De Souza & Long, 2018). The effects of whitefly infestation need to be studied in detail as proof for susceptibility, tolerance or resistance of the existing germplasm. This information will facilitate essential discernment on which cultivars to be sued in the breeding programs for better results of improved cultivars to be planted by the farmers.

1.3 Justification of the study

Productivity in green plants is heavily dependent on the photosynthetic efficiency of a plant. In cassava, the leaves are the photosynthetic machinery and yet the hosts of the pests. This calls for the relevant research to determine how the infestation of the whitefly influences the photosynthetic efficiency which ultimately has a direct link to the productivity component of the cassava plant. This knowledge will aid the design of effective, environmental compatible and sustainable management strategies, as well as guide the development and deployment of future improved cassava genotypes (Katono et al., 2021). According to physical monitoring, a few adults feeding on plants will usually not cause significant damage and do not warrant treatment which is what has been the norm to control the pest in the several farms. A certain number of whiteflies may be tolerated when they do not cause significant damage. This tolerance level variability is what this research centres around as knowledge needed by breeders to produce improved cultivars.

1.4 Objectives

1.4.1 Main objective

To examine the effect of whitefly on the performance of infested cassava leaves of selected varieties

1.4.2 Specific objective

1. To determine the effect of whitefly on the photosynthetic efficiency of selected cassava varieties

2. To assess the tolerance of selected cassava varieties to the whitefly

1.4.3 Hypotheses

I. There is no significant effect of whitefly infestation on the photosynthetic efficiency of the selected cassava varieties.

II. There is no variation in the tolerance of selected cassava varieties to the whitefly.

1.5 Significance of the study

The study provides knowledge on the direct significant impact that the whitefly has on the cassava productivity through its influence on the photosynthetic capacity. This knowledge could facilitate the development of control measures that significantly subdue the whitefly effects on the relative yield of the cassava plant. The study could also foster adoption of environmentally friendly control measures through breeding programs as opposed to use of inorganic pesticides that are not sustainable and cost ineffective. With the diverse advantages of cassava couples with its adaptability in marginal agricultural areas, this study could contribute in solving food insecurity.

CHAPTER TWO

LITERATURE REVIEW

2.1 Whitefly species diversity

The cassava whitefly belongs to

Class: Insecta,
Order: Hemiptera,
Sub-order: Sternorrhyncha,
Super-family: Aleyrodoidea,
Family: Aleyrodidae, (Whiteflies)
Genus: *Bemisia*
Species: *tabaci.*

Among the 1556 known whitefly species in 161 genera, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is particularly important because of its ability to infest more than 1000 plant species (Chen et al., 2016). Five genetically distinct groups of *B. tabaci* putative species have been reported for cassava in Africa, namely sub-Saharan Africa 1 (SSA-1 to 5), SSA-2, SSA-3 SSA-4 and SSA-5). Sub-Saharan Africa 1 (SSA-1) is the most prevalent species and has been reported to occur throughout sub-Saharan Africa and has further been divided into five subgroups (SG) namely SSA1-SG1, SSA1-SG2, SSA1-SG3, SSA1-SG4 and SSA-SG5. Sub-Saharan Africa 2 (SSA-2) has been reported to occur in East and West Africa, SSA-3 and SSA-4 in Central and West Africa and SSA5 in South Africa. In East and Central Africa, the most common cassava B. tabaci species have been identified to belong to the three sub-Saharan Africa taxa (SSA1, SSA2 and SSA3) with SSA1 as the most prevalent species in the region(Munguti et al., 2021).

2.1.2 Whitefly description and lifecycle

Whiteflies are small insects (1 to 3 millimeter). Adults have four broad wings of approximately the same size. The adult is the most mobile stage and is responsible for colonizing the host plant. Whiteflies can move and disperse over long distances by flying upward and being picked up and carried by air currents. Eggs are usually laid on the underside of young leaves and may be deposited randomly throughout the leaf, in circles, arcs or spirals, depending on the species. The eggs hatch into mobile first instars called crawlers, which search and find a suitable feeding site. They then insert their mouthparts and remain in one place for the rest of their immature stages.

Second to fourth instars are called nymphs and resemble small scale insects. Nymphs are oval and may be pale yellow to black, depending on the species. The late fourth instar is also known as “pupa” because mobile adults emerge from the exoskeleton of sessile nymphs after development is complete. Silver leaf whitefly fourth instars are also known as “red-eye nymphs” because the relatively large eyes of the developing adult are already visible through the nymph integument (skin) during this stage. The complete life cycle varies from about 21/2 to 5 weeks according to temperature conditions and species. Many overlapping generations can occur each year. Adults and nymphs feed by inserting their mouthparts into plant tissue and sucking phloem sap.(Bográn & Heinz, 2006).

2.1.3 Effect of whitefly on cassava

B. tabaci causes three types of damage to plants. The first two, direct damage through the ingestion of phloem sap and indirect damage from the excretion of honeydew onto the surfaces of leaves and fruit and subsequent growth of sooty mould fungi which reduce both photosynthesis and quality, are largely limited to a very small number of species in the complex. By far the most widespread damage caused by many members of the complex is through the transmission of a large number of economically important viral plant pathogens (Polston et al., 2014).

2.2 Cassava photosynthetic machinery

The cassava physiology section intensively studied photosynthesis of cassava, as the basic process underlying primary productivity and yield, based on the concept that efforts to improve net productivity (P_N) in relation to productivity might lead to the increasing crop yield via selection of germplasm with high P_N, in combination with other yield determinant trait (Mabrouk A El-Sharkawy, 2004). Most notable is cassava inherent high capacity to assimilate carbon in near optimum environments that correlates with both biological productivity and root yield across a wide range of germplasm grown in diverse environments. Cassava leaves possess elevated activities of the C4 phosphoenolpyruvate carboxylase (PEPC) that also correlate with leaf net photosynthetic rate (P_N) in field-grown plants, indicating the importance of selection for high P_N. Under certain conditions such leaves exhibit an interesting photosynthetic C3-C4 intermediate behaviour which may have important implications in future selection efforts(M. A. El-Sharkawy, 2006)

2.3. Effect of sooty mold fungi on cassava Ieavcs

Sooty mold development on the leaf surface is characterized by dark pigmented hyphae that grows on adaxial leaf surface. The heavy infestation on the leaf has a potential to reduce sunlight penetration and inhibition of photosynthesis (Insausti et al., 2015). This triggers sooty mold to negatively impact on leaf through shading, reduction of photosynthetic capacity and premature leaf abscission. According to Lemos *et al*., (2006), 70% inhibition of photosynthesis was observed in pecan leaves due to blockage of photosynthetically active radiation caused by sooty mold. Sooty molds are a group of over 200 epifoliar fungal species that live on plant surfaces where sap-feeding insects feed on plant foliage. The sap-sucking insects excrete honeydew as a waste product which drips on the foliage below and covers leaves, soil and rocks below with a sticky sugary coating. Honeydew is largely composed of sugars and smaller amounts of amino acids, proteins, minerals, vitamins and other organic compounds (VanDoorn *et al*., 2015). The sooty mold grows on this and produce a thin superficial network of dense, dark hyphae. Sooty mold is most common in tropical and subtropical regions around the world. Most of the sooty molds have hyphae with mucilaginous outer walls. Sooty molds are fungi that grow on honeydew secreted by insects in the Order Hemitera, suborder Homoptera, which includes aphids, whiteflies, soft scales, mealy bugs and leafhoppers, living on plants and other surfaces, such as rocks below plants (Chomnunti *et al*., 2014).

2.2.1. Phenolic compounds and their roles in plant tissues

Phenolic compounds are secondary metabolites, which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization. They contain benzene rings, with one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Lin et al., 2016). Phenolics are constitutively produced during the infection process of a certain pathogen to play a pivotal role in the host defence. This could be primarily because of their effect on cell wall lignification, antimicrobial activity, modulation of plant hormones involved in defence signalling pathways and scavenging of reactive oxygen species (Araujo et al., 2016). Plants therefore need phenolic compounds for pigmentation, growth, reproduction and resistance to pathogens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site description

This study was carried out at the agronometry Station of National Crops Resources Research institute (NaCRRI)- Namulonge located approximately 30kilometres northeast of Kampala in Wakiso district (Central Uganda). It is located in the equatorial region along coordinates 00 31'30'N latitude and 32 36 '54"E longitude and at altitude range between 900-1340m above sea level. The area experiences tropical climate with a temperature range of 24C-30C and about 1293mm of precipitation that is bimodally distributed with two wet seasons (March to May and September to November), while the dry months are January to February and July to August(Nsubuga et al., 2011). It is surrounded by tall grassland vegetation. Soils are ferralitic (red sandy clay loams), with a native pH of 4.9-5.0(Nsubuga et al., 2011).

3.2 Experimental design

Four (4) cassava varieties were used in this experiment. The experiment was setup at the beginning of the rainy season in March 2021. An experimental block measuring 5X34M was prepared. In thisnplot, 6 rows with 10 planting holes (4ft) was prepared. The rows and the holes was spaced from one another by 1m distance to minimise competition. The cassava varieties were then assigned to the rows randomly using random numbers generated in Excel. Cassava stem cuttings of 10 cm long was planted in particular rows. The control experiment was set up in a similar manner 10 meters away from the experimental plot. The adult whiteflies known to be 0.Imm wide were screened off with a net of 0.06mm pore diameters stands to confine control plants within the inside environment.

3.3 Sampling technique

Standard agronomic practices were followed and four cassava genotypes at the National Crops Resources Research Institute (NaCRRI), Uganda, were picked for evaluation. Leaves with visible sooty mould coverage at a scale of 3 (moderate damage) were collected from the mid-point branch from the infected plants. The leaves were then packed in well-labelled sample bags

and transferred to a cold box maintained at 4^0C to minimise the rate of leaf enzyme degradation and biochemical response due to the physical damage inflicted on the leaves. The samples were immediately transported to the bio-analytical and nutritional laboratory at NaCRRI for biochemical analysis.

3.4 Measurement of Photosynthetic efficiency

3.4.1 Determination of Chlorophyll and Carotenoid Content

Chlorophyll and carotenoid content was determined by (Mostofa et al., 2015) method. 0.5g fresh weight of the fifth leaf of cassava plant homogenates of 30ml 80% acetone solution was extracted by grinding with a pestle in a ceramic mortar. This was followed by shaking on an orbital shaker at 200 rpm for 30 minutes in the dark. The extract was centrifuged at 6000 rpm for 10 minutes at 4^0C. Subsequently, 1.0ml of the supernatant was pipetted into a lcm path cuvette and absorbance of chlorophyll *a* at 663nm and chlorophyll *b* at 645nm for carotenoid at 470nm measured with a UV spectrophotometer. Chlorophyll and carotenoid levels was calculated following in mg/g FW as described by (Utsumi et al., 2019).

3.5.0 Assessing tolerance

3.5.1 Determination of salicylic acid

Salicylic acid was determined according to (Warrier et al., 2013). lg of the sooty mold infected leaf sample, was extracted in 3mls of acetone (80%) solvent to assay the solubility of salicylic. Extraction of salicylic acid from the infected leaf sample solutions were through efficient solvent swirled. Then centrifugation at 10,000 Xg for 10 min was used to obtain a clear supernatant. The supernatant for salicylic acid measurement, was pipetted off into an empty clean test of the supernatant and mixed with 0.1% freshly prepared 'ferric chloride. The volume of the reaction mixture made up to 3.0ml with 0.1% freshly prepared ferric chloride. The complex formed between Fe^{3+} and salicylic acid (violet in colour) was determined spectrophotometrically, measuring the absorbance of the complex in the visible region at 540 nm. Salicylic acid measurement was carried out against a standard curve with salicylic acid synthetic plant hormone.

3.5.2 Determination of FRAP (ferric reducing antioxidant power) assay

The principle of this assay was based on one-electron reduction of Fe (Ill)/ferricyanide complex to the ferrous form Fe (Il). In brief, I mL of 3 mg/mL compound was mixed with 2.5 mL of

phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of a 10 g/L and incubated at 500C, for 30 min. After the incubation, 2.5 mL of a 100 g/L TCA solution was added to terminate the reaction and the mixture was centrifuged for 10 min (1800 rpm). Finally, 2.5 mL of supernatant was used to mix with 2.5 mL ultra-pure water and 0.5 mL of a I g/L FeC13. The absorbance was recorded at 700 nm and the data presented as ascorbic acid equivalents (Asc.AE; µg ascorbic acid/g sample) (Aminjafari *et al*., 2016).

3.5.3 Determination of total flavonoid content

Total flavonoid content was measured with the aluminium chloride colorimetric assay (Pekal and Pyrzynska, 2014). Iml of aliquots and Iml standard quercetin solution (100, 200, 400, 600, 800, 1000 gg/ml) was pipetted into test tubes and 4ml of distilled water. 0.3mI of 5 % sodium nitrite solution was added into each. After 5 minutes, 0.3ml of 10 % aluminium chloride was added. After 6 minutes, 2ml of 1M sodium hydroxide was added and volume made up to 10ml with distilled water and vortexed. Orange yellowish colour develops and absorbance was measured at 510nm spectrophotometrically using UV-visible Jenway 6305 spectrophotometer. The blank was performed using distilled water and Quercetin used as standard. The samples was performed in triplicates, calibration curve plotted using standard quercetin and flavonoid content expressed as mg of quercetin equivalents/100g of wet mass (Kamtekar *et al*., 2014).

3.5.4 Determination of total phenol content

Total phenolic content was estimated by Folin Ciocalteu's method by Harbone (1998). Iml of aliquots and standard gallic acid (10, 20, 40, 60, 80, 100 pg/ml) was pipetted into the test tubes. 5ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent added and vortexed. After 5 minutes, 1.5 ml of 20% sodium carbonate was added and volume made up to 10ml with distilled water. The mixture was allowed to incubate in a water bath at 400C for 30 min. Intense blue colour develops after incubation. Absorbance was measured at 750nm spectrophotometrically using UV visible Jenway 6305 spectrophotometer. The extracts were performed in triplicates and the blank performed using reagent blank with solvent, Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid and data for total phenolic contents expressed as mg of gallic acid equivalent weight (GAE)/I00g of wet mass (Bhalodia et al., 2011).

3.5.5 Determination of cyanogenic potential

Cyanogenic potential was determined by extracting cassava leaves in phosphate buffer (pH 6) and subsequent hydrolysis of linamarin with linamarase enzyme (Haque and Bradbury, 2002). Twenty-five grams (25g) of fresh cassava leaves was extracted in 1M orthophosphoric acid to immobilise the cyanide prior to quantification. The cyanide generated on hydrolysis with linamarase was quantified spectrophotometrically at 605nm after colouring with chloramine T solution and Isonicotinic/ barbituric acid, at pH 6.5. The cyanogenic potential will be expressed in parts per million (ppm) (Haque and Bradbury, 2002).

3.5.6 Determination of tannins

Total tannin content was determined by folin ciocalteu method by Harbone (1998). 100mg sample was placed into a 2ml eppendorf tube, where 0.5ml of 5% ascorbic acid solution was added to dissolve sample precipitate, and the setup placed on an orbital shaker for 20 minutes. After which, 0.5ml of petroleum ether containing 1% acetic acid, was added to remove pigments and then left to evaporate. 0.3ml of distilled water was added and centrifuged for 10 minutes at 25000 rpm and at 40C. This was followed by placing the solution in a 50ml calibrated tube and 2.4 ml of 5% hydrochloric acid (HCL)-butanol solution added. The solution in the calibrated tube was ran through a 240 mm filter paper. Then 0.5mI of the filtrate made up to I ml with distilled water and 0.5 ml of folin ciocalteau reagent added; followed with 2.5 ml of 20% sodium carbonate solution and mixed. A total of 4ml of the mixture was incubated at 80^0C for 30 minutes. Samples was cooled at 24^0C and spectrophotometric readings taken at 550nm and tannic acid will be used as a standard. The tannin content was expressed as mg of tannic acid equivalent/l00g of sample (Mwila et al., 2017).

4.0 RESULTS

4.1 Data collection and analysis

All the analyses shall be analyzed using the R i3684.1.2(R development core team, 2021) Prior to any analyses, all the data was tested for normality and homoscedasticity. In case the data was not normally distributed and heteroscedastic, it was transformed under log transformation. Data was then subjected to One-way ANOVA followed by Tukeys *Post hoc test* (LSD test) in case of significant variations, with means considered to be significantly different *at P<0.05.* Ordination analysis was be used to relate cassava varieties with variation in photosynthetic efficiency.

4.2 Variation of chlorophyll content

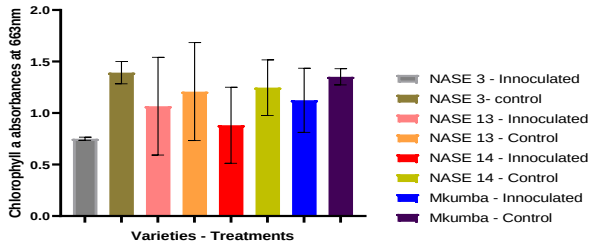
The chlorophyll content for each variety differed significantly ($p < 0.05$) across all chlorophylls i.e. from 0.67mg/g FW to 1.49mg/g FW for chlorophyll *a*, from 0.28mg/g FW to 0.80mg/g FW for chlorophyll *b*, from 0.78mg/g FW to 1.71mg/g FW for carotenoid (table 1). In this study, chlorophyll content was used as an indicator for photosynthetic efficiency and the study clearly demonstrated the effect that the whitefly had on each variety.

Table 1: the variation in chlorophylls of the selected cassava varieties of the controls and after inoculation

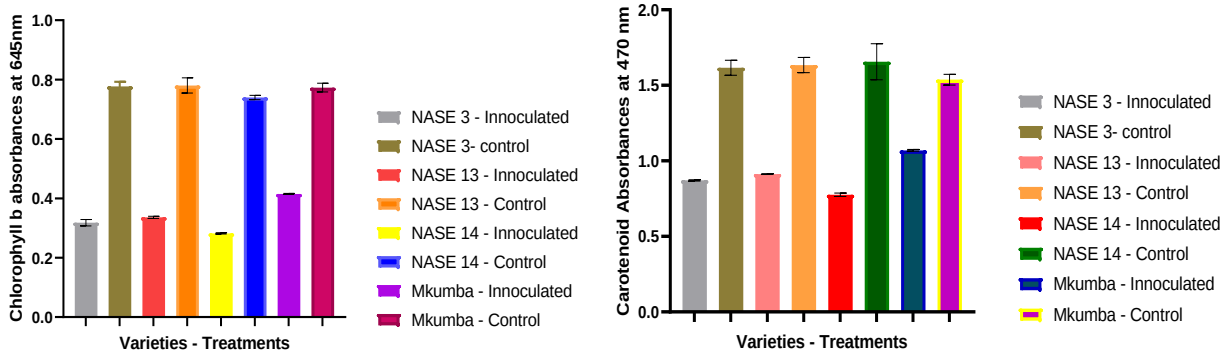
Chlorophyll content	**Varieties and their treatments**							
	NASE 3 - Inoculated	NASE 3 - Control	NASE 13 Inoculated	NASE 13 - Control	NASE 14 Inoculated	NASE 14 - Control	Mkumba - Inoculated	Mkumba - Control
Chlorophyll *a*	0.76±0.01ab	1.43±0.13d	0.79±0.01b	1.49±0.09d	0.67±0.00a	1.40±0.08d	0.94±0.01c	1.42±0.07d
Chlorophyll *b*	0.32±0.01b	0.77±0.02ef	0.34±0.00b	0.80±0.03f	0.28±0.00a	0.72±0.02d	0.41±0.00c	0.76±0.02e
Carotenoid	0.87±0.00b	1.66±0.07de	0.91±0.00b	1.63±0.07d	0.78±0.01a	1.71±0.09e	1.06±0.01c	1.64±0.09de

(means with different letter of alphabet within a particular row indicate significant difference between the values, Turkey's test (P<0.05

Chlorophyll *a* is the major component and plays an active role in photosynthesis as photoenzymes and other pigments and chlorophylls are used as accessory pigments. From graph 1 above, the whitefly had a significant effect on the contents of chlorophyll *a* as above and this ultimately means an effect on photosynthesis and an effect on the productivity. From the graph 1 above Mkumba and NASE 13 were able to exhibit a resistance across all chlorophylls with the highest chlorophyll content after inoculation. (Graph 2 and 3)



Graph 1: A graph of absorbance means of chlorophyll *a* at 663nm



Graph 2 and 3: A gragh of absorbance means of chlorophyll *b* at 645nm and Carotenoids at 470nm

4.3 Variation in the Cyanogenic potential

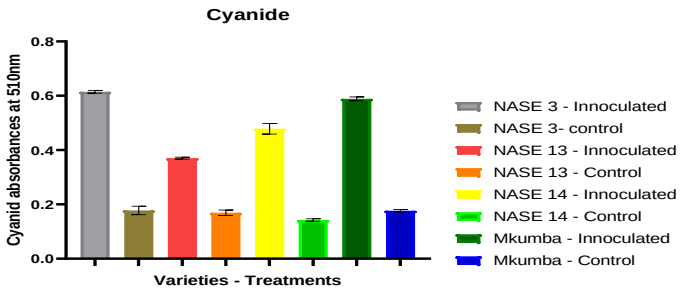
Cyanogenic potential, the ability to generate hydrogen cyanide (HCN), is taxonomically widespread in the plant kingdom: over 1000 plant species are reported to produce cyanogenic glucosides in variable

concentrations. Cassava (*Manihot esculenta Crantz*), is highly cyanogenic species a component that helps cassava as a feeding deterrent to arthropods. This study was able to demonstrate a significant variation in the cyanogenic potential ($p < 0.05$) across all the varieties i.e. 0.14 to 0.62ppm. (Table 2).

Phenolic Acid Derivatives	Varieties							
	NASE 3 - Inoculated	NASE 3 - Control	NASE 13 - Inoculation	NASE 13 - Control	NASE 14 - Inoculation	NASE 14 - Control	Mkumba - Inoculation	Mkumba - Control
Flavonoid	0.29±0.00g	0.08±0.01c	0.25±0.00f	0.02±0.00b	0.21±0.00d	0.00±0.00a	0.23±0.00e	0.01±0.00a
FRAP	0.87±0.30c	0.87±0.02c	0.56±0.02b	0.47±0.00b	0.15±0.00a	0.76±0.17c	0.56±0.018b	0.77±0.02c
Phenolics	0.06±0.00bc	0.13±0.00e	0.02±0.01a	0.11±0.00de	0.05±0.00b	0.04±0.06ab	0.04±0.00ab	0.08±0.00cd
Salicylic Acid	0.68±0.016e	1.08±0.01h	0.28±0.01c	0.86±0.01g	0.18±0.01a	0.54±0.01d	0.21±0.01b	0.74±0.01f
Tannins	0.07±0.00b	0.03±0.02a	0.10±0.00c	0.17±0.00e	0.10±0.00c	0.06±0.00b	1.12±0.01d	0.10±0.00c
Cyanogenic potential	0.62±0.01f	0.18±0.03b	0.38±0.01c	0.16±0.02ab	0.47±0.03d	0.14±0.01a	0.59±0.01e	0.17±0.01b

(means with different letter of alphabet within a particular row indicate significant difference between the values, Turkey's test ($P<0.05$))

Graph 4 shows NASE 3 and Mkumba as highly cyanogenic compared to other varieties. This variation and increase in the cyanogenic potential as demonstrated in the inoculated varieties is solely in response to the whitefly infestation.



Graph 4: A graph of absorbance means of cyanide at 510nm

4.4 Variation in the phenolic acid derivatives

The variation in the phenolic acid derivatives varied significantly ($p < 0.05$) across all the varieties i.e. 0.02 to 0.11 mg of gallic acid equivalent weight (GAE)/I00g of wet mass, 0.03 to 0.17 mg of tannic acid equivalent/l00g of sample, 0.18 to 1.08 for salicylic acid, 0.00 to 0.29 mg of quercetin equivalents/ 00g of wet mass and 0.15 to 0.87 µg ascorbic acid/g sample. From the table 2 above, NASE 13 and Mkumba showed significant response with production of phenolic acid derivatives. This response presented Mkumba and NASE 13 as better varieties over the others for a pick as varieties with tolerance to the whitefly.

5.0 DISCUSSION

This study was intended to: (1) To determine the photosynthetic efficiency of whitefly infested leaves of cassava and (2) To assess the tolerance of selected cassava varieties to the whitefly. The study, therefore, presents the most comprehensive evaluation of the selected varieties and the attained results show that Mkumba and NASE 13 are better varieties from which to breed for tolerance and resistance against the whitefly infestation. This finding agrees with Gwandu et. al. (2019) where in a study about whitefly resistance in African cassava genotypes, NASE 13 among other varieties showed good levels of resistance to whitefly infestation and damage. One Way Analysis of variance was used to demonstrate the variations and Turkeys test to demonstrate the significant differences in these means. Cyanogenic potential was equally average and less significant across all controls of varieties i.e. from 0.14 to 0.18. Upon infestation, the response by the varieties was an elevated cyanogenic potential and this was highest in NASE 3 at 0.62 and in Mkumba at 0.59. these two varieties were able to adapt by increasing their deterrence to the whitefly by increasing their cyanogenic potential which however reduces the palatability of these infested plants. However in the origin of cassava farming communities, farmers preferred to plant cassava varieties with high cynogenic potential due to their ability to deter many pests from them according to Anthony et.al (2000). Across all the other phenolic acid derivatives, NASE 13 was able to significantly adapt in production of these metabolites as a defence mechanism to the whitefly infestation making it generally a superior over the other varieties. These secondary metabolites are produced in plants as defences and in cotrols, ther levels are seen to be significantly low. This makes the increase in the levels of the metabolites acoss all varieties a response to the infestation. The levels of production are what is used as the variation in the tolerance to the whitefly and the NASE 3 and Mkumba were better suited after analyses across all the varieties selected.

Chlorophyll content as an indicator for photosynthetic capacity was able to score highly in Mkumba however a less effect was witnessed in NASE 13. This finding can be explained by Stansly (1986) who showed that *B. tabaci* adults preferentially fed and oviposited more on green than dark green cultivars a significant characteristic of NASE 13 leaves. Chlorophyll content differed less significantly in the controls i.e. from 1.40 to 1.49 for chlorophyll a, from 0.72 to 0.80 for chlorophyll b, and from 1.63 to 1.71 for carotenoid. These contents only were affected by the infestation of the whitefly, which directly feeds on the sap of the leaves. NASE 13 was

able to show a resistance in the chlorophyll degradation which could have further been degraded by the production of the cyanide as a response to the whitefly. This makes NASE 13 to outstand from this study and is seen as a better variety and primary in the breeding program. Less photosynthesis ultimately means low productivity of the cassava plant, and this variety will be undesirable by the farmer. In this stydy, the chlorophyll content was used as an indicator for photosynthetic efficiency and capacity and hence the variety out of the selected varieties that was able to stand out with highest chlorophyll content ater infestation was selected as a better candidate for use in the breeding program as a resistant variety.

6.0 CONCLUSION AND RECOMMENDATION

Given the importance of cassava to the economy of Uganda, and its role as a major crop in alleviating hunger in Africa, breeding for whitefly resistance to address constraints of production is necessary. The crop has to be improved for productivity, proximate composition and safe cyanide content. This study achieved identification of a variety that can meet the above requirements and is the recommendation made to breeding programs towards generating improved varieties across all boards. I therefore recommend as follows:

1. There should be accelerated research to combine resistance to cassava viruses and whiteflies in the same genotype.
2. From this research, a narrow range of germplasm has been tested, there should be more deliberate breeding programs aimed at developing higher levels of resistance in cultivars
3. I also recommend evaluation of related wild species for whitefly resistance for breeding programs

REFERENCES

Act, A. W. (2019). *Title : Pest Control Procedures*. 1–2.

Aminjafari, A., Miroliaei, M., V. T., Emamzadch, R., I)jukic, M. M., Djuric, A., & Saso, L. (2016). Antioxidant activity and protective role on protein glycation of synthetic aminocoumarins, Electronic Journal of Biotechnology, 19(6), 43-48.

Analianasari, A., Hidayat, B., & Trisnanto, T. B. (2020). Functional characteristics and added value siger rice based on cassava as a local food source. *IOP Conference Series: Earth and Environmental Science*, *411*(1). https://doi.org/10.1088/1755-1315/411/1/012055

Bográn, C. E., & Heinz, K. M. (2006). *Whiteflies*. 1–10.

Boykin, L. M., Bell, C. D., Evans, G., Small, I., & De Barro, P. J. (2013). Is agriculture driving the diversification of the Bemisia tabaci species complex (Hemiptera: Sternorrhyncha: Aleyrodidae)?: Dating, diversification and biogeographic evidence revealed. *BMC Evolutionary Biology*, *13*(1), 1. https://doi.org/10.1186/1471-2148-13-228

Chen, W., Hasegawa, D. K., Kaur, N., Kliot, A., Pinheiro, P. V., Luan, J., Stensmyr, M. C., Zheng, Y., Liu, W., Sun, H., Xu, Y., Luo, Y., Kruse, A., Yang, X., Kontsedalov, S., Lebedev, G., Fisher, T. W., Nelson, D. R., Hunter, W. B., … Fei, Z. (2016). The draft genome of whitefly Bemisia tabaci MEAM1, a global crop pest, provides novel insights into virus transmission, host adaptation, and insecticide resistance. *BMC Biology*, *14*(1). https://doi.org/10.1186/s12915-016-0321-y

Chetty, C. C., Rossin, C. B., Gruissem, W., Vanderschuren, H., & Rey, C. (2012). Empowering biotechnology in southern Africa: Establishment of a robust transformation platform for the production of transgenic industry-preferred cassava. *New Biotechnology*, *30*. https://doi.org/10.1016/j.nbt.2012.04.006

De Souza, A. P., & Long, S. P. (2018). Toward improving photosynthesis in cassava: Characterizing photosynthetic limitations in four current African cultivars. *Food and Energy Security*, *7*(2). https://doi.org/10.1002/fes3.130

Doungous, O., Khatabi, B., Hanna, R., Tchuanyo, M., Kuate, A. F., & Fondong, V. N. (2021).

Acibenzolar-S-methyl induces resistance against cassava mosaic geminiviruses in Nicotiana benthamiana and their vector Bemisia tabaci in cassava (Manihot esculenta). *Crop Protection*, *150*, 105796. https://doi.org/https://doi.org/10.1016/j.cropro.2021.105796

Egesi, C. N., Ogbe, F. O., Akoroda, M., Ilona, P., & Dixon, A. (2007). Resistance profile of improved cassava germplasm to cassava mosaic disease in Nigeria. *Euphytica*, *155*(1–2), 215–224. https://doi.org/10.1007/s10681-006-9323-0

El-Sharkawy, M. A. (2006). International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. *Photosynthetica*, *44*(4), 481–512. https://doi.org/10.1007/s11099-006-0063-0

El-Sharkawy, Mabrouk A. (2012). Stress-Tolerant Cassava: The Role of Integrative Ecophysiology-Breeding Research in Crop Improvement. *Open Journal of Soil Science*, *02*(02), 162–186. https://doi.org/10.4236/ojss.2012.22022

El-Sharkawy, Mabrouk A. (2004). Cassava biology and physiology. In *Plant Molecular Biology* (Vol. 56).

Gwandu, C., Ochwo-Ssemakula, M., & Sseruwagi, P. (2019). Whitefly resistance in African cassava genotypes. *African Crop Science Journal*, *27*(2), 213-228.

Haque, M. R., & Bradbury, J. H. (2002). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food chemistry, 77(1), 107-114.

Kamtekar, S., Keer, V., & Patil, V. (2014). Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. Journal ofappliedpharmaceutical Science, 4(9), 61.

Katono, K., Macfadyen, S., Omongo, C. A., Odong, T. L., Colvin, J., Karungi, J., & Otim, M. H. (2021). Influence of cassava morphological traits and environmental conditions on field populations of bemisia tabaci. *Insects*, *12*(7). https://doi.org/10.3390/insects12070604

Moreno-Cadena, P., Hoogenboom, G., Cock, J. H., Ramirez-Villegas, J., Pypers, P., Kreye, C., Tariku, M., Ezui, K. S., Becerra Lopez-Lavalle, L. A., & Asseng, S. (2021). Modeling growth, development and yield of cassava: A review. *Field Crops Research*, *267*, 108140.

https://doi.org/https://doi.org/10.1016/j.fcr.2021.108140

Mostofa, M. G., Rahman, A., Ansary, M. M. U., Watanabe, A., Fujita, M., & Tran, L.-S. p. (2015). Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. Scientific Reports, 5, 14078.

Munguti, F. M., Kilalo, D. C., Nyaboga, E. N., Wosula, E. N., Macharia, I., & Mwango'mbe, A. W. (2021). Distribution and Molecular Diversity of Whitefly Species Colonizing Cassava in Kenya. *Insects*, *12*(10), 875. https://doi.org/10.3390/insects12100875

Mwila, N., Rubaihayo, S., Kyamanywa, S., Odong, T., Nuwamanya, E., Mwala, M., Badji, A. (2017). Biochemical factors associated with cassava resistance to whitefly infestation. African Crop Science Journal, 25(3), 365-385.

Nsubuga, F. W., Olwoch, J. M., & Rautenbach, C. J. de W. (2011). Climatic Trends at Namulonge in Uganda: 1947-2009. *Journal of Geography and Geology*, *3*(1), 119–131. https://doi.org/10.5539/jgg.v3n1p119

Oliveira, M. R. V, Henneberry, T. J., & Anderson, P. (2001). History, current status, and collaborative research projects for Bemisia tabaci $. In *Crop Protection* (Vol. 20).

Omongo, C. A., Kawuki, R., Bellotti, A. C., Alicai, T., Baguma, Y., Maruthi, M. N., Bua, A., & Colvin, J. (2012). African Cassava Whitefly, Bemisia tabaci, Resistance in African and South American Cassava Genotypes. In *Journal of Integrative Agriculture* (Vol. 11, Issue 2, pp. 327–336). https://doi.org/10.1016/S2095-3119(12)60017-3

Pekal, A., & Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. Food Analytical Methods, 7(9), 1776-1782.

Polston, J. E., De Barro, P., & Boykin, L. M. (2014). Transmission specificities of plant viruses with the newly identified species of the Bemisia tabaci species complex. *Pest Management Science*, *70*(10), 1547–1552. https://doi.org/10.1002/ps.3738

Pratama, S. N., Sudarsono, Ardie, S. W., Khumaida, N., & Sukma, D. (2021). Development of phenotypic markers and contrast genotype candidates of target minerals related to cassava.

Biodiversitas, *22*(6), 3049–3056. https://doi.org/10.13057/biodiv/d220606

Stansly, T.-X.L. and P.A. 1986. Life history of Bemesia Argentifolii (Homoptera: Aleyrodidae) on Hibiscus Rosa-Sinensis (Malvaceae). Florida Entomology 437–45.

Utsumi, Y., Utsumi, C., Tanaka, M., Van Ha, C., Takahashi, S., Matsui, A.,Seo, M. (2019). Acetic Acid Treatment Enhances Drought Avoidance in Cassava (Manihot esculenta Crantz). Frontiers in plant science, 10.

Wang, X. W., Li, P., & Liu, S. S. (2017). Whitefly interactions with plants. In *Current Opinion in Insect Science* (Vol. 19, pp. 70–75). Elsevier Inc. https://doi.org/10.1016/j.cois.2017.02.001

Warrier, R., Paul, M., & Vineetha, M. (2013). Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genetics and Plant Physiology*, 3(1-2), 9097.