INFLUENCE OF NITROGEN, PHOSPHOROUS AND POTASSIUM ON CYANOGENIC GLUCOSIDE PRODUCTION IN CASSAVA GROWN IN SOME SOILS OF MTWARA REGION, TANZANIA

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EXTENDED ABSTRACT

Poor soil nutrient supply is often overlooked as a contributing factor of increased cyanogenic glucoside accumulation in cassava produced in areas affected by konzo, despite the low soil fertility in these areas. High cyanogenic glucosides in cassava roots is one reason attributed to cassava cyanide intoxication that sometimes leads to a health disorder called konzo. Various studies were hence carried out to investigate the role of nutrient supply on cyanogenic glucoside production in cassava varieties grown in areas affected by konzo, using konzo-affected Mtwara region as the study area. The first study was a survey, carried out using a questionnaire. In this study farmers' perceptions on the causes of cassava root bitterness were investigated, with the interest of finding out whether farmers had observed that soils in the region were a contributing factor of increased cassava toxicity and to learn from them the characteristics of these soils. The second study was carried out to investigate whether soil nutrient levels of soils in konzo-affected areas of Mtwara region was inadequate for cassava production and hence capable of causing increased cassava cyanogenic glucoside levels. The third study determined whether soil nutrient supply could equally influence cyanogenic glucoside production in cassava grown in these areas just like variety and moisture supply. A 2×3×4 factorial pot experiment which consisted of two cassava varieties, three soil moisture treatments and four fertiliser treatments and laid-out as a randomised complete block design was used to carry out this study. The fourth study was carried out as a field experiment using four cassava varieties and six N-P-K fertiliser treatments, it was arranged using a split-plot design and was repeated in two consecutive years. This study was carried out to investigate

the beneficial effects of improved plant nutrition (with N-P-K use) on root HCN levels and yields of commonly grown cassava varieties in the region.

About 14.2% of the farmers perceived that soils, particularly nutrient poor soils, were a contributing factor to cassava root bitterness and therefore probably of increased cassava root cyanide toxicity. In agreement with farmers, the survey results showed that soils in the region were severely inadequate for cassava production, with 99.1%, 34.8%, 84.3%, 13.9%, 84.3%, 63.5% and 93.0% of the cultivated soils in konzo-affected villages of Mtwara region being deficient in N, P, K, Ca, Mg, S and Zn, respectively. Adequate levels of most of the deficient nutrients in these soils are known to reduce cyanogenic glucoside production in cassava. Soil nutrient supply ($p < 0.001$) was also found to have an equally important influence on cyanogenic glucoside levels as variety ($p < 0.001$). On the other hand, water was found to have no influence ($p = 0.080$) and was only important for the variety *Kiroba* whose cyanogenic glucoside production was influenced differently by water supply depending on the soil nutrient conditions ($p = 0.033$). Leaf HCN levels in the two varieties used ranged from 44.4 to 310.4 mg/kg. The study finally revealed that effects of fertiliser use on the total root cyanide (HCN) content (a measure cyanogenic glucoside levels) of some commonly grown cassava varieties was greatly dependant on variety and was mainly non-responsive to fertiliser. Inconclusive results were obtained on the effects of N-P-K on yields. With N-P-K applied at the rate of 25-10-50 kg N-P-K/ha, the fertiliser responsive variety *Kiroba*, gave the lowest root HCN level of 43.6 mg/kg under water stress conditions and attained fresh root yields of 26.0 t/ha. The important role of soil fertility on cyanogenic glucoside accumulation was clearly demonstrated.

DECLARATION

I, **MATEMA L.E. IMAKUMBILI**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has neither been submitted nor being concurrently submitted for a degree award in any other institution.

Matema L.E. Imakumbili (PhD Candidate)	Date

The above declaration is confirmed by:

Prof E. Semu (Supervisor)	Date
Prof J.M.R. Semoka (Supervisor)	Date
Dr A. Abass (Supervisor)	Date

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DEDICATION

I dedicate the work firstly to God and to everyone that believed in me.

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ABBREVIATIONS

Al	aluminium
ANOVA	analysis of variance
AusAID	Australian aid
C2	coastal agroecological zone 2
Ca	calcium
cmol	centimoles
CuO	copper oxide
DAP	days after planting
DTPA	diethylenetriaminepentaacetic acid
F	fertiliser treatment
FC	field capacity
Fe	iron
h	high
HCN	total hydrogen cyanide
K	potassium
l	low
m	medium
M	moisture level
MAP	months after planting
Mg	magnesium
$MgSO_4$	magnesium sulphate
MOP	muriate of potash

N	nitrogen
NARI	Naliendele Agricultural Research Institute
No.	number
N-P-K	nitrogen, phosphorous and potassium fertiliser combination
OC	organic carbon
P	phosphorous
PCD	Planning Commission Dar es Salaam
P-value	probability value
r	correlation coefficient
r^2	coefficient of determination
RAND	randomise function in Microsoft Excel.
RCBD	randomised complete block design
RCOM	Regional Commissioner's Office Mtwara
S	sulphur
SD	standard deviation
TAN	Tropical ataxic neuropathy
TFNC	Tanzania Food and Nutrition Centre
TRCS	Tanzania Red Cross Society
TSP	triple superphosphate
V	variety
vh	very high
vl	very low
Zn	zinc
ZnO	zinc oxide

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta* Crantz) is an important food crop, it however contains potentially harmful cyanogenic glucosides which can hydrolyse to release toxic hydrogen cyanide (Burns *et al*., 2010). Due to the cyanogenic glucosides, cassava has been commonly associated with cyanide intoxication in a few areas where it constitutes the main component of the diet (Poulton, 1990). In a few major cassava consuming areas, cassava cyanide intoxication has often resulted in a health disorder called konzo, which causes an irreversible paralysis of legs (Bradbury and Denton, 2010; Nzwalo and Cliff, 2011). A number of Sub-Saharan African countries have been affected by konzo, and the disorder is reported as persistent in very deprived areas of Mozambique, the Democratic Republic of Congo (DRC), Tanzania (Banea *et al*., 2012), Central African Republic (Tylleskär *et al*., 1994; Ernesto *et al*., 2002; Mbelesso *et al*., 2009) and in eastern Cameroon (Cigleneki *et al*., 2011; Agbor *et al*., 2014).

Water stress, due to drought and dry season periods, is usually the main factor attributed to causing increased cyanogenic glucoside levels in the bitter cassava varieties commonly grown in areas affected by konzo (Nzwalo and Cliff, 2011; Akinpelu *et al*., 2011). This may be true in cases of large konzo epidemics or with smaller outbreaks, however where konzo persists its occurrence is speculated to be caused by high cyanogenic glucoside levels in cassava plants outside periods of

water stress as well (Ernesto *et al*., 2002; Cliff *et al*., 2011). This suggests that other factors in the agronomic environment of konzo-affected areas could be additionally contributing to increased cyanogenic glucoside levels in cassava plants. Moreover, higher cyanogenic glucoside levels have been reported in cassava when it was not water stressed as opposed to when it was water stressed, showing that water stress alone may not be enough to explain increased cyanogenic glucoside levels (Bokanga *et al*., 1994).

A characteristic common to all areas affected by konzo is their low soil fertility. Except for the konzo-affected areas in DRC and Cameroon, all other affected areas are situated along the coast or along a lake shore; they include Nampula and Zambézia districts in Mozambique (Cliff *et al*., 2011), Newala and Mtwara districts in Tanzania (Mlingi *et al*., 2011) and the lake shore Tarime district in Tanzania (Howlett *et al*., 1992). All mentioned areas predominantly have sandy soils which have very low soil fertility (Bennett *et al*., 1979; Howlett *et al*., 1992; Mowo *et al*., 1993; World Bank, 2006). Although not coastal, konzo affected areas in Bandundu Region in DRC are located in the Savannah zone which primarily also consists of relatively infertile sandy soils (Banea *et al*., 1992; Kisangani and Bobb, 2009). Molua and Lambi (2006) additionally describe the soils in the non-coastal Eastern region of Cameroon as having a sandy clay texture and as being largely degraded. Soils in konzo-affected western Central African Republic bordering the eastern region of Cameroon (Cigleneki *et al*., 2011) are probably just as degraded. There is thus a possibility that poor nutrient supply from these infertile soils could be influencing cyanogenic glucoside production in cassava produced in these areas.

The possible effects of soil fertility on cyanogenic glucoside accumulation in cassava, is further consolidated by the fact that both low and high soil fertility may cause nutrient stress in cassava (Howeler, 2014). Like water stress, nutrient stress is an abiotic form of plant stress that can also probably lead to increased cyanogenic glucoside accumulation in cassava (Jørgensen *et al*., 2005). Marschner (2012) mentions that a sufficient supply of all essential soil nutrients is needed to maintain adequate plant nutrition, as the biosynthesis of all quality determining organic compounds (like cyanogenic glucosides) in plants and thus their desirable proportions depends on this. Changes in cyanogenic glucosides with nutrient supply have already been demonstrated (Obigbesan, 1977; Endris, 2006; Adekayode and Adewumi, 2013).

The role of soil nutrient supply on cyanogenic glucoside production in cassava grown in areas affected by konzo cannot be overlooked and needs to be investigated. Being an important and permanent part of the growing environment, the influence of soil nutrient supply on cyanogenic glucoside accumulation either alone or when interacting with other factors, is essential in understanding cyanogenic glucoside accumulation in cassava in areas affected by konzo.

Hardly any research has been carried out in konzo-affected areas to find out whether the soils there are able to supply nutrients in amounts adequate enough to support the non-stressful growth of cassava. In addition to variety and moisture supply, the importance of soil nutrient supply as contributing factor of changes in cassava cyanogenic glucoside also needs to be established. Knowledge of the role of nutrient supply on cyanogenic glucoside accumulation in cassava plants both during and outside water stress periods is also lacking. There is also hardly any knowledge on

the role of soil nutrient supply on cassava cyanogenic glucoside production as experienced by farmers who cultivate and consume the produced cassava. Furthermore, very little research has been carried out on how improved plant nutrition could help reduce cyanogenic glucoside levels in cassava varieties grown in these areas. To fully understand the influence of soil nutrient supply on cyanogenic glucoside accumulation in cassava produced in some konzo-affected areas of Mtwara region all these questions need to be answered, as was done in the present study.

Even though konzo and other cassava cyanide related problems are not restricted to Mtwara region in Tanzania, the study focuses on this region. The results may however still be applicable to other areas affected by konzo. With cassava production becoming more widespread due to the marginalisation of more areas by climate change, problems associated with its toxicity are likely to spread into new areas as well, with rural poor areas being most at risk (Lubulwa, 1995; Nhassico *et al*., 2008). The information derived from the study will hence be useful not only in areas where konzo is already encountered but in areas where cassava is likely to spread.

1.2 Toxicity Associated with Cassava

Cassava is a staple food for about 800 million people in tropical countries (Iwuoha *et al*., 2013) and it is just one out of the several thousand plant species, that synthesize cyanogenic glucosides (Poulton, 1990). All organs, except seeds of this important food crop contain cyanogenic glucosides (Alves, 2002). Cyanogenic glucoside molecules are themselves not toxic but they release toxic hydrogen cyanide gas (HCN) when they breakdown in a process known as cyanogenesis (Burns *et al*., 2010). The presence of these cyanogenic compounds in ingested cassava or its

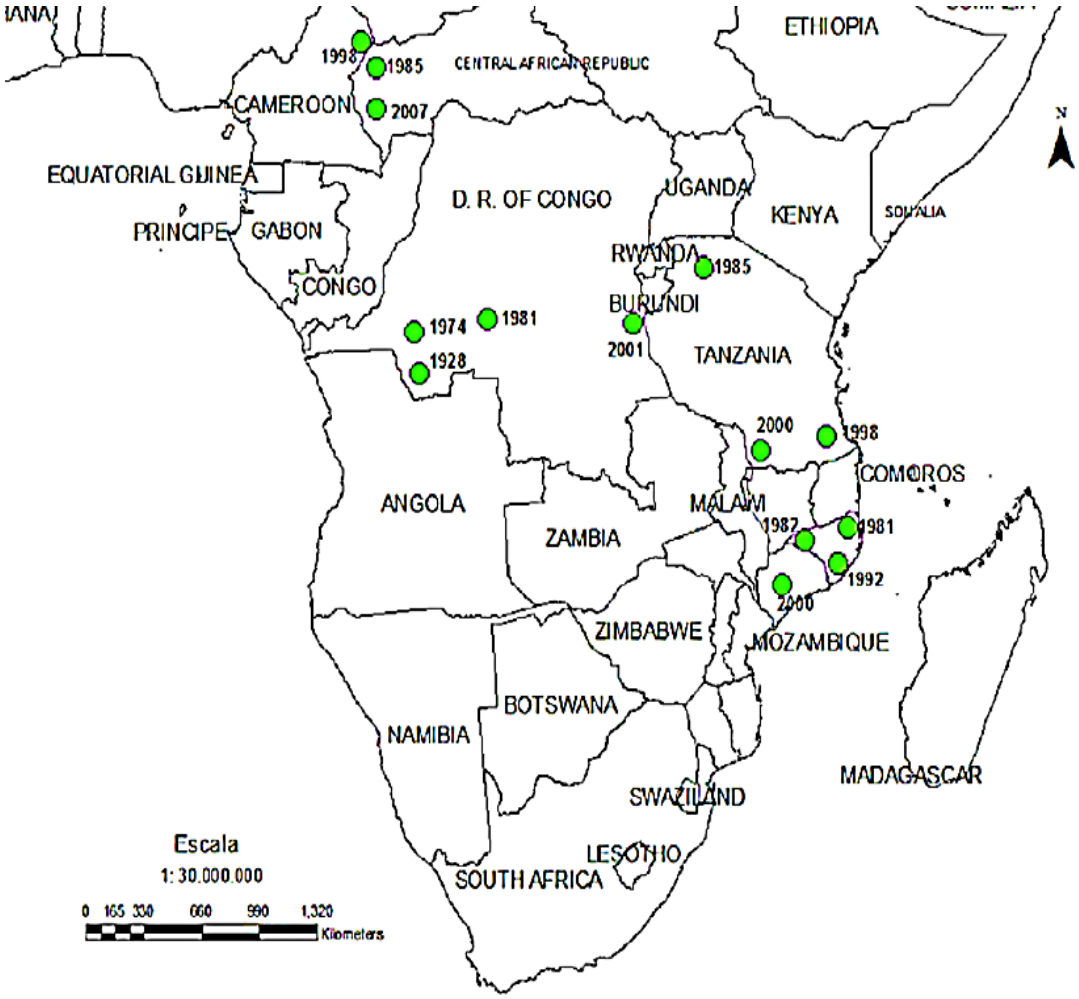
products is what leads to intoxication due to their continued cyanogenesis in the human body (Bradbury and Denton, 2011).

Acute or chronic intoxication may result depending on the levels and frequency of cyanogen exposure and also on the state of nutrition of the consumer (Siritunga and Sayre, 2004). Symptoms of acute intoxication resulting from a high dose within a short period include; dizziness, headache, nausea, vomiting, stomach pain, diarrhoea and sometimes death (Mburu *et al*., 2012). On the other hand chronic intoxication from long-term exposure to low cassava cyanide levels (Speijers *et al*., 2010) is associated with neurological diseases like tropical ataxic neuropathy (TAN) and the more common spastic paraparesis which is also called konzo. Only about 1% of the cassava consuming world population is affected by konzo and TAN, and all cases occur only in sub-Saharan Africa (Lubulwa, 1995).

Countries in Africa where konzo has been reported are shown in Fig. 1.1. Although the number of affected people is small, cassava cyanide health related disorders are a burden to communities that heavily depend on cassava as their main food. Like cases of acute cassava cyanide intoxication, most cases of konzo may also probably often go unreported due to the difficulty in collecting information from mainly remote affected areas (Nzwalo and Cliff, 2011; Cliff, 2012). A list of some konzo cases that have occurred in Tanzania are shown in Table 1.1.

1.3 Cyanogenic Glucoside Accumulation in Relation to Genotype

The amount of cyanogenic glucosides produced by cassava is genetically controlled (Vetter, 2000) and it is thus a varietal (cultivar) characteristic (Onwueme and Charles, 1994).



Source: Nzwalo and Cliff (2011)

Figure 1.1 **Countries in Africa where epidemics of konzo have been reported and the year of their occurrence**

Table 1.1 Summary of konzo cases in Tanzania

Date	District	Region	Number of cases
1985	Tarime	Mara	39
1989	Tarime	Mara	118
1988	Masasi	Mtwara	3
2001 - 2002	Mbinga	Ruvuma	24
2002 - 2003	Mtwara rural	Mtwara	195
2002 - 2003	Newala	Mtwara	19
2008 - 2009	Mbinga, Mtwara rural and Newala	Mtwara	4
Total			363

Source: Mlingi *et al.* (2011)

Depending on the concentrations of total cyanides found in a certain amount of fresh root, cassava can be classified as innocuous (< 50 mg/kg), moderately poisonous (50 - 100 mg/kg) and dangerously poisonous (> 100 mg/kg) (Burns *et al*., 2010). Due to their normally sweet taste, innocuous varieties are commonly called 'sweet' varieties, while the dangerously poisonous varieties are normally called 'bitter' varieties due to their bitter taste (Wilson and Dufour, 2002). Cyanide levels in cassava generally range from 10 to 490 mg/kg (Iwuoha *et al*., 2013).

Varietal differences in cyanogenic glucoside production further vary depending on different physiological or environmental factors (Vetter, 2000). Water stress due to drought is an environmental factor mainly attributed to increased cyanogenic glucoside levels in cassava during outbreaks of konzo (Howlett *et al*., 1992; Ernesto *et al.*, 2002; Mlingi *et al*., 2011; Banea *et al*., 2013). During these periods cyanide levels are higher than the 50 mg/kg and are thus above the level required for reduced cyanide exposure from fresh cassava (Codex Alimentarius Commission, 2013).

1.4 Strategies Proposed to Reduce Root Cyanogenic Glucoside Levels

One strategy needed to reduce cyanide intake from the bitter or toxic cassava varieties is improved processing (Cardoso *et al*., 2005; Cliff *et al*., 2011). The mainly used traditional processing methods may not always be effective especially when cassava cyanide levels are too high (Mckey *et al*., 2010). Modern cassava processing methods which are more effective have been introduced in Mtwara region (Mlingi, 2011). A flour wetting method has also been developed to reduce total cyanide content of cassava flour in konzo-affected areas (Bradbury and Denton, 2010; Banea *et al*., 2012). In the flour wetting method, cassava flour is mixed in a little water and left to soak in it for five hours. This process allows for the complete breakdown of any residual cyanogenic glucosides the realised as hydrogen cyanide gas escapes through the thin layer of water above wet flour. Modern processing and the cassava flour wetting method however still need to be widely disseminated and adopted.

The introduction of low cyanide cassava varieties that are also well-adapted and high yielding is another strategy required to reduce cyanide intake (Cardoso *et al*., 2005; Nzwalo and Cliff, 2011). It can address some short-falls from the non-adoption of other strategies needed to reduce cassava cyanide intoxication in these communities. Planting improved low cyanide varieties may also be an easier strategy to adopt as nothing new must be learned, only wide cassava cutting dissemination is needed. Breeders nonetheless need proper information if they are to breed for cassava varieties that maintain low cyanide production under a range of environmental conditions (Bokanga *et al*., 1994). Knowledge of cyanogenic glucoside production in varieties under various environmental conditions and not only with water stress is thus needed. The interactive influence and not only the single effect of factors also

becomes important as factors occur simultaneously and not independently in agronomic environments (Zhang, 1996). Most genotype by environment studies which could address the interaction of factors and their influence on cyanogenic glucoside production in varieties have been done but most have been biased to differences caused only by the atmospheric environment (Bokanga *et al*., 1994; Burns *et al*., 2012). Although it is a very important part of the agronomic environment, the influence that the soil or edaphic environment has on cyanogenic glucoside production in cassava has been less explored (Burns *et al*., 2012).

A short-fall of the strategy of introducing new low cyanide varieties despite them being high yielding and well adapted to these environments, is the greater preference that farmers have for the bitter tasting cassava varieties they plant. This is because the toxicity of bitter cassava varieties is useful for reducing crop losses due to attacks by herbivores or theft (Siritunga and Sayre, 2003; Siritunga and Sayre, 2004). They are thus a necessary part of these farming systems. Cassava however produces other bitter tasting compounds which sometimes influence its taste more than cyanogenic glucosides (King and Bradbury, 1995). Bitter tasting cassava varieties may thus not always be toxic and sweet tasting varieties not always innocuous. This calls for knowledge that is inclusive of the actual cyanogenic character of varieties preferred by farmers in the konzo-affected areas and how cyanogenic glucoside production in these varieties is influenced by not only water stress but by other environmental factors like soil nutrient supply. A possible selection of suitable varieties already grown could then be done, solving the problem of variety preference. Realistic cultural practices that minimise plant stress and reduce cyanogenic glucoside production should also be encouraged (Bokanga *et al*., 1994).

An agronomic solution, based on a more holistic understanding of all major environmental factors affecting cyanogenic glucoside production in cassava, is needed to effectively reduce cassava cyanide intoxication cases. Such solutions would help overcome problems related with the adoption of other interventions, like the food based strategies that are dependent on the consumption of foods that are difficult to find in these food insecure areas (Gnonlonfin *et al.*, 2011).

1.5 Soil Nutrient Supply and Cyanogenic Glucoside Production

Being tolerant to low pH, high levels of exchangeable aluminium (Al) and low concentrations of phosphorus (P), cassava is able to still give reasonable yields even on poor or degraded soils (Howeler, 2002; Howeler, 2009). It is thus not surprising that the crop can still be successfully grown without fertiliser application as is done by poor farmers (Howeler, 2007). However, despite being well adapted to infertile soils cassava is highly responsive to fertiliser applications and also does very well on naturally fertile soils (Howeler, 2014). It thus thrives under optimal soil nutrient conditions. Cassava is actually nutrient stressed under poor soil nutrient conditions and also with an oversupply of nutrients (CIAT, 2011; Howeler, 2014). Just like any other abiotic stress, nutrient stress can probably also lead to increased cyanogenic glucoside production in cassava (Jørgensen *et al*., 2005; Nhassico *et al*., 2008). It is thus possible that soil infertility in konzo-affected areas could be causing nutrient stress and increasing cyanogenic glucoside production in cassava grown.

A number of studies have demonstrated that nutrient supply does indeed influence cyanogenic glucoside production. A study by Gosh and Nair (1986) found that cyanogenic glucoside production in cassava decreased with an increased supply of calcium (Ca), sulphur (S) and zinc (Zn) through fertiliser application. Significant

reductions in cyanogenic glucosides have been frequently reported with potassium fertiliser application (Mohankumar *et al*., 1988; Susan John *et al*., 2007), but some studies have not reported significant effects with it (Obigbesan, 1977; Cuvaca *et al*., 2015). A common observation made is the increase in cyanogenic glucosides with increased soil nitrogen (N) (De Bruijn, 1973; Amanullah *et al*., 2007; Uyoh *et al*., 2007; Adekayode and Adewumi, 2013), this again is not always the case (Mohankumar *et al*., 1988; Cuvaca *et al*., 2015). Differential responses in cyanogenic glucoside production with nutrient supply observed in various varieties indicate that the response is varietal dependent. Soil nutrient levels observed to reduce or increase cyanogenic glucoside production in cassava need to be investigated in order to fully understand the role that soil nutrient supply could have in contributing to high cyanogenic glucoside levels leading to persistent konzo.

According to Marschner (2012) the synthesis of organic biomolecules in plants depends very much on the adequacy of nutrients in the plant. Being biomolecules, the production of cyanogenic glucosides must thus also be influenced by changes in the nutrient contents in the plant. This further shows the role of nutrient supply in influencing cyanogenic glucoside production in cassava. It hence becomes necessary to find out the contribution of soil nutrient supply in increasing cassava cyanogenic glucoside levels in areas affected by cyanide intoxication.

1.6 Importance of Farmer Perceptions

Farmers have their own experiences and knowledge on the crops they grow. For this reason it is necessary that scientists learn from farmers so that the interventions they develop can be more appropriately targeted. Farmers mostly associate the bitter taste of cassava with toxicity (Mkumbira *et al*., 2003; Gnonlonfin *et al*., 2011). Using taste

they can thus assess when the toxicity in cassava increases and they probably know when the increase in bitterness is associated with particular soil conditions or with soils located in a particular area. A scientific basis backing the farmers' perceptions can then be investigated and solutions on reducing cyanogenic glucoside production in cassava based on the farmers own understanding of the problem can be better arrived at.

1.7 Objectives

1.7.1 Overall objective

To determine the extent to which soil nutrient supply influences cyanogenic glucoside production in cassava produced in areas affected by konzo in Mtwara region.

1.7.2 Specific objectives

i. To determine from farmers whether they perceive that the soils they farm contribute to bitterness in planted cassava.

ii. To determine whether nutrient levels of soils from konzo-affected areas of Mtwara region are adequate for optimal cassava growth.

iii. To determine whether nutrient supply has a comparable influence on cyanogenic glucoside production in cassava like variety and soil moisture.

iv. To determine the effects of N-P-K fertiliser on root HCN levels and yields of various cassava varieties.

1.8 Organization of the dissertation

The dissertation is organised into six chapters as follows: Chapter one is the general introduction, objectives and justification of the study. It also gives a literature review. Chapter two is a survey of farmer perceptions on the causes of increased cassava bitterness. Chapter three is the survey of soil nutrient levels of cultivated cassava fields in the konzo-affected areas of Mtwara region. Chapter four is a pot experiment demonstrating the effects of water supply, nutrient supply and variety on cyanogenic glucoside production. Chapter five reports results of an experiment on the effects of N-P-K fertiliser application on root HCN and yield of various cassava varieties. Chapter six contains the overall conclusion of the study and the recommendations made.

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CHAPTER TWO

2.0 FARMERS' PERCEPTIONS ON THE CAUSES OF CASSAVA ROOT BITTERNESS IN KONZO-AFFECTED AREAS OF MTWARA REGION, TANZANIA

Abstract

The agronomic factors influencing increased cyanogenic glucoside levels in cassava during periods without water stress in areas where konzo, a paralytic disease, persists are not known. However, through their assessment of bitter taste, farmers may have noticed some non-water stress and variety related factors influencing cassava root cyanogenic glucoside content in these environments. Bitterness in cassava is usually associated with high cyanogenic glucoside levels in cassava. Using some konzo-affected in Mtwara region of Tanzania as a case study, a survey was thus carried out to identify the factors, hitherto overlooked, that may additionally influence cyanogenic glucoside levels in cassava. A total of 120 farmers were interviewed. A number of non-water stress and variety related factors that could be additionally influencing cyanogenic glucoside production in cassava plants were mentioned. These included nutrient poor soils, plant age at harvest, weeds, piecemeal harvesting, and branch pruning; these factors, respectively, constituted 14.2%, 7.5%, 0.8%, 0.8%, and 0.8% of the total responses given.

2.1 Introduction

Cassava (*Manihot esculenta* Crantz) is the seventh most important food crop in the world (Howeler, 2014). It however unfortunately contains cyanogenic glucosides which when hydrolysed release toxic hydrogen cyanide (Iwuoha, 2013). Cyanogenic glucosides are a major quality limitation of cassava and their continuous intake from improperly processed cassava products without access to foods containing sulphur amino acids is known to cause cyanide intoxication (Ndung'u *et al*., 2010). Cases of cassava cyanide intoxication have been reported in a number of countries in Sub-Saharan African, such as the Democratic Republic of Congo (DRC), Mozambique, Tanzania, Cameroon, Central African Republic and Angola (Tylleskär *et al*., 1994; Cigleneki *et al*., 2011; Mlingi *et al*., 2011; Cliff *et al*., 2011; Banea *et al*., 2012). The reports consisted of cases of acute cyanide intoxication but more commonly of the cassava cyanide health disorder called konzo, which is known to result in an irreversible paralysis of legs (Mlingi, 2002; Mbelesso *et al*., 2009; Bradbury *et al*., 2011; Cliff, 2012).

One reason given for the high cyanide intake during konzo epidemics are increased cyanogenic glucoside levels in fresh cassava roots and thus in products produced from them (Mlingi *et al*., 2011; Vandegeer *et al*., 2013). Researchers mostly attribute the increase in cyanogenic glucoside levels in cassava plants to water stress from prolonged droughts that coincide with most epidemics of konzo (Mlingi, 2011; Mlingi *et al*., 2011) and also to the bitter varieties which are preferred by many farmers (Oluwole *et al*., 2007; Mlingi *et al*., 2011). The naturally high cyanogenic glucoside contents of bitter cassava varieties are said to be increased by drought (Vandegeer *et al*., 2013). Water stress during dry seasons (seasonal droughts) is

similarly known to result in increased cyanogenic glucoside levels of cassava plants (Banea-Mayambu *et al*., 1997; Cigleneki *et al*., 2011; Oluwole, 2015). A study by Sriroth *et al*. (2001) found about 9 - 10 times higher cyanogen levels in cassava harvested during the dry season compared to when it was harvested during the rainy season.

Banea *et al*. (2012) mentions that konzo is persistent in some rural areas of Mozambique, DRC and Tanzania. According to Ernesto *et al*. (2002) in areas where konzo persists, high cyanogenic glucoside levels in cassava plants may also occur outside periods of water stress. This suggests that factors other than droughts and dry seasons could also be contributing to increased cyanogenic glucoside concentrations in cassava plants, which may have contributed to the occurrence of persistent cassava cyanide intoxication, or konzo, in some areas. Konzo is additionally alleged to be due to chronic cassava cyanide intoxication (Cliff *et al*., 2011). This thereby suggests that other agronomic factors are a permanent characteristic of these farming systems and thus constantly influence cyanogenic glucoside levels in cassava. Factors like soil fertility (Ndung'u *et al*., 2012; Alou *et al*., 2014), cultural practices, flooding and plant age (Bokanga *et al*., 1994; Alves, 2002) are also known to influence cyanogenic glucoside levels in cassava plants and could hence also be contributing factors to increased cyanogenic glucosides to varied degrees in konzo-affected areas.

Farmers in Africa generally use the bitter taste of cassava roots to perceive the potential toxicity of cassava (Mkumbira *et al*., 2003; Oluwole *et al*., 2007; Gnonlonfin *et al*., 2011). Research has proved that a greater portion of cassava varieties perceived as bitter by farmers do indeed contain higher levels of cyanogenic glucoside levels than sweet cassava varieties (Mkumbira *et al*., 2003). This may

however not always be the case because cassava contains other bitter compounds (King and Bradbury, 1995), making validation necessary. Using the method employed to differentiate between bitter and sweet cassava varieties, changes in the degree of bitterness and thus the perceived toxicity of roots of a particular cassava variety can also be made (Araullo *et al*., 1974; Mohan Kumar *et al*., 1977). In demonstration of the use of bitter taste to determine increased bitterness, families had been reported to complain that cassava roots were more bitter than normal during a season in which a konzo epidemic was experienced (Oke *et al*., 1990).

Small epidemics and sporadic cases of konzo have been observed in some communities, creating near persistent konzo exposures (Ernesto *et al*., 2002; Cliff *et al*., 2011). If non-water stress related, it is more difficult to explain the agronomic factors leading to increased cyanogenic glucoside levels when sporadic cases of konzo occur. However, being able to observe the crop throughout the year, farmers may have knowledge of the agronomic factors influencing cassava root bitterness and thus probably causing increased root cyanogenic glucoside levels. Hence using some konzo-affected communities in Mtwara region, Tanzania, as a study area, this research was carried out to investigate the agronomic factors influencing cassava root bitterness according to the perception of farmers.

2.2 Materials and Methods

2.2.1 Description of the study area

A survey was carried out to investigate the perception of farmers of Mtwara Rural and Newala districts on the causes of increased cassava root toxicity. Three districts

have been reportedly affected by cassava cyanide poisoning in Mtwara region, namely Masasi, Mtwara Rural and Newala districts (Mlingi, 2002; Mlingi *et al*., 2011). This study, however, focused on villages of Mtwara Rural and Newala districts. The two districts covered in the survey are two of the five districts found in Mtwara region (S 10^{o}16'25", E 40^{o}10'58").

The soils in the Mtwara region and in the two districts (Mtwara and Newala) have low natural soil fertility (De Pauw, 1984; Veldkamp, 2001). They are predominantly sandy and have been classified as Ferralic Cambisols (De Pauw, 1984; Mowo *et al*., 1993). The study areas mainly lie in Tanzania's Coastal Lowlands agroecological zone (C 2) (De Pauw, 1984; Mowo *et al*., 1993). The rainfall is mono-modal and ranges from 800 to1000 mm/year and the maximum and minimum temperatures vary from 29 to 31 ^{o}C and between 19 to 23 ^{o}C, respectively (De Pauw, 1984). Both districts lie on the Makonde plateau, with Newala lying to the west and Mtwara Rural to the eastern side of the plateau.

2.2.2 Sampling method

A total of 120 individuals with full knowledge of the cropping history of their farm fields were selected from eight randomly selected konzo-affected villages of Newala and Mtwara Rural districts were interviewed in October 2014. Sixty-one of the respondents were from Newala district and 59 were from Mtwara Rural district. The villages were among the 18 villages visited during a konzo rehabilitation and prevention program that was carried out, from 2008 to 2009, through collaboration between the Tanzania Food and Nutrition Centre (TFNC) and the Tanzania Red Cross Society (TRCS), with technical support from Australian National University and funding from AusAID (Mlingi *et al*., 2011). Using the 2012 census list, 15

households from each village were randomly picked for interviews. Each household was first assigned a unique number and the household numbers was then randomly picked from census list using the 'RAND' function in Microsoft Excel.

2.2.3 Field methods and tools

A questionnaire containing both closed and open ended questions was used to collect information on what farmers perceived to be the causes of increased bitterness of cassava roots. Open ended questions were used to allow the farmer to provide further explanation to closed ended responses. Visits to the fields were also made to observe how the households practice cassava cultivation. The questionnaire is given in Appendix 1.

2.2.4 Data Analysis

Collected data was analysed as frequencies, using GenStat package, Edition 14. Each agronomic factor mentioned had 120 chances of being mentioned, because there were 120 respondents. The sum of responses given was determined. Frequency percentages were then calculated based on the total number of responses given.

2.3 Results and Discussion

2.3.1 Farmer perceptions on factors causing cassava root bitterness

Farmers described variety as being the ultimate contributor to cassava root bitterness. Other factors mentioned as contributors to cassava root bitterness were based on effects of environmental growth conditions and also on effects of agronomic practices used by farmers. The mentioned perceived contributions to the mentioned factors are shown in Table 2.1 below.

Table 2.1 Farmers' perceptions (responses) on factors influencing bitterness in cassava roots

Factors influencing root bitterness	Responses from Mtwara Rural District n = 59	(%)	Responses from Newala District n = 61	(%)	Total (both districts) n = 120	(%)
1. Variety	59	100.0	61	100.0	120	100.0
2. Environmental factors						
a. Soil characteristics	1	1.7	16	26.2	17	14.2
b. Seasons (wet or dry)	5	8.5	0	0.0	5	4.2
3. Farmers' agronomic practices						
a. Plant age at harvest	0	0.0	9	14.8	9	7.5
b. Poor weeding	1	1.7	1	1.6	2	0.8
c. Piecemeal harvesting	1	1.7	0	0.0	1	0.8
d. Branch pruning	1	1.7	0	0.0	1	0.8

2.3.1.1 Variety

All the 120 farmers interviewed insisted that if the community classifies a variety as 'bitter variety' then it means that the roots it produces are naturally bitter and if classified as 'sweet variety' then the roots it produces are naturally sweet. In agreement with the farmers' perception, researchers had attributed the cyanogenic character of a variety as being due to an inheritable trait involving a specific gene (Gleadow and Woodrow 2000; Kizito *et al*., 2007) and that the production of small amounts of cyanogenic glucosides in sweet varieties is regulated by a recessive gene complex (Dixon *et al*., 1994).

2.3.1.2 Soil characteristics

Soil type appeared to be of concern as a contributing factor to cassava bitterness by 17 (14.2%) respondents, with most of them being from Newala district. Although the majority of the farmers were often not specific about which cassava varieties changed taste with soil type, the variety *Kigoma* was however specifically identified as having the tendency of changing from sweet to bitter with soil type. Cassava bitterness was associated with four soil factors or types (Figure 2.1).

Fatigued soils and red clayey soils were identified by the farmers as the major causes of bitterness in cassava roots. Farmers described fatigued soils as soils that had lost their fertility due to being continuously cultivated. They described red clayey soils as being red coloured soils that could be used for building. Fewer respondents mentioned red soils (6.25%) and sandy soils (6.25%). It is however not clear whether the red soils mentioned by some respondents are the same as the red-clayey soils mentioned by others, for some respondents simply described the soils only as being red soils. The two categories of 'red' soils were thus recorded separately here.

However, when combined, 50.00% of the respondents consider this factor as half of the problematic soils causing bitterness in cassava roots.

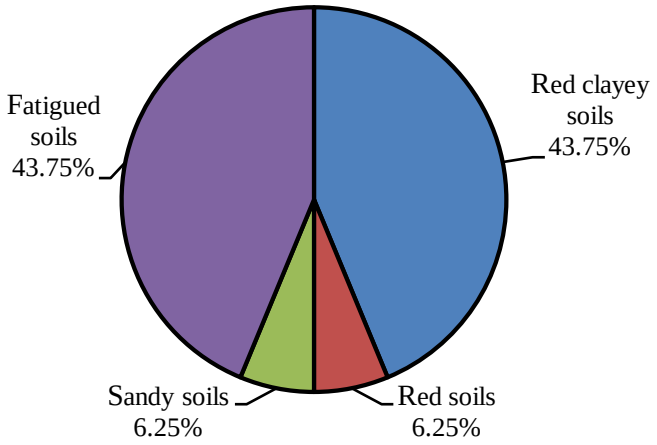

Figure 2.1 Farmers' responses on soil factors or types associated with bitterness in cassava roots

Farmers gave special emphasis to the red-clayey soils located in the Makote area of Newala district. These soils were described as having a thin 'white' top soil layer which was then followed by a thick red coloured soil layer from which it got its description. This description matches the description given by Bennett *et al*. (1979b), where the majority of soils on the Makonde plateau were described as having a thin organic layer overlying a subsoil which increases in redness with depth. Although paler coloured soils are also present, red soils are more common on the Makonde plateau (Bennett *et al.*, 1979b). Bennett *et al*. (1979b) generally describes these soils as being impoverished and as having a poor nutrient holding capacity due to their low cation exchange capacity (< 10 cmol/kg). Being well drained (Bennett *et al.*, 1979b), the red colour of these soils indicates higher concentrations of red coloured

manganese dioxide and unhydrated iron oxide minerals (Chesworth, 2008; IUSS Working GroupWRB, 2014). Both iron and manganese oxide minerals are products of intense weathering and high concentrations of these minerals thus indicate a high degree of weathering in soils (Brady and Weil, 2008). The red soils thus contain higher proportions of nutrient poor minerals, making them low in fertility. Farmers believe that red soils change the taste of sweet cassava to bitter. Some farmers indicated that red soil under anthills caused cassava grown on these soils to become bitter.

In addition, some farmers believe that some farmlands easily became 'tired' or 'fatigued' after being cultivated for only a few years. They attributed the exhaustion to loss of soil fertility. Volosciuc and Josu (2014) define soil fatigue as the exhaustion of the soil through depletion of essential plant nutrients. Hence, like the red soils, fatigued soils are also nutrient poor, and both types of soil are thus capable of causing nutrient stress in cassava (Howeler, 1995; CIAT, 2011). The short fallow periods (4 years on average) practiced in both districts (Bennett *et al*., 1979) further do not allow for proper soil nutrient and carbon restoration (Palm *et al*., 2005), which further enhances nutrient depletion.

Very few studies have been carried out on the role of poor soil fertility on cassava cyanogenic glucoside production. In their studies, Mohan Kumar *et al*. (1977) and Susan John *et al*. (2005) found that improved N, P and K supply, when added in some combinations and rates, could help reduce root total cyanide levels compared to levels in unfertilised plants. Reduced cassava root bitterness was observed as a result of the improved supply of N, P and K in the study by Mohan Kumar *et al*. (1977). These findings probably explain the increased root bitterness observed on nutrient

poor soils. Conversely, higher total root cyanide levels were obtained in cassava grown on more fertile Fluvisols and Andosols as compared to levels found in cassava grown on the highly weathered nutrient poor Nitisols in the study by Ndung'u *et al*. (2012). Increased cassava root bitterness was also observed in cassava grown on more nutrient rich soils with higher levels of basic cations (pH as high as 7.8; K as high as 1.9 cmol/kg) and soil organic matter (as high as 8.2%) in the study by Alou *et al*. (2014). Poor soil fertility may thus not always lead to increased root bitterness as was perceived by the farmers in the present study.

Due to their low CEC and water holding capacity, the problem of sandy soils is also related to poor fertility and to poor water retention (Hartmann and Chinabut, 2005). Increased cassava root bitterness observed on nutrient poor sandy soils such as those in Mtwara region, could thus additionally be influenced by water stress due to the poor water holding capacity of sandy soils.

2.3.1.3 Natural water stress conditions

Five farmers interviewed (4.2%) had observed that natural water stress conditions such as yearly seasons and semi-arid conditions constitute important factors that influence bitterness in cassava roots. All the five farmers were from Mtwara Rural district. Some of the farmers were able to name the varieties whose taste changed with season. The varieties, identified by different respondents, were *Liwoyoka, Kigoma mafia, Musa Saidi, Nachinyanya, Badi, Vincenti, Mnalile Kuchumba and Mtukane*. All the varieties mentioned, except for *Musa Saidi*, were sweet varieties that eventually became bitter. Most respondents mentioned that the bitter taste arose in the dry season; a few respondents however mentioned that the bitter taste arose in

the wet season. One respondent attributed the occurrence of bitterness in cassava as being due to the semi-arid environment in which it is grown.

As previously mentioned, water stress caused by seasonal dry periods is able to increase cyanogenic glucoside levels in cassava roots (Tan, 1995; Sriroth *et al*., 2001; Hular-Bograd *et al*., 2011; Vandegeer *et al*., 2013). This is possibly the reason why the farmers in Mtwara Rural district observed increased root bitterness during the dry season period. The farmers' observation agrees with the findings of Banea-Mayambu *et al*. (1997) and Cigleneki *et al*. (2011) who found that konzo (and thus high cyanogenic glucoside levels in cassava) occurred in a seasonal pattern, with most cases occurring during the dry season. Oluwole (2015) additionally found a high coefficient of determination ($r^2 = 0.9$) between normal yearly cyclic changes in precipitation and cassava cyanide intoxication that resulted in konzo.

It was also observed by one respondent that since the survey area is semi-arid, cassava plants were exposed to harsh environmental or water stress conditions that caused the roots to become bitter. Mtwara region receives rainfall amounts that are barely sufficient to sustain crop growth in most years (Schechambo *et al*., 1999; Morris *et al.*, 2002). The long inter-seasonal dry spells (normal dry seasons) and droughts characteristic of semi-arid areas (Morris *et al*., 2002; Mongi *et al*., 2010) contribute to water stress. In addition, the three to four weeks intra-seasonal dry spell characteristic of the rainy period in Mtwara region (De Pauw, 1984) could constitute another source of water stress in cassava plants.

Higher frequency of konzo that occurs during periods of water stress may be connected to a reduction in food availability (e.g. resulting from poor maize harvest), which pressures farmers to adopt short-cut methods of processing cassava roots that

led to unsafe foods. The farmers' opinion documented in this study suggest that, through taste perceptions, most farmers became conscious that an increase in bitterness (associated with high cyanogen content) occurs in some cassava varieties during dry season.

2.3.2.4 Farmers' agronomic practices

After soil type, the length of time matured roots are left un-harvested was claimed by nine (7.5%) respondents to be the other contributing factor to cassava bitterness. Two sweet cassava varieties were pointed out as being particularly prone to becoming bitter when left unharvested for long. The varieties *Kigoma* and *Kifuru* were mentioned in this connection.

Alves (2002) and McMahon *et al*. (1995) both mention that cyanogenic glucoside production in cassava plants may also depend on their growth stage (plant age). A reduction in cyanogen levels was observed in flour produced from roots of a bitter cassava variety with increased age (six, eight, 10 and 12 MAP) (Chotineeranat *et al*., 2006). Conversely, no differences were observed in the cyanogen content of fresh cassava roots of a sweet cassava variety harvested at three month intervals, starting from 12 to 24 MAP (Tan, 1995), similarly no significant differences were observed in root cyanogen levels of root parenchyma of a bitter cassava variety at different growth stages in the study by Hular-Bograd *et al*. (2011). On the contrary, a strong inverse correlation was seen with growth stage in the cyanide content in peels. These findings suggest that the age dependent influence on cyanogenic glucoside production may depend on variety. Unlike the farmers' observations, the research findings do not restrict age dependent root bitterness to only sweet varieties. The

observed increase in root bitterness with increased plant age in the sweet varieties may probably be due to a loss of root quality (Bokanga, 1999).

The farmers additionally claimed that roots of late maturing bitter cassava varieties, when harvested early, were immature and very 'toxic'. They explained that cassava flour produced from roots of young bitter cassava varieties was toxic, even when produced using their more efficient traditional forms of processing. Leaving bitter cassava varieties to grow for a longer time period was, thus, one method claimed by farmers in Southern Tanzania to reduce cassava toxicity. The bitter varieties were usually left unharvested until 24 - 36 months after planting (MAP) while the sweet varieties that were said to generally mature early were usually harvested at 12 - 18 MAP.

Sweet cassava varieties are also harvested early in other parts of Africa because they bulk early, while bitter varieties can be left unharvested for even up to 39 MAP because they store longer in the field (Nweke *et al*., 2000). If root bitterness in bitter varieties is well correlated with increased cyanogenic glucosides, then reduced root bitterness believed to occur with increased plant age in the bitter cassava varieties agrees to the findings by Chotineeranat *et al*. (2006). Not all bitter varieties were, however, late maturing. For example, the variety *Mohammed Mfuame*, commonly grown in Mtwara Rural district, was a bitter cassava variety that matured within a year and was thus harvested early.

Poor weeding practices were a concern to farmers as an additional factor contributing to cassava bitterness. Two farmers explained that when cassava was poorly weeded, the roots it produced tended to be bitter. Weeds are a form of biotic plant stress. They tend to grow faster and seriously compete with cassava for light, water and nutrients

(Howeler, 2014). According to Jørgensen *et al*. (2005), biotic stress factors are able to influence cyanogenic glucoside production in cassava plants. In agreement with the farmers' perception, a study by Alou *et al*. (2014) found that delayed weeding or no weeding at maturity resulted in increased cassava root bitterness.

Some farmers carried out piecemeal harvesting, although it was also common for them to harvest all the cassava in a field all at once. Piecemeal harvesting is a traditional cassava harvesting method used by farmers to achieve longer storage (in the field), by harvesting only the roots needed at a time while leaving the rest un-harvested (Nduwumuremyi *et al*., 2016). One farmer noted that once the first roots had been removed from a cassava plant the remaining roots in the soil still attached to the plant eventually became bitter. Hardly any studies have been done on the effects of piecemeal harvesting on cyanogenic glucoside production. It can however be assumed that just like actively growing cyanogenic young plants or plant parts (Knight and Walter, 2002), the probably rapidly developing smaller roots left behind after piecemeal harvesting (Onwueme and Charles, 1994; Ntawuruhunga *et al*., 2007) may also contain high cyanogenic glucoside levels. It is however unclear as to the time period after piecemeal harvesting that the root bitterness started. Plants that are piecemeal harvested may go on to form new roots before being harvested again (Onwueme and Charles, 1994) and could thus have high cyanogen levels while still developing.

A common agronomic practice among cassava farmers in Southern Tanzania is branch pruning (cutting-back or debranching) of cassava plants. Branch pruning was mentioned by one farmer to contribute to bitterness in cassava roots. One reason why farmers pruned their cassava plants was to shorten cassava plants to make browsing

easier for goats; this was done because goats tended to damage tall cassava plants as they struggled to reach for the leaves. Secondly, cassava stems are cut from the plant to be used as a mat for drying cassava chips (or *makopa*) in the field. To avoid placing the freshly peeled clean cassava roots on the bare ground, cassava stems are used. Elsewhere pruning is known to be done to increase light for interception by intercrops in multiple cropping systems (Fakir *et al*., 2011). Despite intercropping being commonly practiced by farmers in the survey districts, it was not the reason why they pruned cassava plants. This is because cassava plants were pruned when all other crops had already been harvested and thus no longer causing plant competition. Branch pruning may however also be carried out to encourage rapid re-growth of cassava shoots (Hue *et al.*, 2012) that may be used to provide foliage for use as vegetable or as ruminant feed (Dada and Oworu, 2010).

Research has shown that cyanogenic glucosides are synthesised in the leaves of cassava plants and transported to the roots (Siritunga and Sayre, 2003; Jørgensen *et al*., 2005). Increased cyanogen levels in leaves could hence result in increased root cyanogen levels. Since regrowth contains higher levels of cyanogenic glucosides (Knight and Walter, 2002), it can be assumed that more cyanogenic glucosides are transported to roots from regrowth following pruning. This is what possibly leads to the perceived root bitterness observed by farmers when cassava is pruned.

2.4 Conclusions

The study revealed a number of non-water stress and variety-related factors that additionally were claimed to contribute to bitterness in cassava roots; they include factors such as soil type (especially nutrient low red soils), farmers' agronomic

practices like cassava harvesting stage, poor weeding practices, piecemeal harvesting, and branch pruning. These factors may thus be influencing cyanogenic glucoside production in cassava grown in the region, contributing to the konzo episodes observed in the area in the past and probably still occurring although not being reported.

2.5 Recommendations

In view of the results, the following recommendations are given:

i. Further investigations should be carried out to validate the likely influence of the agronomic factors mentioned by farmers on cassava cyanogenic glucosides.

ii. The degree to which each of the mentioned factors influences cyanogenic glucoside production in cassava plants also needs to be investigated in order to assess their effects on bitterness and cassava safety.

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CHAPTER THREE

3.0 SOIL SAMPLING SURVEY TO REVEAL RELATIONSHIPS OF SOIL NUTRIENT LEVELS TO CYANOGENIC GLUCOSIDE PRODUCTION BY CASSAVA IN MTWARA REGION

Abstract

The influence of nutrient supply on cyanogenic glucoside production in cassava grown on the predominantly infertile soils of Mtwara region is hardly known. A survey of soil nutrient levels in these areas was hence carried out to determine their adequacy for optimal cassava growth and thus their ability to cause plant stress in cassava. Relationships were also explored between soil nutrient levels and root total cyanide (HCN) levels in cassava produced in these areas. The survey was carried out in konzo-affected villages of Mtwara rural and Newala districts which are both situated in Mtwara region. The results of the soil survey revealed that 99.1% of the fields were deficient in N, 34.8% in P, 84.3% in K, 13.9% in Ca, 84.3% in Mg, 63.5% in S and 93.0% of these fields were deficient Zn. Most fields had multiple nutrient deficiencies indicating that cassava in the region grows under conditions of severe nutrient stress. The only associations obtained between the soil nutrient levels and fresh root HCN levels of sampled cassava varieties were for soil S ($r = 0.443$, $p = 0.045$) and soil P ($r = 0.451$, $p = 0.040$).

3.1 Introduction

Soils of Mtwara region have low soil fertility (Bennett *et al*., 1979; Mowo *et al*., 1993). The soils may thus be unable to supply crops grown on them, like cassava (*Manihot esculenta* Crantz), with adequate quantities of nutrients needed for their normal growth. Nonetheless the region is one of the main cassava producing regions of Tanzania (Mkamilo and Jeremiah, 2005). Howeler (2014) mentions that low soil fertility is able to cause plant stress. Thus, being an abiotic form of plant stress, poor soil nutrient supply could also be influencing cyanogenic glucoside accumulation in cassava (Jørgensen *et al*., 2005; Ndung'u *et al*., 2010; Ndung'u *et al*., 2012) produced in the region.

Some areas in the region have experienced cases of cassava cyanide intoxication that resulted in a health disorder called konzo (Mlingi *et al*., 1992; Mlingi, 2002; Mlingi, 2011; Mlingi *et al*., 2011). Drought related water stress is the main agronomic factor attributed to causing the increased cyanogenic glucoside levels in cassava plants that exacerbate cyanide intoxication during konzo outbreaks (Mlingi *et al*., 2011). Thus, increased occurrence of cassava cyanide intoxication is associated with drought periods (Omonona and Akinpelu, 2010; Mlingi *et al*., 2011; Cliff *et al*., 2011). The additional influence of poor nutrient supply from the predominantly nutrient poor soils, which are a permanent feature of the agronomic environment in Mtwara region, can however not be ignored.

How soil nutrient supply could be additionally influencing cyanogenic glucoside production in Mtwara region and the nutrients particularly responsible remains to be known. A survey was thus carried out to determine the adequacy of various soil nutrient levels for optimal cassava growth in Mtwara region. The study was carried

out in areas previously reported to have been affected by konzo. Relationships between root HCN levels and soil nutrient levels were additionally investigated.

3.2 Materials and Methods

3.2.1 Description of study area

Three districts have been reportedly affected by konzo in Mtwara region, namely Masasi, Mtwara Rural and Newala districts (Mlingi *et al*., 2011). This study focused on konzo-affected villages of Mtwara Rural and Newala districts, which are two of the five districts of Mtwara region (S 10^{o}16'25", E 40^{o}10'58"). The two districts studied (Mtwara Rural and Newala) lie in Tanzania's, Coastal Lowlands agroecological zone (De Pauw, 1984; Mowo *et al*., 1993). Soils in the region are generally classified as Ferralic Cambisols and are predominantly sandy (De Pauw, 1984; Mowo *et al*., 1993). The rainfall in the region is mono-modal and ranges from 800 to 1000 mm/year and the maximum and minimum temperatures vary from 29 to 31 ^{o}C and 19 to 23 ^{o}C, respectively (De Pauw, 1984). Both districts lie on the Makonde plateau, with Newala lying to the west and Mtwara Rural to the eastern side of the plateau.

3.2.2 Selection of household fields surveyed

In order to investigate soil nutrient adequacy and root HCN levels in the region, soil and root samples were collected from fields from konzo-affected villages in Mtwara Rural and Newala districts. The survey was carried out from 7th to 16th October, 2014, which was during the hot-dry season which is the common harvest time for cassava in the region. A total of 112 fields, 57 from konzo affected villages of

Newala district and 55 from konzo-affected villages of Mtwara Rural district were sampled. The villages were selected from 18 villages visited during a konzo rehabilitation and prevention program that was carried out in 2008 to 2009, through collaboration between the Tanzania Food and Nutrition Centre (TFNC) and the Tanzania Red Cross Society (TRCS), with technical support from the Australian National University and funding from AusAID (Mlingi *et al*., 2011).

Four villages in Mtwara Rural and Newala districts were randomly selected. Mdimba, Ngalu, Songambele and Mkunjo were the villages from Newala district whereas for Mtwara Rural district the villages were Njengwa, Nyundo, Niyumba and Kiromba. From each village 15 households were randomly picked and soils from their farm fields sampled. The 15 households from each village were randomly selected from a 2012 census list of the villages. Each household was given a unique number and the 15 households were randomly picked using the 'RAND' function in Microsoft Excel. Household representatives with full knowledge of the farm fields gave direction to the location of the households' field for sampling.

3.2.3 Soil sample collection and chemical analyses

Composite soil samples of about 500 g were collected from every household's field. This was done by collecting soil from at least 10 randomly selected points on the cassava fields from the top 20 cm layer of the soil (CIAT, 2011). Soil samples were first air-dried and later analysed for soil pH, organic carbon (OC), total nitrogen (N), available phosphorous (P), exchangeable potassium (K), calcium (Ca) and magnesium (Mg), available sulphur (S), available zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) and exchangeable aluminium (Al).

All laboratory methods used were as outlined by Møberg (2001), as follows: Soil pH was determined in a 1:1 soil to water solution. The Bray No. 1 method was used to extract P. The exchangeable cations K, Ca and Mg were determined using 1 N ammonium acetate (pH 7) extracting solution, S was determined using the calcium phosphate extraction method while Zn, Cu, Fe and Mn were determined using diethylenetriaminepentaacetic acid (DTPA). Nitrogen was analysed using the micro Kjeldhal method and OC was analysed using the Walkley and Black method. Exchangeable Al was extracted using 1 N potassium chloride (KCl), and it was done only on soil samples that had pH of less than five. The value obtained for exchangeable Al and the respective Ca, Mg and K levels for the soils were then used to calculate the soils' Al saturation, using equation (i) (CIAT, 2011).

$$Al\ saturation\ (\%) = \left(\frac{Al}{Al+Ca+Mg+K}\right) cmol/kg \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

3.2.4 Root sampling and total hydrogen cyanide determination

Root samples of bitter and sweet cassava varieties commonly grown in the villages were collected from fields where soil samples were taken to assess root cyanogen content. Root sampling was done as outlined by Essers (1994). Cassava root samples were collected from four randomly selected plants of the same variety from a farmers' field. Only plants identified by famers as being ready for harvest were selected. Three roots were sampled per plant for analysis. Total cyanide content in fresh cassava roots was determined within 24 hours after harvest using the picrate paper method (Egan *et al.*, 1998; Bradbury *et al*., 1999). A 100 mg section of fresh

cassava root taken from the middle of the root was placed in a vial, with buffer solution and a picrate paper. The contents in the vial were then left to incubate in the dark at room temperature for 16 – 24 hours. The picrate papers darkened by the cyanide liberated from the fresh root sections were then eluted in 5 ml of distilled water. The absorbance of the solution so obtained was then determined using a spectrophotometer at 510 nm. The total hydrogen cyanide (HCN) content on a fresh weight basis was then calculated using equation (ii).

$$\text{Total HCN (mg/kg)} = 396 \times \text{absorbance} \quad \text{(ii)}$$

where 396 is a value derived from the calibration curve between picrate solutions placed in known standard cyanide solutions and their absorbance values (Egan *et al.*, 1998; Bradbury *et al*., 1999).

3.2.5 Data Analysis

Descriptive statistics (means, frequencies, and maximum and minimum values) were used to analyse the collected data. Mean differences between soil chemical characteristics of soils between the two districts were determined using a t-test of independent samples. Relationships between soil nutrients and root HCN levels were determined using the Pearson's correlations coefficient test at 5% probability level. A one-way analysis of variance (ANOVA) was also carried out on the fresh root HCN levels of the sampled cassava varieties. All analyses were done using GenStat Edition 14.

3.3 Results and Discussion

3.3.1 Soil chemical characteristics in the cassava producing areas and implications for cyanogenic glucoside accumulation in cassava

Table 3.1 shows the proportion of sampled fields in Mtwara region that are deficient in each essential nutrient required for the optimal growth of cassava. The proportions of fields outside the optimal range and other important soil chemical characteristics like pH, OC and Al saturation, are also included in Table 3.1. In Table 3.2 mean differences of the soil chemical characteristics between the two districts are shown.

Soil pH levels in the region were mainly in the desirable range (4.5 – 7.0) and were hence not too low or too high for good cassava growth (CIAT, 2011). Only 0.9% of the fields had soil pH levels below 4.5, while none had pH levels above 7.0. Bennett *et al*. (1979b) mentions that the pH of soils in Mtwara region is not as low as might be expected for highly weathered soils and is usually > 5.0 because of the presence of limestone in the parent material of these soils. Limestone has reducing effects on soil pH (Brady and Weil, 2008). Aluminium toxicity similarly posed no limitations on cassava growth with all fields having none or low levels of Al saturation. Soils in Mtwara rural had more favourable pH levels than soils in Newala district (Table 3.2).

Almost all (99.1%) the fields sampled had N levels below what is considered as sufficient (0.20 – 0.50 %N) for a broad range of tropical crops (Landon, 2014). The low OC levels (< 2%) in all the fields' supports the low levels of N. Soil organic carbon is a main source of N for crops grown without fertiliser application (Landon, 2014). Despite the significantly similar N levels in soils from the two surveyed districts, soils in Newala district had higher OC levels.

Table 3.1 Levels of some nutrients and other chemical characteristics in soils from Mtwara Region and their rating for suitability for cassava production

Soil parameter	Nutrient deficient fields (%)	Mean n = 112	Minimum		Maximum		Range/level rated as suitable for cassava production	Rated according to:
			Value	Rating	Value	Rating		
pH*	0.9	5.29	4.44	l	6.96	m	4.5 – 7.0	CIAT (2011)
OC (%)	100.0	0.63	0.31	vl	1.22	vl	4.0 – 10.0	Landon (2014)
N (%)	99.1	0.07	0.01	vl	0.33	m	0.20 – 0.50	Landon (2014)
P (mg/kg)	34.8	8.21	1.19	l	34.52	h	< 4.2	Howeler (2002)
K (cmol/kg)	84.3	0.09	0.02	vl	0.32	h	0.15 – 0.25	CIAT (2011)
Ca (cmol/kg)	13.9	2.02	0.46	l	4.49	m	1.0 – 5.0	CIAT (2011)
Mg (cmol/kg)	84.3	0.26	0.02	vl	0.76	m	0.40 – 1.00	CIAT (2011)
S (mg/kg)	63.5	5.30	0.60	l	23.51	h	< 6.0	Landon (2014)
Zn (mg/kg)	93.0	0.34	trace	vl	1.79	m	1.0 – 3.0	Motsara and Roy (2008)
Cu (mg/kg)	95.7	0.01	trace	vl	0.43	m	0.3 – 0.8	Motsara and Roy (2008)
Fe (mg/kg)	0.9	32.87	3.19	l	83.45	vh	4.0 – 6.0	Motsara and Roy (2008)
Mn (mg/kg)	0.0	9.74	1.29	m	71.71	vh	1.2 – 3.5	Motsara and Roy (2008)
Al Saturation (%)ǂ	0.0	9.27	0.0	m	32.94	m	< 75.0	CIAT (2011)

Where: vl, l, m, h and vh stand for very low, low, medium, high and very high.

*Percentage of fields with low pH reported.

ǂPercentage of fields with high levels of saturated Al reported and n = 72

Table 3.2 Mean differences between nutrient levels and other chemical characteristics of soils from Mtwara rural and Newala districts

Soil parameter	Mtwara rural district n = 55					Newala district n = 57					t-test
	Mean	Minimum	Rating	Maximum	Rating	Mean	Minimum	Rating	Maximum	Rating	p-value
pH	5.48	4.50	l	7.00	m	5.10	4.40	l	6.60	m	**< 0.001**
OC (%)	0.55	0.31	vl	1.22	vl	0.71	0.35	vl	1.12	vl	**< 0.001**
N (%)	0.06	0.01	vl	0.13	l	0.07	0.04	vl	0.33	m	0.147
P (mg/kg)	3.98	1.19	l	23.86	h	12.29	3.72	l	34.52	h	**< 0.001**
K (cmol/kg)	0.13	0.05	vl	0.32	h	0.05	0.02	vl	0.11	l	**< 0.001**
Ca (cmol/kg)	2.14	0.68	l	4.49	m	1.90	0.46	l	4.04	m	0.173
Mg (cmol/kg)	0.29	0.02	vl	0.76	m	0.23	0.06	vl	0.66	m	**0.018**
S (mg/kg)	6.28	1.27	l	23.51	h	4.34	0.60	l	11.56	h	**< 0.001**
Zn (mg/kg)	0.28	0.01	vl	1.07	m	0.39	0.09	vl	1.79	m	**0.034**
Cu (mg/kg)	0.00	0.01	vl	0.08	vl	0.03	0.01	vl	0.43	vl	**0.010**
Fe (mg/kg)	22.30	3.19	l	76.83	vh	43.26	13.95	vh	83.45	vh	**< 0.001**
Mn (mg/kg)	12.99	1.82	m	71.71	vh	6.54	1.29	m	35.58	vh	**< 0.001**
Al Saturation (%)*	7.02	0.00	m	25.27	m	10.62	0.00	m	32.94	m	0.108

Numbers in bold are significant at $p < 0.05$. Where: vl, l, m, h and vh stand for very low, low, medium, high and very high.

*Where: n = 27 and n = 45 for Al Saturation in Mtwara rural and Newala districts, respectively.

An improved supply of N, on N deficient soils, was found to reduce root HCN levels in the study by Mohankumar *et al*. (1988). An improved supply of N could thus be beneficial in reducing cassava root HCN levels on these N deficient soils. An increased supply of N is however mainly associated with increasing cyanogenic glucoside levels in cassava plants (Allem *et al*., 2002; Howeler, 2009). In the study by Susan John *et al*. (2005) higher HCN levels were mainly observed in roots of plants supplied with N containing fertilisers.

Soil P levels varied from very low to high in the sampled fields with more fields having sufficient P. Only 34.8% of the sampled fields had soil P levels below the critical level of 4.2 mg/kg reported by Howeler (2002). Not much research has been done on the relationship between soil P availability and root HCN accumulation. In the study by Susan John *et al*. (2005) sole P fertiliser application decreased root HCN levels (85.7 mg/kg) compared to levels obtained in roots of unfertilised plants (107.1 mg/kg). De Bruijn (1973) however reported that P had no influence on cassava root HCN levels. Root development increases when P is adequate in soils, enabling greater water uptake particularly in dry conditions (Shaxson and Barber, 2003). With adequate P, cassava plants would thus be able to mitigate water stress which commonly occurs in the region. Water stress in the region is associated with increased HCN levels in cassava roots (Mlingi *et al*., 2011). Soil P levels were much higher in Newala district, increased cyanogenic glucoside levels associated inadequate P would thus be less in this district.

One important nutrient limitation in the sampled soils was low soil K. About 84.3% of the sampled fields had soil K levels below 0.15 – 0.25 cmol/kg, which is the amount of K in soils considered adequate for healthy cassava growth (CIAT, 2011).

An adequate supply of soil K through fertiliser application has been shown to often reduce HCN levels in cassava roots (Howeler and Kawano, 1988; Susan John *et al*., 2005; Endris, 2006; Susan John *et al*., 2007). The low soil K levels could thus contribute to increased root cyanogenic glucoside levels in cassava produced in the region. No effects on root HCN were however found with K fertiliser application in the study by Cuvaca *et al*. (2015). Problems of increased cyanogenic glucosides in cassava would probably be greater in Newala district, due to the lower levels of K in its soils.

The nutrients Ca, Mg and Zn were deficient in 13.9%, 84.3% and 93.0% of the fields, respectively. An increased supply of the nutrients Mg and Zn in soils, has been shown to reduce cassava root HCN levels (Howeler and Kawano, 1988; Susan John *et al*., 2005). Soil Ca levels were mainly adequate (> 1 cmol/kg) for cassava production (CIAT, 2011) on most farm fields. Like pH the adequate Ca can probably also be attributed to the presence of limestone in the parent material of these soils (Bennett *et al*., 1979b). De Pauw (1984) additionally mentions that the soils in Mtwara region are calcareous and thus have high levels of Ca (Brady and Weil, 2008).

Reduced root HCN levels have been reported with improved Ca supply (Gosh and Nair, 1986; Mohankumar *et al*., 1988). Reduced root HCN levels have also been reported with improved Zn supply to cassava (Mohankumar *et al*., 1988). In the study by Susan John *et al*. (2005) root HCN reduction with organic fertilisers was only achieved when ash was applied with them, the reduction in root HCN was attributed to the presence of K, Ca and Mg in the ash. Adequate supplies of the nutrients K, Mg, Zn (Cakmak, 2006; Marschner, 2012) and also of N and Ca

(Cakmak 2006) are also known to help plants mitigate abiotic stress. Abiotic stress in cassava is known to influence cyanogenic glucoside production (Jørgensen *et al*., 2005) by mainly increasing it, as observed with water stress (Vandegeer *et al*., 2013). Deficiencies of N, K, Ca, Mg and Zn could thus result in increased cyanogenic glucoside production in cassava produced in the region. Only Ca was adequate in most soils from both districts out of the rest of the nutrients.

Sulphur was deficient in 63.5% of the sampled fields; these fields all had S levels below the critical level (< 6 mg/kg) recommended by Landon (2014). Mohankumar *et al.* (1988) and Gosh and Nair (1986) reported reductions in root HCN levels with an increased supply of soil S. There is thus a possibility that low levels of S in Mtwara region could have an increasing effect on root cyanogenic glucoside production, particularly in Newala district.

Copper was additionally deficient in many fields (95.7%). The levels of soil Cu considered as adequate for crop growth range from 0.3 – 0.8 mg/kg. On the other hand Fe and Mn were mainly sufficient to very high in all fields; no fields had low Mn levels and only 0.9% of the fields had low Fe levels. There are hardly any reports on the influence of Cu, Fe or Mn on root HCN levels. The very low soil Cu levels could however cause nutrient stress in cassava grown in the region. High levels of Fe and Mn were expected as soils in Mtwara region are predominantly Ferralic cambisols (De Pauw, 1984; Mowo *et al*., 1993) and are thus dominated by Fe and Mn oxides and hydroxides (IUSS Working GroupWRB, 2014).

3.3.2 Total hydrogen cyanide levels of sampled varieties

Root HCN levels for the varieties sampled at harvesting time are given in Table 3.3.

Table 3.3 Total hydrogen cyanide levels of fresh cassava roots of sampled cassava varieties from both districts

Variety	Type	n	HCN levels (mg/kg)		
			Minimum	Maximum	Mean*
Badi	Sweet	1	16.8	.	.
Kigoma	Sweet	4	43.1	58.1	49.5[b]
Mnalile Kuchumba	Sweet	3	25.9	109.8	62.2[ab]
Limbanga	Bitter	2	114.4	181.9	148.2[ab]
Mohammed Mfaume	Bitter	1	79.4	.	.
Musa Saidi	Bitter	3	51.0	131.1	85.15[ab]
Namanjele	Bitter	1	44.3	.	.
Nanjenjeha	Bitter	3	54.3	144.3	98.1[ab]
Salanga	Bitter	3	111.3	191.9	147.5[a]

*Means with the same letter are not significantly different at $p < 0.05$ using the Tukey's test, and means based on n = 1 were not included in the analyses.

All root samples of the bitter cassava varieties *Limbanga* and *Salanga* had HCN levels above 100 mg/kg; these levels exceeded the 50 mg/kg limit safe for fresh cassava consumption (Codex Alimentarius Commission, 2013). The two varieties thus had a great potential of causing cyanide poisoning. Cassava varieties with total HCN levels less than 50 mg/kg are classified as innocuous while those with levels between 50 - 100 mg/kg are classified as moderately poisonous; those with levels above 100 mg/kg as dangerously poisonous (Burns *et al*., 2010). Some root samples collected of the bitter varieties *Nanjejeha* and *Musa Saidi* were however below 100 mg/kg. This suggests that some bitter varieties can also at times contain lower HCN levels and may not always pose a danger.

All root samples from the sweet cassava varieties except for some from the variety *Mnalile Kuchumba* had total HCN levels below 100 mg/kg. This suggests that the sweet variety, *Mnalile Kuchumba*, and probably others like it, may also be capable of

accumulating high levels of cyanogenic glucosides. Omitting the varieties that were not replicated (Table 3.3), the ANOVA results showed that only the variety *Salanga* had significantly higher root HCN levels than the variety *Kigoma.*

3.3.3 Associations between soil nutrient contents and total root HCN content

Correlations were carried out to determine whether there were any associations between fresh root HCN levels of bitter and sweet cassava varieties and levels of different nutrients in soils on which they were grown (Table 3.4).

Table 3.4 Correlations between root HCN levels in bitter, sweet and all cassava varieties and various soil properties

Soil parameter	Bitter varieties		Sweet varieties		All varieties	
	r	p - value	r	p - value	r	p - value
pH	0.078	0.800	-0.174	0.681	0.100	0.667
OM	0.486	0.092	0.516	0.191	0.055	0.814
N	-0.140	0.648	0.116	0.784	-0.012	0.958
P	0.355	0.234	0.349	0.397	0.451	**0.040**
K	-0.404	0.171	-0.382	0.351	-0.139	0.548
S	0.677	**0.011**	0.617	0.103	0.443	**0.045**
Ca	-0.029	0.925	0.112	0.792	0.074	0.749
Mg	-0.394	0.183	0.210	0.619	-0.096	0.679
Zn	0.226	0.459	0.217	0.606	0.235	0.305
Cu	0.000	1.000	-0.064	0.880	-0.186	0.420
Fe	-0.067	0.829	0.061	0.885	-0.135	0.561
Mn	-0.367	0.217	-0.290	0.486	-0.114	0.621
Al Saturation	-0.277	0.596	-0.024	0.970	-0.217	0.522

Numbers in bold are significant at $p < 0.05$.

One significant correlation was observed between the soil S and root HCN levels in the sampled bitter cassava varieties. The positive coefficient of correlation (r) of 0.677 ($p = 0.011$) indicated that HCN levels in the sampled fresh roots of bitter cassava varieties increased with soil S levels. Two significant associations were also obtained between soil S ($r = 0.443$, $p = 0.045$) and soil P ($r = 0.451$, $p = 0.040$) levels with root HCN levels in all sampled cassava varieties (bitter and sweet combined). This again shows that root HCN levels in all varieties generally increased with increased soil S and P levels, contrary to the findings by Susan John *et al*. (2005) and Mohankumar *et al*. (1988), respectively.

The correlations were probably affected by the combined influence of the multiple nutrient deficiencies in these soils, which interfered with the single influence of each nutrient. The small sample size, and the heterogeneity of varieties sampled, together with differences in how farmers managed their crop, also probably affected the results observed. Cultural practices like time of planting and weed management may influence cyanogenic glucoside accumulation in cassava (Bokanga *et al*., 1994). A controlled experiment would be needed to properly determine the influences of these individual nutrients on root cyanogenic glucoside accumulation in cassava. Although the results were contradicting, the existence of associations between some soil nutrients and root HCN levels was nonetheless established.

3.4 Conclusions

Soils in Mtwara Region are deficient in N, P, K, Ca, Mg, S, Zn and Cu and are thus unable to supply these nutrients in amounts needed to support the optimal growth of cassava. Nutrient stress is thus expected to occur in cassava grown in the region due

to the inadequate levels of these nutrients. Increased cyanogenic glucoside production in cassava grown in the region could be expected, particularly from deficiencies of K, Ca, Mg and Zn, whose sufficiency contributes to reduction in cyanogenic glucoside production. Relationships between low soil nutrient levels and higher root HCN were however not established.

3.5 Recommendations

The following recommendations are given from the results of this study:

i. Nutrient management practices that will increase levels of N, P, K, Ca, Mg, S, Zn and Cu in soils should be encouraged, to eliminate nutrient stress and the negative effects that stress might have on cyanogenic glucoside accumulation in cassava grown in Mtwara region.

ii. Controlled experiments should be carried out to precisely determine the influences of the individual nutrients, including S and P, on HCN accumulation in cassava varieties commonly grown in Mtwara region.

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CHAPTER FOUR

4.0 EFFECTS OF VARIETIES, SOIL MOISTURE AND NUTRIENT SUPPLY ON CYANOGENIC GLUCOSIDE PRODUCTION IN CASSAVA: A POT EXPERIMENT

Abstract

Soil nutrient supply is often not considered as a contributing factor to increased cyanogenic glucoside accumulation in the edible portions of cassava plants. To investigate its role, a pot experiment was carried out to compare the effects of nutrient supply together with the effects of varieties and soil moisture levels on cyanogenic glucoside production in cassava. Total cyanide (HCN) levels (a measure of cyanogenic glucoside content) in leaves were used as an indicator of cyanogenic glucoside production in cassava plants. The nutrient treatments included a Control, which was designated as $N_0P_0K_0$ where N, P and K were not applied, $N_0P_0K_{25}$ where 25 mg K/kg was applied, $N_{25}P_5K_{25}$ where 25-5-25 mg N-P-K/kg was applied and $N_{50}P_{13}K_{50}$ where 50-13-50 mg N-P-K/kg was applied. The soil moisture treatments included soil moisture levels at 30%, 60% and 100% of field capacity (FC). The two varieties used included an improved sweet variety called *Kiroba* and a local bitter variety called *Salanga*. Results revealed that variety and soil nutrient supply were important (both at $p < 0.001$) in influencing cyanogenic glucoside accumulation while moisture level was not ($p = 0.080$). The interaction between cassava varieties, soil moisture and nutrient supply was also significant ($p = 0.035$), revealing that the

cassava varieties responded differently to soil moisture levels and nutrient supply. Leaf HCN in the variety *Salanga* was mainly dependent on nutrient supply ($p < 0.001$) with values of 157.2, 118.2, 178.2 and 278.3 mg/kg, obtained under the nutrient treatments $N_0P_0K_0$, $N_0P_0K_{25}$, $N_{25}P_5K_{25}$ and $N_{50}P_{13}K_{50}$, respectively. For the variety *Kiroba* all nutrient treatments maintained significantly similar leaf HCN levels at all moisture stress levels, except for the sole K treatment which had significantly higher leaf HCN levels at 30% FC (142.8 mg/kg) than at 60% (51.7 mg/kg) and 100% (44.4%) FC. Nutrient supply was found to have an important influence on cyanogenic glucoside production as did variety.

4.1 Introduction

Cyanogenic glucoside accumulation in cassava (*Manihot esculenta* Crantz) is influenced by several agronomic factors occurring during the growing period (Bokanga *et al*., 1994). The agronomic factors can be both edaphic and atmospheric (El-Sharkawy, 2004; Hue *et al*., 2012). When agronomic conditions are harsh plant stress occurs and for cassava this leads to an undesirable increase in cyanogenic glucosides (Jørgensen *et al*., 2005; Burns *et al*., 2010). Cassava roots containing high cyanogenic glucoside concentrations usually have higher residual amounts of the potentially toxic cyanogenic glucosides retained in processed cassava products (Sriroth *et al*., 2001). Humans consuming such products or the fresh roots without adequate intake of protein consequently ingest more cyanogens, which leads to the occurrence of a paralytic disease called konzo (Siritunga and Sayre, 2004; Cardoso *et al*., 2005). Konzo occurs in many rural-poor African communities where the population mainly relies on improperly processed cassava products combined with a

lack of or inadequate access to sulphur amino acid containing foods that help with detoxification (Nzwalo and Cliff, 2011).

Water stress in cassava plants during droughts or long dry periods increases cyanogenic glucoside levels in cassava (Nzwalo and Cliff, 2011). A number of research findings on the effects of drought on cyanogenic glucoside production by cassava have confirmed that drought related water stress increases cyanogenic glucoside levels (Sriroth *et al*., 2001; Vandegeer *et al*., 2013; Oluwole, 2015). Another cause of high cassava cyanogenic glucoside levels experienced in konzo-affected areas are the bitter genotypes that dominate the farming systems (Mlingi *et al*., 2011; Cliff *et al*., 2011; Cigleneki *et al*., 2011). Bitter genotypes are known to naturally contain high levels of cyanogenic glucosides (Bokanga *et al*., 1994), which can be further increased by environmental stress. Bitter varieties are, however, often preferred due to their high yielding ability (Mckey *et al*., 2010).

Besides the frequent droughts or long dry seasons, another feature characteristic of konzo-affected areas is low soil fertility. Except for the konzo affected areas of Central African Republic, Democratic Republic of Congo (DRC) and Cameroon (Cigleneki *et al*., 2011; Banea *et al*., 2012), all other affected areas are situated along the coast or along lake shores. Some of these areas include Nampula and Zambézia districts in Mozambique (Cliff *et al*., 2011; Akinpelu *et al*., 2011). In Tanzania, the konzo-affected areas include Mtwara and Tarime districts (Howlett *et al*., 1992; Mlingi *et al*., 2011). Soils in these areas are predominantly sandy with low natural fertility (Bennett *et al*., 1979; Howlett *et al*., 1992; Mowo *et al*., 1993; World Bank, 2006). Also, although not situated along the coast but in the Savannah zone, konzo-affected areas in Bandundu Region in DRC are also primarily dominated by

relatively infertile sandy soils (Banea *et al*., 1992; Kisangani and Bobb, 2009). Molua and Lambi (2006) similarly reported that the soils in non-coastal Cameroon's Eastern region, where konzo-affected villages are located, have a sandy clay texture and are largely degraded. Soils of low soil fertility are capable of causing nutrient stress in cassava (Howeler, 1995). Being a form of environmental stress, there is a possibility that low soil fertility could also cause increased cyanogenic glucoside levels (Jørgensen *et al.*, 2005).

The contribution of low soil fertility to cyanogenic glucoside accumulation is, however, commonly overlooked in konzo-affected areas. This is mainly because of greater association of konzo with periods of soil moisture stress. Despite this association being a major factor, sporadic cases of konzo still occur in the absence of drought or dry months, in areas where konzo is persistent (Ernesto *et al*., 2002; Cliff *et al*., 2011). Ernesto *et al.* (2002) pointed out that persistent konzo that also occurs outside the harvest season could partly be due to consumption of cassava containing high cyanogen content outside these main harvest time periods. This observation suggests that other agronomic factors besides water related stress in konzo-affected areas may also be influencing cassava cyanogenic glucoside production. Furthermore, higher cyanogenic glucoside levels have been reported in cassava when it is not water stressed as opposed to when it is water stressed, showing that water stress alone may not be enough to explain increased cyanogenic glucoside levels (Bokanga *et al*., 1994).

The objective of the study was to determine whether nutrient supply has an influence on cyanogenic glucoside production in cassava comparable to that of variety and soil moisture.

4.2 Materials and Methods

The effects of varieties, nutrient supply and soil moisture levels on cyanogenic glucoside production in cassava were investigated using a pot experiment. Total cyanide (HCN) levels in cassava leaves were used to indicate the effects of the three factors on cyanogenic glucoside production.

4.2.1 Soil sampling

An infertile soil was used in the pot experiment in order to mimic the soil conditions in most konzo-affected areas. Carrying large amounts of soil from Mtwara or Newala districts to Sokoine University of Agriculture (SUA) (S 6°51'13", E 37°39'26") in Morogoro district, Tanzania, where the experiment was carried out, proved to be impossible. Soils of similar characteristics as those of Mtwara were thus collected from Soga village (S 6°49'54", E 38°51'49") in Kibaha district. Like most parts of Mtwara and Newala districts, Kibaha district is also located in the coastal agro-ecological zone. The soils in the district are also predominantly Ferralic Cambisols and are similarly associated with low natural soil fertility (De Pauw, 1984; Veldkamp, 2001).

Ten different points were selected prior to soil collection from the field where the soil for the pot experiment was to be obtained. After removing surface litter, top soil was collected from the selected points from the depth of 0 - 20 cm and placed in sacks before being transported to SUA. The collected soil was then thoroughly mixed together and passed through a 0.80 cm sieve to remove clods and trash after which it was left exposed to air-dry. It was turned from time to time to facilitate quicker and even drying. When sufficiently dry, the moisture content of the soil was determined gravimetrically. The value obtained was then used to calculate the amount of air-dry

soil equivalent to 5 kg of the oven-dry soil, which was placed in plastic pots of 5 L capacity, just before planting.

4.2.2 Soil analysis

Before setting the experiment, the soil was analysed for organic carbon (OC), soil reaction (pH), total N, available P, available K, exchangeable calcium (Ca), exchangeable magnesium (Mg), available sulphur (S), extractable zinc (Zn), copper (Cu), iron (Fe) and for soil texture. All soil analysis procedures were carried out as outlined by Møberg (2001) as follows: pH in H_2O 1:1; OC using the Walkley and Black method; N was determined by micro-Kjeldahl digestion; P using the Bray No. 1 method; Sulphate-S using calcium phosphate extracting solution; K, Ca and Mg in 1 N ammonium acetate; extractable Zn, Cu and Fe in diethylenetriaminepentaacetic acid (DTPA); Texture by the hydrometer method. The results obtained are shown in Table 4.1, together with their respective ratings.

4.2.3 Treatments used in the pot experiment

4.2.3.1 Cassava varieties

Two cassava varieties, one bitter and the other sweet, were used. The sweet variety was an improved cassava variety called *Kiroba*, which is widely grown by farmers in Mtwara region due to its high yielding ability and also for its pest and disease tolerance. The bitter variety used was *Salanga*, which is commonly associated with cyanide intoxication in konzo-affected areas of Mtwara and Newala districts. *Kiroba* was collected from Naliendele Agricultural Research Institute (NARI) (S 10°21'22", E 40°09'59") while *Salanga* was collected from Kitangari village (S 10°39'01", E 39°20'01") in Newala district. Kitangari village is, however, not a konzo-affected

village, but was selected only due to its close proximity and also because the bitter variety known to cause toxicity in konzo-affected areas could also be found there.

Table 4.1 Soil chemical and physical properties of the pot experiment soil

Parameter	Value	Rating	Medium range	Reference
pH	5.80	m	4.5 – 7.0	CIAT (2011)
OC (%)	0.35	vl	4.0 – 10.0	Landon (2014)
N (%)	0.06	vl	0.20 – 0.50	Landon (2014)
P (mg/kg)*	3.54	l	< 4.2	Howeler (2002)
K (cmol/kg)	0.14	l	0.15 – 0.25	CIAT (2011)
Ca (cmol/kg)	3.04	m	1.0 – 5.0	CIAT (2011)
Mg (cmol/kg)	0.08	vl	0.40 – 1.00	CIAT (2011)
S* (mg/kg)	1.27	l	< 6.0	Landon (2014)
Zn (mg/kg)	0.82	l	1.0 – 3.0	Motsara and Roy (2008)
Cu (mg/kg)	0.70	m	0.3 – 0.8	Motsara and Roy (2008)
Fe (mg/kg)	25.12	vh	4.0 – 6.0	Motsara and Roy (2008)
Sand (%)	86.00	Loamy sand		Soil Survey Division Staff (1993)
Clay (%)	11.00			
Silt (%)	2.00			

*Critical levels but not medium ranges are indicated for P and S. Where, vl, l, m and vh stand for very low, low, medium and very high.

4.2.3.2 Soil moisture treatments

The soil moisture treatments were begun at 70 days after planting (DAP) and were maintained for the next 20 days, that is, until 90 DAP. The selected soil moisture treatments were intended to keep some plants well-watered, others moderately stressed and the rest severely stressed, and this was achieved by irrigations which kept the soil in the respective pot at 100%, 60% and 30% field capacity (FC), respectively (Alves and Setter, 2004; Nezar, 2005; Ngugi *et al*., 2013).

The amount of water needed to bring the soils in the pots to complete field capacity was determined by merging the methods used by Alves and Setter (2004), Nezar

(2005) and Somasegaran and Hoben (2012). Three transparent pots were filled with equal amounts of the air-dried soil. The pots were then tapped to ensure a similar consistency as that of soils packed in the actual experimental pots. The pots had holes in the bottom allowing for free drainage. The soil in the pots was saturated by gently pouring water until they begun to leak. The pots were covered with plastic sheets and left standing for three days, ensuring that the water moved only downwards, filling up every empty pore spaces. After the third day 50 g of soil was collected from the middle 5 cm portion of the transparent pots and placed on a moisture can. The moist soil was then immediately weighed and the can placed in the oven to dry at 105 ^{o}C for 24 hours to determine the moisture held at field capacity. The equivalent amount of water needed to bring 5 kg of the oven-dry soil to field capacity was then determined.

At 70 DAP, pots under the 100% FC moisture treatment were brought to field capacity every day while pots under the 30% FC and 60% FC moisture levels were allowed to lose water until they were slightly below their respective moisture levels. Once each pot had attained slightly lower moisture content than 30% FC or 60% FC it was re-watered and maintained at the respective moisture level by daily replacement of the lost water and this was done for 20 days. Daily weighing of the pots was done at 0700 h to determine the amount of water lost in each pot before re-watering. The amount of moisture replaced in each pot took into consideration the weights of the pot, soil and plant as outlined by Alves and Setter (2004) as follows: The mean initial weights of the pots with soil at FC was recorded. On the day of planting the weight of pot + soil at FC + plant was determined and the plant weight at day 70 estimated using equation (i).

$$C_0 = (P + S_{FC} + C_0) - (P + S_{FC}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (i)$$

Where:

- C_0 is the initial plant weight at day 70
- S_{FC} is the weight of soil at FC (100%)
- P is the weight of pot

The water content at time t relative to field capacity was then calculated using equation (ii).

$$FC_t(\%) = \left[{((P + S + C_t) - C_0)} \Big/ {(P + S_{FC})} \right] \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (ii)$$

Where:

- FC_t (%) is the percent FC at time t

4.2.3.3 Fertiliser treatments

As the most deficient nutrients in most African soils are nitrogen (N), phosphorous (P) and potassium (K) (Sommer *et al*., 2013), the role of nutrient supply was evaluated using different levels of these nutrients in soils. Fertiliser treatments were formulated based on the low, moderate or high supply of N, P or K on the soils. This allowed the investigation of the effects of improved N, P and K supply on

cyanogenic glucoside accumulation in cassava. Field based N-P-K rates were transformed to pot based rates to formulate the treatments used in the experiment. The first treatment was a control, referred to as $N_0P_0K_0$ in which no nutrient was applied. The second treatment was a K only treatment referred to as $N_0P_0K_{25}$ in which K only was applied at the rate of 25 mg/kg. The fertiliser treatment containing K alone was included given the effect of K fertiliser in reducing cyanogenic glucoside production (Susan John *et al.*, 2005; Endris, 2006; Susan John *et al.*, 2007). The third fertiliser treatment was a moderate N-P-K fertiliser treatment, $N_{25}P_5K_{25}$, where N, P and K were applied at rates of 25 mg/kg, 5 mg/kg and 25 mg/kg, respectively. The fourth treatment was a high N-P-K fertiliser treatment, referred to as $N_{50}P_{13}K_{50}$, in which N, P and K were supplied at rates of 50 mg/kg, 13 mg/kg and 50 mg/kg, respectively. The applied pot N-P-K rates expressed on a per kg soil basis, are shown in Table 4.2 together with their field based rates.

Table 4.2 Fertiliser treatments tested in the study

		Pot based rates (mg/kg)			Field rate (kg/ha)		
No.	Treatment	N	P	K	N	P	K
1	$N_0P_0K_0$	0	0	0	0	0	0
2	$N_0P_0K_{25}$	0	0	25	0	0	50
3	$N_{25}P_5K_{25}$	25	5	25	50	10	50
4	$N_{50}P_{13}K_{50}$	50	13	50	100	25	100

NB: Each pot contained 5 kg of oven-dry soil, pot rates are indicated as nutrients applied per kilogram of soil

Although there are slight differences the rates of N-P-K fertilisers used were based on the moderate and full levels of the general recommended rate for cassava

production, which is, 100–22–83 kg N-P-K/ha or 100-50-100 kg N-P_2O_5-K_2O/ha) (CIAT, 2011; Howeler 1995). The fertilisers urea ($CO(NH_2)_2$), triple super phosphate (TSP) ($Ca(H_2PO_4)_2.H_2O$) and muriate of potash (MOP) (KCl), were used to supply N, P and K. All the KCl and TSP were mixed into the soil before planting. Urea was applied in solution in two split applications at two and six weeks after planting. The solutions were made as outlined by Johnston and Askin (2005).

4.2.4 Experimental design

The experiment was a factorial combination of two genotypes, three soil moisture levels and four different N-P-K treatments. It was laid out in the Randomised Complete Block Design, with six replicates. Each block thus had 24 treatments. Blocking was necessary due to the differential lighting in the screen house.

4.2.5 Cultural Management

4.2.5.1 Planting material

All cassava stem cuttings were collected from mature, ready to harvest, plants. Previously rooted cassava plantlets (Fig. 4.1) from mature cassava plants were used to establish the pot experiment. This was done to avoid any delayed response to applied nutrients in the experiment, due to the large nutrient reserves found in the 20 – 30 cm long mature stem cuttings if these were to be used as planting material (CIAT, 2011). To produce the rooted plantlets the collected mature stem cuttings of the two varieties were first grown in a nursery using rapid cassava multiplication methods as outlined by CIAT (2011) and Otoo (1996). Small cuttings 5 - 10 cm long of each cassava test variety were densely planted at a spacing of 10 cm x 10 cm in beds with soil of low fertility. The cuttings were allowed to sprout until the shoots

became 15 cm long, at which stage they were cut off and rooted in distilled water. It generally took about one month for all shoots to develop roots, although it took less time for the variety *Kiroba* which displayed a higher rooting ability. The day they were transplanted marked the first day of planting.

Figure 4.1 Rooted cassava plantlets developed and used in the experiment

4.2.5.2 Water management and quality of water used for irrigation

Tap water was used to irrigate the plants throughout the entire experiment. The electrical conductivity of the water (EC_w) was 0.007 dS/m and it had a pH of 6.58. The water had a nitrate-nitrogen (NO_3 - N) content of 5.60 mg/L and only traces of phosphate-phosphorous (PO_4 – P). It also had 0.01, 0.16, 0.25 and 0.03 meq/L of K, Na, Ca and Mg, respectively. All parameters measured were within permissible levels required for irrigation water (Ayers and Westcot, 1985; Phocaides, 2007). In particular, there were negligible levels of N, P and K in the water (Ayers and Westcot, 1985; Phocaides, 2007) making their additional contribution to nutrient supply very minimal. During the first 69 days after planting (DAP) the soils in all

pots were kept well-watered to field capacity. Each pot had a saucer to collect any excess water that drained.

4.2.5.3 Correction of nutrient deficiencies

The deficiency in Mg and S was corrected using magnesium sulphate ($MgSO_4.7H_2O$), applied at a rate of 50 kg Mg/ha (Howeler, 2014) by mixing 25 mg Mg/kg (simultaneously adding 32.5 mg S/kg) to the soil of each pot before planting, at the same time as the TSP and MOP. A 2% solution of YaraVita Zintrac (700 g Zn/L, as ZnO), a chelated foliar fertiliser was used to correct Zn deficiency at one month after planting and again at two months after planting.

4.2.5.4 Pest control

To keep insect pests away, the broad spectrum insecticide Dursban ($C_9H_{11}Cl_3NO_3PS$) was used. The Dursban was mixed with the ZnO foliar solution.

4.2.6 Screen house environmental conditions

The temperatures in the screen house ranged between a minimum of 23^oC to a maximum temperature of 33^oC. Low temperature around 21^oC and sometimes high temperatures of about 37^oC were at times also experienced.

4.2.7 Data collected

4.2.7.1 Determination of total hydrogen cyanide (HCN) in leaves

Plants were grown in the pots for 90 days before leaf sampling for cyanide analyses. Leaf sampling was carried out early in the morning around 07:00 h. Sample collection was done by picking the first fully-expanded leaf from the top of each plant plus two leaves below it (Essers, 1994). To avoid damage to the leaf blade,

leaves were picked together with their petioles. The picked leaves were immediately placed in labelled plastic bags and were temporarily placed in a cool box until all leaves from all plants were picked. At the laboratory leaf samples were placed in a fridge and were kept refrigerated until right before sample preparation for cyanogen extraction.

The picrate paper method was used to determine the total cyanogen content of cassava leaves (Egan *et al*., 1998; Bradbury *et al*., 1999). Leaf blades without petioles were chopped using scissors and the small pieces immediately ground using a mortar and pestle. A 100 mg sample of the ground leaves was then left to incubate in a pH 6 solution at room temperature for 24 hours in an airtight container. A picrate paper placed in the container reacted with the liberated cyanide during this time. The picrate paper was later eluted in 5 ml of water and the absorbance of the picrate solution produced was measured at the wavelength of 510 nm using a spectrophotometer. The total hydrogen cyanide (HCN) levels determined were expressed in mg/kg, on a fresh weight basis.

$$HCN\ (mg/kg) = 396\ \times absorbance \ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

4.2.8 Statistical analysis

The data collected was first analysed using a three-way analysis of variance (ANOVA). The data was then split by variety type and analysed using a two-way ANOVA, where the effects of moisture level and nutrient supply on each variety were investigated. Mean separation was done using the Tukey's mean separation test

at the 5% probability level. All statistical analyses were carried out using GenStat Edition 14.

4.3 Results and Discussion

4.3.1 Influence of varieties, soil moisture and soil nutrient supply on cyanogenic glucoside accumulation in cassava leaves

The F-test probability values obtained after the three-way ANOVA carried out to determine the effects of varieties, soil moisture and soil nutrient supply on cyanogenic glucoside accumulation in cassava leaves is shown in Table 4.3.

Table 4.3 F-test probability values (p-values) for the three-way ANOVA on the effects of varieties, soil moisture and nutrient supply on leaf HCN levels

Factor	p-value
Variety (V)	**< 0.001**
Moisture supply (M)	0.080
Nutrient supply (F)	**< 0.001**
V × M	0.772
V × F	**< 0.001**
M × F	0.123
V × M × F	**0.035**

Numbers in bold are significant at $p < 0.05$ using the Tukey's test.

4.3.1.1 Effects of variety on leaf HCN levels

Leaf HCN levels varied significantly with cassava varieties ($p < 0.001$) (Table 4.3), highlighting a genetic control on leaf HCN levels. The genetic control of cyanogenic glucoside production was similarly evident in the study by Tan (1995) as indicated

by HCN levels in cassava shoots and roots. The overall mean leaf HCN level for the bitter variety *Salanga* (181.3 mg/kg) was much higher than the mean leaf HCN levels of the sweet variety *Kiroba* (97.6 mg/kg) (Table 4.4), confirming the fact that bitter varieties have relatively higher HCN levels the sweet varieties. The leaf HCN levels obtained in this study were on average higher than the 65 mg/kg (fresh weight) reported by Jørgensen *et al*. (2005) in a wild-type variety. They were also higher than the 71.3 mg/kg and 33.0 mg/kg (fresh weight) leaf HCN levels reported by Ndung'u *et al*. (2010) in an improved and local cassava variety, respectively.

Table 4.4 Main effect means and standard deviations for the effects of varieties, soil moisture levels and nutrient supply on leaf HCN levels

Treatment	Level	HCN (mg/kg)	SD (mg/kg)
Variety	*Salanga*	181.3[a]	88.1
	Kiroba	97.6[b]	54.0
Moisture level (%FC)	30	153.2[a]	94.2
	60	138.2[a]	79.2
	100	126.9[a]	75.8
Nutrient level	$N_0P_0K_0$	121.1[bc]	57.3
	$N_0P_0K_{25}$	99.2[c]	78.9
	$N_{25}P_5K_{25}$	138.2[b]	69.9
	$N_{50}P_{13}K_{50}$	199.1[a]	94.9

Within each measured parameter and for each factor, means in the same column followed by the same lowercase letter are not significantly different at $p < 0.05$ using the Tukey's test. SD is the standard deviation.

4.3.1.2 Effects of soil moisture on cassava leaf HCN levels

The overall influence of soil moisture level on HCN levels was not significant ($p = 0.080$) (Table 4.3). Soil moisture level on its own, thus, had no role in influencing

leaf HCN levels in the present cassava varieties in this experiment (Table 4.4). This is in contrast with findings found by Vandegeer *et al*. (2013) where water stress significantly increased cassava leaf and root hydrogen cyanide levels, resulting in, respectively, 2.9 and 4 times higher than HCN levels found in well-watered plants. A study by Tan (1995) also showed that water stress increased cyanogenic glucoside levels in cassava when imposed for different periods during the crops' growth period. The role of water in the present study was however not completely absent as revealed by the significant V x M x F interaction ($p = 0.035$) (Table 4.3).

4.3.1.3 Effects of N, P and K supply on cassava leaf HCN levels

Like variety, significant effects on leaf HCN levels were also observed with soil nutrient supply at $p < 0.05$ (Table 4.3). Application of K alone ($N_0P_0K_{25}$) led to the lowest HCN level (99.2 mg/kg) while of N, P and K together, especially at relatively high rates ($N_{50}P_{13}K_{50}$), resulted in the highest leaf HCN level (199.1 mg /kg) (Table 4.4). However, the application of only potassium is known to decrease cyanogenic glucoside levels in cassava (Bokanga *et al*., 1994; Susan John *et al*., 2005; Endris, 2006; Susan John *et al*., 2007). Application of N and P, in addition to K, generally increased leaf HCN levels (Table 4.4). Increased N-P-K levels have also been observed to increase cassava HCN levels in other studies (De Bruijn, 1973; George *et al*., 2000; Susan John *et al*., 2005; Adekayode and Adewumi, 2013; Cuvaca *et al*., 2015). This could be probably due to the increased production of cyanogenic glucosides with high rates of N (Susan John *et al.*, 2005), since P has little influence on cyanogenic glucoside production (De Bruijn, 1973; Mohan Kumar *et al*., 1977; Susan John *et al*., 2005). It seems that the presence of N tends to override the effect

of K in reducing HCN levels. Hence, N should be used only to the extent that it does not override the effect of K.

4.3.2 Interactive effects of varieties, soil moisture levels and nutrient supply on cyanogenic glucoside levels in cassava

4.3.2.1 The V × M, V × F and M × F interaction effects on leaf HCN levels

Amongst the three interaction effects V × M, V × F and M × F, only the V x F interaction was significant (Table 4.3). The V x F interaction suggests that the two varieties responded differently to N-P-K fertiliser application, as shown in Table 4.5.

Table 4.5 Mean leaf HCN levels and standard deviations for the V × M and V × F interaction effects

Treatment	Level	Variety			
		Salanga		*Kiroba*	
		HCN (mg/kg)	SD (mg/kg)	HCN (mg/kg)	SD (mg/kg)
Moisture level (%FC)	30	201.5[a]	104.0	109.5[a]	62.6
	60	175.3[a]	84.1	95.9[a]	52.9
	100	172.1[a]	76.1	81.8[a]	41.4
Nutrient level	$N_0P_0K_0$	157.2[bc]	53.3	85.2[a]	47.4
	$N_0P_0K_{25}$	118.2[c]	86.1	79.6[a]	57.1
	$N_{25}P_5K_{25}$	178.2[b]	74.3	98.1[a]	34.0
	$N_{50}P_{13}K_{50}$	278.3[a]	81.3	120.1[a]	59.8

Within each measured parameter and for each factor, means in the same column followed by the same lowercase letter are not significantly different at $p < 0.05$ using the Tukey's test. SD is the standard deviation.

The interaction is due to the significant and greater variations in leaf HCN content with incremental additions of the N containing N-P-K fertilisers in the variety *Salanga* as compared to the variety *Kiroba*. The variations are more evident between

the lowest leaf HCN levels obtained under the $N_0P_0K_{25}$ treatment and the two significantly higher leaf HCN levels obtained under the $N_{25}P_5K_{25}$ and $N_{50}P_{13}K_{50}$ nutrient treatments in the variety *Salanga*. Leaf HCN levels of *Salanga* plants under the $N_{25}P_5K_{25}$ and $N_{50}P_{13}K_{50}$ nutrient treatments were respectively 1.5 and 2.4 times greater than levels in *Salanga* plants under the $N_0P_0K_{25}$ treatment. For the variety *Kiroba*, leaf HCN levels of plants under the $N_{25}P_5K_{25}$ and $N_{50}P_{13}K_{50}$ treatments were respectively 1.2 and 1.5 times greater than levels in *Kiroba* plants under the $N_0P_0K_{25}$ treatment.

The differences in leaf cyanogenic glucoside production in the two varieties, even with the same level of supplied nutrients could be due to their genotypic differences. In their study, Gleadow and Woodrow (2000) found that even if cyanogenic glucoside production depended on N supply and N leaf content, differences were seen in how varieties allocated their N; due to genotypic differences, some cultivars were observed to allocate over 5% of leaf nitrogen to cyanogenic glucosides, while others allocated less than 0.5%.

The stronger influence of nutrient supply on leaf HCN production observed with the variety *Salanga* may not, however, imply that bitter varieties are always influenced more by nutrient supply as compared to sweet varieties. For instance, using a sweet variety, Susan John *et al*. (2005) found significant differences in root HCN levels with increased N-P-K application. On the other hand, no significant differences were observed in root HCN levels with increased N-P-K application on a bitter cassava variety in the study by Cuvaca *et al*. (2015).

4.3.2.2 Effects of moisture level and nutrient supply on the leaf HCN content of each cassava variety due to the V x M x F interaction effect

The significant three-way interaction between variety, soil moisture and soil nutrient supply ($p = 0.035$) in the three-way ANOVA indicates that the two varieties were influenced differently by moisture and nutrient supply (Table 4.3). Separate two-way ANOVA were hence carried out to show how leaf HCN production differed in the two varieties in response to moisture levels and nutrient supply. The results obtained are shown in Table 4.6.

Table 4.6 Effects of variety, soil moisture level and nutrient supply on leaf HCN levels

		Variety			
		Salanga		*Kiroba*	
FC level (%)	Nutrient level (N-P-K)	HCN (mg/kg)	SD (mg/kg)	HCN (mg/kg)	SD (mg/kg)
30	$N_0P_0K_0$	215.7^{abcd}	56.2	69.6^{ab}	35.4
	$N_0P_0K_{25}$	135.2^{cd}	142.7	142.8^{a}	78.5
	$N_{25}P_5K_{25}$	144.9^{bcd}	64.5	100.6^{ab}	51.4
	$N_{50}P_{13}K_{50}$	310.4^{a}	109.8	125.0^{ab}	70.5
60	$N_0P_0K_0$	140.8^{cd}	49.4	97.7^{ab}	51.4
	$N_0P_0K_{25}$	95.6^{d}	14.0	51.7^{b}	30.0
	$N_{25}P_5K_{25}$	191.8^{abcd}	78.3	105.2^{ab}	23.1
	$N_{50}P_{13}K_{50}$	273.0^{ab}	82.9	129.2^{ab}	75.4
100	$N_0P_0K_0$	115.0^{d}	23.2	88.2^{ab}	63.3
	$N_0P_0K_{25}$	123.8^{cd}	32.2	44.4^{b}	20.2
	$N_{25}P_5K_{25}$	198.0^{abcd}	80.0	88.6^{ab}	21.7
	$N_{50}P_{13}K_{50}$	251.4^{abc}	65.3	106.0^{ab}	26.0

Within each measured parameter, means in the same column followed by the same lowercase letter are not significantly different at $p < 0.05$ using the Tukey's test. SD is the standard deviation.

For the variety *Salanga* the interaction between moisture level and nutrient supply was not significant ($p = 0.120$); instead only nutrient supply had a significant effect ($p < 0.001$) on leaf HCN levels in this variety. The overall effect of nutrient supply on leaf HCN levels in the variety *Salanga* is shown above, in Table 4.5. Just as was observed with the overall influence of nutrient supply, the lowest and highest leaf HCN levels were again obtained under the sole K ($N_0P_0K_{25}$) treatment and the highest under the N-P-K ($N_{50}P_{13}K_{50}$) treatment. In Table 4.5 it can also be seen that the overall response to nutrient supply observed in Table 4.4, had mainly been due to the bitter variety *Salanga* and not due to the sweet variety *Kiroba*.

The results obtained showed that there was a significant two-way interaction between soil moisture levels and nutrient supply for the variety *Kiroba*. The interaction implies that at least one nutrient level influenced leaf HCN levels differently depending on soil moisture level for this variety. From Table 4.6 it can be observed that the interaction was due to the $N_0P_0K_{25}$ treatment. Amongst all other nutrient treatments, the sole K ($N_0P_0K_{25}$) treatment had given *Kiroba* plants the lowest leaf HCN levels both at 60% and 100% FC, while it gave the highest leaf HCN level at 30% FC. Thus, when *Kiroba* plants were fertilised with only K, their leaf HCN levels increased under severe moisture stress. The results probably explain why inconsistent results have been obtained with sole K application, with it either reducing or having no effect on cassava HCN production (Bokanga *et al.*, 1994; Cuvaca *et al*., 2015). Similarly, although no significant responses were observed with N-P-K application in the study by Cuvaca *et al*. (2015), sole K application still gave slightly higher root cyanogenic glucoside levels than the other N-P-K treatments.

The high HCN levels observed by Cuvaca *et al.* (2015) with sole K application were also obtained when cassava was under moisture stress, caused by waterlogging conditions for most of the year and from the excessive rains that had also occurred right before harvest. Both extremes of water stress (to wet or too dry) have been reported to increase HCN levels, although water stress caused by dry conditions may give higher HCN levels (Bokanga *et al*., 1994). Being an unbalanced nutrient treatment, sole K application probably caused plant stress which manifested increased leaf HCN levels under severe moisture stress. Increased root bitterness in cassava harvested during the wet season was also positively correlated ($r = 0.320$, $p = 0.045$) to soil K levels in the study by Alou *et al*. (2014). Root bitterness is mostly associated with high cyanogenic glucoside levels (Burns *et al*., 2010).

The study shows that making general conclusions on the expected effects of environmental conditions on cyanogenic glucoside accumulation can indeed be difficult. De Bruijn (1973) also commented on the complexity of the nature of cyanogenic glucoside accumulation in different cassava varieties, mentioning that different varieties do not react in the same way under a given set of ecological conditions.

4.4 Conclusions

Using leaf HCN levels, the study established that nutrient supply has an important role as had variety, and a greater role than water stress, in influencing cyanogenic glucoside production in cassava. While cyanogenic glucoside production is solely influenced by nutrient supply in some varieties, it can also be influenced by water stress in other varieties depending on soil nutrient conditions.

4.5 Recommendations

From the results presented herein, the following are recommended:

i. Soil nutrient management practices that help to balance the amount of K relative to N need to be adopted by farmers in these areas if increased root HCN levels are to be avoided.

ii. Breeders must also strive to breed for varieties that remain innocuous under the adverse soil and climatic conditions of konzo-affected areas.

iii. To help validate the present research findings, further studies on how nutrient supply and moisture levels interact to affect cassava cyanogenic glucoside accumulation need to be carried out under field conditions.

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CHAPTER FIVE

5.0 EFFECTS OF N, P AND K ON TOTAL ROOT CYANIDE LEVELS AND YIELDS OF CASSAVA VARIETIES GROWN IN MTWARA REGION, TANZANIA: A FIELD EXPERIMENT

Abstract

The effects that N-P-K fertiliser use would have on cyanogenic glucoside production and on yields of cassava grown in Tanzania's Mtwara region are unknown. A field experiment, laid in a split-plot design and repeated in two consecutive years was thus carried out to investigate how N, P and K application influenced cyanogenic glucoside accumulation and yields of cassava varieties grown in Mtwara region. The four cassava varieties used included the improved cassava variety *Kiroba* and the local cassava varieties: *Salanga, Kalinda* and *Supa*. The six fertiliser treatments used included; $N_0P_0K_0$ (0-0-0 kg/ha), $N_0P_0K_{50}$ (0-0-50 kg/ha), $N_0P_{10}K_{50}$ (0-10-50 kg/ha), $N_{25}P_{10}K_{50}$ (25-10-50 kg/ha), $N_{50}P_{10}K_{50}$ (50-10-50 kg/ha) and $N_{100}P_{25}K_{100}$ (100-25-100 kg/ha). The variety *Kiroba* was the only variety which had its root HCN levels influenced by N, P and K fertiliser application. The highest root HCN levels (67.9 mg/kg) in the variety *Kiroba* were obtained under the $N_{100}P_{25}K_{100}$ treatment. The lowest root HCN levels (42.3 mg/kg) obtained under the $N_0P_0K_{50}$ treatment by *Kiroba* plants however increased to 63.1 mg/kg when water stress occurred prior to harvest. Except for the variety *Kalinda*, yield increases were possible with N, P and K fertiliser application in the other three varieties this was however only when soils

had other nutrient deficiencies. Fresh yields for the varieties *Salanga*, *Kalinda*, *Supa* and *Kiroba* ranged from 7.8 - 13.2, 7.6 - 15.8, 7.2 - 20.6 and 5.2 - 18.1 t/ha, respectively in Year 1 when other soil nutrient deficiencies were uncorrected, and ranged from 14.3 - 29.3, 23 - 28.5, 18.2 - 25.0 and 18.5 - 34.1t/ha, respectively in Year 2 when soil nutrient deficiencies were corrected.

5.1 Introduction

Apart from processing, fertiliser use practices is one other strategy that can be used to reduce the cyanogenic glucoside content of cassava in order to make the crop safer to consume (Bokanga *et al*., 1994). However, the use of fertilisers is limited by the several conflicting reports on their influence on cassava (*Manihot esculenta* Crantz) cyanogenic glucoside production. For instance, using a local cassava variety and two improved varieties, a definite influence of K application on the total root cyanide (HCN) content (a measure of cyanogenic glucosides) could not be established in the study by Obigbesan (1977). On the other hand, significant reductions in root HCN with K fertiliser application were reported by Susan John *et al*. (2007). Also, although the use of N containing fertilisers is commonly associated with increasing cassava root cyanogenic glucoside levels (De Bruijn, 1973; Susan John *et al*., 2005; Nur *et al*., 2013), other studies, for example those reported by Amanullah *et al*. (2006) and Cuvaca *et al*. (2015), observed no effects of N-P-K fertiliser on root cyanogenic glucoside levels. In contrast, Cadavid *et al*. (1998) reported significant reductions in root HCN levels with the use of moderate rates of N-P-K fertiliser.

Similar contrasting reports have also been reported on the influence of organic fertilisers on cassava cyanogenic glucoside production. In the study by Mohan

Kumar *et al.* (1977), cassava root HCN increased with the use of farmyard manure. Contrary to this, farmyard manure reduced cassava root HCN in a study carried out by De Bruijn (1973). Furthermore, Susan John *et al*. (2005) found that N-P-K fertiliser together with farmyard manure could reduce root cyanogenic glucosides in cassava.

If reductions of cyanogenic glucosides in cassava are to be guaranteed with use of either organic or inorganic fertiliser, an initial investigation of how fertiliser would actually influence cyanogenic glucoside accumulation in cassava varieties grown in a particular area would be necessary. As using fertiliser for yield improvement could still influence cassava cyanogenic glucoside accumulation, knowledge on the influence of a particular fertiliser on cyanogenic glucosides is essential even if the primary aim of fertiliser use in most cassava production systems is not for reducing cyanogenic glucoside production but for yield improvement.

Appropriate fertiliser use could probably help reduce cyanogenic glucoside levels of cassava grown in Mtwara region. The region has experienced a number of recurrent cassava cyanide intoxication cases and increased levels of cyanogenic glucosides are pointed out as contributors of increased cassava toxicity (Mlingi *et al*., 2011; Banea *et al*., 2012). Although larger epidemics of cyanide intoxication related health disorders occur during drought and dry seasons, sporadic cases still arise outside these periods, implying that other factors (Cardoso *et al*., 2005), like soil nutrient supply could also be influencing cyanogenic glucoside production (Burns *et al*., 2012) in the region. Fertiliser application could help improve the nutrition of cassava plants (CIAT, 2011) enabling them to mitigate soil nutrient related abiotic stress (Cakmak, 2006). Abiotic stress is known to influence cassava cyanogenic

glucoside production (Jørgensen *et al*., 2005). Due to the region's low soil fertility, amongst other reasons, cassava yields are very low, being on average approximately 5 t/ha (Bennett *et al*., 1979b). Fertiliser use in Mtwara region could therefore also help to increase cassava yields in addition to reducing cyanogenic glucoside accumulation.

Hardly any research has been carried out on the effects of fertiliser use on cyanogenic glucoside production and on yields of cassava grown in Mtwara region. This study was hence carried out to investigate the effects of N, P and K use on both root HCN levels and yields of some cassava varieties grown in Mtwara region.

5.2 Materials and Methods

A field experiment was carried out to test the effects of N, P and K application on cyanogenic glucoside production and yields of some cassava varieties grown in Mtwara region.

5.2.1 Location and climate

Two field experiments were carried out in consecutive cropping seasons on two different but close sites at Naliendele Agricultural Research Institute (NARI). The first (Year 1) field experiment, located at S 10°22'56", E 40°10'00", was planted in February 2014 and harvested in December 2014. The second field experiment (Year 2), located at S 10°23'03", E 40°09'49", was planted in February 2015 and harvested in February 2016. The experimental sites were located on the Eastern Makonde Plateau in Mtwara Rural district, Tanzania.

Mtwara Rural district lies in the coastal agro-ecological zone 2 (C2) which is characterised by one short growing season of 3 - 4½ months in a year (De Pauw, 1984; Mowo *et al*., 1993). The rainy season begins in November/December and ends in April/May. The rainfall has unreliable onset dates and a three to four week mid-season dry spell period or seasonal interruption, experienced in February (De Pauw, 1984; Bennett *et al*., 1979a). The total rainfall ranges from 800 to 1000 mm/year; mean minimum and maximum temperature range from 21.7 ºC to 30.5 ºC and the relative humidity ranges between 79% and 87% (PCD and RCOM, 1997; Bennett *et al*., 1979a; De Pauw, 1984). The field experiments were carried out under rain-fed conditions. Fig. 5.1 shows the mean annual rainfall, temperature and relative humidity data collected during the experimental period.

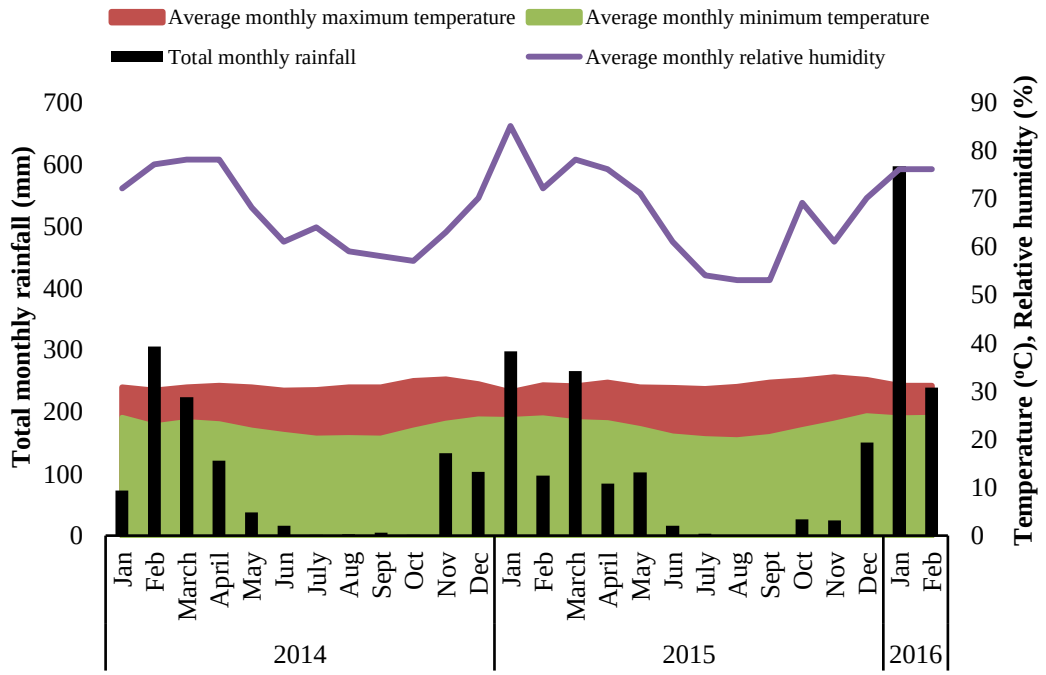

Figure 5.1 **Mean monthly rainfall, relative humidity, and maximum and minimum temperature data at NARI**

The total rainfall received during the first and the second field experiment periods was 1019 mm and 1600 mm, respectively. Above normal rains were hence received in the 2015 - 2016 cropping season, which was the period of the second year (Year 2) field experiment.

5.2.2 Soils

A composite surface (0 to 20 cm) soil sample was collected from each site in each year before planting for the determination of any nutrient deficiencies. The soils were analysed for organic carbon (OC), soil reaction (pH), total N, available P, available K, exchangeable Ca, exchangeable Mg, available S, Zn, Cu and Fe and for soil texture. All soil analysis procedures were carried out as outlined by Møberg (2001) as follows: pH was determined in H_2O using a 1:1 soil to water ratio; organic carbon was determined using the Walkley and Black method; N was determined by micro-Kjeldahl digestion; P using the Bray No. 1 method; Sulphate-S using the calcium phosphate extraction method; available K, exchangeable Ca and Mg in 1 N ammonium acetate; extractable Zn, Cu and Fe in diethylenetriaminepentaacetic acid (DTPA); and soil texture by the hydrometer method. Table 5.1 shows the soil analysis results obtained for the two sites used in the two years.

Beneffectivet *et al*. (1979) describes the soil of the area as being predominantly sandy and of low soil fertility. The soil was classified as a Haplic Ferralsol (Dystric, Rhodic) according to the World Reference Base (IUSS Working GroupWRB, 2014) (See Appendix 2 and 3 for soil profile description data).

Table 5.1 Soil chemical and physical properties of field experimental sites used in Year 1 and 2 prior to planting cassava

Parameter	Year 1		Year 2		Medium range	Reference
	Value	Rating	Value	Rating		
pH	5.40	m	6.10	m	4.5 – 7.0	CIAT (2011)
OC (%)	0.27	vl	0.53	vl	4.0 – 10.0	Landon (2014)
N (%)	0.04	vl	0.05	l	0.20 – 0.50	Landon (2014)
P (mg/kg)*	1.49	vl	2.24	l	< 4.2	Howeler (2002)
K (cmol/kg)	0.12	l	0.03	vl	0.15 – 0.25	CIAT (2011)
Ca (cmol/kg)	2.25	m	2.51	m	1.0 – 5.0	CIAT (2011)
Mg (cmol/kg)	0.12	vl	0.41	m	0.40 – 1.00	CIAT (2011)
S*(mg/kg)	3.33	l	1.27	l	< 6.0	Landon (2014)
Zn (mg/kg)	0.16	vl	0.31	vl	1.0 – 3.0	Motsara and Roy (2008)
Cu (mg/kg)	trace	vl	0.13	l	0.3 – 0.8	Motsara and Roy (2008)
Fe (mg/kg)	9.81	h	9.40	h	4.0 – 6.0	Motsara and Roy (2008)
Sand (%)	86.00	Loamy sand	86.00	Loamy sand		Soil Survey Division Staff (1993)
Silt (%)	11.00		11.00			
Clay (%)	3.00		2.00			

*Critical levels but not medium ranges are indicated for P and S. Where, vl, l, m and h stand for very low, low, medium and high

5.2.3 Treatments used in the field experiment

5.2.3.1 Cassava varieties

Four cassava varieties, two bitter and the other two sweet, were used in the experiment. One sweet variety was an improved cassava variety called *Kiroba* while the other sweet variety, *Supa,* was a local variety. The two bitter varieties used were the local cassava varieties *Salanga* and *Kalinda*. The local varieties were collected from Kitangari village (S 10°39'01", E 39°20'01") in Newala district, Tanzania. *Kiroba* was obtained from NARI.

5.2.3.2 Fertiliser treatments

The fertiliser treatments consisted of a control treatment ($N_0P_0K_0$) that received no fertiliser; a sole K treatment ($N_0P_0K_{50}$) with only K applied at a rate of 50 kg/ha; a P+K only ($N_0P_{10}K_{50}$) treatment with only P and K applied at rates of 10 kg/ha and 50 kg/ha, respectively; and three N-P-K treatments, that is, $N_{25}P_{10}K_{50}$, $N_{50}P_{10}K_{50}$ and $N_{100}P_{25}K_{100}$, which consisted of N-P-K fertiliser applied at rates of 25-10-50 N-P-K kg/ha, 50-10-50 N-P-K kg/ha and 100-25-100 N-P-K kg/ha, respectively. The $N_{50}P_{10}K_{50}$ and $N_{100}P_{25}K_{100}$ treatments were, respectively, moderate and high N-P-K fertiliser rates. The treatments used are shown in Table 5.2.

Urea ($CO(NH_2)_2$), triple super phosphate (TSP) ($Ca(H_2PO_4)_2.H_2O$) and muriate of potash (MOP) (KCl) were used to supply N, P and K. All the TSP was applied at planting together with one-third of urea and MOP. Urea and MOP were applied in two split applications; the remaining two-thirds were applied at two MAP. All basal fertiliser was banded below the stake at planting. This was done by placing the

fertiliser in individual planting holes made using a hand-hoe. The fertiliser was then covered with soil before placing the stake above it.

Table 5.2 Fertiliser treatments

No.	Treatments	N (kg/ha)	P (kg/ha)	K (kg/ha)
1	$N_0P_0K_0$	0	0	0
2	$N_0P_0K_{50}$	0	0	50
3	$N_0P_{10}K_{50}$	0	10	50
4	$N_{25}P_{10}K_{50}$	25	10	50
5	$N_{50}P_{10}K_{50}$	50	10	50
6	$N_{100}P_{25}K_{100}$	100	25	100

The second fertiliser application was applied by side banding the fertiliser. A shallow hole was made using a sharp stick or machete on the side of a cassava plant at a 10 cm distance (CIAT, 2011). The remaining two-thirds of the urea and MOP was then placed in the hole and covered with soil.

5.2.4 Experimental design

The field experiment was a split-plot laid out in the randomized complete block design (Gomez and Gomez, 1984). Varieties were placed in the main plots while the fertiliser treatments were assigned to the sub-plots. All treatments were replicated three times.

5.2.5 Land preparation, planting and weed control

The field was initially ploughed using a tractor and later harrowed using hand-hoes before marking out the blocks, plots and sub-plots of the experiment. Each sub-plot

had six rows, each with six plants. Plants in the sub-plot were planted at the spacing of 1 m × 1 m, giving a plant population of 10 000 plants/ha. Sub-plots were hence 6 m × 6 m in size and had a total of 36 plants. When border rows were ignored, the net (effective) plot of each sub-plot contained 16 plants. The 1 m long cassava cuttings for each variety were cut into 20 cm long planting stakes. The stakes were planted inclined on the flat, with no ridges.

The field was maintained weed free for the first three MAP by hand-hoe weeding. This was achieved by weeding the field twice, first at just before two MAP and then at three MAP.

5.2.6 Correction of nutrient deficiencies

Unlike the second year experiment, the first year experiment was carried out without correcting for nutrient deficiencies other than N, P and K. This was because the analysis of the soil of the site used in Year 1 was delayed. Soils at the experimental site in the second year were corrected for S, Zn and Cu. Magnesium sulphate was used to correct S deficiency. It was applied at the rate of 20 kg S/ha (Howeler, 2014) at two months after planting together with the second application of urea and MOP. Zinc deficiency was corrected using a 1% solution of a product called YaraVita Zintrac (700 g Zn/L; ZnO), while Cu deficiency was corrected using a 0.05% solution of YaraVita Coptrac (500 g Cu/L; CuO). Two foliar applications of Zn and Cu were done, each at two and three months after planting.

5.2.7 Pest management

Dursban was used to control insect pests; it was mixed with the foliar fertiliser solutions before spraying.

5.2.8 Data collected

5.2.8.1 Total cyanide content in fresh cassava roots

Four plants were selected from each sub-plot, and from each plant three roots were collected for the determination of total HCN levels (Essers, 1994). The picrate paper method was used to determine the total cyanide levels in fresh cassava roots (Egan *et al*., 1998; Bradbury *et al*., 1999).

5.2.8.2 Determination of fresh root yield

Having been affected by moisture stress, due to insufficient rainfall in the first month after planting, the plant population of a number of sub-plots in the second year experiment were quite poor; because of this, the root mass of cassava plants that had had less plant competition were also included when calculating fresh root yields. This was done for both field experiments to make results comparable. Roots from every plant in the effective sub-plot were weighed and recorded at harvest. The average root weight was then used to calculate yield per hectare as shown in equation (i).

$$\text{Fresh root yield (t/ha)} = \frac{\text{X kg per plant}}{1\ \text{m}^2} \times \frac{10\,000\text{m}^2}{1\ \text{ha}} \times \frac{1\ \text{t}}{1000\ \text{kg}} \quad \text{..... (i)}$$

where:

X is the average root weight per plant in effective plot and 1m^2 is the area occupied by each plant

The method used to determine fresh root yields was as outlined by CIAT (2011), but a slight adjustment was made to the fresh root yield formula (equation (i)) to accommodate the calculation of fresh root yield from the average root mass per unit area due to the inclusion of sub-plots with missing plants.

5.2.8.3 Determination of dry root yield

In order to determine the dry root yield, root dry matter content had to be determined first. Two different methods were used to calculate root dry matter content (DM) in Year 1 and Year 2. The specific gravity (SP) method (CIAT, 2011) was used in the Year 1 field experiment, but was not used in the second years experiment. In Year 1, approximately 5 kg of randomly selected roots from a sub-plot were weighed first in the air and then later weighed when submerged in water. Three sets of roots were weighed for each sub-plot. Using the values recorded the specific gravity (SP) was then determined using equation (ii).

$$\text{SP (kg/L)} = \frac{\text{Weight in air (g)}}{\text{Weight in air (g)} - \text{weight in water (g)}} \quad \text{..... (ii)}$$

The calculated SP value was then used to calculate the root DM content using equation (iii).

$$\text{DM content (\%)} = (158.3 \times \text{SP}) - 142.0 \quad \text{..... (iii)}$$

In Year 2, four roots were randomly selected from each sub-plot at harvest time and a sub-sample of approximately 30 g was taken from the top end, middle and bottom end of the roots. The sub-samples were then chopped, air dried and later oven dried at 70^{o}C to constant weight (Ebah-Djedji *et al.*, 2012; Fermont *et al*., 2009). The DM content was then calculated using equation (iv).

$$\text{DM}\ (\%) = \frac{W_2}{W_1} \times 100 \quad \text{..... (iv)}$$

where:

W_1 is the weight of fresh pulp of tuberous roots

W_2 is the weight of dried pulp of tuberous roots

Dry root yield was then determined using equation (v).

$$\text{Dry root yield}\ (\text{t/ha}) = \frac{\text{DM}(\%)}{100} \times \text{Fresh root yield}\ (\text{t/ha}) \text{..... (v)}$$

5.2.9 Statistical analyses

Data collected from the field experiments were subjected to Analysis of Variance (ANOVA). Mean separation in both analyses was done using the Tukey's means separation test at the 5% probability level. Correlations between N supply and root HCN levels were done using the Pearson's correlation method. Significance for the

correlation was also tested using the 5% probability level. All statistical analyses were done using GenStat Edition 14.

5.3 Results and Discussion

5.3.1 Effects of variety and application of N, P and K on root HCN levels and yield in field experiments in Years 1 and 2

Table 5.3 shows the F-test probability values from the ANOVA carried out on the yield (fresh and dry) and root HCN results obtained from the field experiments in Years 1 and 2.

Table 5.3 Summary of F-test probability values for the split-plot ANOVA on the effects of variety and N, P and K application on root HCN levels and yield in field experiments in Years 1 and 2

Experiment	Factor	Root HCN p-value	Fresh root yield p-value	Dry root yield p-value
Year 1	Variety (V)	**< 0.001**	0.294	0.301
	Fertiliser (F)	**< 0.001**	**< 0.001**	**< 0.001**
	V x F	0.162	0.158	0.071
Year 2	Variety (V)	**< 0.001**	0.388	0.075
	Fertiliser (F)	**0.020**	**0.024**	**0.046**
	V x F	**< 0.001**	0.844	0.899

Numbers in bold are significant at $p < 0.05$ using the Tukey's test.

Significant effects are observed for the influence of variety, fertiliser and their combined effects. The significant effects obtained for the influence of variety on root HCN in years 1 and 2 indicates that at least one variety influenced root HCN levels

differently in both years. Similarly, the significant effects of fertiliser on root HCN and fresh and dry yields in years 1 and 2, also indicate that at least one fertiliser level influenced root HCN, and fresh and dry yields differently in both years. Responses due to fertiliser and variety were thus obtained on the mentioned measured parameters. Lastly, the significant effects obtained for the combined influence of variety and fertiliser on root HCN, indicates that at least one variety had its root HCN levels influenced differently by fertiliser supply in the second year.

The ways in which yield and root HCN were influenced by variety are shown in Table 5.4.

Table 5.4 Effects of varieties on root HCN levels and yields in Years 1 and 2

Variety	Year 1			Year 2		
	Root HCN (mg/kg)	Fresh yield (t/ha)	Dry yield (t/ha)	Root HCN (mg/kg)	Fresh yield (t/ha)	Dry yield (t/ha)
Salanga	128.2[a]	9.5[a]	2.6[a]	140.9[a]	21.1[a]	5.0[a]
Kalinda	30.6[b]	11.3[a]	3.1[a]	42.3[b]	25.8[a]	7.6[a]
Supa	18.5[b]	11.4[a]	3.2[a]	19.1[b]	22.0[a]	7.1[a]
Kiroba	49.1[b]	11.4[a]	3.2[a]	55.2[b]	26.8[a]	8.3[a]

Within each measured parameter and for each factor, means in the same column followed by the same lowercase letter are not significantly different at $p < 0.05$ using the Tukey's test.

In both years, the bitter cassava variety *Salanga* gave significantly higher root HCN than the other three varieties (Table 5.4). Root HCN levels of the variety *Salanga* were consistently on average above 100 mg/kg, while root HCN levels in the varieties *Kalinda*, *Supa* and *Kiroba* were consistently on average much below 100 mg/kg. With root HCN levels above 100 mg/kg, the variety *Salanga* classified as

bitter while the other three varieties are classified as sweet (Burns *et al*., 2010). The non-significant effects of variety on yields indicates that all varieties had similar yield potentials. The ways in which yield and root HCN, in each variety, were influenced by N, P and K application are discussed in the next sections (section 5.3.2 and 5.3.3).

5.3.2 Effects of N, P and K fertiliser application on the root HCN of each variety in Years 1and 2

Table 5.5 shows how the root HCN levels and yields of each variety were influenced by N, P and K under field conditions in Years 1and 2. In Years 1 and 2, due to the greater influence of variety on cyanogenic glucoside accumulation in the varieties *Salanga*, *Kalinda* and *Supa*, their root HCN levels were not significantly different in response to fertiliser application. Only in the sweet variety *Kiroba* were the root HCN levels influenced by fertiliser application. Similar to the findings of the present study, other studies have also reported significant increases in root HCN levels with N-P-K fertiliser application (Uyoh *et al*., 2007), while other studies reported no effects (Adekayode and Adewumi, 2013; Cuvaca *et al*., 2015). Okwu and Awurum (2001) reported reductions in root HCN with increased N-P-K fertiliser application. Effects of N, P and K fertiliser on root HCN levels are thus largely dependent on variety type.

From its consistently low root HCN levels, of less than 100 mg/kg, both in Years 1 and 2, the bitter local variety *Kalinda* appears to be wrongly considered by farmers as being toxic. It may simply be an innocuous bitter tasting variety containing in its tissues bitter compounds other than the bitter cyanogenic glucosides (King and Bradbury, 1995).

Table 5.5 Effects of N, P and K fertiliser application on root HCN levels and yields of each variety in Years 1 and 2

Variety	Fertiliser treatment	Year 1			Year 2		
		Root HCN (mg/kg)	Fresh yield (t/ha)	Dry yield (t/ha)	Root HCN (mg/kg)	Fresh yield (t/ha)	Dry yield (t/ha)
Salanga	$N_0P_0K_0$	108.7^{a}	7.8^{a}	2.1^{ab}	122.8^{a}	14.3^{a}	3.2^{a}
	$N_0P_0K_{50}$	97.6^{a}	5.0^{a}	1.3^{b}	131.7^{a}	16.4^{a}	3.9^{a}
	$N_0P_{10}K_{50}$	-	7.7^{a}	2.1^{ab}	135.7^{a}	29.3^{a}	7.2^{a}
	$N_{25}P_{10}K_{50}$	-	10.6^{a}	2.9^{ab}	151.7^{a}	23.8^{a}	6.4^{a}
	$N_{50}P_{10}K_{50}$	147.2^{a}	13.2^{a}	3.9^{a}	127.6^{a}	17.4^{a}	4.1^{a}
	$N_{100}P_{25}K_{100}$	159.3^{a}	12.7^{a}	3.4^{ab}	175.6^{a}	25.1^{a}	5.6^{a}
	CV (%)	23.0	31.7	33.0	13.7	25.9	35.2
Kalinda	$N_0P_0K_0$	23.1^{a}	7.6^{a}	1.8^{a}	32.0^{a}	24.0^{a}	7.1^{a}
	$N_0P_0K_{50}$	17.9^{a}	8.5^{a}	2.3^{a}	47.4^{a}	23.7^{a}	6.7^{a}
	$N_0P_{10}K_{50}$	-	11.3^{a}	3.1^{a}	43.8^{a}	27.6^{a}	7.8^{a}
	$N_{25}P_{10}K_{50}$	-	12.1^{a}	3.3^{a}	45.0^{a}	24.0^{a}	7.6^{a}
	$N_{50}P_{10}K_{50}$	44.3^{a}	15.8^{a}	4.4^{a}	45.8^{a}	28.5^{a}	8.8^{a}
	$N_{100}P_{25}K_{100}$	37.3^{a}	12.5^{a}	3.5^{a}	39.8^{a}	27.1^{a}	7.4^{a}
	CV (%)	32.9	28.0	33.8	14.4	37.7	41.4
Supa	$N_0P_0K_0$	17.2^{a}	7.2^{b}	1.8^{bc}	17.7^{a}	19.9^{a}	6.3^{a}
	$N_0P_0K_{50}$	11.5^{a}	6.7^{b}	1.1^{c}	20.7^{a}	18.2^{a}	6.0^{a}
	$N_0P_{10}K_{50}$	-	6.0^{b}	1.3^{bc}	18.6^{a}	22.8^{a}	8.1^{a}
	$N_{25}P_{10}K_{50}$	-	14.0^{ab}	4.5^{ab}	16.3^{a}	25.0^{a}	7.1^{a}
	$N_{50}P_{10}K_{50}$	18.4^{a}	14.1^{ab}	4.1^{abc}	18.4^{a}	23.9^{a}	7.7^{a}
	$N_{100}P_{25}K_{100}$	27.0^{a}	20.6^{a}	6.4^{a}	22.8^{a}	21.9^{a}	7.6^{a}
	CV (%)	39.6	28.0	38.2	19.7	18.4	16.2
Kiroba	$N_0P_0K_0$	41.5^{b}	5.2^{b}	1.4^{b}	54.4^{ab}	18.5^{a}	6.1^{a}
	$N_0P_0K_{50}$	42.3^{b}	5.9^{b}	1.7^{b}	63.1^{a}	24.7^{a}	7.6^{a}
	$N_0P_{10}K_{50}$	-	9.1^{ab}	2.5^{ab}	63.5^{a}	31.5^{a}	9.8^{a}
	$N_{25}P_{10}K_{50}$	-	12.6^{ab}	3.5^{ab}	43.6^{b}	26.0^{a}	7.3^{a}
	$N_{50}P_{10}K_{50}$	44.9^{b}	17.6^{a}	5.1^{a}	55.0^{ab}	26.0^{a}	8.4^{a}
	$N_{100}P_{25}K_{100}$	67.9^{a}	18.1^{a}	5.2^{a}	51.2^{ab}	34.1^{a}	10.6^{a}
	CV (%)	11.9	29.8	33.1	12.1	25.2	24.0

Within each measured parameter and for each variety, means in the same column followed by the same lowercase letter are not significantly different at $p < 0.05$ using the Tukey's test. Plants under the $N_0P_{10}K_{50}$ and $N_{25}P_{10}K_{50}$ fertiliser treatments in Year 1 had not been analysed for their root HCN content.

Considering the effects of fertiliser, *Kiroba* plants had the lowest root HCN levels under the control ($N_0P_0K_0$), K only ($N_0P_0K_{50}$) and the moderate N-P-K ($N_{50}P_{10}K_{50}$) treatments in Year 1 (Table 5.5). Its highest root HCN levels were obtained under the highest N-P-K treatment ($N_{100}P_{25}K_{100}$). Contrary to this, Mohan Kumar *et al*. (1977) and Susan John *et al*. (2005) found higher root HCN levels in unfertilised cassava compared to those fertilised with N-P-K fertiliser. Root HCN levels of 103.0 mg/kg, 68.0 mg/kg and 98.0 mg/kg (fresh weight basis), were, respectively, obtained under the Control (0-0-0 N-P-K kg/ha), sole K (0-0-83 N-P-K kg/ha) and N-P-K (100-44-83 N-P-K kg/ha) treatments in a study by Mohan Kumar *et al.* (1977), while root HCN levels of 107.1 mg/kg, 41.7 mg/kg and 77.5 mg/kg (fresh weight basis), were, respectively, obtained under the same treatments in a study by Susan John *et al*. (2005). The highest root HCN treatments in the studies by Mohan Kumar *et al.* (1977) and Susan John *et al*. (2005) were both obtained in fresh roots of unfertilised cassava plants. Thus, unlike the reported findings, cyanogenic glucoside production in the variety *Kiroba* was not increased by poor soil nutrient conditions. However, like the variety *Kiroba*, in Year 1, the lowest root HCN levels were also obtained under the sole K treatment in the studies by Mohan Kumar *et al*. (1977) and Susan John *et al*. (2005).

In Year 2, unlike in Year 1, *Kiroba* plants under the sole K treatment gave one of the highest root HCN levels for this variety. Contrary to these findings, lower cassava root HCN levels are usually obtained with sole K fertiliser (De Bruijn 1973; Susan John *et al.*, 2005; Endris, 2006; Susan John *et al*., 2007). Root HCN levels under sole K were statistically similar to levels under P+K fertiliser application. Root HCN levels under the P+K treatment were thus also as high. The observed increase in root

HCN levels in the variety *Kiroba* under the sole K and P+K fertiliser may have been caused by the excessive rainfall received prior to harvest (Fig. 5.1). In the study of Cuvaca *et al*. (2015), although no significant differences had been obtained with fertiliser use, the highest root HCN levels had also been found in cassava under sole K fertilisation after the crops had experienced excessive rainfall prior to harvest. Excessive rainfall prior to harvest appears to have a similar effect of increasing cyanogenic glucoside levels as that experienced in a dry season or a drought (Sriroth *et al*., 2001; Vandegeer *et al*., 2013). There is a tendency to increase HCN levels due to water stress, which can be associated with both low and excessive moisture conditions (Bokanga *et al*., 1994).

Root HCN levels (67.9 mg/kg) of *Kiroba* plants under the highest fertiliser rate had exceeded the 50 mg/kg safe limit for fresh cassava consumption (Codex Alimentarius Commission, 2013), in Year 1. Given the low effect of P on root HCN accumulation (De Bruijn, 1973), the resulting root HCN levels in *Kiroba* plants were, thus, mainly dependent on the balance between the supply of N and K. The limited influence of P is also seen by the similar root HCN levels obtained under sole K and P+K fertiliser as was observed in the present study and in the studies by Mohan Kumar *et al*. (1977) and Susan John *et al*. (2005). An imbalance between N and K, where N was higher, could have hence resulted in the increased root HCN levels observed in *Kiroba* plants under the highest N-P-K fertiliser rate. A suitable balance between N and K is needed to control the effects of excess N in increasing HCN production in cassava (Susan John and Imas, 2013). The higher root HCN levels observed under sole K and P+K in the variety *Kiroba* in Year 2, suggests that an

imbalance between N and K, this time caused by more K relative to N, could also be undesirable when water stress (excess water) is experienced prior to harvest.

Unlike in Year 1, *Kiroba* under the control, K only and moderate N-P-K ($N_{50}P_{10}K_{50}$) treatments had just as high root HCN levels as those obtained under the highest N-P-K rate ($N_{100}P_{25}K_{100}$) in Year 2. This shows that when *Kiroba* plants are unfertilised they too can attain undesirable levels of cyanogenic glucosides when water stressed. Plants under the $N_{50}P_{10}K_{50}$ had the lowest root HCN levels in Year 2; this treatment probably had a better N to K balance that lowered root HCN production more efficiently even with moisture stress as a result of excess water.

5.3.3 Effects of N, P and K fertiliser application on the fresh and dry yields of each variety in Years 1 and 2

Only the varieties *Supa* and *Kiroba* had significant increases in fresh root yields in response to N, P and K fertiliser in Year 1 (Table 5.5). Dry root yields of the variety *Salanga* were, however, also significantly increased by N, P and K fertiliser. The increase in yield in Year 1 seems to be largely due N. A similar trend has been reported by Mohan Kumar *et al*. (1977). On the contrary, in Year 2, the dry and fresh root yields of each variety in response to N, P and K were all not significantly different (Table 5.5). Unfertilised cassava plants thus had similar root yields as optimally fertilised plants in Year 2. The effects of N, P and K on yield for the varieties *Salanga, Supa* and *Kiroba* were thus different in Year 1 as compared to Year 2. This was probably due to the presence of nutrient deficiencies in Year 1. Fermont *et al*. (2010) mentions that varied yield responses to N, P and K can occur when some fields have uncorrected nutrient limitations. On the other hand, the non-response to the N, P and K by the variety *Kalinda* in both years could be because of

its better adaption to low soil nutrient conditions (CIAT, 2011), which lead it to give yields that are reasonably high even when unfertilised.

In Year 1, sole K was mostly associated with low yields in the fertiliser responsive varieties. Contrary to these findings, sole K is often attributed to increasing cassava yields (Adekayode and Adeola, 2009). The observed low yields suggest that other nutrients were more limiting in soils than K (Tisdale *et al*., 1993). The soils in the field experiment in Year 1 was uncorrected for deficiencies of Mg, S, Zn and Cu (Table 5.1). The treatments $N_{50}P_{10}K_{50}$ and $N_{100}P_{25}K_{100}$ were both associated with the highest yield values in fertiliser responsive varieties in Year 1. This highlights the importance of N and P (Howeler, 1995) and adequate and balanced fertiliser use in improving cassava yields (Kamaraj *et al*., 2008).

Yields reported for the variety *Kiroba* under the $N_{50}P_{10}K_{50}$ treatment (17.6 t/ha) in Year 1 were lower than the 20.5 - 37.9 t/ha reported by Shekiffu (2011) when the variety *Kiroba* was supplied with N, P and K at rates of 40 kg N/ha, 15 - 30 kg P/ha and 40 kg K/ha. With the correction of soil nutrient deficiencies fresh root yields increased to 26.0 t/ha in Year 2 and were thus now within the range reported by Shekiffu (2011). Cassava yields are often on average about 10 - 15 t/ha (Roy *et al*., 2006). However, as indicated by the high coefficients of variation for each variety (Table 5.5), the effects of N-P-K on yield were masked by high variability amongst treatment replicates. Clear differences in yields in response to N-P-K fertiliser application were hence not seen.

5.3.4 Relationships between root HCN levels and N supply in Years 1 and 2

Table 5.6 show the results of the correlations between root HCN and N supply (0, 25, 50 and 100 kg N/ha) for each variety in both years. Even though all correlations were

positive, no significant correlations were observed between root HCN levels and N supply in all varieties in both years. Also, no correlations between soil N levels and root HCN level were found in the study by Burns *et al*. (2012).

Table 5.6 Correlations between cassava root HCN and N supply in Years 1and 2

Period	Value	*Salanga*	*Kalinda*	*Supa*	*Kiroba*
Year 1	r	0.616	0.502	0.206	0.552
	p-value	0.078	0.168	0.594	0.123
	r^2 (%)	37.9	25.2	4.2	30.5
Year 2	r	0.584	0.178	0.515	0.021
	p-value	0.059	0.579	0.087	0.948
	r^2 (%)	34.1	3.2	26.5	0.0

Where 'r' is the Pearson's correlation coefficient and 'r^2' the coefficient of determination.

5.4 Conclusions

Root HCN reduction can be achieved with N-P-K use in Mtwara region but only in a few cassava varieties and only with certain fertiliser combinations and rates. Inconclusive results were obtained on the effects of N-P-K on yield. Yields of a few varieties may however remain unaffected by N-P-K, as they are well adapted to the poor soil nutrient conditions of the region.

5.5 Recommendations

The following are recommended:

i. Moderate additions of N-P-K fertiliser (mainly 25-10-50 N-P-K kg/ha and 50-10-50 N-P-K kg/ha to a lesser extent) should be adopted as they can reduce root HCN levels and increase cassava yields in the region.

ii. Breeders should aim to breed cassava varieties that will not have their root HCN levels increased by changes in nutrient and moisture supply.

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CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The study established the influence of soil nutrient supply on cyanogenic glucoside production in some cassava varieties grown in konzo-prone areas of Mtwara region. The influence of nutrient supply on cyanogenic glucoside production in cassava has been observed by farmers. In their observations cassava root bitterness occurs on nutrient depleted soils, which implies that cyanogenic glucoside levels in cassava increase with decreased soil fertility. Most farm fields in the konzo-prone villages have inadequate levels of many soil nutrients needed for the optimal growth of cassava and are thus capable of causing nutrient stress and the associated increase in cassava cyanogenic glucosides. According to the farmers' perceptions, the predominance of soils of low fertility could increase imply that its influence on cassava cyanogenic glucoside production may be widespread. This is however not the case as, contrary to the farmers' perceptions, it was found that cassava varieties grown in the region are adapted to nutrient poor conditions and thus attain the lowest root HCN levels when soils have low soil fertility. However, poor soil nutrient conditions where higher K levels occur relative to N can result in increased root HCN levels in a few cassava varieties when water stress occurs prior to harvest. High K levels in these soils are quite common, while N is severely deficient on almost all fields in konzo-affected areas of Mtwara region. Soil nutrient conditions of higher K

relative to N are thus quite common and could be responsible for the increased cassava root HCN contents observed during periods of water stress.

In general two kinds of cassava varieties are found in the region; those with their cyanogenic glucoside production influenced and those not influenced by nutrient supply. Increased nutrient supply, particularly with the addition of high N, could increase root HCN levels in varieties affected by nutrient supply. The increased proportion of N to K supply is what causes the increase in cyanogenic glucoside production, which could reach levels toxic for human consumption. Caution must thus be taken with the use of N containing fertilisers in these konzo-affected areas. On the other hand, root HCN levels are maintained at fairly constant levels in cassava varieties whose cyanogenic glucoside content is not influenced by nutrient supply because of a strong genetic control of cyanogenic glucoside production in these varieties. These varieties hence retain their inherent varietal characteristics which make them either sweet, moderately bitter or bitter.

On the overall, nutrient supply has a greater influence on cassava cyanogenic glucoside production by the varieties than has soil moisture but a comparable influence to that of variety. Effects of soil moisture in the moisture sensitive varieties are mainly dependent on soil nutrient conditions of high K relative to N. For these varieties, sole K fertiliser should not be recommended. Moderate applications of N in N-P-K fertilisers (25-10-50 kg/ha or 50-10-50 kg/ha) is however able to minimise the effects of water stress in these cassava varieties. Moderate rates of N in N-P-K fertilisers also reduce root HCN levels of water stress insensitive varieties and give low HCN levels comparable to those obtained when these cassava varieties are not

fertilised or when they are supplied with only K. Sole K fertiliser is not beneficial for these varieties.

With improved nutrient supply cassava yields of most varieties would increase with N-P-K use; however, this is only when there are deficient nutrients other than N, P ad K in soils. Varied effects of N-P-K on yields however occur not only between varieties but also within a particular variety depending on soil nutrient conditions. Yields of a few varieties may however not be influenced by N-P-K at all, showing their greater adaptation to the low soil nutrient conditions of konzo affected areas.

Although the present findings were based on Mtwara region in Tanzania, similar trends may be observed in other konzo-affected areas in Africa.

6.2 RECOMMENDATIONS

In view of the results obtained, the following recommendations are given:

i. Farmers need to better manage soil nutrients on their fields to help minimise nutrient stress and its associated effects on cyanogenic glucoside production in cassava produced in konzo-affected areas.
ii. Moderate applications of N in N-P-K at rates of 25-10-50 kg/ha or 50-10-50 kg/ha are recommended in the region in order to reduce root HCN levels but also for improving cassava yields.
iii. When breeding for low cyanide varieties, low cyanogenic glucoside levels under both moisture and nutrient stress conditions should be the target.
iv. To ensure that cassava cyanide related toxicity problems are not introduced to new areas, effects of both water and nutrient supply on cyanogenic glucoside

accumulation need to be investigated on varieties before introducing them into new areas.

APPENDICES

Appendix 1 Questionnaire used to obtain the perception of farmers about the causes of cassava bitterness

1. Which of these factors causes cassava to become bitter?

Reason (Don't read out suggested responses)	Yes/No
1. Type of variety	
2. Drought	
3. Soil type	
4. Length of time matured cassava is left in ground	
5. Time of the year (e.g. wet or dry season)	
6. Other (specify):	

2. If 3 = Yes, then describe soil characteristics thought to bring about cassava bitterness. If soils are located in a certain location, mention this?

_____

_____

_____

_____

_____

_____

3. If 5 = Yes, then specify the time of the year that bitter and sweet tastes are observed and mention the variety?

Variety	Season when it becomes bitter
1.	
2.	
3.	

Appendix 2 Soil profile description

Profile number: P-NAL Date: 2015/05/02

Region: Mtwara District: Mtwara Area: Mikindani

Location: Naliendele Agricultural Research Institute, Cassava research fields

Coordinates: S 10°22'58", E 040°10'01" Elevation: 145 m

Season/Weather conditions: End of rainy season

Soil Name: Classification FAO: Ferrosol Soil Taxonomy: Oxisol

Soil moisture regime: Ustic

Soil temperature regime: Isohyperthermic

Landform: Dissected Plateaux
Macro relief: Upper slope on ridge
Micro relief: None

Parent material: Quaternary, Neogene, Jurrasic and Cretaceous sediment

Geological formation: Developed on Quaternary, Neogene, Jurrasic and Cretaceous sediment

Site characteristics: Slope gradient: 0 - 2%
Slope type of: very gently sloping
Length of slope: 75m
Position on slope: Lower slope

Natural vegetation: Shrubs: 15%, Herbs: 20%, Grasses: 60%, Bare ground: 5%

Land use: Rain fed arable cultivation of cassava as a mono-crop; land now under fallow but still has some cassava outcrops interspersed between grass, shrubs and herbs.

Surface characteristics: Rock outcrops: None; Surface stoniness: None; Sealing/crusting: None; Erosion: by water; Type: Sheet and rill erosion; Degree: Slight; Deposition: Not evident; Natural drainage class: Well drained.

Ap 0 - 12 (7 - 16) cm; dark brown (5YR 3/4) moist. Sand. Very friable moist, non-sticky and non-plastic wet. Weak medium crumbs. Common very fine pores. Many very fine roots; clear wavy boundary.

Bs1 16 - 48 (43 - 53) cm; red (5YR 3/6) moist. Loamy sand. Very friable moist; non-sticky and non-plastic wet. Weak medium sub-angular blocks. Common very fine pores. Many very fine roots; gradual smooth boundary.

Bs2 48 (43 - 53) - 104 (84 – 124) cm: red (5YR 4/6) moist. Sandy clay loam. Friable moist, slightly sticky and slightly plastic wet. Weak to moderate medium sub-angular blocks. Common very fine pores. Common very fine roots, few open burrows; diffuse smooth boundary.

Bs3 104 (84 – 124) - 160+ cm: red (5YR 4/8) moist. Sandy clay loam. Friable moist, slightly sticky and slightly plastic wet. Moderate medium sub-angular blocks. Common very fine pores and very few medium roots. Common very fine roots.

Appendix 3 Analytical data for soil profile

	Horizon			
Parameter	Ap	Bs1	Bs2	Bs3
Depth (cm)	0 – 12	12 – 48	48 – 104	104 – 160 +
Clay %	9	19	26	32
Silt %	0	1	0	0
Sand %	91	80	74	68
Texture class	Sa	SaL	SaCL	SaCL
Silt/clay ratio	0.00	0.05	0.00	0.00
Bulk density g/cm^3	1.54	1.50	1.43	1.34
pH H_2O 1:1	5.2	5.0	4.8	5.0
pH KCl 1:1	4.1	3.8	3.7	3.7
pH H_2O 1:2.5	5.5	5.4	4.8	5.0
pH KCl 1:2.5	4.2	4.0	3.8	3.9
NaF	7.7	8.0	8.3	8.7
Organic C%	0.20	0.20	0.15	0.10
Total N%	0.03	0.03	0.03	0.03
C/N	6.67	6.67	5.00	3.33
Avail. P mg/kg	0.48	0.20	trace	trace
CEC (1 *M* NH_4OAc cmol/kg)	8.6	3.6	3.0	3.6
Exch. Ca cmol/kg	0.31	1.10	0.31	0.05
Exch. Mg cmol/kg	0.15	0.10	0.04	0.85
Exch. K cmol/kg	0.05	0.07	0.16	0.15
Exch. Na cmol/kg	0.05	0.03	0.02	0.02
Total Reserve Bases cmol/kg	0.56	1.3	0.53	1.07
Base saturation %	6.5	36.1	17.7	29.7
Exch. Al (cmol/kg)	0.08	0.22	0.40	0.36
Exch. H (cmol/kg)	0.09	0.19	0.17	0.23
ECEC cmol/kg	0.73	1.71	1.10	1.66
Fe mg/kg	16.02	10.86	4.72	2.01
Cu mg/kg	0.19	0.19	0.05	0.19
Zn mg/kg	0.18	0.10	trace	trace
Mn mg/kg	12.68	9.22	4.92	2.01