Development of cassava (*Manihot esculenta* Crantz) cultivars for resistance to cassava mosaic disease in Zambia

By

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THESIS ABSTRACT

Despite the increasing number of farmers growing cassava in Zambia, yield per hectare has remained low at 5.8 t ha^{-1}. The major constraints contributing to low yields are pests and diseases of which cassava mosaic disease (CMD) caused by East Africa cassava mosaic virus (EACMV), Africa cassava mosaic virus (ACMV) and South Africa mosaic virus (SACMV) is the most important. Breeding of cassava is restricted by limited information on viruses and associated satellites, and farmer preferences. Most of the farmers cannot manage to institute control strategies that require buying of chemicals. The most feasible option remains improving existing cultivars through resistance breeding. The study therefore was conducted to: i) establish farmers' perception and knowledge of CMD; ii) to identify viruses of cassava occurring in Luapula province; iii) evaluate the performance of local and improved cultivars for agronomic traits; iv) evaluate the performance of F_1 progenies for CMD resistance; and v) determine general combining ability and specific combining ability for CMD resistance. The studies were carried out between 2008 and 2011 at different locations in Zambia. The information generated was important in formulating a local breeding strategy for CMD resistance.

A participatory rural appraisal and a structured survey was conducted in Mansa, Samfya and Mwense districts in Luapula province involving farmers to ascertain farmers' perceptions of CMD. The results of the study showed that the majority of the respondents (97.6%) were not aware of CMD. Most of the farmers grew landraces on small pieces of land. Although, the cultivars (local and improved) were widely grown, they were susceptible to CMD. The farmers preferred cultivars with high yielding and early bulking characteristics among others.

A CMD survey conducted between April and May 2009 in Samfya, Mansa, Mwense, Kawambwa and Nchelenge districts in Luapula province established East Africa cassava mosaic virus (EACMV), and Africa cassava mosaic virus (ACMV) as the most prominent viruses in the area. Symptoms of satellites were also observed in the farmers' fields in most of the areas visited. Satellite II and III were detected in leaf samples. The CMD incidence (59.1%) and severity (2.4) was moderate across the districts surveyed. The CMD symptoms on the cassava plants were variable with plants showing mild and severe symptoms characterised with narrowing and reduced leaf blades. The transmission of CMD infections was mainly through cuttings rather than via whitefly infection which means that most of the planting materials used by the farmers were infected.

Evaluation of cassava cultivars for CMD resistance was conducted in 2009/2010 and 2010/11 seasons at Mansa Research Station in Luapula province using a 4 x 4 α lattice design. Both introduced and locally grown cultivars had significant ($P<0.001$) differences in their reaction to CMD. Bangweulu, Namuyongo, Kalaba, Chikula, Mwakamoya, Chila7 and Chila11 were the most susceptible genotypes. Mweru, Tanganyika, and Nalumino were moderately tolerant to CMD.

Eight hundred F_1 genotypes developed using a North Carolina II mating design were evaluated in a 4 x 5 α lattice design in 2011 at Mansa Research Station, Luapula province to determine combining ability for reaction to CMD, yield and yield components. The plants were harvested 7 months after planting (MAP). Significant ($P<0.001$) general combining ability and specific general combining ability were recorded for CMD. The SCA effects were more important for CMD than GCA effects suggesting that non-additive gene action was more prominent than the additive gene action in determining CMD reaction. Parent lines with desired significant, negative GCA effects for reaction to CMD were Bangweulu, Kampolombo, Nalumino and TME2.

In general, the survey and participatory rural appraisal established CMD as one of the constraints to cassava production and created a basis for the research study. The findings indicate opportunities that exist in creating genotypes with tolerance to CMD. The study identified cassava lines with resistance to CMD. The lines that expressed the above trait should be selected and tested further for release to the farmers in Zambia. Since the clonal evaluation trial was harvested at 7 MAP, there is need to investigate further for earliness trait in best performing lines in different locations.

Declaration

I Patrick Chiza Chikoti hereby declare that the research work in this thesis, prepared for the Doctor of Philosophy degree in Plant Breeding, submitted by me to the University of KwaZulu-Natal, is my own original work and has not previously, in its entirety or in part, been submitted to any other university. This thesis does not contain data, pictures, and graphs from other peoples work nor text, graphics, tables from the internet. It also does not contain persons writing. Where other persons work has been sourced, the words have been rewritten and information attributed to them referenced.

Signed on ……..

……..

Signature

Patrick Chiza Chikoti

Supervisors approval

As the candidate's supervisors we agree to the submission of this thesis.

Professor Rob Melis …….

(Principal supervisor) Signature Date

Dr Paul Shanahan …….

(Co-Supervisor) Signature Date

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Dedication

This thesis is dedicated to my late father (Edward Chikoti), mother (Mary Nankala), late brother (Mushota Chikoti) and the greater family for the pivotal role there have played in encouraging me throughout my educational life.

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General introduction

Cassava (*Manihot esculenta* Crantz) is a shrub widely grown in the tropical and sub-tropical regions of the world between latitudes 30^{o} north and 30^{o} south. It is native to South America (Nassar, 2003) and was introduced to Africa by the Portuguese in the 16th Century (Cock, 1985). The crop is highly valued in marginal agricultural environments due to its ability to grow under conditions of drought (Jennings, 1970) and/or low soil fertility (Howeler, 2002). It is also able to grow in areas with less than 600 mm in semi-arid tropics to more than 1000 mm in sub-humid and humid tropics (Alves, 2002). In addition, it can go without rainfall for four to six months. Furthermore its storage roots may be stored underground for over two years, thereby allowing farmers to harvest on demand. Alongside maize (*Zea mays* L.), rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.), cassava is among the most important sources of energy in most tropical countries of the world (Allem, 2002). Compared to rice, maize or sorghum (*Sorghum bicolor* L.), calories from cassava per hectare are much higher (in non-stress environments) (El-Sharkawy, 1993).

On the global scale, cassava ranks fourth as the most important basic food crop after rice, wheat and maize (Scott et al., 2000). With its diverse use, global production has increased over the years particularly from sub-Saharan Africa. Worldwide, production has increased in the last 40 years and it is anticipated that by the year 2020 production will reach 291 million metric tons (Scott et al., 2000) due to expanded acreage, especially in Africa. Equally sub-Saharan Africa has witnessed an increase in cassava production. Between 2001 and 2009, 996 million tons fresh mass was produced, with Africa accounting for 50.8% (FAO, 2009). Most of the cassava is grown in West Africa adjoining the Congo basin, tropical South America, and South East Asia. According to FAOSTAT (2009), world cassava production in 2009 was estimated at 240 million tons (Table 1), eight million tons more than the 2008 production, with Africa contributing about half of the world production. In Africa cassava is one of the most widely grown staple crops with harvests reaching 124 million tons in 2009 (FAOSTAT, 2009). Though the crop is grown widely in Africa, average yield vary from one country to another for example in Ghana 12 t ha^{-1}, Nigeria 11.8 t ha^{-1} and in Angola 12.8 t ha^{-1} (FAOSTAT, 2009).

Table 1.1: Production of cassava in the world and selected countries

Country	Quantity (million tons)	Yield (t ha^{-1})
World	240	12.6
Africa	124	10.1
Nigeria	45*	11.8
Tanzania	6.5*	9.7
Democratic Republic of Congo	15*	8.1
Angola	9*	12.8
Zambia	0.9	5.8

Source: FAOSTAT (2009); estimates for 2009 production, FAO (2009)*

In Zambia, the crop is grown on 200 000 ha with an average output of 5.8 t ha^{-1}, an amount that is substantially below the Africa's average (10.1 t ha^{-1}) and about one-third that of Malawi (19.1 t ha^{-1}) (FAOSTAT, 2009) which borders Zambia to the east. The crop is the second most important food crop after maize and supports about 30% of the estimated 13.8 million Zambians. Most of the cassava is grown by the small scale farmers and the majority (in major cassava growing areas) consider cassava as the most important crop (Kuseka, 2011). The crop is grown in many parts of the country. However, the main producing areas are in the Luapula, Northern, North-Western and Western Provinces. Cassava forms an important component of the cropping system in Zambia. As in most parts of Africa, cassava in Zambia is used in various ways: as a raw material for industry and livestock and as a staple food. In the paper industry, cassava is used as a source of starch (in Zambia). As one of the principal foods, it is blended with maize meal, millet or sorghum depending on local traditions and customs. In addition to it being a basic food crop, the stems are used as planting materials. Furthermore, in the event of drought, cassava is used as a hedge against famine when all other crops fail.

Despite the many attributes (drought tolerance and low input requirement), coupled with the sizable land planted under cassava in Zambia each year, yield per hectare has been on the decline or has remained the same over the years. Between 1990 and 2009 cassava yield has declined from 6.2 to 4.5 t ha^{-1} (Figure 1). The decline in yield occurred despite the release of improved cultivars in 1993 and 2000 by the Zambia Agriculture Research Institute (ZARI). Apparently, the cultivars were developed for yield potential and have inadequate levels of CMD resistance. The increasing area under production and demand for cassava justifies the development of improved cassava cultivars for the farmers.

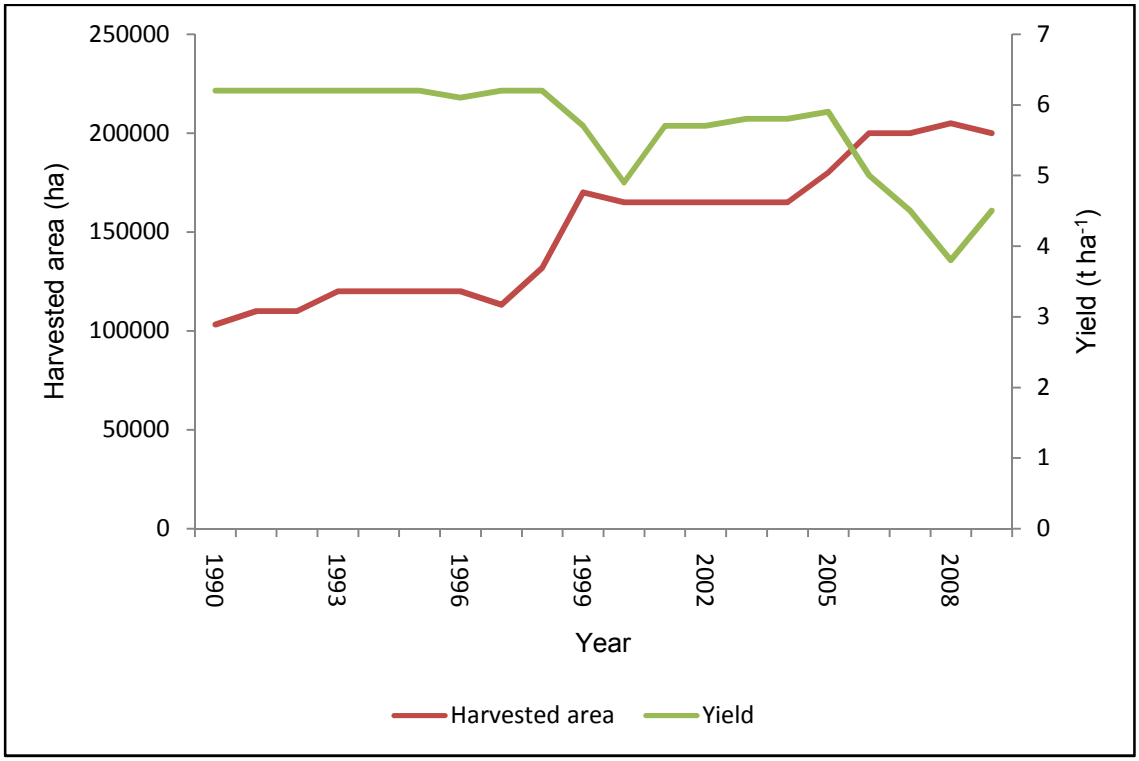

Figure1. 1: Trend in yield and area harvested of cassava in Zambia, 1990-2009 (FAOSTAT, 2009)

The poor performance of cassava can be attributed to a number of challenges of which abiotic and biotic factors are some of the constraints. Other limitations include unsustainable farming system owing to intensification of cassava production without innovation (Goossens, 1997). Abiotic stresses limiting productivity include low soil fertility, acidic and alkaline soils, drought, and low temperatures. In addition to abiotic stresses, lack of improved cultivars and post-harvest losses have been suggested to impact negatively on cassava production (Hillocks, 2002). Insect pests and diseases are the major biotic constraints. A wide range of insect pests are now found in Africa and important ones are cassava green mite (CGM: *Mononychellus tanajoa* Bondar), cassava mealy bug (CMB: *Phenacoccus manihoti* Matile-Ferrero), whiteflies (*Bemisia tabaci* Genn.), termites (*Cubitermes tenuiceps*), and variegated grasshopper (*Zonocerus variegates* (L)). The major diseases include cassava bacterial blight (CBB) (*Xanthomonas axonopodis* pv. *manihotis*), cassava mosaic disease (CMD), cassava anthracnose disease (CAD), cassava brown streak disease (CBSD) and root rots. The most important of these diseases is CMD and it is a serious problem in Africa and Zambia in particular. It is caused by either African cassava mosaic virus (ACMV), East

African cassava mosaic virus (EACMV) or South Africa mosaic virus (SAMV) and is transmitted by whiteflies. Of these three virus strains, EACMV and ACMV are most common and important in Africa and are also prevalent in Zambia. In addition to viruses, sub-viral catalysts known as satellites cause undesirable effects in cassava plants through virus accumulation and increase the severity of the expression of the symptoms of their helper virus (Mansoor et al., 2003). The satellites associated with CMD were recently discovered and have been reported to enhance disease symptoms in CMD infected cassava plants (Ndunguru et al., 2008). It is known that cassava infected plants with either ACMV or EACMV show severe symptoms depending on the cultivar. With the presence of satellites in plants with CMD, symptoms are more severe depending on the virus/combination and host plant (Briddon et al., 2008). The interaction of the virus and satellite in the host plant may cause CMD resistance to be broken (Ndunguru et al., 2008).

Cassava mosaic disease occurs as a mixed or single infection. Dual infections with two different cassava mosaic germiniviruses (CMGs) cause more severe symptoms than either virus alone (Fondong et al., 2000; Pita et al., 2001). Cassava losses are in the form of reduced storage roots and stunted plants. In Africa yield losses have been estimated between 15 to 40% (Fargette et al., 1988; Legg and Thresh, 2000; Legg and Thresh, 2003). In monetary terms it is estimated that US $440 million worth of cassava is lost due to CMD annually (Thresh et al., 1997). In Zambia, CMD is the major threat to cassava and is found in all major cassava producing areas (Haggeblade and Zulu, 2003). It causes yield losses of 50 to 70% per year (Muimba-Kankolongo et al., 1997). The yield loss is a result of viruses interfering with photosynthetic process in the leaves thereby reducing storage root size and quality.

With the majority of farmers trading, growing and exchanging infected planting materials in the country without proper phytosanitary controls, it is unrealistic to expect higher yields with the present susceptible and infected cassava cultivars. To enhance sustainable cassava productivity, development of cassava cultivars with improved resistance to cassava mosaic disease is essential. Developing cultivars with reasonable levels of resistance forms an integral part of disease management and reduces yield losses experienced by the farmers.

To mitigate the impact of CMD, the government of Zambia, through the Department of Agriculture has been encouraging small scale farmers to use disease free cassava cuttings. Cultural practices such as rotation and intercropping have also been encouraged. However, the measures have not helped in reversing or solving the extent of CMD infection in the crop. The majority of the economically disadvantaged farmers are unable to use the above control measures because of financial constraints. The long term solution appears to be the development of resistant cultivars. Cassava mosaic disease resistant cultivars exhibit less symptoms (Thresh and Cooter, 2005) and consequently low or no yield reduction. In areas where CMD has been reported, resistant materials have proved to be reliable and effective in combating CMD. In East Africa, the incidence of CMD has significantly been reduced as a result of the multiplication and distribution of resistant cultivars to farmers (Bua, 1999; IITA, 2001; Obiero et al., 2007).

To ensure increased and sustainable cassava production in Zambia, small and medium scale farmers need to be provided with cultivars resistant to CMD. Given the low cassava yields in Zambia, it is also important that Zambia develops its own adapted cultivars through a local breeding programme which incorporate farmer preferred traits such as early bulking and high yield. With the complex nature of the viruses that cause CMD, there is a need to generate more information on the satellites and CMD in Zambia. The appropriate breeding strategy must be employed in view of multiple virus strains and satellites. Therefore the purpose of this research was to strengthen and sustain cassava production through the development of cultivars with CMD resistance.

Research objectives

The objectives of the study were to:

i) establish farmers' key production constraints and desired cassava traits
ii) identify viruses of cassava occurring in Luapula province of Zambia
iii) evaluate cassava genotypes for resistance to CMD
iv) evaluate the performance of F_1 progenies for CMD resistance
v) determine general combining ability and specific combining ability for resistance to CMD

Thesis organization

This thesis is made up of seven chapters as follows:

1. Chapter 1: Literature review
2. Chapter 2: Farmers' perceptions of cassava mosaic disease on cassava cultivar grown in Luapula province
3. Chapter 3: Cassava mosaic geminviruses occurrence in Luapula province
4. Chapter 4: Evaluation of cassava genotypes for resistance to cassava mosaic disease
5. Chapter 5: Evaluation of F_1 cassava progeny performance for agronomic traits
6. Chapter 6: Combining ability analysis of cassava to cassava mosaic disease
7. Chapter 7: Overview of research findings and implications of cassava breeding

All the chapters with the exception of chapters 1 and 7 follow the IMRAD format, i.e. Introduction, Materials and Methods, Results and Discussion.

Chapters 2 to 6 are written as discrete chapters, therefore the text and references may overlap.

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CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

This review provides relevant current background information for a breeding study on cassava (*Manihot esculenta* Crantz). Particular attention is given to characteristics of the cassava plant, production environment, flowering and pollination habit, production constraints (abiotic and biotic), breeding methods, and cassava selection cycle. Furthermore the chapter reviewed current knowledge on cassava mosaic disease (CMD), its spread, symptoms, management and detection. It has also given attention to recent studies of CMD associated satellites and their effects on plant growth. The chapter also looks at the mechanism of CMD resistance, genetics of resistance and sources of resistance.

1.2 Taxonomy of the genus *Manihot*

Cassava is an amphidiploid allopolyploid (2n=36 chromosomes) (El-Sharkawy, 2004), has regular bivalent pairing and behaves as a diploid. It is a perennial shrub of the Euphorbiaceae (spurge) family and native to South America (Allem, 2002; Olsen and Schaal, 2001). Cassava is believed to have originated by hybridisation between wild cassava species (Nassar, 2000). The cassava plant grows to a height of 1.5-3 m. In some cultivars it can reach heights of up to 4 m (Alves, 2002; FSANZ, 2004). The genus includes a large number of different species of which only *Manihot esculenta* is nutritionally and economically important. Hybrids between cassava and other Manihot species occur spontaneously in Africa and South America (Nassar, 1994) and have been found growing in diverse environments. Interspecies crosses have been widening the cassava genetic base of traits such as CMD resistance, yield and low cyanide content.

1.3 Production requirements of cassava

Compared to other crops such as maize, soybean, and wheat, cassava tolerates a wide range of environmental conditions. It grows in a variety of geographical regions from sea level to elevation as high as 2000 m (Kawano, 1980). Cassava has been reported to grow in regions receiving below 600 and over 1500 mm of rainfall per year (Alves, 2002).

Though it is able to grow under a wide range of rainfall conditions, the optimum is approximately 1 000 mm (Kawano, 1980). Cassava can withstand soils with pH of up to 8.0 while some cultivars are able to grow on acidic soils. On acidic soils the crop encounters a host of problems (Howeler, 2002). In certain soils a low pH will lead to high concentrations of aluminium (Al) or magnesium (Mg), which in turn may result in low availability of calcium (Ca), and potassium (K), which are important elements for plant growth. Temperatures between 25 and 35^{o}C are suitable for cassava growth (El-Sharkawy, 1993). The implication of the wide environmental tolerance of cassava is that the crop can be found in many regions of the world, especially in tropical and subtropical areas.

In Africa cassava is mostly cultivated by small scale farmers who have low capital base for inputs such as fertilizers and pesticides. Furthermore most of the crop is continuously grown on marginal lands for many years. Although, cassava is able to give good yields compared to maize under low soil nutrient conditions, root yield performance declines over time if nutrients are not replaced. For example continuous cultivation of cassava for 31 years in Thailand at Ranyong and Banmai Samrong and 30 years at Khonkaen showed yield reduction in the absence of fertilizer application (Nakviroj et al., 2002).

1.4 Reproduction in cassava

Cassava can either be propagated by stem cuttings or by seed, but the former is the most common method (Alves, 2002). The stakes (15-30 cm) are either planted horizontally, vertically or inclined on ridges (El-Sharkawy, 2004). Cassava plants grown from true seed are highly heterozygous (Ng and Ng, 2002) and plants derived from seed can be found growing in farmers' fields. Although cuttings provide rapid establishment, diseases easily build up in infected cuttings (Nassar, 2007). On the other hand storability, ease of transportation, long seed viability and relative absence of insect pests and diseases make seed propagation an option. However, the major limitation is the heterogeneous nature and variation of the seedlings (Nair and Unnikrishnan, 2007). At international research organizations and national research centres, botanical seed is produced for creating new genetic variation in breeding programmes through controlled or uncontrolled pollination. At the Centro Internationcional de Agricultura Tropical (CIAT),

the use of tissue culture techniques have been developed to accelerate cassava production among the small scale farmers (Escobar et al., 2006).

1.4.1 Flowering and pollination

Cassava is monoecious with male and female flowers found on the same inflorescence. The stigma and anthers occur in different flowers on the same plant (Kawano, 1980). The female flowers are located near the base and open 10 to 14 days earlier than the male flowers. However, male and female flowers on different branches can open at the same time (Alves, 2002). Early opening of female flowers facilitates outcrossing through insect pollination. The pollen grains are large and sticky and adhere to insect bodies and this facilitates cross pollination. Due to the size of the pollen grains, natural pollination by wind is not common (Kawano, 1980).

Flowering in cassava depends on the genotype and the environmental conditions, and varies from 6 to 18 months after planting (MAP). In tropical regions, most of the cassava cultivars flower from 8 to 16 MAP. During the first 6 MAP, the flowers are rarely receptive (Kawano, 1980). With the lengthy flowering period, obtaining F_1 seedlings from a cross may take more than a year.

Environmental conditions that affect flowering include soil moisture, photoperiod, and temperature. Long dry weather spells have been reported to inhibit flowering (Kawano, 1980). Apart from environmental conditions mentioned above, flowering in cassava is also affected by day length. Long days favour flowering, while, short days slow down flowering (Keating, 1982). The optimum temperature for flowering is 24^{o}C (Alves, 2002). In regions north of the equator, cassava has been reported to flower between July to January and between January to July south of the equator (Hahn et al., 1979).

Under natural conditions cassava is cross-pollinated, mostly by insects such as several species of wasps and bees. Simultaneous opening of male and female flowers on different branches or different plants belonging to the same genotype can result in self-pollination (Jennings and Iglesias, 2002). Seed produced through self-pollination is considered inbred (Kawano, 1980). Kawano (1980) observed that one cycle of selfing results in some plants becoming weak such that production of female and male flowers

is inadequate for future hybridization. Following self or cross-pollination the amount of seeds produced varies depending on the cultivar.

Controlled pollination can be achieved by covering unopened flowers in a muslin bag and then applying pollen to the stigma of the female flower once it opens (Jennings and Iglesias, 2002). After pollination, netting bags are placed around the fruit to trap the dehiscing seeds from the mature fruit. On average between one to two seeds are obtained per cross using the above technique (Ceballos et al., 2004; Kawano, 1980). The advantage of controlled pollination is that the source of pollen is known and studies can be done on the specific combining ability.

The polycross method developed by Wright (1965), through a mating design, can also be used to cross-pollinate different genotypes. Superior parental lines are randomly distributed and replicated to maximize the frequency of crosses. The method requires critical understanding of flowering capacity in order to achieve synchronized flowering. Though less laborious compared to hand-pollination, avoiding self-pollination is difficult. The advantage is that more seeds from the crosses are obtained than with hand-pollination. Once the harvest is completed seeds from each cross are then bulked to form half-sib families.

1.4.2 Seed germination

After maturation of cassava fruit, the seeds remain dormant and require 3 to 6 months storage at room temperature before they germinate (Jennings and Iglesias, 2002). Under field conditions viable cassava seeds take about 2 to 4 months to germinate (Nartey, 1978). Under these circumstances, the long period it takes for cassava to germinate makes the seed susceptible to infection. High temperatures (35^{o}C) have been found to promote seed germination, while lower temperatures (25^{o}C) reduce germination (Pujol et al., 2002). In addition to high temperatures, mechanical scarification and dry heat treatment enhance seed germination.

Cassava seed germinates optimally at 35^{o}C. Ellis and Roberts (1979) observed that at constant temperature of 35^{o}C, seed germination was higher than alternating temperatures of 25 or 30^{o}C. At either 20 or 40^{o}C no germination was recorded. Treating

cassava seeds with 1% or 300 ppm potassium nitrate (KNO_3) promotes uniform germination (Rajendran et al., 2005). According to Rajendran et al. (2005), more than 60% seed germination can be achieved within 17 days after sowing using KNO_3 treatment. Dark conditions have also been reported to enhance germination (Rajendran et al., 2005). Earlier, Rajendran et al. (2004) reported high seedling vigour and germination of seeds soaked in 1% KNO_3 and in 300 ppm gibberellic acid. Dry heat and complete darkness have also been reported to promote seed germination (Halsey et al., 2008). Under field conditions, good germination is obtained by making holes in the soil and covering the seeds with a thick layer of soil. At CIAT in Colombia, seed germination is done in the screenhouses; seedlings are transplanted to the field when they are 20 to 25 cm tall (Jennings and Iglesias, 2002). At the International Institute of Tropical Agriculture (IITA-Nigeria), seeds from different crosses are planted directly in the field taking advantage of irrigation and high temperatures (30-35^{o}C).

1.5 Cassava mosaic disease

Cassava mosaic disease is the most important viral disease of cassava in Africa. It is widely distributed wherever cassava is grown. In Africa, especially in East Africa where epidemics of the virus have been experienced, its importance has increased in the last two decades (Legg and Fauquet, 2004). The disease is caused by whitefly transmitted begomoviruses (family Geminiviridae). Cassava mosaic geminiviruses have genomes with two circular, single stranded DNA molecules (DNA-A and DNA-B) enclosed in a coat protein (Stanely and Gay, 1983; Stanely et al., 1986). The DNA-A is required for virus replication and encapsidation, while the DNA-B component is responsible for virus movement (Hanley-Bowdoin et al., 1999). DNA-A has six open reading frames (ORFs) and each ORF encodes a specific protein, while DNA-B consists of two ORFs (Patil et al., 2007).

At least three geminiviruses cause CMD (Hillocks and Thresh, 2000). These are African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), and South Africa cassava mosaic virus (SACMV) (Berrie et al., 2001; Berrie et al., 1998). Within the species mentioned, a number of variants have been described and the most widely reported is the Ugandan variant (Ogbe et al., 2006) form of the East African cassava mosaic virus (EACMV: EACMV-UG) (Zhou et al., 1997). EACMV-UG is a recombinant of

EACMV and ACMV which has developed through interspecific recombination (Zhou et al., 1997). In West Africa (Nigeria), increase in the spread of recombinant type of EACMV-UG was observed between 1998 and 2003 (Ogbe et al., 2006).

The first report on CMD in Africa was in 1894 in Tanzania (Jameson, 1964; Fauquet and Fargette, 1990; Legg and Fauquet, 2004). Today the virus is found almost in all major cassava producing areas in sub-Saharan Africa. The countries where cassava viruses, including Ugandan variant (UgV), are found include Burundi (Bigirimana et al., 2004), Uganda (Sseruwagi et al., 2004a), Rwanda (Legg et al., 2001), Kenya (Were et al., 2004), Democratic Republic of Congo and Tanzania (Legg, 1999).

In Zambia little information exists on CMD (and nothing specifically on this disease is in the published literature). The Southern Africa root crops research network (SARRNET) surveys carried out in the mid-1990s reported moderate levels of CMD incidence (41%) in Zambia. A survey of East and Central African countries by Ogbe et al. (1997) used enzyme linked immunosorbent assay (ELISA) based diagnostics to report the occurrence in Zambia of both ACMV (predominant) and EACMV (localized).

1.5.1 Geminivirus satellites

Satellites are sub-viral catalysts composed of nucleic acids which depend on co-infection with a helper virus for their reproductive replication, movement and encapsidation (Briddon et al., 2008). In return, the helper virus benefits through virus accumulation and symptom expression (Mansoor et al., 2003). There are two classes of DNA satellites, alphasatellites and betasatellites. Alphasatellites (formerly known as DNA-1) are 1.3 kb in size and in some cases suppress viral symptoms (Briddon et al., 2008). While betasatellites (previously referred to as DNA-β) are also 1.3 kb in size and associated with monopartite begomoviruses enhance symptom expression. Satellites can be associated with RNA or DNA viruses and differ in size from less than 200 nucleotides to more than 1500 nucleotides. However, most of the satellites have RNA and are associated with viruses with RNA genomes (Briddon and Mansoor, 2008). Although, the satellites are closely associated with the helper virus, the nucleotide sequences for the satellites and that of the helper viruses genomes differ substantially (Briddon et al., 2008; Mansoor et al., 2006). For example, the betasatellites molecules posses a highly

conserved structure and are typically in the region of 1350 nt in length, approximately half the size of the helper begomoviruses (Briddon and Mansoor, 2008).

Relative to their small size, satellites can exacerbate the symptoms induced by their helper virus (Collmer and Howell, 1992; Simon et al., 2004). In addition, the majority of the satellites interfere with replication of their helper virus (Mansoor et al., 2003). Depending on the host plant, symptoms may vary ranging from mild to severe (Briddon et al., 2008). The severity of symptoms may vary depending on the host, helper virus and satellite combinations. Patil and Fauquet (2010) have reported differential interaction between cassava geminiviruses and DNA satellites and also changes of symptom expression by satellites. Similarly, Mansoor et al. (2006) have reported an association of DNA-β (referred to as ssDNA) components with diseases caused by begomoviruses. The DNA satellites are widespread and economically significant especially in developing countries. One example is the cotton leaf curl disease (CLCuD) which was epidemic in the 1990s in Pakistan and India (Briddon et al., 2008).

To demonstrate the effect of satellites on symptom expression, Guo (2008) infected malvastrum yellow vein virus (MYVV) alone in *Nicotiana benthamiana* Domin*, N. glutinosa* L. *and Petunia hybrid*, and no symptoms developed. However, co-inoculation with MYVV and MYVV DNA-β resulted in development of downward curling of leaves associated with yellow vein and leaf curling. The implication is that satellites have a role to play in disease expression. In addition, DNA-β of the satellite encodes a dominant symptom determinant (Saeed et al., 2008). From the works of Briddon and Mansoor (2008), the importance of satellites in disease expression has been demonstrated. When the dimeric construct of DNA-β component associated with ageratum yellow vein virus (AYVV) and cotton leaf curl multan virus (CLCuMV) was integrated into *N. benthamiana*, the transgenic plants developed severe abnormalities demonstrating a pathogenicity determinant that is active in the absence of the helper virus. Reviews by Simon et al. (2004) suggest that the majority of satellites such as sat RNAs of cucumber mosaic virus (CMV sat RNAs) and sat RNAs of groundnut rosette virus (GRV sat RNAs) intensify symptoms in their hosts.

Recently the discovery of ‘satDNA’, associated with CMD in Tanzania and able to break resistance, raises challenges in the breeding for resistance to CMD (Ndunguru et al.,

2008). Furthermore, during the routine surveys in Tanzania, 'satDNA' was found in severely diseased plants (J. Ndunguru personal communication). It is known that ACMV and EACMV co-infected in cassava plants result in more severe symptoms than single infection of either ACMV or EACMV (Chellappan et al., 2004b). In the presence of satDNA the symptoms are even more severe as a result of interaction of suppressor proteins. Betasatellites have been reported to enhance disease symptoms for ACMV, East Africa cassava mosaic Kenya virus (EACMKV) and East African cassava mosaic Zanzibar virus (EACMZV) isolates (Patil and Fauquet, 2010). The implication of the discovery of satellites and more specially the effects of the satellites on host plant is that satellites have to be considered when breeding for resistance to viruses. In addition to widespread distribution and diversity coupled with movement of diseased planting materials, the virus-satellite complex poses threats to the agro-ecological systems (Mansoor et al., 2003). A small number of satellites are known to exacerbate symptoms or produce novel symptoms in groundnuts, tobacco, and turnip among others (Collmer and Howell, 1992).

1.5.2 Other viruses affecting cassava

Cassava brown streak disease (CBSD), a member of the genus ipomovirus and potyviridae family, which is also transmitted by the whitefly (Hillocks and Thresh, 2000), is also becoming an important cassava disease in Africa, especially in East and Southern Africa. The disease was first reported in 1936 in Tanzania (Hillocks and Jennings, 2003; Thresh, 2002). The disease is important as it is associated with root necrosis in cassava (Hillocks et al., 2001). Cassava brown streak disease has been reported in Mozambique, Kenya (Njeru and Munga, 2002), Uganda and Malawi (Shaba et al., 2002). Other viruses of less importance in Africa include cassava virus X (potexvirus), cassava ivorian bacilliform virus and cassava Q virus (Calvert and Thresh, 2002).

1.6 Transmission and spread of cassava mosaic disease

Cassava mosaic disease is widely distributed in Africa and India, however, it is not found in South America. The disease is transmitted by adult whitefly (*Bemisia tabaci* Genn.) (Dubern, 1994) in a persistent manner and retained for at least 9 days. Transmission

efficiency differs depending on the *B. tabaci* biotypes and the germinivirus (Maruthi et al., 2002). Cassava mosaic disease can also be transmitted by grafting and biolistic inoculation (Ariyo et al., 2003). However, the virus is not mechanically transmitted. In farmers' fields, CMD is also transmitted through stem cuttings. The implication is that virus spread is enhanced to areas previously disease free when farmers exchange and plant infected cuttings. Where whitefly populations are low, the spread of CMD has been attributed to the use of infected stem cuttings. In a survey conducted in West Africa, Okao-Okuja et al. (2004) reported infection rates of 86% in Senegal and 83% in Guinea Conakry respectively despite low populations of *B. tabaci* (1.7 adults per shoot). *Bemisia tabaci* has been reported to transmit ACMV, EACMV, EACMV-UG and Indian cassava mosaic virus (ICMV) (Maruthi et al., 2002).

1.6.1 Effect of *Bemisia tabaci* and age of cassava plants on cassava mosaic disease

Crop plants are often more vulnerable to plant pathogens and insect damage during early stages of plant growth compared to later stages. Fargette et al. (1994) reported higher rates of infection in two month old plants than in six month old plants. The yield reduction induced by whitefly infection is consistent with reports by Fargette et al. (1988). Plants infected by *B. tabaci* within 120 days after planting showed a significant yield reduction (Fargette et al., 1988). However, reduction in yield is higher in cuttings infected from the outset than in plants infected by *B. tabaci* at later stages of growth (Fargette et al., 1988). This is consistent with Calvert and Thresh's (2002) observation that plants grown from infected cuttings are more severely affected than those of the same cultivar infected at an early stage by whiteflies. This has considerable implications for small scale farmers who often exchange diseased planting materials within their communities.

1.6.2 Symptoms of cassava mosaic disease

Symptoms of CMD infected cassava plants vary depending on the virus strain, variety and season (Hillocks, 2002). In addition, infection due to the virus is characterised by initial onset of symptoms from which the plant may or may not recover (Patil and Fauquet, 2009). In resistant cultivars few leaves or branches show disease symptoms. Infected leaves are characterised by chlorotic mosaic pattern. In severe infections,

leaves exhibit abscission, necrosis, crumpling, distortion and reduced size (Pita et al., 2001; Sseruwagi et al., 2004a; Zhou et al., 1997), while in moderate infections symptoms consist of patchy green or yellow mosaic without leaf distortion or abscission. As a result of a decrease in photosynthesis in the leaves resulting from chlorosis, tuberous root formation is affected.

Cassava mosaic disease occurs either in mixtures (EACMV with ACMV) or as single infections. Cassava plants with mixed infections (ACMV and EACMV) show more severe symptoms than plants with single infections (Fondong et al., 2000; Legg et al., 2004; Ogbe et al., 2003; Pita et al., 2001). Lokko et al. (2004) reported severe symptoms in plants infected with ACMV and EACMV-UG2. The intensity of symptoms in plants with two or more viruses could be attributed to synergism of two viruses. The variability in symptoms has been reported to be as a result of variations in virus strains, virus virulence, host susceptibility, or vector activity (Patil and Fauquet, 2009). Using the southern blot analysis, Pita et al. (2001) reported a positive correlation between symptom severity and virus accumulation, signifying a possible synergistic interaction between ACMV and EACMV-UG. Under laboratory conditions, cassava plants simultaneously infected with ACMV and EACMV showed severe symptoms (Chellappan et al., 2004b). Cassava plants infected early with ACMV showed higher yield losses than plants infected at later stages of growth (Fargette et al., 1988). In plants infected with EACMV-UG, the symptoms are more severe than plants infected with ACMV (Legg et al., 2004). Although EACMV-UG has caused severe infections in East Africa (Legg and Fauquet, 2004), no such symptoms have been reported in Southern Africa. Resistant varieties may display mild or no symptoms at all when infected with the viruses. However, plants without symptoms can have latent infection. Using specific primers (ACMV-F1/ACMV-R1, ACMV-ALF/ACMV-ARO/R), Lokko et al. (2005) reported the presence of ACMV in resistant and moderately resistant genotypes. Similarly Fargette et al. (1996) reported ACMV in highly resistant cassava cultivars.

1.7 Detection of cassava mosaic viruses and satellites

Cassava geminiviruses are detected using different serological and nucleic methods each with varying levels of sensitivity. One of the serological methods commonly used is the enzyme-linked immunosorbent assay (ELISA). It is robust and quick. In addition to its

robustness, the ELISA method can also quantify the amount of virus in the plant tissue. Although widely used it is less sensitive compared to nucleic methods (Narayanasamy, 2001). Other limitations include failure to distinguish cassava viruses with similar coat protein epitopes such as EACMV and ACMV in mixed infections (Sseruwagi et al., 2004b) or differentiate ACMV from EACMV-UG. Using ELISA technique, cassava mosaic begomoviruses (CMBs) cannot be detected from the symptomless plants. To overcome the limitations of ELISA methods, nucleic acid based diagnostic techniques have been developed which use the polymerase chain reaction (PCR) with specific designed primers. Studies conducted in the 1990s on CMD prevalence and distribution in Zambia, used ELISA method (Ogbe et al., 1997) and physical observation technique (Muimba-Kankolongo et al., 1997).

Polymerase chain reaction is more sensitive as it is able to detect at lower concentrations than the ELISA method. Several workers have used PCR based methods, for example in a study of geminiviruses associated with epidemics of CMD in Uganda (Sseruwagi et al., 2004a; Zhou et al., 1997); synergism studies between ACMV and ECMV in Cameroon (Fondong et al., 2000); molecular variability of cassava mosaic begomoviruses and their distribution in Nigeria (Ariyo et al., 2005).

1.8 Mechanism of cassava mosaic disease resistance

Cassava like many other crops depends on various defence mechanisms for protection against diseases such as CMD. Six categories of resistance to CMD have been suggested (Hahn et al., 1980): 1) immunity; 2) resistance to virus infection; 3) resistance to establishment and spread of virus in host plants; 4) resistance to virus multiplication; 5) tolerance; and 6) resistance to vectors. The above mentioned mechanisms are interrelated to each other in their function. Studies by Ogbe et al. (2002) and Winter et al. (2004) showed that movement of ACMV into cassava plants of resistant and moderately resistant genotypes is restricted. The restriction in the virus movement and multiplication in resistant cultivars, result in appearance of inconspicuous or no disease symptoms. In resistant and susceptible cultivars, there is a correlation between virus titre and symptom intensity (Fargette et al., 1996).

Resistance to the insect vector is also a resistance mechanism (*B. tabaci*) (Ogbe et al., 2002). An understanding of the resistance mechanisms has led to the development of resistant cultivars at IITA and at many National Agriculture Research Organisations across sub-Saharan Africa.

Although defence mechanisms have evolved over time, viruses also developed ways to overcome host plant defences. Work by Chellapen et al. (2004a) has shown that in infected plants, cassava mosaic geminiviruses trigger post-transcriptional gene silencing (PTGS) with the production of virus specific short interfering RNAs (siRNAs).

1.9 Genetics of resistance to cassava mosaic disease

Resistance to CMD was previously thought only to be inherited polygenically. Furthermore, inheritance was considered to be controlled by recessive genes that are additively inherited (Hahn and Holland, 1972; Hahn et al., 1980). However, Akano et al. (2002) have reported qualitative resistance controlled by single dominant gene (CMD2). Using the bulk segregant analysis, Akano et al. (2002) reported a resistant gene associated with SSY28 marker that explains 68% of phenotypic variance of CMD resistance at $P < 0.001$. Earlier, Akano et al. (2000) reported resistant gene CMD1, associated with SSY40 marker on linkage group D TMS 30572 derived genetic map. Unlike CMD2, CMD1 is recessive. Lokko et al. (2005) identified three markers from a cross between resistant landrace TME7 and susceptible line TMS30555. The markers accounted for different levels of total phenotypic variation for resistance, SSRY28-180 (57.4%), SSRY106-207 (35.59%) and E-ACC/M-CTC-225 (22.5%).

Although genes with resistance to CMD have been identified, small scale farmers in Zambia and other countries such as Kenya (Were et al., 2004), continue to get low yields. Lack of CMD resistant varieties with farmer preferred traits explain why susceptible cultivars are still grown in some areas (Hillocks and Thresh, 2000). In Uganda between 1990 and 1994, a CMD pandemic continued to spread rapidly due to availability of few resistant genotypes (Otim-Nape et al., 2001). However with multiplication and distribution of resistant materials, the pandemic in East African countries such as Uganda and Kenya has since been reversed.

1.10 Sources of resistance to cassava mosaic disease

Cassava landraces are cultivated in many parts of Africa and some have been reported to be sources of resistance (Akano et al., 2002; Fregene and Puonti-Kaerlas, 2002). In addition to the landraces, wild species of cassava including *M. glaziovii* have been used since the 1930s for resistance to CMD. At IITA, Nigeria, TME3 and TME4 landraces have revealed a major source of resistance conferring dominant gene (CMD2) (Akano et al., 2002). Cassava mosaic disease resistance has also been identified from a cross (TMS 1330572 x TME 7) involving local germplasm (Lokko et al., 2004) in Nigeria.

The levels of resistances in landraces vary from moderately resistant to resistant (Jennings and Iglesias, 2002). In a study to evaluate CMD resistance involving landraces, 40% of the materials evaluated were regarded as resistant (Egesi et al., 2007). In another study, Raji et al. (2008) evaluated 12 cassava landraces for resistance genes and one landrace 'Atu' with farmer acceptable qualities had 12% incidence (least amount of disease) and severity of 1.8 on a scale of 1-5. The above studies indicate that landraces can be sources of CMD resistance.

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1.11 Economic impact of cassava mosaic disease

Various studies have been conducted on the impact of CMD in Africa. Thresh et al. (1997) observed that the effect of CMD on cassava varies depending on the location and genotype. Yield losses are also dependant on the number of viruses infecting cassava plants. In Uganda, field experiments conducted in 1999-2000 and 2000-2001 showed reduced tuberous root mass of 42%, 12% and 68% in plants infected with ACMV, EACMV 'mild' and EACMV 'severe' respectively (Owor et al., 2004). Fauquet and Fargette (1990) reported yield losses between 20 and 95%. In India, Nair and Unnikrishnan (2007) reported 80% losses due to CMD. In co-infected plants (ACMV and EACMV-UG2) no root yield was obtained. In other studies, Thresh et al. (1997) estimated 15-24% yield loss equivalent to 15-28 million tonnes. On the other hand the Food and Agriculture Organisation (FAO) estimate 84 million tonnes yield loss for the same year. The time of infection is important in determining yield losses (Osiru et al., 1999). The earlier the infection the greater the losses (Osiru et al., 1999) in susceptible cultivars. Early stages of growth in cassava are vulnerable to the virus as they are critical for the physiological process that influence yield.

Although the CMD has been recognised for more than a century, only a small number of severe epidemics have been reported. Most of these epidemics occurred in West Africa (Sierra Leone, Ivory Coast, Ghana, Nigeria), East Africa (Uganda) and Madagascar during the latter part of the 20th century (Legg and Fauquet, 2004). In monetary terms more than US$ 60 million worth of cassava was lost annually in Uganda between 1992 and 1997 (Otim-Nape et al., 1997). Losses of similar value in United States dollar (US$) terms have also been reported in the eastern Democratic Republic of Congo (DRC), Sudan, Tanzania, and Kenya (Legg, 1999). Thresh et al. (1997) estimated losses amounting to US $440 million annually on the entire African continent. Manyong et al. (2000) estimated yield losses of US$ 2 billion per year as a result of both ACMV and EACMV infections in Africa. Furthermore Fargette et al. (1988) estimates yield loses due to CMD in Africa to be more than UK £1 billion. Susceptible cultivars show high yield losses when infected during early growth stages. In localities where farmers plant susceptible cultivars alongside resistant genotypes, the spread of CMD is minimal (Osiru et al., 1999).

1.12 Management of cassava mosaic disease

Several approaches have been developed in managing CMD, including genetic engineering, breeding of resistant cultivars, and cultural practices. However, cassava mosaic disease has principally been managed by host plant resistance (Thresh et al., 1998) and phytosanitation (Thresh et al., 1988). Phytosanitation involves the use of CMD free planting material, crop hygiene, and roguing. Effectiveness of sanitation depends on the availability of CMD free cuttings and at prices affordable by farmers (Thresh and Cooter, 2005). Choosing cassava cuttings without disease symptoms is simple; however, problems arise when the supposedly disease free cuttings have latent infections (Hillocks and Thresh, 2000). Growing different cultivars in the same field has also been reported to slow down the spread of CMD and results in the reduction in the size of the whitefly population (Osiru et al., 1999).

However, the measures appear to be temporary in halting the spread of CMD. Host plant resistance in cassava is believed to be the most reliable long term strategy in minimizing adverse effects of the disease. Integrated management approach has been suggested (Ogbe et al., 2002). The approach is based on combining different control methods i.e.

planting disease free cuttings, using resistant/tolerant materials, and inspecting cassava fields regularly for disease symptoms. In countries where CMD has been a major problem, the use of virus resistant cultivars is the main method of control (Thresh and Cooter, 2005). In East Africa, use of resistant cultivars developed at IITA has assisted in managing the CMD epidemic (Legg and Thresh, 2000).

Likewise Latin American elite germplasm susceptible to CMD has been improved through introgression of the CMD2 gene conferring resistance (Okogbenin et al., 2007). The improved material released to African breeding programmes have additional traits such as increased dry matter content, low cyanide content and resistance to post harvest deterioration (PPD). Cassava mosaic disease has also been managed using improved antisense RNA technology (Zhang et al., 2005). In developing countries such as Zambia, where testing of cassava viruses in planting materials is not carried out, managing CMD through developing resistant cultivars may be the most appropriate approach. Use of NPK fertilizers at rates of 90 kg ha^{-1} N, 15 kg ha^{-1} P and 75 kg ha^{-1} K have been reported to enhance cassava growth without increasing CMD severity (Ogbe et al., 1993). However, most of the small scale farmers do not use fertilizer as the crop has been reported to grow fairly well on marginal lands (Howeler, 2002).

1.13 History of cassava breeding

In Africa cassava breeding first began in Tanganyika (now Tanzania) in 1953 at Amani Station during the early part of the 20^{th} century (Jennings and Iglesias, 2002). During that period little work was done until the early 1970s (Jennings and Iglesias, 2002). Production of hybrids started in 1973 when a great proportion of hybrids were produced through controlled pollination. According to Kawano (2003) the size of the germplasm variation that existed then was the basis for the growth of cassava production. The landraces were improved for yield potential, pest and disease tolerance. Although the breeding programme did not continue for long, useful cassava clones resistant to CMD, were developed. In 1970, IITA was established in Nigeria with the mandate of creating improved cultivars. The aim was to integrate exotic germplasm from different places while maintaining desirable genes and removing recessive genes. As cultivation of the cassava expanded and the need for improved cultivars arose in different parts of the world, it necessitated the establishment of other research institutes i.e. CIAT in South

America, Africa and Asia in 1970s and 1980s respectively. The overall objective was to increase both yield per unit area and area under cultivation (Jennings and Iglesias, 2002). The CIAT breeding programme had the aim of providing economic benefits among the less privileged people in rural communities (Kawano, 2003).

1.13.1 Breeding for cassava mosaic disease resistance

Breeding for CMD resistance started during the early part of the 20th century at Amani Station, Tanzania (Legg and Fauquet, 2004). It was then recognized as the long term solution in combating the disease (Legg and Fauquet, 2004). Since then several clones have been produced that are resistant to CMD. These include: Tropical Manihot Selections (TMS) 4(2)1425, TMS 30337, TMS 91934, TMS 30001, TMS 60142, and TMS 30572. Breeding for disease resistance has been without difficulty due to the relative ease of crossing cassava with closely related species such as, *M. glaziovii*. The first resistance to CMD was recognised in backcross derivatives of *M. glaziovii* (Hahn et al., 1989). Resistant TMS and Tropical Manihot Evaluations (TME) clones are now being used in countries such as Uganda, Kenya and Tanzania, previously ravaged by CMD. Tropical Manihot Evaluations clones from Nigerian landraces have been developed at IITA conferring a single dominant gene (CMD2) for resistance to CMD (Akano et al., 2002). The advantage of the dominant gene is that it can be detected in the F_1 unlike CMD1 (considered to be polygenic) which was described earlier (Fregene, 2000). For CMD1 to be detected, a backcross has to be performed. CMD2 would be preferred where resistant CMD genotypes are urgently required as less time is spent on selection. The CMD1 and CMD2 genes conferring resistance can be combined since they are complementary (Thresh and Cooter, 2005).

Though several studies have been made on breeding for CMD resistance in Africa and elsewhere, research in this area is still limited. Moreover, the viruses continue to mutate resulting in potent variants. Efforts to develop control strategies such as phytosanitary measures, cultural practices, planting date, use of cultivar mixtures and insecticides have had limited success. Besides CMD resistance, other equally important traits such as tuberous root yield and low cyanide content have also received more attention in breeding programmes.

1.13.2 Breeding for high root yield

In the last 30 years, international research centres (IITA and CIAT) have spear-headed cassava breeding programmes with the objective of improving yield potential and tolerance to insect pests and diseases (Kawano, 2003). To this effect breeders have focused on number of storage roots per plant, average fresh root weight, and root dry matter content as these are the major components of cassava root yield. However, what determines root yield is crop growth rate (CGR) in relation to leaf area index (LAI); radiation use efficiency; and partitioning of assimilates between shoots and roots. Genetic variability of cassava performance on root yield has been observed in many different agro-ecologies (Aina et al., 2007). To obtain clones with high root yield, Aina et al. (2007) suggest considering clones number of roots, root size, and harvest index. However, this requires investigating and eliminating environmental factors[1] that may reduce the number and size of roots. On farmers fields the root yields are not comparable to those obtained at research stations. To bridge the differences in yield performance, several options have been suggested including exploiting heterosis between landraces and introductions. At IITA (Nigeria) hybrid vigour has been enhanced through interspecific crosses between cassava and *Manihot* spp (Jennings and Iglesias, 2002). Kamau (2006) has also reported hybrid vigour (selected genotypes yielding three times more than the parents) from crosses between local landraces and introduction.

1.13.3 Breeding for low cyanide content

All cassava cultivars, either bitter or sweet, have appreciable amounts of cyanide. About 2650 species of plants, including cassava, are known to produce cyanogenic glucosides (CG) (FSANZ, 2004). The cyanogenic potential (CNP) has been reported to be controlled by two quantitative trait loci (QTL) found on linkage group 10 and 23 (Kizito et al., 2007). The bitter cultivars, having more than 1000 mg hydrogen cyanide (HCN) equivalent per kg dry weight, are regarded as toxic while sweet cultivars, with less than 200 mg HCN equivalent per kg dry weight of tuberous roots, are regarded as safe for human consumption. However, Jennings and Iglesias, (2002) classified sweet cultivars as having less than 10 mg $100g^{-1}$ cyanogenic glucoside. Genotypes with low HCN content are often preferred by breeders for incorporation into their breeding programmes (Jennings and Iglesias, 2002). The cyanide in cassava plants exists in the form of

[1] Factors such as insect pests, diseases and soil fertility that may have direct or indirect effect on yield

cyanogenic glucosides which is made up of linamarin (95%) and lotaustralin (5%) (Siritunga and Sayre, 2005). These compounds are produced in the leaves and distributed to other parts of the plant. All plant parts of cassava with the exception of the seed contain cyanogenic glucosides (Ceballos et al., 2004). The amount of CG in different plant parts (roots, leaves, stems) varies. For example leaves have higher (3800-5900 mg HCN kg^{-1}) amounts of CG than the roots (4-113 mg HCN kg^{-1}) (Ceballos et al., 2004). Cyanogenic potential in the roots ranges from below 10 mg kg^{-1} to over 500 mg kg^{-1} (O'Brien et al., 1994). The HCN potential in the leaves is 10% higher than what is found in the roots (FSANZ, 2004).

However, in roots the amounts vary depending on the genotype, environmental conditions, and crop management (Dufour, 2007; El-Sharkawy, 1993). Total cyanide in the root has been reported to increase in drought stressed environments or areas experiencing low rainfall in a season (Tan and Chan, 1993). Selecting genotypes with low HCN potential during the early stages of breeding is essential. No barrier appears to exist in integrating low HCN with other farmer preferred traits (Jennings and Iglesias, 2002).

1.14 Cassava selection cycle

Cassava breeding involves the collection of germplasm and hybridising the different genotypes either through controlled or open pollination. A typical selection cycle (Table 1.1) for cassava involves crossing of elite clones in the first year and ending with few clones surviving the rigorous selection process after several years (Jennings and Iglesias, 2002). As selection progresses to later stages the number of surviving genotypes reduces significantly. Up to 100 000 genotypes are produced in the first year through open pollination. In the second year, the first selections are based on high heritability traits such as reaction to diseases, branching habits and plant type. Due to the large number of genotypes involved during the early stages of the selection cycle, choosing the breeding materials is done visually (Ceballos et al., 2004). The selections are carried out from the nurseries where botanical seeds have been raised. At CIAT in Colombia between 40 to 60 000 botanical seeds are produced per year (Kawano, 2003). The amount of seed is dependent on the adaptation of parents to different ecological

zones. In the second generation, traits with high heritable characteristics such as plant height, reaction to disease, branching habits, are selected (Jennings and Iglesias, 2002).

Table 1.2: Typical selection cycle in cassava breeding, beginning with the crossing of elite clones through the different stages of the selection process (from Jennings and Iglesias, 2002).

Year	Activity	Number of genotypes	Number of plants per genotype
1	Crosses among elite	Up to 100,000	1
2	F1: evaluation of seedlings From botanical seeds strong selection for CMD in Africa	100,000[a] 50,000[b],17,500[c]	1
3	Clonal selection trial (CET)	2000-3000[ab], 1800[c]	6-12
4	Preliminary yield trial (PYT)	100[a], 300[b], 130[c]	20-80
5	Advanced yield trial (AYT)	25[a], 100[b], 18-20[c]	100-500
6 – 8	Regional trials (RT)	5-30[abc]	500-5000

Figures for cassava breeding at [a]IITA (Ibadan, Nigeria); [b]CIAT (Cali Colombia) and CIAT-Rayong Field Crops Research Centre (Thailand).

The second selection stage is the clonal evaluation trial (CET). In the past, breeding at earlier stages was based on mass phenotypic recurrent selection and little information was collected. As such the opportunity of establishing the general combining ability (GCA) of the parental lines, whose progenies are evaluated, is missed (Ceballos et al., 2007). Usually in most cassava breeding programmes, the first two stages of selection are not replicated (Ojulong et al., 2008). Hence the procedure lacks organised information on the breeding values of parental lines used in the breeding programme (Ojulong et al., 2008). The selection criteria at this stage depend on the F_1 genotype to produce quality vegetative cuttings. At the CET stage, between 2000 and 3000 genotypes are normally evaluated and selection is made for highly heritable traits such as root dry matter, harvest index and HCN content. Six to ten cuttings can be obtained from a single plant at the CET (Ceballos et al., 2004). Lenis et al. (2006) selected 1350 best clones of 3000 seedlings that produced eight or more stakes. From the initial 100 000 genotypes during the first year to between 2000 to 3000 during the CET it means therefore that over 95% of the genotypes discarded (Kawano, 2003).

To capture information on the performance of each clone at the CET, Ceballos et al. (2004) suggests modifications to the breeding programmes by keeping records for each and every genotype; selection within each block; and dividing each family into three groups. Using the new cassava breeding scheme as proposed by Ceballos et al. (2007), Ojulong et al. (2008) obtained high broad sense heritability values, which are

comparable to those at advanced stages as environmental effects were minimised. Following the CET, preliminary yield trial (PYT) follows in year four. Up to 300 genotypes are tested in preliminary yield trial. In year five up to 100 genotypes are tested in large trials involving several sites. In year six 5 to 30 genotypes are evaluated in multlocation regional trials. Table 1.2 illustrates the differences in the old and new breeding scheme used at CIAT.

Table 1.3: Old breeding scheme verses the new breeding scheme at CIAT (Ceballos et al., 2007)

Time (mo)	Stage (old system)	Stage (new system)	Time (mo)
0	Crossing of selected parental genotypes	Crossing of selected parental genotypes	0
6	F1 (3000-5000) (6 mo) 1 plant/1 site/ 1replication	F1 (3000-5000) (10 mo) 1 plant/1 site/1 replication	10
18	F1C1 (2000-4000) (1 year) 1 plant/2 sites/1 replication	Clonal evaluation (1000-1500) (1 year) 6-8 plants/1 site/1 replication	22
30	Clonal evaluation (500-1500) (1 year) 6 plants/1 site/1 replication	Preliminary yield trial (150-300) (1 year) 10 plants/1 site/3 replication	34
42	Preliminary yield trial (100-200) (1 year) 20 plants/1-2 site/1 replication	Advanced yield trial (40-80) (2 years) 25 plants/2-3 sites/3 replication	58
66	Advanced yield trial (30-60) (2 years) 25 plants/2-3 sites/3 replication		
	Elite germplasm		
	Germplasm collection	Regional trials	Crossing block	Participatory research	

1.15 Mating designs

Several designs have been used in cassava breeding, namely diallel (I, II and III), North Carolina (NC: I, II, III) and polycross. Polycross mating design is suited to out-breeding

species such as cassava and good for determining general combining ability. However, advanced general selection cannot be carried out since inbreeding may result because of relatedness of cassava (van Buijtenen, 1982). The diallel mating design consists of crossing three or more parents in all possible combinations (Stuber, 1980). The design is important in the analysis of dominant, additive and epistatic gene action in breeding programmes. Although the diallel mating scheme is useful in studying gene action, the number of crosses goes up as the square of the number of parents. Plant breeding procedures can be costly and time consuming. Choosing the right design that gives accurate and reliable information should be taken into consideration before experimentation. Managing a large number of crosses and the costs involved restrict the number of parents to between eight or 10 in the diallel design (Stuber, 1980). North Carolina designs can handle more parents with fewer crosses.

North Carolina II (NCII) is a factorial design which provides considerable information for estimation of all parameters (additive and non-additive variances) (Hill et al., 1998) (Table 1.3). Half-sib family relationships are obtained through the common male and common female. The progeny families are generated through mating male (m) and female (f) parents in all the possible crosses. In the NCII design mean square for males and females provide individual and separate estimates of the additive component of variance (GCA m and SCA f) which is an added advantage over the diallel design. General combining ability (GCA) and specific combining ability (SCA) and type of gene action are important in cassava improvement. General combining ability refers to mean performance when expressed as a deviation from the mean of all crosses. Specific combining ability is the deviation of a cross from the mean of GCA of parent lines (Falconer and Mackey, 1996).

In NCII both random and fixed entries can be used to determine genetic effects. When entries are regarded as fixed effects, attention is focused on estimating genetic effects rather than genetic variances.

Table 1.4: Analysis of variance for NCII mating design (Hill et al., 1998)

Item	DF	Expected mean square
Replication	r-1	
Between males	m-1	$\delta^2_w + p\delta^2_{mf} + f\delta^2_m$
Between females	f-1	$\delta^2_w + p\delta^2_{mf} + m\delta^2_f$
Males x females	(m-1)(f-1)	$\delta^2_w + p\delta^2_{mf}$
Within families	mf(m-1)	δ^2_w

The additive gene effects or GCA is important for establishing the performance of progenies. For example, CMD resistance negative GCA effects are important estimates for resistance as it signifies large input of a parent in resistance. In addition, interaction between male and female mean square generate specific combining ability (SCA, non-additive effects). NCII also allows for estimation of maternal effects through reciprocal crosses.

Diallel mating designs suggested by Griffing (1956) provide considerable information for estimating GCA and SCA (Hill et al., 1998) and can be used to identify superior parents for use in hybrid development (Yan and Hunt, 2002). Hayward (1979) suggested that application of diallel mating design in F_1 hybrid development should be confined to a limited number in specific combinations. The genetic variance in diallel is partitioned according to the methods of Griffing (1956) into GCA of the parents and SCA of the crosses. In general diallel mating designs are used for determining genetic effects for a fixed set off parental lines (fixed effects). Although the two designs, diallel and North Carolina mating designs, are different, genetic information obtained from the two is similar (Hallauer and Miranda, 1988). In addition, NCII can adequately provide for selection of parents for the next generation as long as there are enough male parents. Information obtained from the diallel and NCII are more less the same, however, differences lie in the parents used (Hallauer and Miranda, 1988). In diallel mating design the parents can interchangeably be used as males and females where as in NCII, the design requires different sets of males and females. North Carolina II design has been used in genetic studies on cassava mosaic disease to generate segregating F_1 populations (Lokko et al., 2005).

1.16 Summary

Cassava is a very important food security crop in Africa especially among the resource poor farmers. Its ability to grow under marginal conditions (low soil fertility, acidic and alkaline soils, and low soil moisture) makes cassava cultivation attractive to rural communities. However, insect pests and diseases are a major drawback to cassava production in Africa. Although there are many and different diseases, CMD is considered to be the most important disease in sub-Saharan Africa.

The discovery of satellites which interact with CMD and are capable of breaking resistance has necessitated the need to develop plants with suitable resistance. The current management options such as use of disease free planting material, phytosanitation, and roguing have not helped much in reducing yield losses. Significant differences in cassava resistance to CMD have been observed in landraces; therefore genetic improvement of cultivars with adequate levels of resistance should be possible. In Uganda and Tanzania, the use of CMD resistant materials has resulted in reduced yield losses. In Zambia CMD is still a major threat to thousands of small scale farmers whose livelihoods are dependent on cassava. There is therefore an urgent need to integrate CMD resistant genotypes with the locally available cassava landraces while at the same time maintaining the existing farmer preferred traits.

To generate progenies with CMD and satellite resistance, the choice of mating design is important. Despite the many mating designs available for estimating GCA and SCA, the diallel design provides more information for estimating GCA and SCA (Hill et al., 1998). However, the diallel results in more crosses and requires more labour than the NCII. North Carolina II, which also provides similar combining ability information as the diallel requires decreased number of crosses and labour.

The existing information on flowering, pollination and hybridization, insect pest and disease management is of paramount importance to Sub-Saharan Africa. Of particular interest is the CMD2 dominant gene which is expressed in F_1. This allows for early selection of genotypes resistant to CMD without backcrossing F_1 to the parents. CMD2 can be complemented with CMD1 in susceptible local cultivars. Therefore with this theoretical background information, breeding cassava for CMD resistance will significantly reduce yield losses among small scale farmers in Zambia.

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CHAPTER 2: FARMER'S PERCEPTION OF CASSAVA MOSAIC DISEASE, PREFERENCES AND CONSTRAINTS IN LUPAULA PROVINCE

Abstract

Cassava is the principal staple root crop for rural and urban households in Luapula province. It constitutes a major portion of the diet and provides a substantial amount of calories. However, the yields on smallholder farms are low. The study was therefore conducted to: i) establish farmers' perceptions and knowledge of cassava mosaic disease (CMD), ii) evaluate farmers knowledge on the management of CMD, iii) establish farmers' preferred traits and constraints, and iv) assess sources of cassava planting materials. Focus group discussions (FGD) and structured interviews involving 156 farmers in Mwense, Mansa, and Samfya districts were conducted from December 2008 to March 2009. Knowledge of CMD was limited among the respondents. Only 2.4% of the respondents were aware of the disease despite high CMD incidence in farmers' fields. Though CMD was evident in the fields, there were no control strategies put in place by the farmers. The majority of the farmers were aware of the importance of insect pests; however, they could not differentiate between damage due to diseases or insect pests. High yield and early bulking traits were highly ranked. Most of the farmers planted local landraces on small fields (<1 ha) using on farm planting. Cassava was planted either as a sole crop or intercropped with maize, sweet potato or beans. Intercropping was the most practiced method of growing cassava with other crops in all the three districts. It was evident that a local breeding programme developing locally adapted, disease and pest resistant cassava cultivars is a pressing requirement.

2.1 Introduction

Cassava is one of the most highly valued root crops in Zambia. It is mostly grown in Northern, Luapula, North-Western and Western provinces of the country, the so called cassava belt, accounts for 95% of total production (Chitundu et al., 2006). Thirty percent of the population in Zambia is directly or indirectly dependent on cassava for their livelihood, with the majority from the cassava belt region (FAO, 2006). In the last few years, cassava promotion and production has spread to other parts of the country such as Central, Eastern, and Southern provinces.

Cassava mosaic disease has been reported to be one of the most limiting constraints to cassava production in Africa (Thresh et al., 1994). The disease in Zambia is prevalent in most of the farmers' fields (Muimba-Kankolongo et al., 1997), affecting both local and improved cultivars. Despite the availability of improved cassava cultivars in some of Zambia's agricultural research institutions and non-governmental organisations (NGOs) nurseries, production is still low, with an average yield of 5.8 t ha^{-1} (FAOSTAT, 2009). Despite farmers being aware of the availability of improved cultivars, distribution, dissemination and adoption has been slow. Adoption of a cultivar depends on the qualities that are preferred by the farmers and the available information upon which decisions are based).

To assess the usefulness of any given cultivar, there is a need to determine the attributes and constraints that are responsible for farmers' choices through participatory approaches. Farmers have local knowledge on the attributes of their cultivars. Studies by Agwu and Anyaeche (2007) indicated that a farmer's decision to use a particular cassava cultivar was influenced by a number of factors, some of which are quality based (high yield, low cyanide, early maturity, and colour of roots).

Improving cassava production in smallholder agriculture in Luapula province requires farmer involvement in the early stages of breeding. In many national breeding programmes where the farmers have been involved in the breeding process, improvements have been observed in the adoption and release of cultivars. For instance, in Ghana, scientists working in collaboration with farmers identified 129 superior accessions from a total of 1350 seedlings (Manu-Aduening et al., 2006). The participatory approach improves the adoption rates by way of integrating indigenous

knowledge into research through dialogue between farmers and scientists. Furthermore, it is necessary for evaluating traits most preferred by the farmers. Therefore, a PRA was conducted to gather information on farmers' preferences, perception, and knowledge of CMD and other production constraints and to lay the foundation for the development of CMD resistant cultivars in Zambia.

The objectives of the PRA were to:

i) assess farmers' knowledge and perceptions of CMD;
ii) evaluate farmers' knowledge on the management of CMD;
iii) establish farmers' preferred traits and various constraints to cassava production; and
iv) assess sources of cassava planting material

2.2 Materials and methods

2.2.1 Description of study area

The study was carried out in Mansa, Mwense and Samfya in Luapula province, Zambia. The province is located between latitude 8 to 12^{o} south of the equator and longitude 28 to 30^{o} east of Greenwich Mean Time (Joy, 1993). The districts are located in the high rainfall agroecological zone (AEZ III) (Figure 2.1) and receive above 1000 mm of rainfall per year and does not experience drought. The rainfall pattern is monomodal and lasts from November to April. The mean annual minimum temperature is 10^{o}C and mean annual maximum is 31^{o}C. The length of growing season is approximately 160 to 170 days (November to April) for rain grown crops.

The altitude varies from 900 m above sea level in the lower Luapula valley to over 1300 m at Kawambwa (Joy, 1993). The districts are characterised by different vegetation types. For instance, Mwense and Mansa districts have Miombo[2] forest interspersed with *hyperennia* spp, while Samfya district has Chipya[3] type of vegetation (Joy, 1993). Much of the landuse is Chitemene[4].

[2]Light vegetation with closed canopy, deciduous woodland dominated by leguminous tress of the genera *Brachystegia* and *Julbernardia* usually 12-15 m tall
[3] Consists mainly of perennial grasses with small trees
[4] slash and burn shifting systems

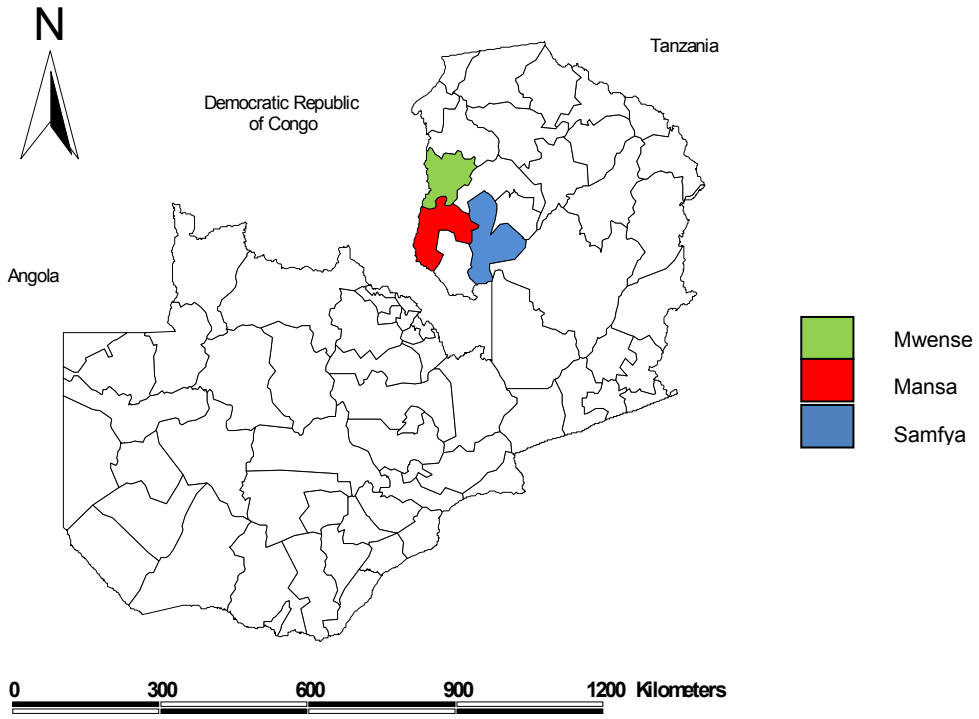

Figure 2. 1: Districts in Zambia surveyed for the participatory rural appraisal study

In terms of soil characteristics, Mansa and Samfya districts are characterised by acrisols which are well drained, deep to very deep, yellow red to strong brown, friable and fine loamy to clay soils (MACO, 1991). Mwense district is characterised by well drained, very deep, strong brown to red, friable, and fine loamy, to clayey soils having clear clay increase with depth. The soils are acidic.

2.2.2 Sampling procedures and selection of participants

The study was conducted between December 2008 and March 2009 in collaboration with the Department of Agriculture which falls under the Ministry of Agriculture and Cooperative (MACO). Following discussions between the principal investigator and District Agriculture Coordinators (DACO) in each district, a list of cassava farmers was drawn up and 20 to 25 farmers were randomly selected for the focus group discussion (FGD). The selected farmers were asked if they were willing to participate in the FGD and the essence of the study was explained to the participants. In each district, six to eight villages were targeted for the FGD. Adult men (56.1%) and women (43.9%) farmers (Table 2.1) were involved in the study.

Table 2.1: Number of farmers by gender participating in focus group discussions

District	Number of villages	Male	Female	Total
Mwense	6	12	10	22
Mansa	6	17	7	24
Samfya	8	8	12	20
Total	20	37 (56.1%)	29 (43.9%)	66

In the second stage, 90 randomly selected farmers (30 per district) (Table 2.2). were involved in the semi-structured interview. The farmers were asked similar questions as in the FGD on their perceptions of pests and diseases, production and marketing constraints, control strategies and cropping system using a semi-structured questionnaire (Appendix 1).

Table 2.2: Total number of farmers by gender participating in the structured interviews

District	Male	Female	Total
Mwense	21	9	30
Mansa	23	7	30
Samfya	23	7	30
Total	67 (74.4%)	23 (25.6%)	90

2.2.3 Data collection

Prior to data collection a multi-disciplinary team was constituted comprising the principal investigator, three assistants, extension officer (from DACO's office), and a camp officer from each study area. All the team members underwent training on administering questionnaires and handling of FGD. In addition, the participatory appraisal team reviewed interviewing techniques and questions in the questionnaire. Furthermore, the group also discussed various options to use in order to extract maximum information from the farmers. Mostly open-ended questions allowed farmers to give their opinions freely. The interviews and discussions were conducted in the local language (Bemba) as most of the farmers were conversant in it and also to encourage wide participation.

To collect as much information as possible, farmers were presented with plants having CMD symptoms or infested with insect pests. This was done to get farmers reaction on the diseases and insect pests. In addition, probing and iterative techniques were used

during FGD discussion and structured interviews. Some of the questions asked were repeated and rephrased to enable farmers understand and respond fully. Repeating and rephrasing of questions is often necessary when the study group comprises semi-literate respondents. Other techniques used were listing and ranking of constraints, observations of cultivars in the field, listing of traits, and direct matrix ranking.

Data on farmers' knowledge and perception of insect pests and diseases were collected from the FGD and structured interviews. The questions centred on farmers' awareness of insect pests and diseases, cultivars grown, production and marketing constraints, cropping system used and cropping calendar. Farmers were asked to list cultivars and provide their attributes. Comparisons between factors under discussion e.g. constraints, insects and diseases, were done using the pair-wise ranking (matrix) method. The factors mentioned in pairs were compared and the totals for each were countered. The factor with the highest number of points was ranked as first and that with lowest total last. In Mwense and Samfya districts, farmer training centre's (FTCs)[5] were used as sites for the FGD as the study was done during the rainy season (Figure 2.2).

Figure 2.2: Researcher with farmers during group discussion session in Samfya district

[5] FTCs are places where farmers are trained by experts on different agricultural subjects

Additional information on CMD incidence and severity was also collected. The incidence was determined by observing 30 plants in the 'Z" configuration. Ten plants were observed on each side of the field and another 10 plants on the diagonal, making a total of 30 plants. Cassava plants within the same transact or line, were sampled equidistant from each other. Three to six month old plants were targeted for disease incidence and severity observations. Severity scores for each plant were collected on each of the 30 plants using a 1-5 scale (Hahn et al., 1980), where: 1) no symptoms observed; 2) mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only with the remainder of the leaflets appearing green and healthy; 3) moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets; 4) severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size; and 5) severe mosaic distortion of the entire leaf.

2.2.4 Data analysis

Statistical analysis for quantitative survey data was analysed using the statistical package for social sciences (SPSS) (SPSS, 2006). Descriptive statistics, analysis of variance and mean comparisons for each district were generated.

2.2 Results

2.3.1 Land size

Most of the farmers (46.3%) in the three districts had an average cassava field of less than 1 ha. The rest of the cassava fields were between 1.0 to1.5 (22.5%) and 1.5 to 2.0 ha (31.2%) in size. In Samfya district (Figure 2.3) 38.8% of the respondents had cassava fields less than 1 ha. In Mansa, 44.4% of the respondents had fields measuring between 1.0-1.5 ha.

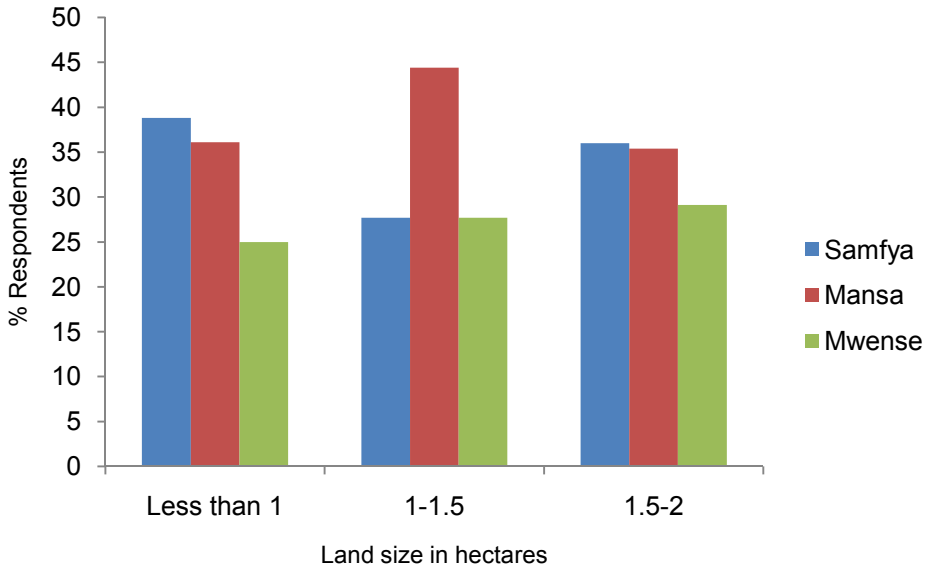

Figure 2.3: Land size of cassava fields in Samfya, Mansa and Mwense districts

2.3.2 Knowledge and perception of cassava mosaic disease

In all the three districts, the majority of the respondents (97.6%) were not familiar with the symptoms of CMD. Only few respondents (2.4%) knew CMD by virtue of working within Mansa Research station[6]. When farmers were shown cassava plants with CMD symptoms (Figure 2.4), the majority could not identify the disease. A number of reasons were given as to the probable cause of CMD. The majority of the respondents (73.6%) thought the symptoms were as a result of harvesting of cassava leaves. On the other hand, 12.6% were of the view that CMD was caused by mealybug infestation. The association of CMD with harvesting of leaves by the farmers was common across the districts. Other respondents were of the view that CMD was caused by old age of the plants (4.6%), cold (3.4%), and lack of rain (3.4%). The rest of the respondents attributed the cause of CMD to lack of hygiene (non-removal of affected plants). Infection of CMD was also poorly understood. In Samfya district some farmers were able to differentiate between symptoms of mealybug infestation and CMD. However, the farmers did not have a name for the condition in plants that exhibited CMD syptoms.

[6]Research Station for Luapula province

Figure 2.4: Farmers identifying disease symptoms on cassava plant

2.3.3 Incidence and severity of cassava mosaic disease in farmers' fields

Although the farmers were not aware of CMD, symptoms of the disease were present in most of the fields (Figure 2.5). However, there were no significant differences in CMD incidence in Samfya, Mansa and Mwense districts. Average CMD incidence within the three districts was 61.2%. Samfya (68.6%) and Mwense (62.6%) districts had high CMD incidence (Figure 2.6). Mansa had the least incidence (57.8%). The overall mean CMD severity was 2.5. CMD severity was moderate in the three districts Samfya (2.4), Mansa (2.5) and Mwense (2.5).

Figure 2.5: A cassava plant with CMD symptoms in a farmers' field

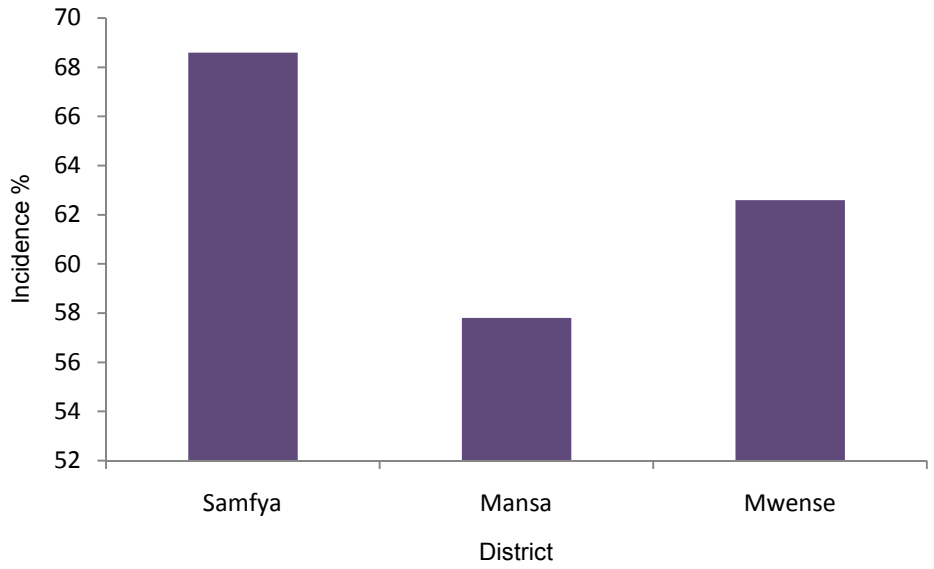

Figure 2.6: Cassava mosaic disease incidence in Samfya, Mansa and Mwense districts in 2009.

2.3.4 Insect pests of cassava

All the fields visited had mealybug (*Phenacoccus manihoti* Matile-Ferrero), whitefly (*Bemisia tabaci* Gennadius, a vector of CMD) and cassava green mite (*Mononychellus tanajoa* Bondar). In all the three districts farmers revealed that no cultivar was resistant to pests and that all the cassava cultivars were equally affected. When asked about the pests in general, the farmers could not differentiate between damage due to diseases versus insects. The majority (91.1%) of the respondents recognised insect pests and diseases as important in their cassava fields. On the other hand a few farmers (8.9%) thought that insect pests and diseases were less important. Across the three districts, cassava mealybug was regarded as the most important (71.0%) pest by the farmers. Fifteen percent of the respondents in the three districts viewed termites[7] as least important. Though termites were generally viewed as less important, in Samfya (57.1%) and Mansa (35.7%) they were regarded as important. However, in Mwense district, respondents (7.1%) regarded termites to be least important. Other pests that farmers mentioned were moles (6.5%), grasshoppers (4.3%) and cutworms (3.2%). Mealybugs were also mentioned as important pests in Mansa (32.3%), Mwense (30.8%) and Samfya districts (36.9%).

[7] Termites, according to the farmers occur during the dry season (April-October)

Most of the farmers indicated that high levels of mealybugs were noticed during August to November (hot and dry season). During the FGD, termites and mealybugs were also mentioned by the farmers (Table 2.3). However, whiteflies which transmit CMD were not mentioned in both FGD and structured interviews despite their presence in the cassava fields. Equally CGM was also not mentioned during FGD. Not a single farmer used pesticides to control insects.

Table 2.3: Cassava insect pests and diseases identified by farmers (2009)

District	Local name	English name	Control strategy
Mwense	Cholera[8], Namukoko	Mealybug	Removing affected parts
	Ubuchimbele	Root rot	Harvesting early
Mansa	Cholera	Mealybug	Removing affected parts
	Ubuchimbele	Root rot	none
	Infuko	Mole rat	Trapping, inserting red ants in hole Inserting chili in holes and planting Ulusinga in field
	Ububenshi	Termites	Leaving weeded weeds in the field
Samfya	Kalenshi	Mealybug	Removing affected parts
	Ububenshi	Termites	None
	Infunko	Mole rat	Trapping, digging trench around the field
	Imbeba	Rats	Weeding

When asked to rank the pests affecting cassava during the FGD, farmers ranked mealybug as the most important insect pest (Table 2.4). Moles were ranked second in Mwense and Mansa district as the pest directly affected the cassava storage roots. However, in Mansa, moles were ranked third.

Table 2.4: Ranking of important pests and diseases by the farmers (2009)

	District		
Pest	Mwense	Mansa	Samfya
Mealybug	1	1	1
Termites	3	4	2
Mole	2	2	3
Rats	-	-	5
CMD	-	-	4
Grasshopper	4	5	-
Root rots	-	3	-
Goats	5	-	-

- Not a criterion

[8] Cholera, named after the bacterial disease which ravaged Luapula province in 2003

2.3.5 Sources of cassava planting materials

Across the three districts, 56.2% of the farmers sourced the planting materials from their own fields, while 32.3% of the respondents obtained planting materials from their fellow farmers. A few farmers (11.5%) obtained the planting materials from the Ministry of Agriculture and Cooperatives (MACO). In Mansa district (Figure 2.7), 40% of the farmers obtained planting materials from MACO, while 36.1% of the respondents from the same district accessed the planting materials from their own fields. In Mwense district 41.5% of the farmers accessed the planting materials from fellow farmers and 26.7% of the farmers obtained the cuttings from MACO. In Samfya district, 39% obtained the planting materials from their colleagues. The rest of the farmers in the district got the planting materials from MACO (33.3%) and own fields (34.7%).

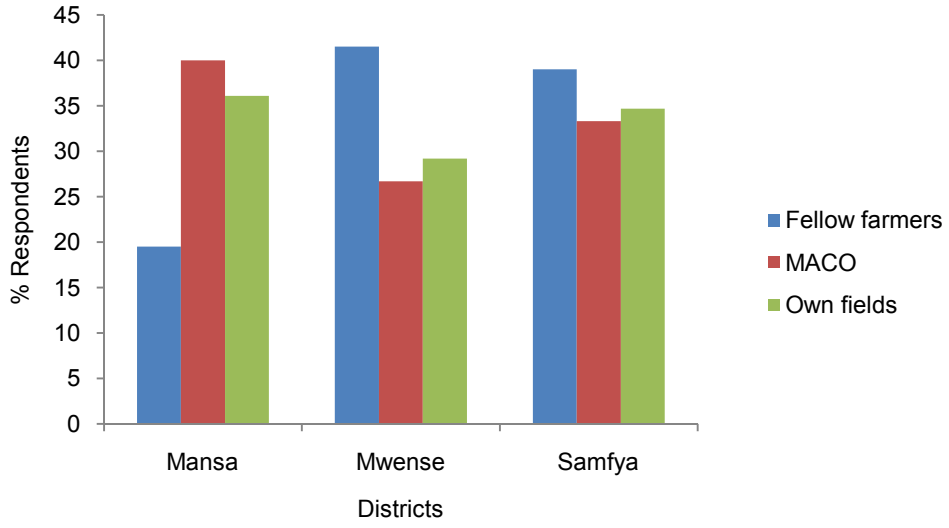

Figure 2.7: Sources of cassava planting material. Percentages are from multiple responses

2.3.6 Management of cassava mosaic disease

During both the FGD and structured interview not a single farmer had a strategy for CMD. Since the disease was poorly understood by most of the farmers, management options were equally not mentioned. Some farmers practised field sanitation through the removal of affected leaves, although this was ideally meant for the control of cassava mealybug.

2.3.7 Cassava cultivars grown

In the three districts surveyed, about 22 different cultivars were grown by the farmers. The cultivars were mostly (>90%) local landraces. Across the three districts, 58.1% of the respondents grew local cultivars, 19.8% grew improved ones, while 22.1% grew both local and improved cultivars. On average four to five different cultivars were grown on each farm. The most popular cultivars included Bangweulu (20.1%), Katobamputa (19.1%), and Kabala (11.1%) (Figure 2.8). Farmers indicated that the improved cultivars (Mweru, Chila, Kapumpa, Kampolombo and Tanganyika) were not readily available in their localities.

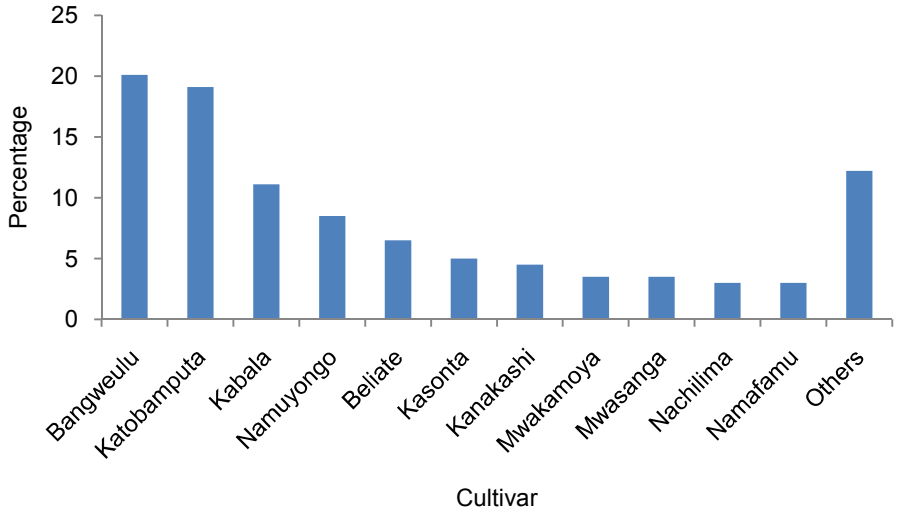

Figure 2.8: Cassava cultivars grown by the farmers

2.3.8 Farmers' preferred characteristics

Across the three districts, 37% of the respondents preferred cultivars that were high yielding, while 36% preferred early bulking cultivars[9] (Figure 2.9). Very few respondents (1.2%) preferred cultivars with insect pest resistance. Few respondents (13.4%) based their preference on the colour of cassava flour. Some respondents preferred local cultivars to improved ones as they produce better flour in terms of colour. A minority (2.4%) grew specific cultivars because the plants gave them more cuttings and leaves for relish.

[9] Early bulking according to the farmers was between 2 to 3 years

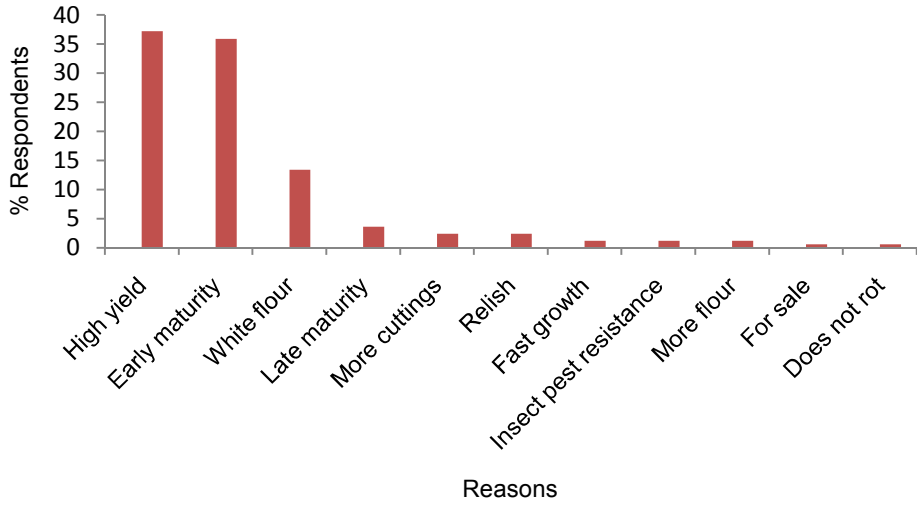

Figure 2.9: Characteristics of cassava preferred by the farmers. Percentages are from multiple responses

2.3.9 Production and marketing constraints

During the FGD in each district, farmers highlighted a number of production and marketing constraints. The constraints included insect pests, lack of capital, late bulking of cassava, and lack of market for cassava. In Mwense and Mansa districts, lack of capital was considered most important (Table 2.5), while in Samfya lack of planting material was ranked as the major constraint. However, for the structured survey (Figure 2.10) 16% in Mansa district viewed lack of capital as a hindrance to cassava production. Fifty two percent of the respondents in Mwense district considered lack of capital as the major production constraint. Seventy-five percent of the respondents in Samfya district regarded drought as an important production constraint.

Table 2.5: Pair-wise ranking of cassava production constraints identified during the focus group discussion in three districts of Luapula province (2009)

Constraint	Farmers' score per district		
	Mwense	Mansa	Samfya
Capital	1	1	7
Late maturing	-	4	-
Market	6	3	-
Insect pests and diseases	3	5	3
Cassava cuttings	4	-	1
Shortage of labour	-	2	6
New varieties	-	-	4
Extension information	-	-	2
Transport to market	5	6	-
Drying of cassava	2	8	-
Low soil fertility	-	-	5
Implements	-	7	-

- Not a criterion

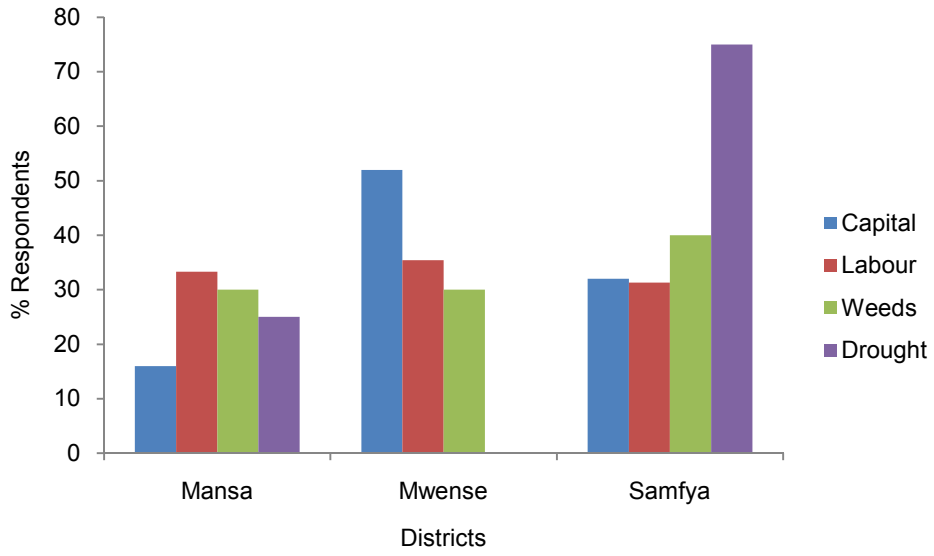

Figure 2.10: Constraints affecting cassava production as identified by farmers during the structured interviews. Percentages are from multiple responses

For the marketing contraints, 41% of the respondents in all the three districts (Figure 2.11) considered distance to market as a major constraint. About 32% of the respondents felt that lack of transport to move the cassava to the market was a problem.

Poor roads were recognised by 21.7% of the respondents as being one of the marketing constraints. Very few famers (4.3%) regarded lack of market as a problem.

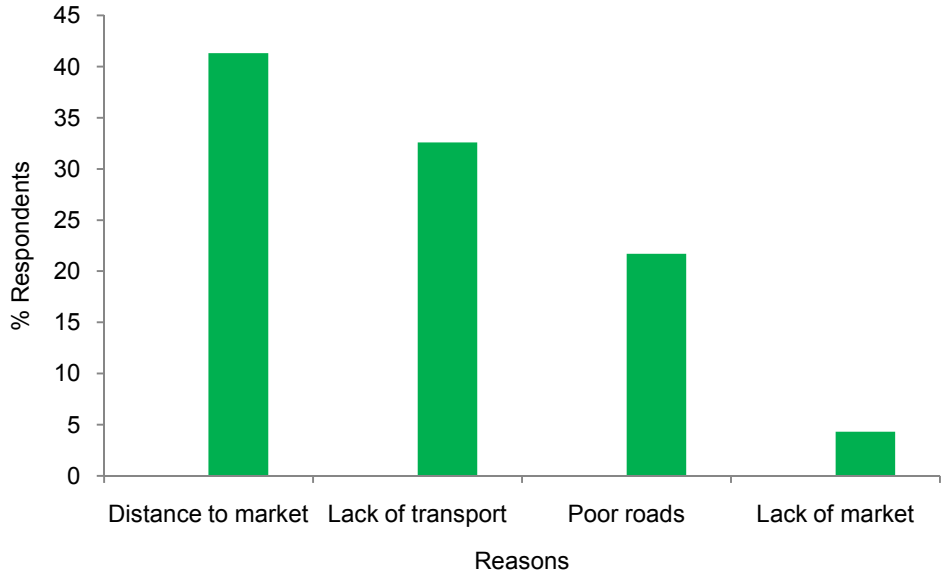

Figure 2.11: Percentage of farmers suggesting market constraints. Percentages are from multiple responses

2.3.10 Other crops grown and cropping system

The results of the FGD revealed that apart from cassava, farmers grew a number of crops during the rainy season (November – April). Crops such as maize, groundnuts, bambara nuts, rice, bean, cowpea, sweet potato, finger millet, sorghum, pumpkin were planted during the early part of the rainy season (November-December). For cassava, planting was staggered throughout the rainy season. Of all the respondents in the three districts (Samfya, Mansa and Mwense), besides growing other crops, 34% percent also grew groundnut. Although maize is not a major crop in the province, 30% grew the crop. Few farmers grew sweet potato (14.8%) and dry bean (10.5%). In Mansa and Samfya districts, in addition to cassava, 50% and 38%, respectively of the respondents grew beans (Figure 2.12). Growing of groundnut in addition to other crops was more or less the same in Mansa (31.9%), Mwense (33.3%) and Samfya (34.7%) districts.

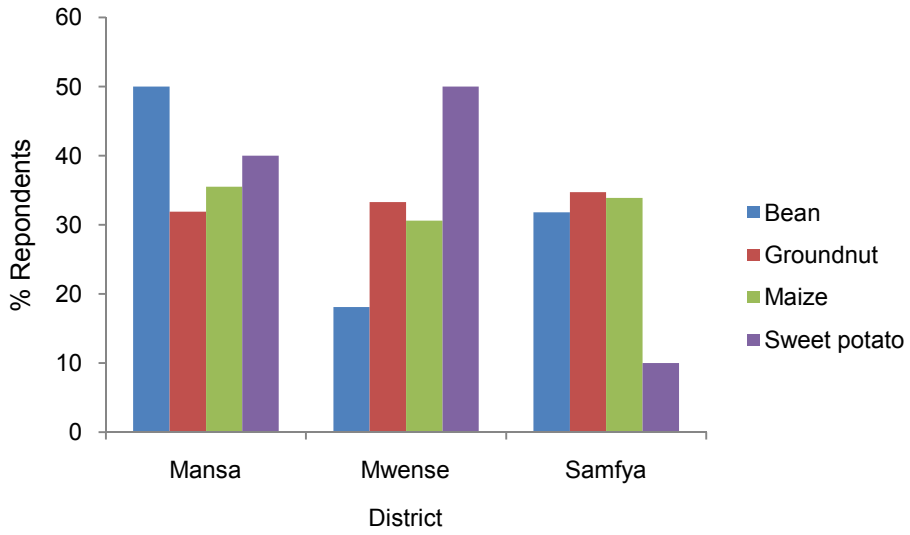

Figure 2.12: Crops grown by the farmers in the three districts. Percentages are from multiple responses

In most of the farmers' fields cassava was intercropped with maize, groundnut or beans (Figure 2.13). Field observations showed that most of the farmers grew maize for food security. Since the local cassava cultivars took long (2 to 3 years) to give appreciable yield, maize and other crops served as the immediate food source. A large proportion of the farmers (65.5%) in Mansa district intercropped cassava with other crops (maize, dry bean and sweet potato). About 27.6% cultivated sweet potato alone, while 6.9% practised both systems, intercropping and sole cropping (Figure 2.14). In Mwense district there were more farmers (72.4%) intercropping cassava than in Mansa district. However, few farmers (3.4%) grew cassava alone. In Samfya district, intercropping (83.9%) was the mostly practiced system followed by sole cropping (9.7%). For farmers growing cassava as a sole crop, the reason given was that intercropping affected root development of cassava especially when harvesting sweet potato or groundnut.

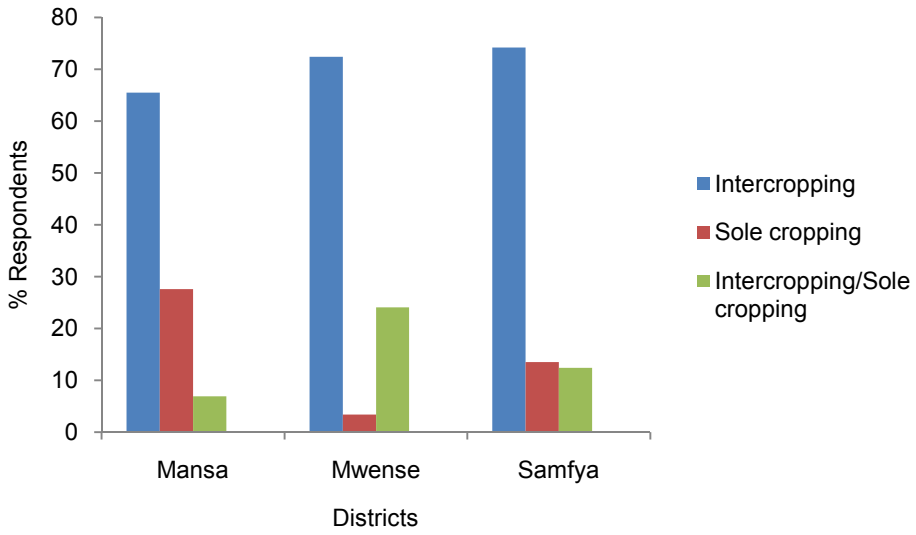

Figure 2.13: Cropping system practised by farmers in Samfya, Mansa and Mwense districts. Percentages are from multiple responses

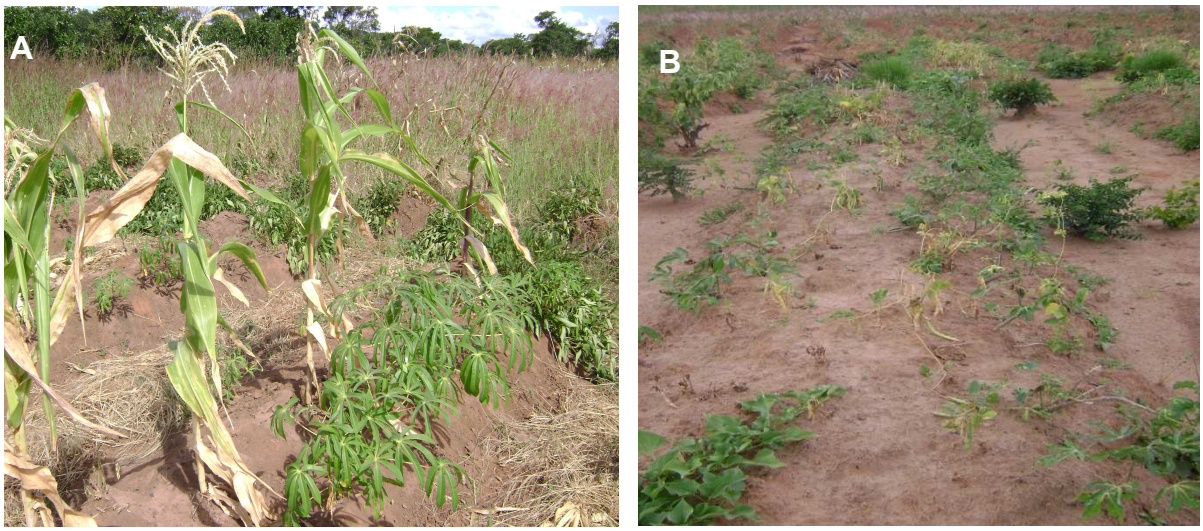

Figure 2.14: A) Field intercropped with maize, sweet potato and cassava. B) Field intercropped with bean,

2.3.11 Cropping calendar

Farmers had several activities throughout the year, and most of the activities were centred on agriculture (Table 2.7). Similar agricultural activities were observed in the three districts. For example, Chitemene was practiced in all the three districts from June to September. In addition to growing cassava, the crops cultivated in the three districts were similar (maize, bean, groundnut, bambara nut, cowpea and sweet potato).

Table 2.6: Cropping calendar for the farmers in the three districts surveyed

Month	Activity		
	Mwense	Mansa	Samfya
January	Planting bean, weeding	Planting cassava, bean Maize, sweet potato	Tilling land, planting groundnuts, maize, bean weeding maize, groundnut
February	Planting cassava, weeding	Weeding, harvesting bean Planting bean,	Weeding, planting cassava, bean, sweet potato
March	Weeding, harvesting cassava	Weeding cassava, harvesting groundnut	Weeding all crops. Winter ploughing
April	Harvesting groundnut, cassava	Harvesting groundnut	Harvesting bean, bambara nut, groundnut, Cowpea, pumpkin, cassava. Weeding cassava planted in January
May	Tilling land, chitemene	Harvesting groundnut, tilling land	Harvesting all crops (maize, cowpea, cassava)
June	Drying cassava	Chitemene, tilling land	Weeding cassava, harvesting rice, finger millet Chitemene system
July		Chitemene, tilling land	Heaping leaves, stems, tilling land
August	Selling cassava	Chitemene, tilling land	Tilling land
September	Selling cassava	Chitemene, tilling land	Tilling land
October	Selling cassava	Discing, planting cassava, maize groundnut	Tilling land, clearing land
November	Clearing land Planting	Planting, tilling land, planting Maize, groundnut	Planting cassava, groundnut, clearing land
December	Planting maize, groundnut Rice, finger millet, pumpkin	Planting cassava, maize	Planting all crops

2.4 Discussion

The findings of the study revealed that the farmers did not know CMD as a disease in spite of its presence in most of the fields. Farmers associated CMD with mealybug infestation. The lack of knowledge on CMD by the farmers could be attributed to perceived damage to cassava plants. Usually, farmers tend to view damage to pathogens and insect pests as a whole and not separately. The susceptibility of improved and local cultivars to CMD and other pests in the three districts could be because the cultivars were not specifically bred for pest and disease resistance. In addition, the failure by the majority of the farmers to recognise CMD as a disease necessitates effort on the part of researchers and extension officers to educate the farmers on diseases of cassava.

Technical support from the Ministry of Agriculture and Cooperatives, and extension was almost non-existent in the three districts visited. The few extension officers that participated in the group discussions were also ignorant of the disease. For the farmers to recognise CMD symptoms on leaves or differentiate between diseased or healthy cassava plants, requires properly trained extension officers both public and private. The level of education may have contributed to the poor understanding of the disease as most of the respondents were semi-literate. Although high CMD incidence (61.2%) was recorded, the disease was not reported by the majority of farmers. Studies by Muimba-Kankolongo (1997) estimated 50 to 70% yield loss per year in Zambia. The high levels of CMD incidence in the three districts visited need intervention by the breeders.

Cassava mealybug was regarded as the major insect pest of cassava. However, no mention of whitefly (a vector of CMD) and cassava green mite was made. This could have been due to the relatively small size of the insects. The observations are comparable to findings reported by Poubom et al. (2005) where farmers only mentioned large sized insects as the major constraints affecting cassava in West Africa. The farmers indicated that termites and mealybugs caused the most damage to cassava plants. The importance of the mealybugs was raised in all the three districts as they were more easily noticed unlike whitefly.

There was no clear management of CMD by the minority farmers who were aware of the disease. Although the farmers removed the affected top leaves (not specifically for CMD), this

clearly did not constitute effective management of CMD. Most of the cultivars grown by the farmers were susceptible to CMD.

The majority of the farmers in Mansa, Samfya and Mwense districts obtained cassava planting materials from either their own fields or from fellow farmers. The exchange of cassava cuttings is not only restricted within farming communities but also across districts. Consequently planting materials are often contaminated with viruses, a situation which contributes to the high incidence of CMD. In Mansa district, few farmers obtained cuttings from their colleagues, probably because farmers farm in close proximity to Mansa Research Station, which has a cassava multiplication programme. The planting materials are mostly distributed to farmers in Mansa district by MACO (M. Chiona, *personal communication*). In contrast, farmers in Mwense and Samfya districts sourced the planting materials mainly from their fellow farmers, because MACO and the various NGOs are less involved in these districts in the distribution of cuttings. Although MACO is one of the sources for cassava cuttings, the materials are also contaminated with viruses as indexing is not done prior to distribution to the farming community. In addition, farmers were not aware of the planting materials being sources of the cassava viruses in the fields.

Important traits mentioned by the farmers included high yield, early bulking, low cyanide content, branching type and white flour. Yield was the principal consideration when farmers choose a cultivar. However, they required yield to be complemented by earliness, low cyanide content, branching type and white flour. This was reflected in the growing of local landraces, even though they were susceptible to insect pests and diseases.

A few farmers preferred late bulking cultivars as a way of ensuring food security for their households. One of the attributes of local cultivars is long underground storability which allows the farmers to harvest when convenient. The improved cultivars which are early bulking (16 MAP) were not readily available in the farmers' communities.

Farmers in all the three districts understood the constraints affecting cassava production and marketing. Some of the constraints included weeds, labour, capital, distance to market and lack of transport. Shortage of labour, lack of working capital and distance to market, were the most commonly mentioned constraints.

Farmers grew a number of cultivars in their fields, most of them low yielding and susceptible to pests and diseases. Though the cultivars were susceptible, farmers often unknowingly, protected the crop against diseases and pests with very little outside technical assistance. However, the crop protection methods applied were based on “trial and error” with little impact on addressing the CMD problem.

The local economies of the three districts surveyed were largely driven by agricultural activities. Growing of more than one crop and at different times of the year assured the farmers of food security. This is the underlying reason why intercropping was practised in the three districts. Most of the farmers intercropped cassava with annual crops e.g. bean, maize, groundnut, sweet potato and bambara nut.

None of the farmers applied fertiliser although some of them acknowledged the low fertility levels of the cassava fields. Most of the farmers weeded their fields three times in a growing season. It is, therefore, imperative for breeders to consider developing cultivars that have high competitive ability over weeds. Although, most of the farmers acknowledged the presence of insect pests, not a single farmer used pesticides. The high cost of pesticides in Zambia and technical know-how, probably restricted smallholder cassava farmers from using the chemicals. Farmers indicated that cassava production was labour intensive which is perhaps why most farmers had fields of not more than 1 ha. These issues highlight the need to encourage farmers to adopt efficient farming methods and use high yielding cultivars.

In final conclusion: the study established that the farmers have little knowledge of CMD if any. High yield and early bulking were some of the traits preferred by the farmers. Most of the farmers prefer growing local cultivars because they have the desired attributes. Farmers’ preferred traits need to be integrated into the objectives of a cassava breeding programmes to meet the farmers’ needs and expectations. The farmers pointed out a number of constraints with regard to cassava production, namely capital, labour and drought. Therefore, farmers’ preferences, such as high yielding and early bulking traits which were widely mentioned by the farmers have to be given attention in the breeding process. The participation of farmers in the breeding programme, from early to advanced stages, will facilitate the adoption of new cultivars.

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Appendix

Participatory rural appraisal questionnaire used for the 2008 to 2009 survey

District:…… Name of farmer:…….. Date:……
Village:…… Field size:…… GPS:…….

Cropping system

What crops do you grow apart from cassava?.....
……
Why do you grow cassava?.....
……
Name the cultivars that are grown in your area.....
……
Where do you get planting material?.....
- Fellow farmers ☐
- Ministry of Agriculture ☐
- NGOs ☐
- Other ……

Give the reasons why you grow or prefer the mentioned cultivars…….....
- Low cyanide…….....
- Insect pest resistance…….....
- Disease resistance…….....
- Early maturity…….....
- Late maturity…….....
- High yield…….....
- Ability to suppress weeds…….....
- Resistant to drought…….....
- Easy of harvest…….....
- Colour of storage roots.....
- Palatability of storage roots…….....
- High starch content…….....
- Other…….....

Do you like erect or branching plants?.....
Why?.....
How do you grow cassava and why?
- Sole crop…….....
- Intercropping…….....
- Sole crop and intercrop…….....

Production marketing and constraints

What are the production and marketing constraints you face in growing cassava?.....

Production constraints
……
Marketing constraints
……

Farmer preferred characteristics

Do you grow improved or local cultivars?…….....
Why?…….....…
What are the characteristics you like for the cultivars you grow?.....

Improved ……
Local……

Farmers' perception of insect pests and diseases

Are pests and diseases important in your cassava crop?.....
……
What are the insect pests that affect your cassava crop?.....
……
……
What are the diseases that affect your cassava crop?.....
……
……
……
How are your cultivars affected by the diseases you have mentioned?.....
……
How are insects and diseases transmitted?.....

Insect pests……
……
……
Diseases……
……
……

Are there any cultivars grown in your area resistant to the pests and diseases you mentioned?
……
……
Do you grow resistant cultivars?.....
How do you control the insect pests, diseases and weeds you have mentioned?.....

Insect pests……
……
……
Diseases……
……
……
Weeds……
……
Do you apply fertiliser?……

CHAPTER 3: CASSAVA MOSAIC GEMINIVIRUSES OCCURRING IN LUAPULA PROVINCE

Abstract

Cassava plays a significant role in many family households in Africa. However, its production is affected by a number of insect pests and diseases including cassava mosaic disease (CMD), which is a major contributor to low yields. A survey of the prevalence of the viruses and associated satellites of cassava was conducted between April and May 2009 in 52 fields in Samfya, Mansa, Mwense, Kawambwa, and Nchelenge districts. The objectives of the study were to: i) determine the sources of CMD; ii) assess the disease incidence and severity of CMD and adult whitefly population; and iii) detect and identify the viruses and associated satellites. Cassava mosaic disease incidence and severity were recorded on 3-6 month old cassava plants. The cassava plants were characterised by mild to severe symptoms. Cassava mosaic disease incidence was high in Nchelenge, Mansa, Mwense, Samfya and moderate in Kawambwa. Moderate CMD severity was recorded in Nchelenge, Mansa, Mwense, and Samfya districts. The mean whitefly population per plant was low in all the five districts. Most of the CMD infection recorded was due cutting infection (55.5%). Samples collected from the field and subjected to polymerase chain reaction revealed the presence of African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV). Cassava mosaic diseases associated satellites were also detected in all the districts surveyed. Single infections of ACMV and EACMV were detected in 43.6 and 30.5% from the leaf samples, respectively. Mixed infections of ACMV+EACMV were detected in 14.6% of the leaf samples. Mansa district had the highest number of ACMV positive samples, while Mwense district recorded the highest EACMV positive samples. The EACMV-UG (Ugandan strain) of CMD and cassava brown streak virus (CBSV) were not detected in any of the districts surveyed.

3.1 Introduction

Cassava mosaic disease caused by a group of begomoviruses namely: African cassava mosaic virus (ACMV); East African cassava mosaic virus (EACMV); and South Africa cassava mosaic virus (SACMV) is one of the most important constraints to cassava production in sub-Saharan Africa. On the African continent, the disease has been reported to cause yield losses of 19 to 27 million tonnes (Legg and Thresh, 2003) and much of the losses have been reported through infected cuttings rather than whitefly infested plants (Fargette et al., 1988). The disease is transmitted by whiteflies and spread by cassava cuttings. Cassava cuttings are the major source of planting material and these are exchanged between farmers within the communities or in other parts of the country, thus facilitating the spread of pests and diseases to previously pest free areas. Compounding the problem is that most of the local and improved popular cassava cultivars grown in Zambia are susceptible to CMD (Muimba-Kankolongo et al., 1997).

Cassava plants in farmers' fields show considerable variation in disease symptoms ranging from mild to severe. Where severe symptoms occur, it has been as a result of mixed virus infections (Fondong et al., 2000) indicating synergistic interaction of the viruses. In the Democratic Republic of the Congo (DRC) and Tanzania, where cassava production is higher than Zambia's, ACMV and EACMV have been reported to occur in single and mixed infections (Were et al., 2004). Earlier surveys in DRC by Were (2001) indicated the presence of ACMV only, however, later surveys have shown large numbers of samples testing positive for ACMV and EACMV-UG (Ugandan variant) (Were et al., 2004). The EACMV-UG is a recombinant of ACMV and EACMV (Zhou et al., 1997). The indication, therefore, is that EACMV-UG which is a more virulent virus is spreading southwards towards Zambia. The EACMV-UG is destructive in susceptible cassava cultivars and has been reported to be moving at a rate of 20 km $year^{-1}$ (Otim-Nape et al., 1997). Considering the close proximity of Zambia to DRC and the rate of movement of the virus, cassava in Zambia is at risk of being infected by the virulent strain.

Although the causal viruses of CMD have been known, other subviral agents called satellite DNA molecules have only recently been discovered (Ndunguru et al., 2008). Satellites modulate replication and symptom expression of their helper virus. Near full length sequences of 260 satellites associated with begomoviruses isolated from different geographical regions have been determined and lodged with data bases (Briddon et al., 2008). Recently resistance breaking satellite DNA molecules associated with CMD were isolated in Tanzania (Ndunguru et al., 2008). Symptoms associated with satellites include leaf narrowing (filiform) and reduced leaf

blade on one side. This study was conducted in Luapula province, one of the most important cassava producing provinces in Zambia.

The objectives were:

i) to determine the sources of CMD;
ii) to assess the disease incidence and severity of CMD and adult whitefly population; and
iii) to detect and identify the viruses and satellites affecting cassava

3.2 Materials and methods

3.2.1 Location of the study area

The survey was carried out in Luapula province, Zambia, which borders with Democratic Republic of Congo (DRC) in the west. The province lies between latitude 8 to 12^{o} south of the equator and 28 to 30^{o} east of Greenwich Mean Time (GMT). Luapula province is situated in high rainfall agroecological zone (AEZ III) receiving >1 000 mm of rainfall per year. Annual minimum and maximum temperature in the province range between 10 to 31 ^{o}C. The altitudes vary from 900 m above sea level in the lower Luapula valley to over 1 300 m at Kawambwa. The province experiences monomodal rainfall from November to April and does not experience drought. It also has savannah type of vegetation interspersed with trees.

3.2.2 Field sampling and mapping

The study was carried out between April and May 2009. Fifty-two cassava fields were sampled at average intervals of 5-10 km along the high-way (main road). Occasionally fields closer to the main road were also sampled. In areas where the fields were far apart (>5-10 km), the sampling intervals were made further apart. To gather information regarding the cassava plants, the farmers growing cassava were interviewed on cultivars grown, age of the cassava plants and what they thought about CMD. Sampling was done in Samfya, Mansa, Mwense, Kawambwa and Nchelenge districts (Figure 3.1). Sampling was done by walking through cassava fields in a 'Z' configuration, two sides on either side and along the diagonal. In each field 10 plants per transect were sampled from the predominant cultivar to give a composite sample of 30 plants per field. The plants were sampled at approximately the same distance between one another within the transect.

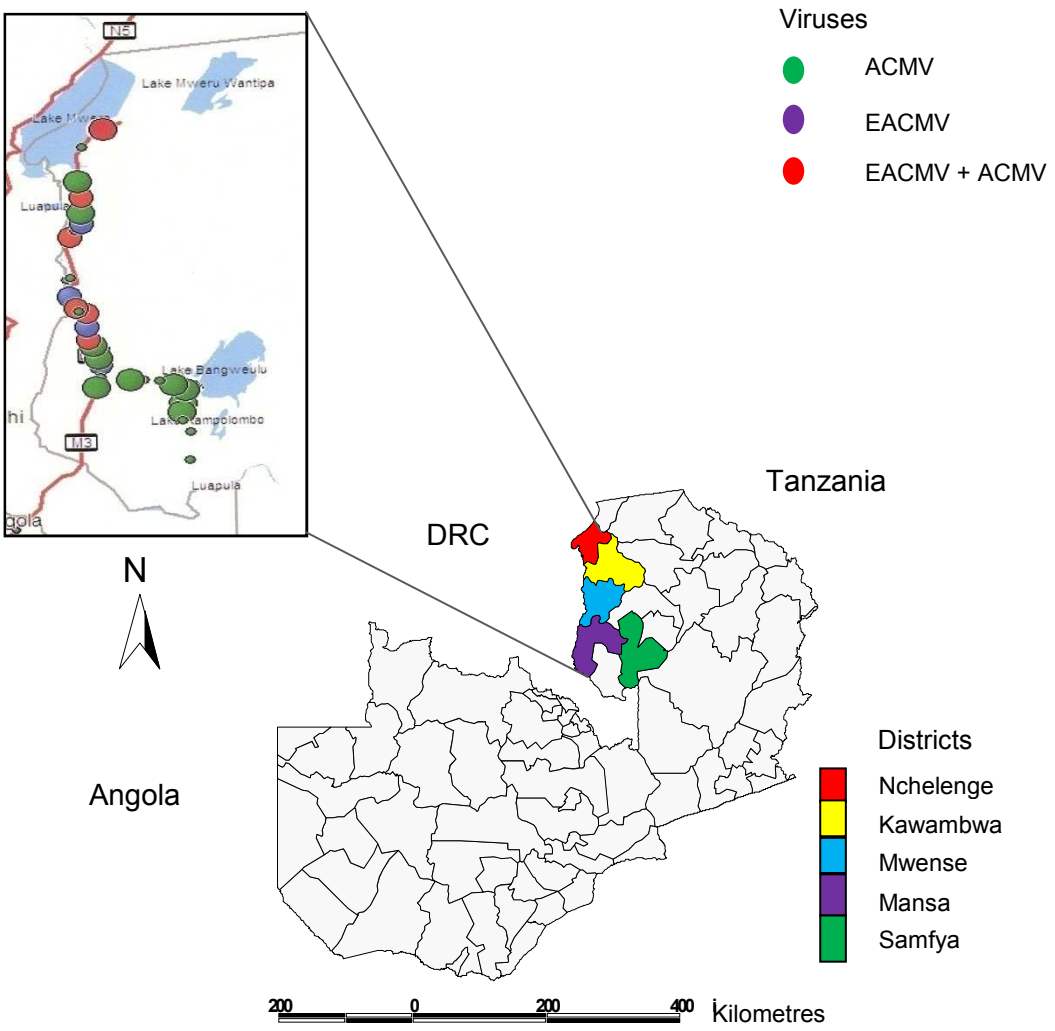

Figure 3.1: Districts in Zambia surveyed for cassava mosaic disease incidence and severity

The total length of a transect varied depending on the field size. In this survey cassava fields which were 3 to 6 months after planting were targeted. The 3 to 6 months old plants allowed for distinguishing plants that were either infected by whiteflies or through the cuttings (Sseruwagi et al., 2004). The type of CMD infection was examined by observing the whole plant. Plants with symptoms on only the upper most leaves were considered as whitefly infected, whereas those with disease symptoms occurring throughout the plant were regarded as having been infected from the cutting.

The coordinates (latitude, and longitude) including altitude for each sampled field were recorded using global positioning system (GPS) equipment (Garmin GPS, model etrex summit HC). The

distribution of cassava geminiviruses were mapped using Arcview software (Environmental Systems Research Institute, Inc., Redlands, CA, USA). Other information related to the CMD survey was collected and recorded on a sample record sheet (Appendix 1). In addition, photographs and symptoms of cassava plants from the field were also described and recorded.

3.2.3 Cassava mosaic disease incidence, severity and adult whitefly population

Disease incidence was determined by assessing the visibly diseased plants (with CMD symptoms) in relation to the total number of assessed plants in each field. Disease severity was recorded for each sampled whole plant using the five point rating scale (Hahn et al., 1980) (Table 3.1).

Table 3.1: Cassava mosaic disease severity rating (scale 1-5)

Scale	Symptom description
1	No symptoms observed
2	Mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only with the remainder of the leaflets appearing green and healthy
3	Moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets
4	Severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size
5	Severe mosaic distortion of the entire leaf

Source: Hahn et al. (1980)

The number of whiteflies was counted on the five youngest leaves of individual plants. The leaves were held gently and turned to count the whiteflies. Counting of whiteflies was done from the same plants that were examined for CMD incidence and severity.

3.2.4 Sample collection

In each field, two young leaves were sampled from plants with CMD symptoms for deoxyribonucleic acid (DNA) extraction. One sample was collected from a plant with severe symptoms and the other sample was from a plant with mild symptoms. The reason for sampling from plants with mild and severe symptoms was to determine whether different virus strains occurred in the same field. In some cases three samples were taken from a field with a third sample taken from a plant exhibiting peculiar symptoms. Young leaves with symptoms were removed from the infected plants and placed in 1.5 ml eppendorf tubes and placed in a cool box containing ice blocks for preservation purposes until DNA was extracted. Each eppendorf tube was labelled indicating the location from where the sample was collected. Furthermore, cuttings

from each field where the young leaves had been sampled were collected, labelled and planted in the screenhouse. This was done to check for symptom variation from the planted cuttings and to guarantee the availability of viral DNA from the sampled fields in case DNA from leaf samples was not recovered. In addition, diseased cuttings were later taken from plants that had grown from the planted diseased cuttings for grafting onto the F_1 progeny (clonal evaluation trial). The planted cuttings were inspected twice weekly and disease symptoms recorded and described.

3.2.5 Viral genomic deoxyribonucleic acid isolation

The genomic DNA was recovered directly from the samples using the method described by Dellaporta et al. (1983). The leaf samples collected from the field were crushed with a mortar and pestle in 500 µl of extraction buffer [100 mM Trizma base, 8.5 mM ethyl-enediaminetetraacetic acid (EDTA), 500 mM NaCl, and 10 mM *β*-mercaptoethanol in 100ml of double distilled water at pH 8.0]. A volume of 33 µl of 20% lauryl sulphate solution was added to each sample, mixed and the tube with contents was then incubated at 65^{o}C in a water bath for 10 min. A volume of 160 µl of 5 M potassium acetate solution was then added to each tube, mixed thoroughly and incubated in an icebox for 10 minutes and tubes centrifuged in a microfuge at 11 600 rpm for 10 minutes. A volume of 450 µl of the resultant supernatant was removed and transferred into a new 1.5 ml microfuge tube to which 450 µl of ice-cold isopropanol was added, mixed by inverting and centrifuging at 13000 rpm for 10 min to precipitate the DNA. The supernatant was then removed and the remaining DNA air dried at room temperature for 1 h after removing the ethanol and thereafter suspended in 300 µl of distilled water and stored at 4^{o}C until used.

3.2.6 Amplification and differentiation of cassava viruses and associated satellites

Amplification of viral DNA and satellites was performed using the Polymerase chain reaction (PCR) machine (Techne – TC500) for each sample. The reaction mixture for the PCR was carried out in 0.5 ml microfuge tubes. The total reaction mixture was 50 µl and was made up of: 41.0 µl, distilled water; 2.5µl, PCR buffer (10X); 1.5 µl, magnesium chloride; 0.5 µl, dNTPs (10mM); 1.0 µl, forward (10mM) and reverse primers (10mM); 0.5 µl, Taq polymerase; and 2.0 µl DNA template. A drop of oil was added to each tube to prevent evaporation.

Universal and satellite primers were used in the amplification of near full length fragments of DNA-A and DNA-β of total DNA. In the PCR assay, differential primers amplifying specific virus species or different strains were also used (Table 3.2).

Table 3.2: Primers used to detect cassava viruses in leaf samples collected from Samfya, Mansa, Mwense, Kawambwa and districts

Primer pairs	Sequences (5'-3')	Specificity	Target
JSP00/F	ATGTCGAAGCGACCAGGAGAT	ACMV	AV1/CP
JSP00/R	TGTTTATTAATTGCCAATACT	ACMV	AV1/CP
EAB555/F	TACATCGGCCTTTGAGTCGCATGG	EACMV	DNA-B
EAB555/R	CTTATTAACGCCTATATAAACACC	EACMV	DNA-B
SatIIF	GCCGCACCACTGGATCTC	Satellite II	DNA-II
SatIIR	CAGCAGCCAGTCAGGAAGTT	Satellite II	DNA-II
SatIIIF	AGGCCTCGTTACTAAAAGTGC	Satellite III	DNA-III
SatIIIR	ACCTGACGGCAGAAGGAAT	Satellite III	DNA-III

F, forward primers; R, reverse primers; sat, satellite

The viral DNA was amplified using the following PCR stages; first cycle of 1 minute at 94^{o}C, followed by 30 amplification cycles of 1 minute at 94^{o}C, 1 minute of primer annealing at 58^{o}C, 2 minutes for strand extension at 72^{o}C and then finally for 10 minutes at 72^{o}C (final extension). The samples were held at 4^{o}C before being loaded into the gel apparatus. Before loading the gel apparatus, the amplified DNA was mixed with 1 µl of loading dye. After carrying out PCR analysis, the reaction was subjected to gel electrophoresis in Tris Acetate EDTA (TAE) buffer. The gel was visualized using the bench top single UV transilluminator and photographed with the gel documentation system (Gel Doc XR: Universal Hood-S.N 765/03363, Bio-rad).

3.2.7 Data analysis

The data were analysed using Genstat version 14 (Payne et al., 2008) based on the following statistical model

$$Y_{ij} = \mu + d_i + f_j + d_i / f_j + \varepsilon_{ij}$$

Where:

Y_{ij} is the CMD score observed at the ij^{th} location

μ is the overall mean recorded for the disease symptom

d_i is the CMD score observed in the i^{th} district

f_j is the CMD score observed in the j^{th} field

d/f_{ij} is the CMD score observed in the jth field nested in the ith district

ε_{ij} is the error term associated with each observation

3.3 Results

3.3.1 Sample collection

About 52 farmers' fields were visited and 112 leaf samples were obtained from Samfya, Mansa, Mwense, Kawambwa and Nchelenge districts. In addition, 104 cuttings from the sampled fields were also collected. The mean age of the cassava plants surveyed was 4 months old and the average altitude above sea level was 1130.21 m (Table 3.3) above sea level (masl). The highest altitude (1295.4 masl) was recorded in Kawambwa district (S10 55.157; E28 47.912) and the lowest (946 masl) was in Mansa district (S9 50.172; E28 45.366).

On average, the field size in all the districts visited was 0.98 ha and 50% of the fields were either intercropped with maize, bean, or sweet potato. The other 50% had either cassava or maize cultivated (sole cropping). In Luapula province cassava was either planted on ridges (predominant) or round mounds. In this survey local cultivars were predominant (77%), even though improved cultivars (13%) were cultivated. The most frequently cultivated local cultivars in all the districts visited were Bangweulu, Beliate, Katobamputa and Kabala while for the improved cultivars, Chila was the most popular. However, it was not possible to get all the local names of the cultivars because in some places the owners of the fields were not present at the time of the survey.

Table 3.3: Mean altitude, mean incidence, symptom severity and adult whitefly number plant^{-1} in Samfya, Mansa, Mwense, Kawambwa and Nchelenge districts surveyed, 2009

District	Number of fields	Mean altitude (masl)	Mean Incidence (%)	Mean severity (scale 1-5)	Whitefly number plant^{-1}
Samfya	7	1195.7	57.1	2.4	0.1
Mansa	19	1259.4	60.8	2.5	0.4
Mwense	13	1061.2	59.5	2.5	1.2
Kawambwa	6	961.7	41.0	1.8	0.3
Nchelenge	7	986.9	70.9	2.6	0.4
Combined mean	-	1130.2	59.1	2.4	0.7
LSD (0.05)	-	-	0.670	0.006	0.001
S.E.D.			4.51	0.041	0.05

Scale (1-5) 1, no symptoms observed; 2, mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only with the remainder of the leaflets appearing green and healthy; 3, moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets; 4, severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size; 5, severe mosaic distortion of the entire leaf; masl (metres above sea level)

3.3.2 Sources of cassava mosaic disease infection

In most of the surveyed fields, the main mode of CMD transmission was through cuttings (55.5%) rather than whiteflies (7.0%). Cutting transmission was highest (70.5%) in Nchelenge district, while infection through whiteflies was highest (13%) in Mansa district (Table 3.4). The lowest infection through cuttings (43.3%) and whitefly (1.4%) were recorded in Kawambwa and Nchelenge districts, respectively.

Table 3.4: Transmission modes of CMD on cassava plants, 2009

District	Number of Fields	Cutting infection (%)	Whitefly infection (%)	Total infection (%)
Samfya	7	50	8.1	58.1
Mansa	19	49.2	13	62.2
Mwense	13	65.9	2.6	68.5
Kawambwa	6	43.3	1.7	45
Nchelenge	7	70.5	1.4	71.9

3.3.3 Incidence of cassava mosaic disease

Though no significant differences in the CMD incidence in all the surveyed districts were observed, there were, however, interesting trends. The CMD incidence ranged from 13.3-93.3%. Incidence in all the five districts exceeded 45% and the average incidence for the five districts was 59.1%. Overall, Nchelenge district (Figure 3.2) had the highest mean incidence (70.9%) and Kawambwa district had the least incidence (41.0%). High disease incidence was also observed in Samfya (57.1%), Mansa (60.8%), and Mwense (59.4%) districts. The highest incidence (93.3%) in all the fields surveyed was recorded near Musonda falls (S10^{o}39.176, E28^{o}43.119) located in Mwense district, while the lowest (13.3%) incidence was recorded in Samfya district, southwest of Lake Bangweulu.

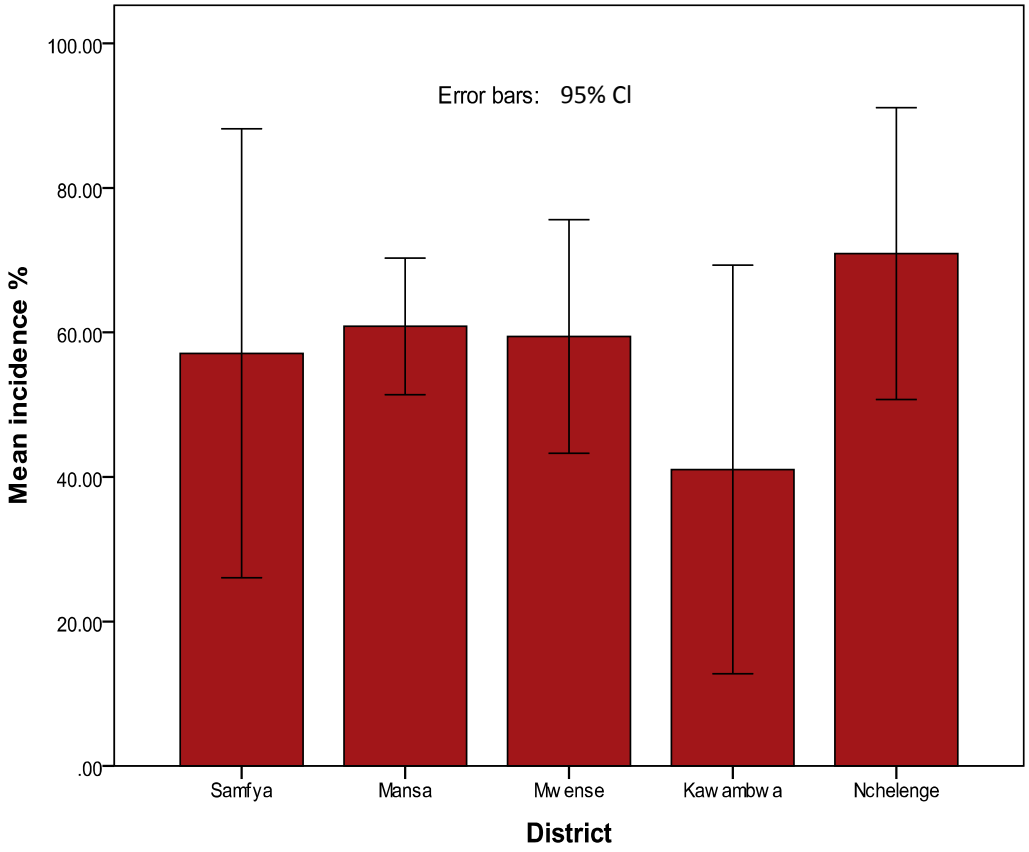

Figure 3.2: Incidence of cassava mosaic disease in five surveyed districts

3.3.4 Severity of cassava mosaic disease

Significant (P<0.05) differences were observed for the CMD severity in the five districts surveyed. The mean disease severity was low (2.46) for all the districts surveyed. Moderate severity scores were recorded in Samfya (2.39), Mansa (2.54), Mwense (2.46) and Nchelenge (2.56) districts. Kawambwa district had the lowest severity (1.82) (Figure 3.3).

The severity rating of up to 5 on the 1-5 scale was recorded in all the districts with the exception of Kawambwa which had 4 as the highest severity score. The disease severity score in Kawambwa district was significantly (P<0.05) lower compared to all the other districts.

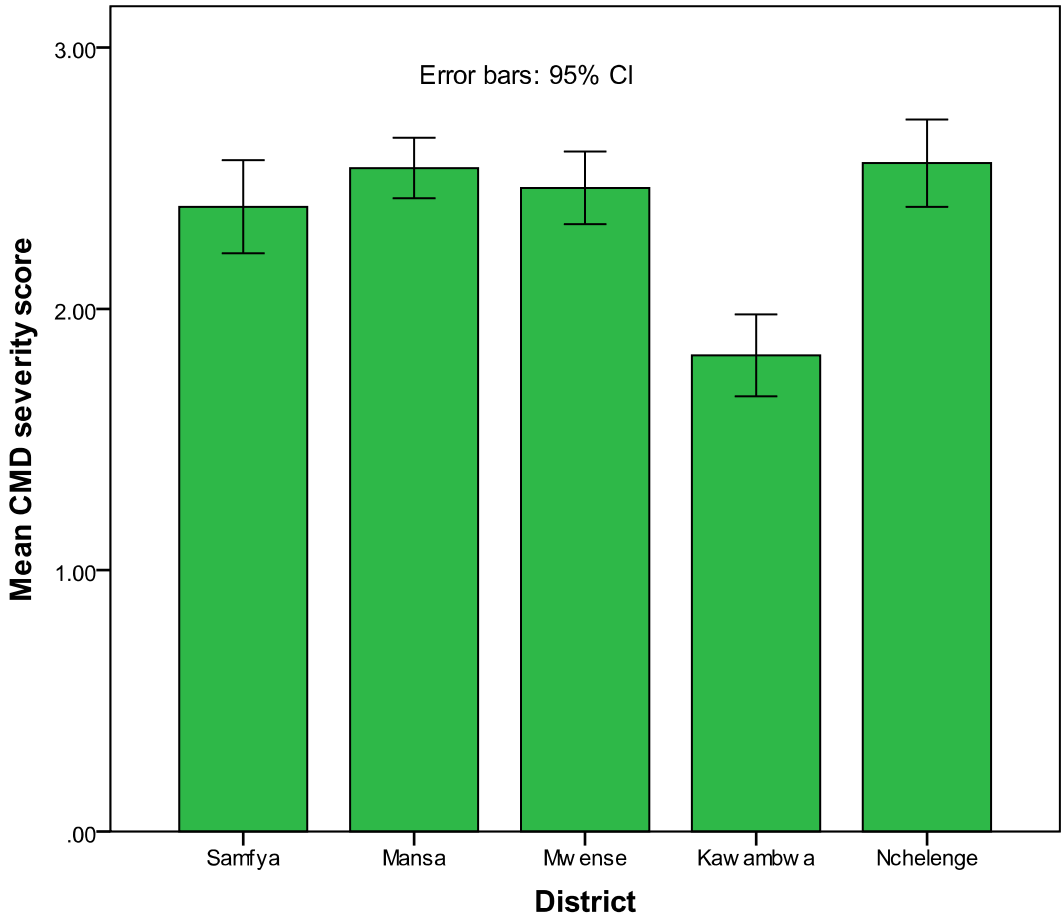

Figure 3.3: Severity of cassava mosaic disease in five surveyed districts of Luapula province

3.3.5 Mean adult whitefly number plant^{-1} in the surveyed areas

Adult whitefly number plant^{-1} was significantly different (P<0.001) between districts. The overall mean adult whitefly plant^{-1} was 0.5. The highest mean whitefly number plant^{-1} was recorded in Mwense district (1.2), while the lowest (0.4) was in Samfya district (Figure 3.4). The mean adult whitefly number plant^{-1} for Mwense district was significantly higher (P<0.001) than Samfya, Mansa, Kawambwa and Nchelenge districts. In Kawambwa district whitefly number plant^{-1}was significantly (P<0.001) higher than Samfya district.

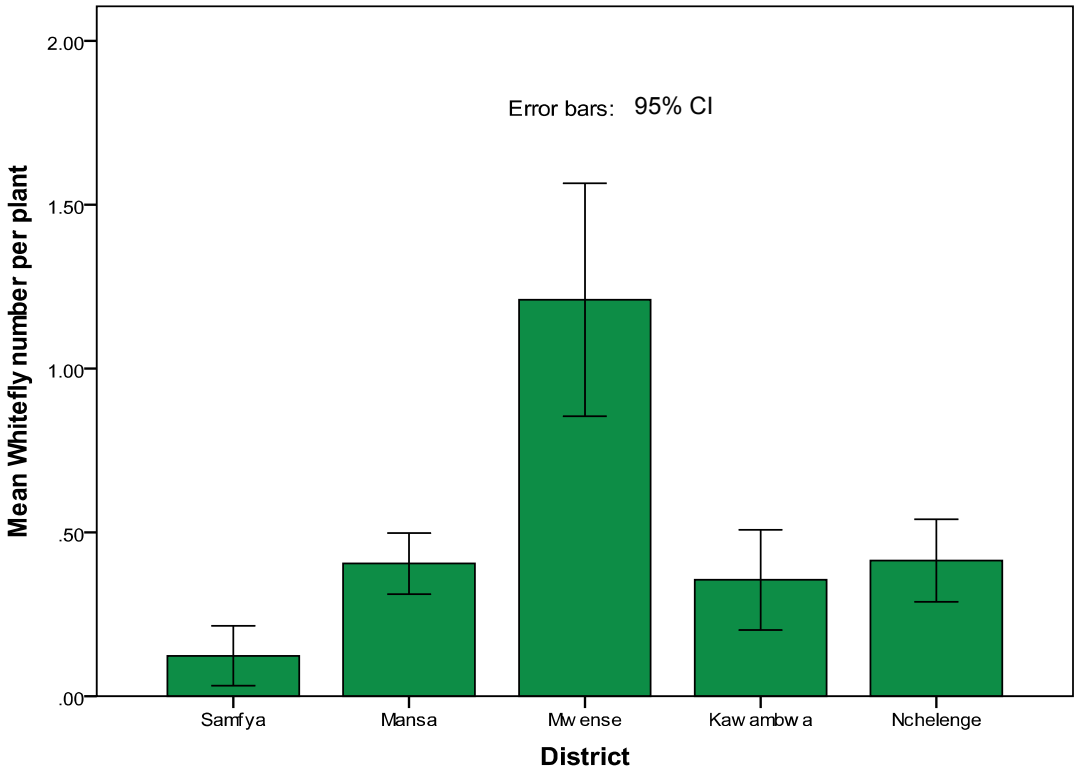

Figure 3.4: Mean number of whitefly plant^{-1} in the surveyed five districts

3.3.6 Cassava mosaic disease symptom expression

The disease symptoms expressed from the infected plants varied widely. The symptoms ranged from barely visible mosaic to severe leaf distortion (Figure 3.5A). The most severe symptoms were observed in Mwense district. In most cases, the symptoms appeared on the base of the leaf and progressed along the primary vein and traversing along the secondary veins. The mild symptoms displayed patches of yellow and green and lacked leaf distortion. With mild infections, symptoms did not appear from the base of the leaf.

Other symptoms observed included stunting and leaf curling (where one side of the leaf blade was highly reduced). Reduced leaf size was also observed in severely affected plants. The leaf narrowing and curling symptoms characteristic of satellites were observed in all the districts irrespective of the cultivars grown. In some fields different symptoms were observed on the same cultivar (Figure 3.5A and 3.5B). Cassava cuttings collected from the survey sites and planted in the screen house (Figure 3.5C) developed similar symptoms 3 to 4 weeks after planting.

Figure 3.5: Symptoms on naturally infected cassava plants (Figures A and B are of cultivar "Beliate" and from the same field, but with different symptoms); (A) leaf curl and distortion, typical of EACMV + ACMV (B) patchy green and yellow mosaic, typical of ACMV; C) Planted in the screen house, leaf blade with both margins reduced; D) Young filiform leaves on the apical end of the stem. Figures C and D are characteristic of satellites.

3.3.7 Detection of viral DNA

The four districts surveyed, tested positive for ACMV and EACMV except for Kawambwa district whose PCR tests were not successful. Of the 82 samples analysed, 75 reacted positively of which 30.5, 43.6 and 14.6% were identified with EACMV, ACMV and EACMV+ACMV, respectively. The EACMV-UG and cassava brown streak virus were not detected in any of the samples. The EACMV was more frequent (12.1%) in Mwense district (Figure 3.6), while Mansa district had more ACMV (15.8%) compared to the rest of the districts. More than three fields with mixed infection of EACMV and ACMV were found in Samfya district near Lake Bangweulu (S11 21.100; E29 29.785). Although, other districts had a combination of EACMV and ACMV, they did not occur in close proximity unlike in Mwense district. In most of the fields single and mixed infections were detected.

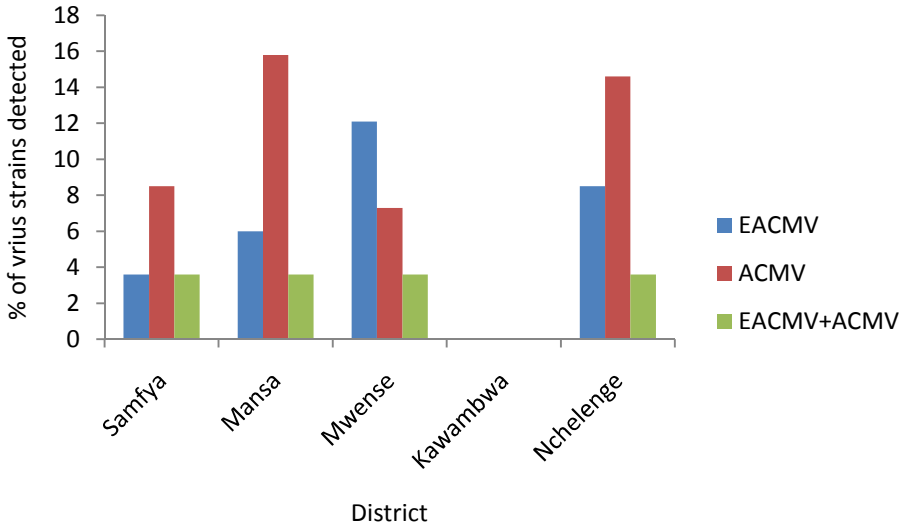

Figure 3.6: Distribution of cassava viruses in five districts of Luapula province

Seventy-five out of 112 leaf samples produced amplification products with the universal primers. Two bands characteristic of ACMV (774 bp) and EACMV (556 bp) were produced (Figure 3.7) indicating the presence of the two virus strains. All the lanes were loaded with equal amounts of nucleic acid. Lanes 26, 28, 31, 33, 34, 36, and 38 up to 44 showed presence of ACMV (Figure 3.7A). East Africa cassava mosaic virus (ACMV) was detected in lanes 74, 76, 79, 83, 85 and 85 (Figure 3.7B). Satellite II (895 bp) was observed in lanes 3, 8, 15, 17, 18, 19 and 20 (Figure 3.7C) while satellite III (306 bp) was seen in lanes 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 33 and 34 (Figure 3.7D).

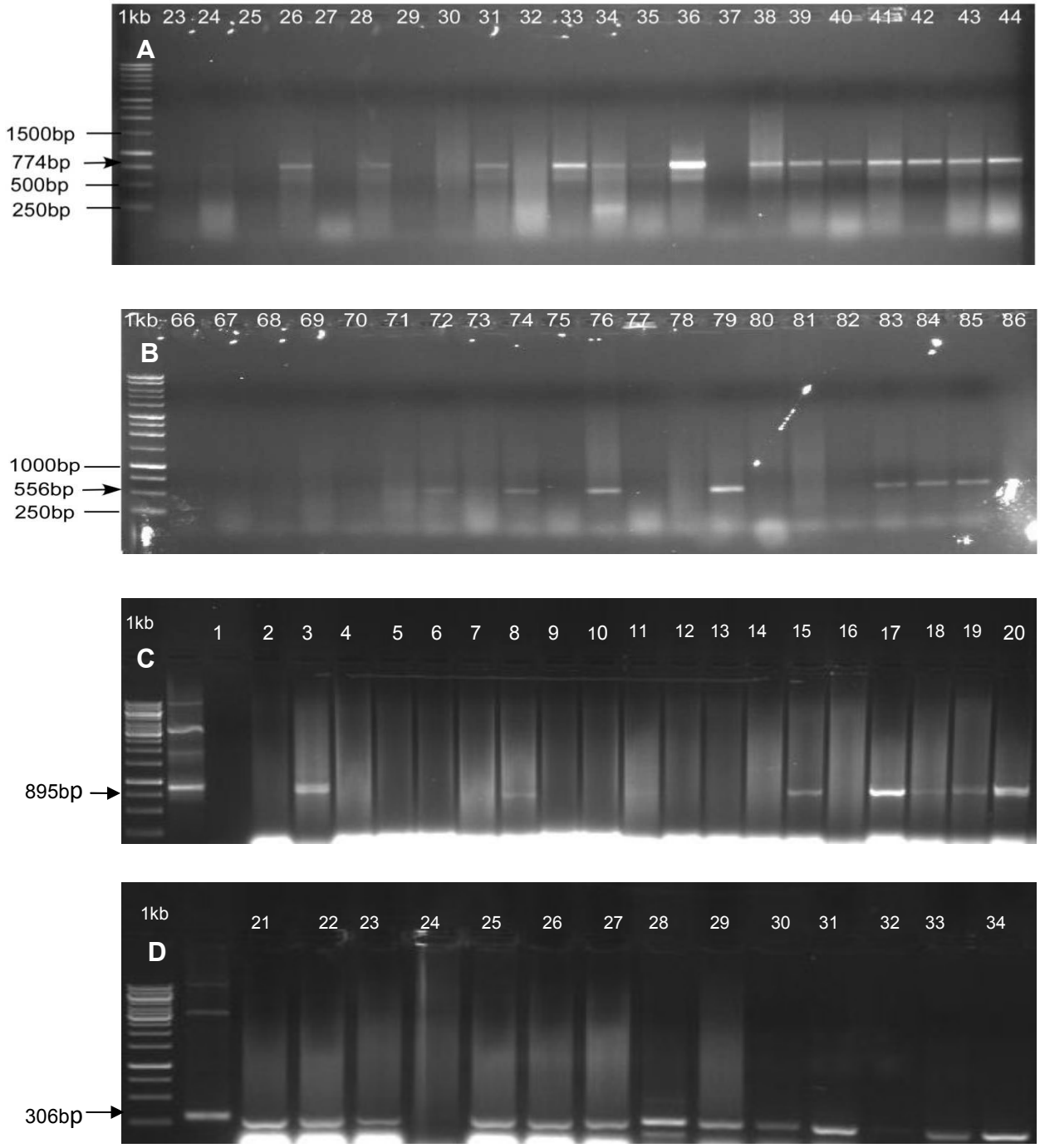

Figure 3.7: Gel electrophoresis of PCR amplified DNA fragments using universal primers; A) JSP001/ JSP002; B) EAB555F/EAB555R, and specific primers; C) Satellite II; and D) Satellite III

3.4 Discussion

The survey revealed that CMD occurred in all the five surveyed districts of Luapula province. The overall mean CMD incidence of 60.5% was higher than the 41.0% previously reported by Muimba-Kankolongo et al. (1997). This may be due to timing of the previous two surveys as they were carried out between June and August (winter season in Zambia). During this period cassava plants shed most of their leaves and some CMD symptoms are masked by leaf senescence. In addition, cassava green mite infestation masks CMD symptoms. The increase in incidence levels could also be attributed to increased usage of "recycled" infected planting materials (cuttings) by the farmers. Most of the farmers in Luapula province obtain planting materials from amongst themselves and from their own fields. The survey revealed no clear differentiation between districts for CMD incidence. The highest incidence of 70.9% was found in Nchelenge district with a mean disease severity of 2.56. This corroborates the earlier study by Muimba-Kankolongo et al. (1997). During the survey differences in CMD severity were observed between the districts. The highest CMD severity was observed in Nchelenge district. Though the whole province had a mean CMD severity of 2.41, this has hardly changed from previous reports (2.4 severity) (Muimba-Kankolongo et al., 1997).

The spread of CMD in Luapula province is predominantly through cuttings (55.5%) rather than through whiteflies (7%). The lack of evidence of spread suggests that roguing of infected plants may be important. The findings on the mode of infection also concur with Okao-Okuja (2004) who reported infection rates by cuttings of 57% and by whiteflies of 11% in West Africa. The surveyed districts registered low mean whitefly number plant^{-1}. Low adult whitefly plant^{-1} in the entire province could be due to unimodal rainfall pattern. For most part of the year, the province experiences dry periods and most of cassava plants shed their leaves leaving few alternative food sources for the whiteflies. This is in contrast to countries in East Africa which experience two rainy seasons per year and have high whitefly numbers per plant (P. Sseruwagi *personal communication*). The other possible reason could be infectiveness of the spread of CMD by whiteflies. Differences in whitefly transmission efficiencies have been reported (Bedford et al., 1994).

Though the whitefly number plant^{-1} was generally low, Mwense district registered the highest mean number of adult whitefly plant^{-1}. This might be due to high temperatures that exist in the district coupled with high rainfall. Climatic factors particularly temperature and rainfall have been found to play a significant role in whitefly abundance (Legg, 1994). In addition, leaf senescence

affects fluctuations of whitefly numbers (Leite et al., 2003). However, the number of adult whiteflies does not correspond with the disease incidence in the districts surveyed. These findings agree with those reported in Senegal and Guinea Conakry by Okao-Okuja (2004). On the other hand, this is contrary to the findings of Fauquet and Fargette (1990), who suggested that CMD incidence is related to movement and activity of whiteflies.

The severe symptoms of cassava plants in Mwense districts and elsewhere within Luapula province could be attributed to mixed infections of ACMV, EACMV and satellites detected in some of the samples. Synergistic interaction of the two viruses and satellites in the same cassava plants could also play a role in severe symptom expression. Mixed infections of ACMV+EACMV have been reported to cause severe symptoms in Tanzania (Harrison et al., 1997), Uganda (Pita et al., 2001) and Cameroon (Fondong et al., 2000). There is also the issue of the susceptibility of cultivars grown by the farmers to the viruses as most of the cultivars grown by the farmers are local landraces. Since the two viruses and satellites were detected in the same cassava plants, yield improvement remains a challenge and phytosanitation could play an important role of countering the effects of the viruses. In view of the fact that the incidence of CMD is mostly as a result of infected cuttings, the probability of the double infection spreading and giving rise to new variants is high in Luapula province.

The failure to detect EACMV-UG could be because the virus is not present yet in the surveyed districts. However, in most of Zambia's neighbouring countries, namely Angola (Kumar et al., 2008), Democratic Republic of Congo (Neuenschwander et al., 2001), and Tanzania (Ndunguru et al., 2005), EACMV-UG has been reported. Moreover, when ACMV and EACMV are found in the same cassava plants they have been implicated in recombination resulting in EACMV-UG (Zhou et al., 1997). Despite the few EACMV detected in the samples, the geographical distribution of the viruses was uniform. The differences in the number of positive reactions for ACMV and EACMV in cassava fields could not be explained. However, use of infected planting materials from different sources could have contributed to uneven distribution. The frequent occurrences of ACMV compared to mixed infections of ACMV and EACMV may be attributed to farmers avoiding selecting severely diseased planting materials.

The study established that high CMD incidence and moderate severity occurred in the five districts of Luapula province. Furthermore, the study demonstrated the presence of viruses (ACMV and EACMV) in single and double infections. Symptoms of satellites were also observed

and detected in all the districts. The presence of ACMV, EACMV and their associated satellites necessitates long term management strategies such as breeding for resistance.

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Appendix

Cassava mosaic disease survey data sheet (2009), (Sseruwagi et al., 2004)

District:		Crop mixture:					
Village:		Cassava cultivars:					
Field size:		Cultivar sampled					
Date and time:		Crop age (months)					
No. of nearby fields		GPS		Latitude	Longitude	Altitude	
Plant no.	CMD infection			CMD severity	CMD incidence	Wf. No	Symptom description
	Cutting inf	Whitefly inf	Healthy				
1							
2							
3							
4							
n							
Mean							

CMD, Cassava mosaic virus; Inf., infection (+/-); GPS, Geographical positioning system; Wf.No, Whitefly adult population number; n, up to 30 plants

CHAPTER 4: EVALUATION OF CASSAVA GENOTYPES FOR RESISTANCE TO CASSAVA MOSAIC DISEASE AND AGRONOMIC TRAITS

Abstract

Sixteen cassava genotypes comprising introductions, local landraces and improved genotypes were evaluated for two seasons in Mansa, Zambia, for their reaction to cassava mosaic disease (CMD). The study was conducted to evaluate the reaction of cassava cultivars to CMD. Cassava mosaic severity and leaf retention was scored at 6 months after planting (MAP) and data on yield and yield components was recorded at harvest (7 MAP). Significant genotype x season interaction for CMD, harvest index, fresh root yield, biomass, plant height, root size and leaf retention was recorded. Bangweulu, Kalaba, Chikula, Mwakamoya and Chila7 were the most susceptible genotypes over the two seasons. Mweru, Kampolombo, TMS190, TMS3001, Tanganyika and Nalumino had low severity scores. Harvest index ranged from 0.36 (Mwakamoya) to 0.55 (Chila7) for the combined seasons. Chila7 had the highest fresh root yield with a mean of 0.87 kg plant^{-1} for the combined seasons. The resistant genotypes may be used to improve the CMD resistance of local cultivars through hybridisation.

4.1 Introduction

Cassava forms an integral part of the farming system in Zambia. A number of cassava landraces are grown by smallholder farmers mainly in Luapula, Northern, North Western, Western and parts of Central provinces. In most of the communities, the crop is grown for its storage root. The cassava roots have variable uses such as fresh food, animal feed (Chhay et al., 2003), starch extraction and alcohol production (Tonukari, 2004). One of the important breeding objectives in many research institutions, for example the International Institute for Tropical Agriculture (IITA) and Centro Internationcional de Agricultura Tropical (CIAT), is producing cultivars which are high yielding, early bulking, resistant to pests and diseases, and with low cyanide glycoside content (HCN). However, many of the cultivars grown in Zambia are susceptible to pests and diseases.

One of the farmers' primary concerns is having planting materials which are resistant to important diseases, as diseases are the major constraints to cassava production (Theiberge, 1985). Without proper management of CMD, the disease may prove difficult to eradicate especially in planting materials perceived to be free from the disease. A practical solution is selecting cultivars that resist CMD infection. Increased resistance to CMD offers hope of achieving higher yields and improving household food security especially in rural communities. Plants that are resistant to CMD, partition more carbohydrates to the storage roots which results in improved yields.

Selecting cassava genotypes that are resistant to CMD requires subjecting the materials to virus infection. Recently, a grafting inoculation method has been used to evaluate the resistance of genotypes to cassava brown streak disease (CBSD) (Munga, 2008). Furthermore, grafting has been reported to be a reliable method of transmitting viruses in cassava (Ariyo et al., 2003). Information on the performance of cassava cultivars to CMD in Zambia is inadequate, especially for landraces. In addition, there is little information available on agronomic traits of cassava cultivars. Evaluation of local cassava cultivars is necessary in order to generate important information that can form the basis of a breeding programme for CMD resistance in Zambia.

The objectives of the study were to evaluate:

i) the reaction of local and improved cassava cultivars to CMD
ii) the cultivars for agronomic traits

4.2 Materials and methods

4.2.1 Location and site description

The trial was carried out at Mansa Research Station in 2009/10 and 2010/11 seasons. The trials were established on 10 December of each season. Mansa experiences a monomodal rainfall pattern and receives between 1 000 and 1 500 mm of rainfall per year primarily from November to April. The annual rainfall for season one was higher than for season two (Figure 4.1). The mean annual minimum temperature is 10°C and mean annual maximum temperature is 31°C (Figure 4.2). The soils are acidic at both sites and have been classified as sandy loam, well drained to imperfectly drained (MACO, 1991). The climatic data and soil composition is presented in Table 4.1. The vegetation of the trial sites is predominantly Miombo [10] and interspersed with grass (Lawton, 1978).

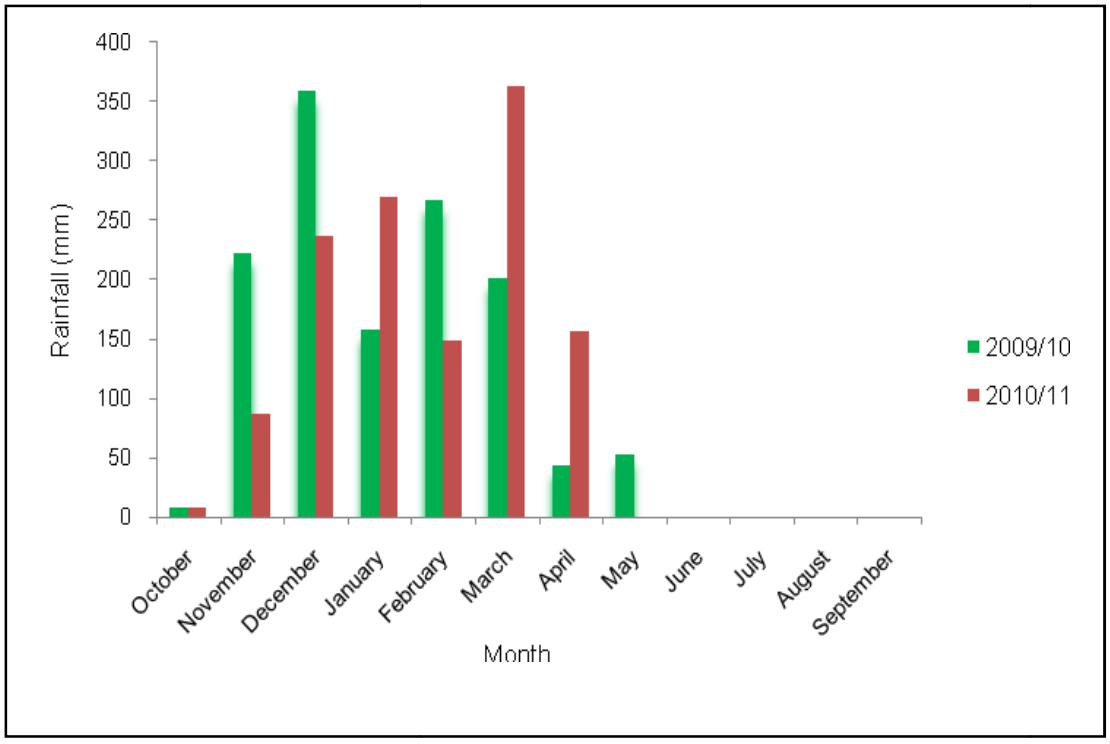

Figure 4.1: Rainfall distribution for 2009/10 and 2010/11

[10] Light vegetation with closed canopy, deciduous woodland dominated by leguminous trees of the genera *Brachystegia* and *Julbernardia,* usually 12-15 m tall

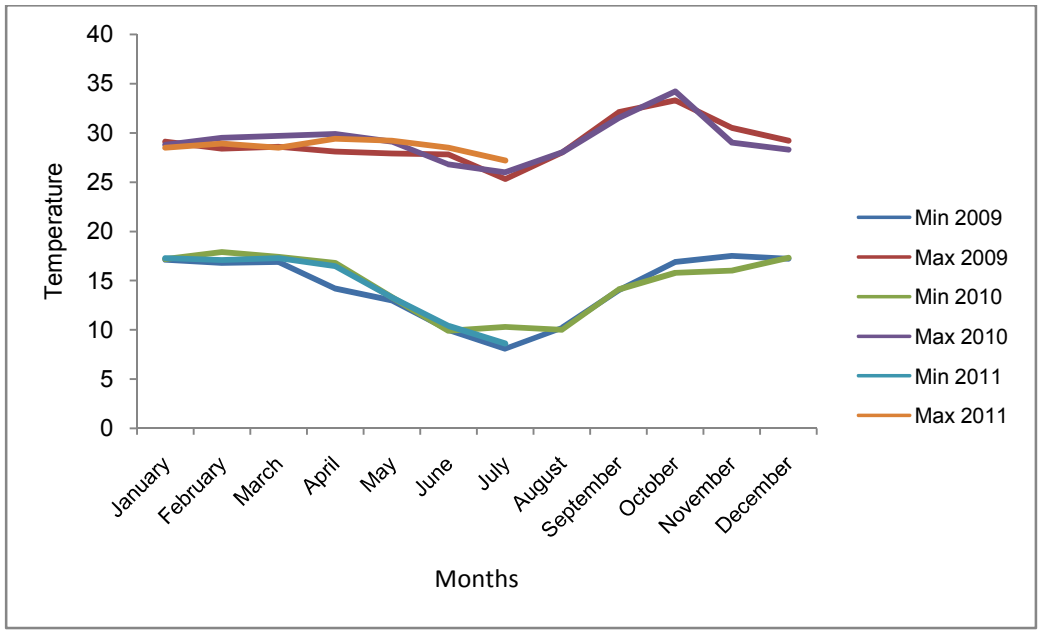

Figure 4.2: Temperature distribution from January 2009 to July 2011

Table 4.1: Climatic data and mineral composition of soil from Mansa research sites

	Mansa	
Site description	2009/10 season	2010/11 season
Altitude (masl)	1237	1199
Latitude (S)	11°13.797'	11°14.416'
Longitude (E)	28°56.727'	28°56.456'
Annual rainfall (mm)	905.7	1155.6
Mean max temperature (°C)	29.2	28.6
Mean min temperature (°C)	14.5	14.3
Soil description		
Soil classification	Acrisols	Acrisols
Soil type	Sandy loam	Sandy loam
pH	5.25	5.3
N%	0.02	0.04
Org C%	0.56	0.55
P (mg kg^{-1})	10.3	7.5
K (mg kg^{-1})	67	121
Ca (mg kg^{-1})	63.3	114.5
Mg (mg kg^{-1})	16.7	30

Source: Zambia Agriculture Research Institute (ZARI) soil advisory unit, Chilanga

4.2.2 Germplasm

The germplasm used in the research study was obtained from Mansa Research Station, Mount Makulu Gene Bank, and IITA (Table 4.2). The local and improved genotypes are widely grown in Zambia especially in Northern, Luapula, North-Western, Western and parts of Central

provinces. The genotypes were not randomly sampled, therefore they were considered as fixed effects.

Table 4.2: List of cassava cultivars evaluated for agronomic traits

Entry	Cultivar	Local landrace/Improved	Source
1	Nalumino	Local landrace	Mansa Research Station
2	Bangweulu	Local landrace	Mansa Research Station
3	Namuyongo	Local landrace	Mansa Research Station
4	Kabala	Local landrace	Mansa Research Station
5	Chikula	Local landrace	Mt. Makulu Gene bank
6	Mwakamoya	Local landrace	Mansa Research Station
7	Chila 7	Local landrace	Mansa Research Station
8	Manyopola	Local landrace	Mansa Research Station
9	Chila 11	Local landrace	Mansa Research Station
10	Chila	Improved	Mansa Research Station
11	Tanganyika	Improved	Mansa Research Station
12	Kampolombo	Improved	Mansa Research Station
13	Mweru	Improved	Mansa Research Station
14	TME2	Improved	IITA[1]
15	TMS190	Improved	IITA
16	TMS3001	Improved	IITA

[1]IITA (international Institute of Tropical Agriculture)

4.2.3 Experimental layout and management

The design used was a 4 x 4 α lattice with three replications. The experimental field was ploughed with a tractor and ridges made manually using hoes at spaces of 1 m between the ridges (height of ridges, 40 cm). Mature cassava cuttings from plants certified to be disease free measuring 30 cm in length were planted vertically, 1 m apart on the ridges. Each cultivar was planted on four ridges per plot. The length of each ridge was 11 m. Weeding was done manually and no fertilizer was applied. The two trials were grown with no supplementary irrigation.

Although Luapula province is considered to be a hot spot for CMD and has a favourable environment (rainfall and high temperatures), the whitefly population is small (Chapter 3). Augmented transmission of CMD to the test plants was necessary. Therefore, additional cassava plants exhibiting CMD and satellite symptoms were collected from farmers' fields within Luapula province (Chapter 3).

The collected diseased plants were planted in the screenhouse (Figures 4.3A and 4.3B). Water was applied regularly and monocrotophos was sprayed to control cassava green mites. Once the plants were ready to be used as source of virus inoculum, grafting was carried out 3 MAP on test plants in the field in 2009/10 and 2010/11 seasons. The scion (diseased plant) was cut to a

tapered shape and the root stock (test plant) cut to a wedge shape. With the scion and the root stock in direct contact and held in position, a plastic strip was firmly wrapped around the graft union (Figures 4.3C and 4.3D). In addition to grafting, five CMD susceptible local cultivars were planted in each of the first and fourth rows between the test plants.

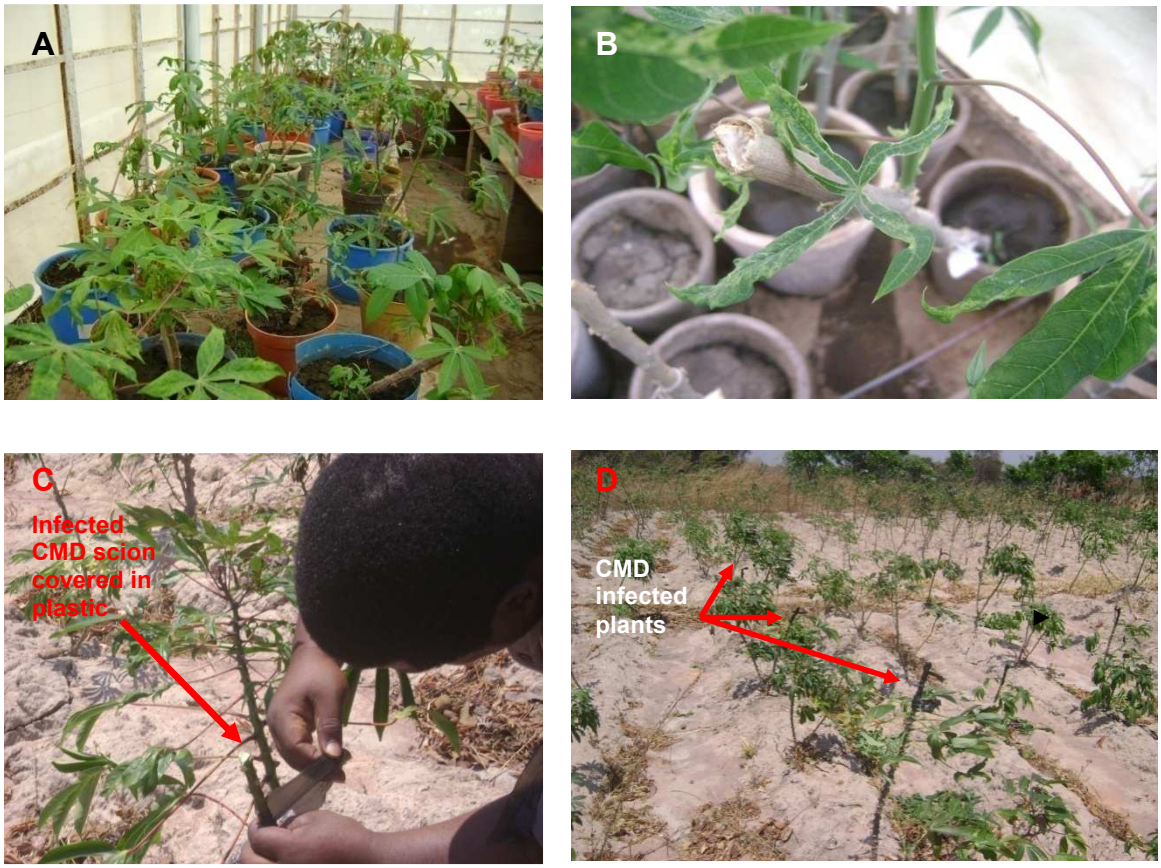

Figure 4.3: A) Plants with CMD and satellite symptoms grown in the greenhouse; B) Close up of a cassava plant showing filiform leaves characteristic of satellites; C) Grafted cassava with an infected scion fused onto a test plant rootstock; D) Grafted plants in the field

4.2.4 Data collection

Data on CMD severity was collected from the two middle ridges using the 1-5 scale (Hahn et al., 1980) where:

1= No symptoms observed

2= Mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only, with the remainder of the leaflets appearing green and healthy

3= Moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets

4= Severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size

5= Severe mosaic distortion of the entire leaf

Data was collected on a monthly basis for three months after grafting.

Fresh root yield and harvest index

At harvest time (7 MAP), the number and mass (kg) of all the storage roots per plant were counted and recorded. In addition, root size was classed as: size 3 (small sized roots); size 5 (medium sized roots); and size 7 (large sized roots). The fresh root mass and the total fresh biomass for each plant were determined. Harvest index (HI) was calculated as the ratio of fresh root yield to fresh total biomass.

Leaf retention

The cassava clones were visually evaluated for leaf retention at 6 MAP (Lenis et al., 2006). The trait was quantified on a scale of 1 to 5, where: 1= very poor retention; 2= less than average retention; 3= average leaf retention; 4= better than average retention; and 5= outstanding leaf retention.

Statistical analysis

Statistics for all the variables evaluated was carried out using Genstat Version 14 (Payne et al., 2011). Analysis of variance (ANOVA) was performed on the two season data. Bartlett's test was also performed on individual seasons. The genotypes were considered as fixed effects, while sites and replications were considered as random effects. Pearson correlation analysis using Genstat procedures was used to determine the relationships among the biotic and agronomic traits. The relative contribution of the different traits towards the genotype performance was estimated by principal component analysis (PCA). The procedure transforms a number of correlated variables into a smaller number of uncorrelated variables called principal components (PCs) (Jollife, 2002).

4.3 Results

The CMD severity scores ranged from 1 to 4 with a mean of 2.0 (Table 4.3). Harvest index was as low as 0.07 to as high as 0.79. Total biomass ranged from 0.1 to 4.1 kg plant^{-1}. Root size varied from 3 to 5 while fresh root yield ranged from 0.02 kg plant^{-1} to 2 kg plant^{-1}.

Table 4.3: Basic statistics of seven traits of 16 genotypes

Variable	Min	Max	Mean	SD	SE	Skew
CMD	1.0	4.0	1.99	0.89	0.029	0.22
HI	0.1	0.8	0.50	0.11	0.004	-0.29
TB	0.1	4.1	0.86	0.64	0.021	1.24
LR	1.0	4.0	2.35	0.73	0.024	0.18
PH	20	190	77.45	23.82	0.770	0.62
RS	3.0	5.0	3.66	0.94	0.030	0.74
FRY	0.02	2.0	0.44	0.34	0.012	1.39

CMD (cassava mosaic disease); HI (harvest index); TB (total biomass, kg plant^{-1}); LR (leaf retention); PH (plant height, cm); RS (root size); FRY (fresh root yield, kg plant^{-1})

The chi-square values for all the traits were not significant, indicating that the season error variances were homogeneous. Analysis of variance (ANOVA) revealed significant differences (P<0.001) among the genotypes across seasons for the biotic and agronomic related traits (Tables 4.4 and 4.5). The mean squares for the genotypes and genotype x season interactions were significant (P<0.001) for CMD, harvest index, total fresh biomass, leaf retention, plant height, root size and fresh root yield.

Table 4.4: Combined analysis of variance for cassava mosaic disease, harvest index and biomass

Source	DF	CMD	DF	HI	DF	TB
Genotype	15	25.91***	15	0.11***	15	4.24***
Season	1	7.66***	1	0.06*	1	34.23***
Genotype*season	15	0.76*	15	0.06***	15	1.71***
Error	917	0.37	855	0.01	900	0.29
Total	950		888		933	

CMD (cassava mosaic disease); HI (harvest index); TB (total fresh biomass, kg plant^{-1}); DF (degrees of freedom); **,***significant at P<0.05 and P<0.001 respectively

Table 4.5: Combined analysis of variance for leaf retention, plant height, root size, fresh root yield

Source	DF	LR	DF	PH	DF	RS	DF	FRY
Season	1	46.46***	1	4329.4***	1	0.58ns	1	11.98***
Genotype	15	5.83***	15	5897.5***	15	12.19***	15	1.37***
Genotype*season	15	6.58***	15	4160.8***	15	4.14***	15	0.59***
Error	915	0.3	919	375.2	905	0.62	855	0.08
Total	948		952		938		888	

LR (leaf retention); PH (plant height, cm); RS (root size); FRY (fresh root yield, kg plant^{-1}); DF (degrees of freedom); *, **, ***significant at P<0.05, P<0.01, P<0.001 respectively; ns (not significant)

4.3.1 Cassava mosaic disease symptom expression

The CMD symptoms appeared three to four weeks after grafting. Appearance of symptoms varied across the genotypes. Mild to severe symptoms of CMD were observed in both seasons on a scale of 1-5. Observations in the field showed that Bangweulu Kalaba, Chikula, Mwakamoya and Chila7 were the most susceptible to CMD. Bangweulu, Kalaba and Chikula are popular cultivars in Luapula and Northern Zambia. Genotypes TME2, TMS190 and TMS3001 expressed mild symptoms, while Chila, Kampolombo, Mweru, and Tanganyika expressed moderate symptoms (Figure 4.3). The ten most resistant genotypes were TME2, TMS190, TMS3001, Nalumino, Kampolombo, Mweru, Chila, Tanganyika, Manyopola and Chila11.

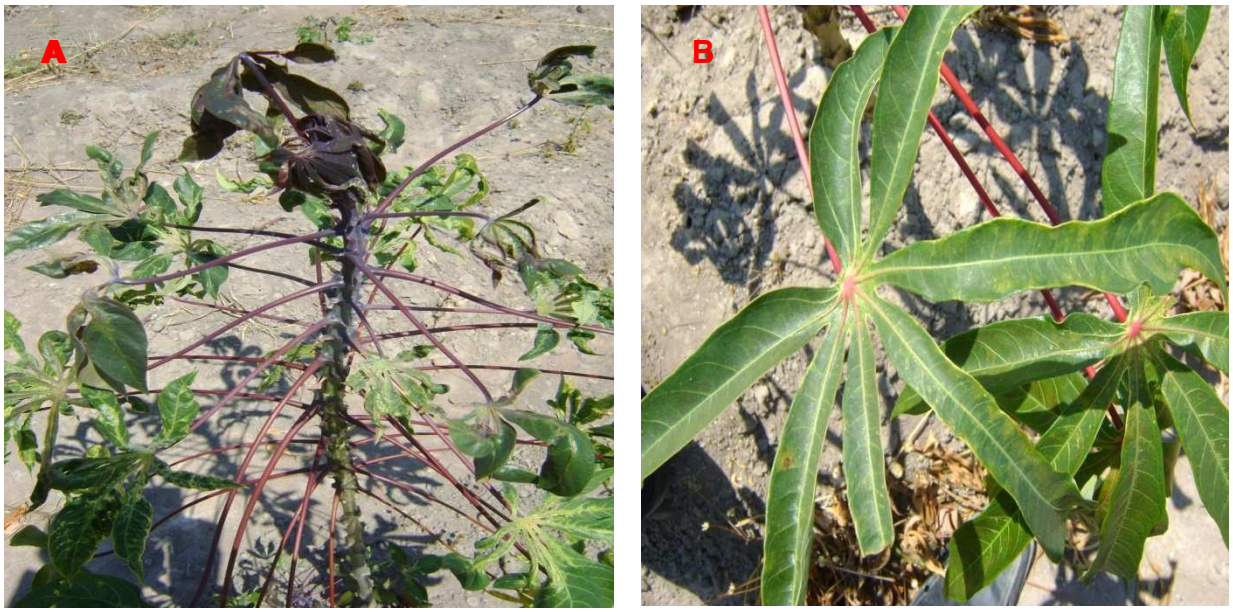

Figure 4.4: Symptoms of CMD on; A) Bangweulu; B) Tanganyika

4.3.2 Cassava mosaic disease and yield components

Reaction of the genotypes to CMD differed significantly ($P<0.001$) (Table 4.6). Harvest index varied significantly ($P<0.001$) among the genotypes for the two cropping seasons. Genotype Chila7 had the highest harvest index (0.55), while Mwakamoya had the lowest (0.36). Bangweulu, one of the popular cultivars had harvest index of 0.45. Leaf retention significantly ($P<0.001$) varied with the genotypes. Genotype TMS190 had the highest leaf retention, while Bangweulu, Chikula, Mwakamoya and Tanganyika had the lowest.

Table 4.6: The main effects of genotypes and cropping season on cassava mosaic disease severity scores, harvest index and leaf retention of 16 genotypes evaluated at Mansa Research Station, Zambia for two cropping seasons, 2009/10 – 2010/11

	Trait		
Genotype	CMD	HI	LR
Nalumino	1.5	0.49	2.6
Bangweulu	3.0	0.45	2.1
Namuyongo	2.2	0.49	2.2
Kalaba	2.6	0.49	2.5
Chikula	2.4	0.45	2.1
Mwakamoya	2.8	0.36	2.1
Chila7	2.7	0.55	2.7
Manyopola	1.8	0.48	2.0
Chila11	2.0	0.50	2.2
Chila	1.7	0.50	1.9
Tanganyika	1.7	0.52	2.1
Kampolombo	1.3	0.51	2.6
Mweru	1.3	0.48	2.4
TME2	1.1	0.44	2.8
TMS190	1.1	0.51	2.9
TMS3001	1.2	0.53	2.5
LSD (0.05)	0.28	0.05	0.28
CV%	11.0	3.0	4.8
F-probability	0.001	0.001	0.001
Season			
2009/10	2.1	0.49	2.1
2010/11	2.0	0.48	2.6
LSD (0.05)	0.11	0.01	0.09
CV%	5.8	2.7	5.0
F-probability	0.003	0.03	0.001

CMD (cassava mosaic disease, scale 1-5); HI (harvest index); LR (leaf retention); LSD (least significance difference); CV (coefficient of variation)

4.3.3 Agronomic traits and yield

Total fresh biomass varied significantly (P<0.001) among the genotypes (Table 4.7). Chila7 had the highest (1.62 kg plant^{-1}) and TMS3001 (0.60 kg plant^{-1}) the lowest total biomass (Table 4.9). There were significant differences (P<0.001) in plant heights for the genotypes. Chila was the tallest (92.6 cm) and TMS190 was the shortest (58.1 cm). Significant (P<0.001) differences were also observed in root size. Root size ranged from 3.0 (Chikula and Tanganyika) to 4.4 (Kampolombo) with a mean of 3.7. Fresh root yield was significantly (P<0.001) different. Fresh root yield ranged between 0.24 kg plant^{-1} (Mwakamoya) to 0.87 kg plant^{-1} (Chila 7).

Table 4.7: The main effects of genotypes and cropping season on total fresh biomass, plant height, root size and fresh root yield of 16 genotypes evaluated at Mansa Research Station, Zambia for two cropping seasons, 2009/10 – 2010/11

	Trait			
Genotype	TB	PH	RS	FRY
Nalumino	0.96	80.1	3.7	0.51
Bangweulu	0.65	83.5	3.8	0.37
Namuyongo	0.97	79.1	4.2	0.51
Kalaba	0.88	73.9	3.4	0.43
Chikula	0.65	81.9	3.0	0.29
Mwakamoya	0.62	84.1	3.3	0.24
Chila7	1.62	90.9	4.5	0.87
Manyopola	0.69	77.0	3.5	0.37
Chila11	1.22	79.6	4.0	0.64
Chila	0.91	92.6	3.2	0.48
Tanganyika	0.79	73.9	3.0	0.39
Kampolombo	0.64	60.8	4.4	0.37
Mweru	1.01	86.2	3.4	0.48
TME2	0.79	73.8	3.5	0.36
TMS190	0.80	58.1	4.0	0.42
TMS3001	0.60	63.9	3.7	0.32
LSD (0.05)	0.27	9.82	0.4	0.15
CV%	11.8	2.50	5.7	15.3
F-probability	0.001	0.001	0.001	0.001
Season				
2009/10	0.67	84.2	3.7	0.33
2010/11	1.05	70.7	3.6	0.55
LSD (0.05)	0.08	2.9	0.11	0.04
CV%	13.1	2.5	6.1	15.4
F-probability	0.001	0.001	0.339	0.001

TB (total fresh biomass, kg plant^{-1}); PH (plant height, cm); RS (root size, scale 1-5); FRY (fresh root yield, kg plant^{-1}); LSD (least significance difference); CV (coefficient of variation)

4.3.4 Phenotypic correlation of cassava mosaic disease and agronomic traits

Correlation analysis for the 16 genotypes is presented in Table 4.8. Total fresh biomass was significantly (P<0.001) and positively correlated to leaf retention, root size, CMD and fresh root yield. A positive and significant correlation was also observed between harvest index and fresh root yield, root size. Leaf retention was significantly (P<0.01) positively correlated to fresh root yield and root size. A weak and negative correlation was observed between leaf retention and CMD. Root size was significantly correlated to fresh root yield. Cassava mosaic disease was significantly positively correlated with plant height, total biomass and fresh root yield.

Table 4.8: Phenotypic correlation of 16 genotypes for biotic and agronomic traits 7 MAP at Mansa Research Station, Zambia

TB	-						
HI	0.02	-					
LR	0.21***	0.08ns	-				
PH	0.07	0.04ns	-0.15***	-			
RS	0.21***	0.13***	0.20***	-0.02ns	-		
FRY	0.93***	0.29***	0.23***	0.05ns	0.26***	-	
CMD	0.14***	-0.06ns	-0.09*	0.13***	0.11*	0.14***	-
	TB	HI	LR	PH	RS	FRY	CMD

TB (total fresh biomass, kg plant^{-1}); HI (harvest index); LR (leaf retention); PH (plant height, cm); RS (root size); FRY (fresh root yield, kg plant^{-1}); *,*** Significantly different from zero at the P<0.05, P<0.001 probability level (two-tailed test); ns (non significant)

4.3.5 Trait contribution to genotype performance

The first three principal components (PCs) accounted for 83.5% of the total variation (Table 4.9). The PC1 accounted for 44.0% total variation with an eigenvalue of 2.20. The major contributors for the first PC were total fresh biomass, root size and fresh root yield. Principal component two and PC3 accounted for 20.5% and 18.9% of variability respectively. The major factors for PC2 were total fresh biomass, harvest index, leaf retention and root size. For PC3 the major factors were harvest index, leaf retention and root size.

Table 4.9: Principal component coefficients of the various traits with loadings of the various yield and yield components

Trait	PC1	PC2	PC3	PC4
TB	0.604	-0.419	0.054	0.017
HI	0.213	0.698	0.624	-0.197
LR	0.292	0.312	-0.647	-0.631
RS	0.312	0.448	-0.375	0.749
FRY	0.639	-0.198	0.219	-0.028
Eigenvalue	2.20	1.03	0.94	0.80
% Variation	44.0	20.5	18.9	15.9

PC (principal component); TB (total fresh biomass, kg plant^{-1}); HI (harvest index); LR (leaf retention); RS (root size); FRY (fresh root yield, kg plant^{-1})

4.4 Discussion

Evaluation of the 16 cassava genotypes showed significant differences in reaction to cassava mosaic disease across the two planting seasons. None of the genotypes exhibited complete resistance to CMD; however, 56.3% of the genotypes were more tolerant. The development of CMD was variable in the two seasons, which resulted in different levels of severity scores. This contributed to the genotype x season interaction. The significant differences between the genotypes and seasons influenced the reaction of the genotypes to CMD infection. The observation agrees with studies by Akainwale et al. (2011).

The significant interaction between genotypes and seasons for all the variables indicates the need for evaluation for more than one season. Harvest index varied significantly, with most of the genotypes having values between 44 to 55% which is high according to CIAT classification (Kawano, 1990). Seven genotypes (Chila7, Chila11, Chila, Tanganyika, Kampolombo, TMS190 and TMS3001) had a harvest index of 50% or more which is very high according to the optimum value of 50 to 60% for cassava (Iglesias et al., 1994). Harvest index is a highly heritable trait (Kawano et al., 1998) and less affected by the environment. Genotypes with high harvest index are considered to be ideal for cultivar improvement.

Most of the genotypes had leaf retention from 1.9 to 2.9 on a scale of 1-5. Leaf retention is a trait in cassava which has been suggested as a possible means of increasing cassava productivity (Lenis et al., 2006). The moderate leaf retention among some of the genotypes evaluated indicates the need for improvement.

Significant variations were observed for fresh root yield indicating wide genetic differences. Yield has been reported as selection criteria for early bulking (Kawano et al., 1978). Several genotypes yielded more than 0.4 kg plant^{-1} (4 t ha^{-1}) at 7 MAP, though lower than what has been reported by Kamau (2006) in Kenya. Harvesting at this stage allowed identification of genotypes with early bulking characteristic. Root yield depends on several climatic and physical factors (rainfall, temperature, soil characteristics) in addition to genetic factors. The rainfall was higher in the 2010/11 season than in the 2009/10 season. This could have influenced the relatively better performance of root yield in the second season. Though cassava is a drought tolerant crop, during initial growth stages moisture content in the soil is important. The low fertility associated with the sandy loam soil, particularly nitrogen, could have also contributed to low yields (Table 4.1) in the first season. Cassava grows well in less fertile soil, however, a considerable amount of nitrogen is required (Howeler, 2002).

The major contributors to the performance of the traits as reflected by the PCs were storage root size, fresh root yield, biomass, leaf retention and harvest index. Leaf retention, harvest index and fresh root yield are among the traits used by the breeders for selecting cassava genotypes.

Significant positive correlation between leaf retention and fresh root yield was observed. Lenis et al. (2006) reported positive relationship between fresh root yield and leaf retention. Fresh root yield correlated positively with all the other traits except for plant height. Positive and weak correlation was observed between CMD and biomass, plant height, and fresh root yield. The genotypes used in the study were fixed and the resistant lines were low yielding, this could have contributed to the positive correlation between CMD and some of the traits. The positive correlation of CMD and yield may have been due to early harvesting of cassava (7 MAP) and low virus titre.The observations agree with Ssemakula and Dixon (2007) who reported significant positive correlation between CMD and yield. On the contrary, studies by Okechukwu and Dixon (2009) reported negative correlations between CMD and yield. In all cases were CMD had a weak positive correlation, it suggested that CMD had no effect on the particular traits. In some of the newly infected cassava planting materials the impact of CMD on yield is low compared to later stages of growth (Muimba and Phuti, 1987).

In conclusion, 16 genotypes were screened for CMD resistance using spreaders and the grafting method. The grafting ensured presence and uniform distribution of the viruses and

associated satellites in the test plants. The study showed that most of the local landraces grown are susceptible while others are tolerant to CMD. This suggests that the susceptible genotypes can be improved while maintaining farmer preferred characteristics (Chapter 2) through hybridisation. Cultivars that showed low or moderate CMD severity could be considered as sources of resistance. The low root yield suggests improvements needs to be made in the genotypes.

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CHAPTER 5: EVALUATION OF F_1 CASSAVA PROGENY PERFORMANCE FOR AGRONOMIC TRAITS

Abstract

The increasing importance of cassava (*Manihot esculenta* Crantz) in Zambia has necessitated the development of improved cultivars. Some traits such as tuberous root yield, dry matter content and leaf retention are recognised as important characters. The study was carried out (i) to develop F_1 segregating populations and (ii) to evaluate the performance of F_1 progenies for agronomic traits. A North Carolina II mating design with nine parents was used to develop F_1 clones. The seeds generated were raised in a growth room at constant temperature (36^{o}C) and humidity (80%) and later transplanted to the field using a row column design in February 2010. Data were taken on leaf retention, fresh root yield, dry matter content, harvest index, plant height and branching height. The trial was harvested 11 months after planting (MAP). The results showed significant ($P<0.001$) differences among the F_1 crosses. Wide significant ($P<0.001$) variation in plant height, leaf retention, root dry matter content, harvest index, branching height, and fresh root yield were observed. Significant ($P<0.001$) correlations were observed between plant height and branch height, root dry matter content, and fresh root yield. Significant ($P<0.001$) positive association was also observed between fresh tuberous root yield and root dry matter content. No correlation was observed between harvest index and fresh root yield. The variability observed within the F_1 cassava progeny for the traits evaluated reflects the potential that can be explored to improve cassava.

5.1 Introduction

Genetic improvement of cassava starts with the collection and evaluation of germplasm followed by creation of new recombinant genotypes from selected elite clones. Most breeding programmes obtain seed through crossing improved cultivars or local landraces in order to creat new genetic variation. At the Centro Internatiocional de Agricultura Tropical (CIAT) and the International Institute for Tropical Agriculture (IITA), the creation of new variants is done through hybridisation using hand-pollination or controlled open-pollination technique. Following these techniques cassava clones with desirable traits can be identified, evaluated and subsequently released to the farmers for possible adoption. The recombinants produced through hand pollination result in full-sib families whereas those produced through open pollination results in half-sib families. Traditionally, creation of genetic variation has been through open pollinated seed. However, recent breeding research has moved towards using hand pollination at low altitudes or high latitudes experiencing high temperatures (Kamau et al., 2010; Mtunda, 2010; Munga, 2008; Zacarias, 2008). Depending on the availability of resources i.e. irrigation, and favourable environmental conditions (for example high temperatures), botanical seed obtained using different crossing schemes can either be planted in the field or greenhouse (Ceballos et al., 2004).

Once plants are established from botanical seed, selection can be carried out. Because of low correlations between the performance in the first generation (seedling stage) and performance in clonal trials, the early seedling stage selections are generally based on highly heritable traits such as plant type, branching habits and reaction to certain diseases (Hahn et al., 1980; Iglesias et al., 1994). Other selection criteria used for selection are stay green, harvest index, reaction to CMD and dry matter content. Harvest index is a useful tool in early stages of selection (Kawano, 2003; Kawano et al., 1998); however, it is also appropriate to pay attention to root yield (Hershey, 1987). In unselected populations with large genetic variation, harvest index is more important than biomass in a genetically diverse population (Kawano, 2003). Plants that branch when they are above 1 m in height are desirable in intercropping farming systems (Hahn et al., 1979; Kawano et al., 1978). More importantly, clones that branch above 1 m are associated with high yield and also promote intercropping, thus leading to maximum food yield per unit land area (Jennings and Iglesias, 2002).

To evaluate the genetic inheritance of various traits requires developing a F_1 population using one of several mating schemes. In cassava various mating designs have been used, among

them the diallel and NCII (North Carolina II) design. North Carolina II has previously been used in developing early bulking cassava cultivars (Kamau, 2006). During the seedling stage of selection in most of cassava breeding programmes, a large number of genotypes are discarded when subjected to biotic stresses such as cassava mosaic disease (CMD). In so doing, useful genetic variability is lost. Furthermore, severely infected plants produce less planting materials (IITA, 1987). To ensure full representation of each family and to maximise the number of cuttings from each plant for the clonal stage trial, the seedling stage trial was conducted in a CMD free area.

The objectives were:

i) To develop F_1 populations
ii) To evaluate the performance of F_1 progeny for agronomic traits

5.2 Materials and methods

5.2.1 Site description

The crossing block was established in July 2008 and evaluation of the F_1 cassava progeny was carried out in the 2009 season at Mount Makulu Central Research Station. The site is located in region II (Appendix 1). Region II is characterised by high rainfall, 800-1000 mm. During the study period the site experienced heavy rains in November and less in December, January and February (Appendix 2). The site is characterised by hot summers (September to November; Appendix 3), and cold winters (May to July). During the winter months minimum temperatures can be as low as 5^{o}C (Figure 5.1). The soils at Mount Makulu Research Station are loamy soils (Table 5.1).

Table 5.1: Climatic data and soil type for crossing block and seedling trial sites, Mount Makulu Research Station

Site description	Crossing block	Seedling trial
	2008	2010
Altitude (masl)	1235	1238
Latitude (S)	15°32.921'	15°32.851
Longitude (E)	28°15.089'	28°15.060
Soil description		
Soil classification	Acrisols	Acrisols
Soil type	Clay loam	Clay loam
pH	6.61	6.74
N%	0.07	0.06
Org C%	2.08	1.95
P (mg kg^{-1})	5	18
K (mg kg^{-1})	162	150
Ca (mg kg^{-1})	2220	3911
Mg (mg kg^{-1})	311	240

Source: Zambia Agriculture Research Institute (ZARI) soil advisory unit, Chilanga

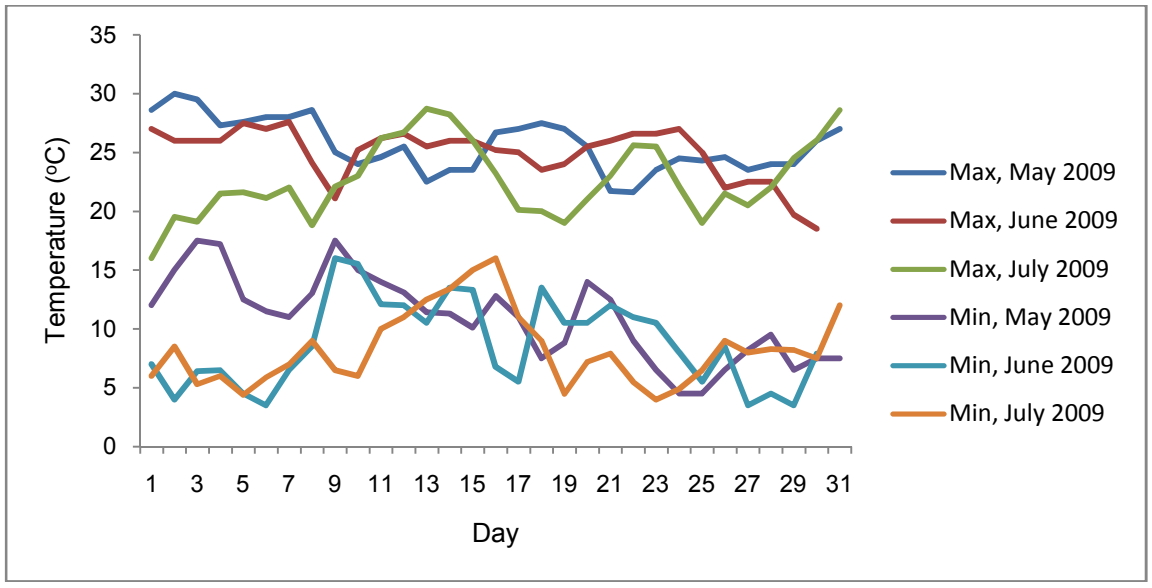

Figure 5.1: Daily maximum and minimum temperatures between May and July 2009, Mount Makulu

5.2.2 Crossing block

A North Carolina mating design (Comostock and Robinson, 1952) was used to produce segregating populations for the full-sib crosses. The cassava parents used for the study were made up of 10 cultivars (Table 5.2). The selection criteria for the cultivars was based on the information obtained from the participatory rural appraisal (Chapter 2), which included resistance to pests and diseases, earliness and yield. The parents were divided into two sets (male and female) and planted in July 2008 (Table 5.3). The female set of four parents were local cultivars, susceptible to CMD. The male set of six parents were both local cultivars and introductions from IITA, resistant to CMD. Cuttings from the parents were planted vertically with two-third of the stems (10-15 cm) buried in the soil. Vertical planting results in rapid establishment and less risk from lodging (Leihner, 2002). The stakes were planted at 2 m between plants and 2 m between rows (Appendix 4). The wider spacing was meant to provide enough room for the plants to branch well and allow for movement during pollination.

Table 5.2: Source and characteristics of parent genotypes used in the North Carolina II mating design

Cultivar	Source and description
Nalumino	Local cultivar, bitter, high dry matter, moderately tolerant to CMD
Chila	Local improved cultivar, high dry matter, sweet, good cooking quality
Kampolombo	Local improved cultivar, sweet, high yield
Bangweulu	Local cultivar, bitter, high yield
Mweru	Local improved cultivar, sweet, high yield, moderately tolerant to CMD
Chikula	Local cultivar, sweet, high yielding
TMS3001	Clone from IITA, resistant to CMD, sweet
TMS190	Clone from IITA, resistant to CMD, sweet
TME 2	Clone from IITA, resistant to both CMD and green mite, sweet
Tanganyika	Local cultivar, resistant to CMD

CMD= cassava mosaic disease, IITA= International Institute for Tropical Agriculture
TMS=Tropical Manihot Species, TME= Tropical Manihot evaluation

Table 5.3: Cross combinations of the 4 x 6 North Carolina II mating design

	Parent (male)					
Parent (female)	TMS190	TMS3001	Nalumino	TME2	Mweru	Tanganyika[1]
Chikula	X	X	X	X	X	X
Bangweulu	X	X	X	X	X	X
Chila	X	X	X	X	X	X
Kampolombo	X	X	X	X	X	X

[1]Very few flowers were produced and no seed was obtained

5.2.3 Hand pollination

Pollination was carried out as described by Kawano (1980) over a period of four months (December 2008 to March 2009). Female flowers earmarked for pollination were identified in the morning between 11h00 and 12h00 and bagged prior to pollination using mosquito netting. The male flowers identified were collected between 10h00 and 11h00 and stored at room temperature on pieces of paper (Figure 5.2).

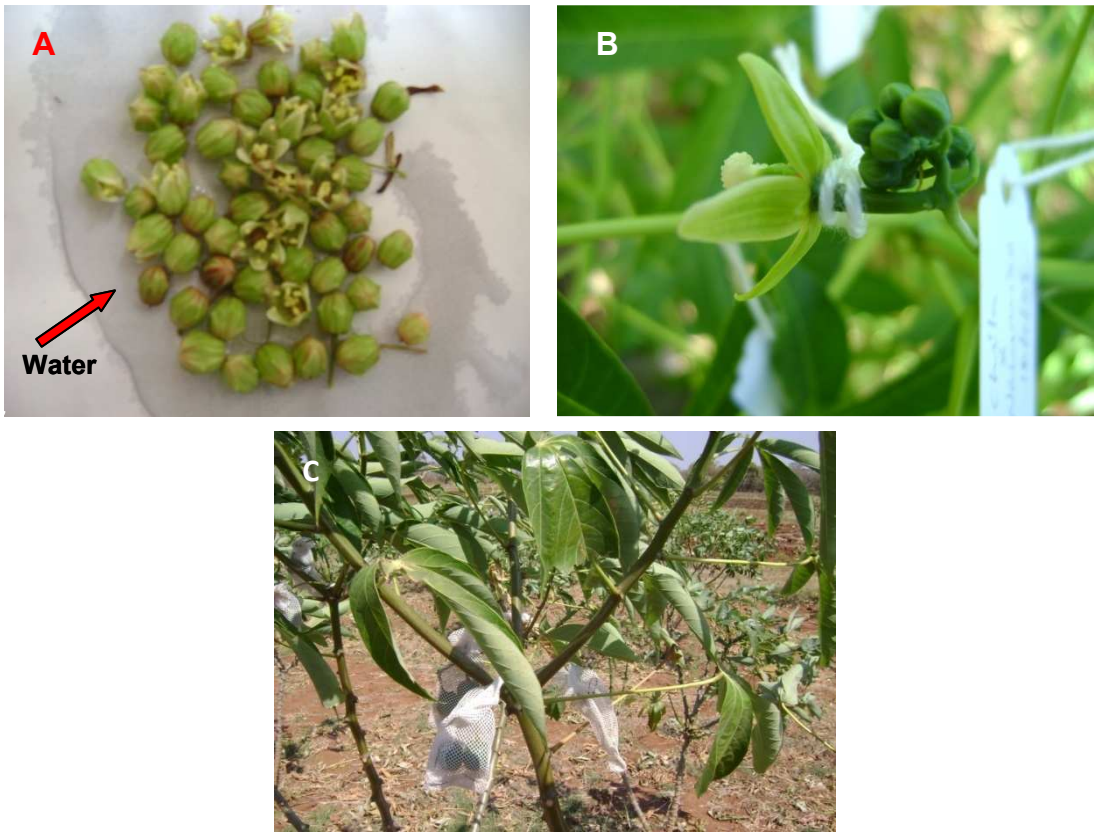

Figure 5. 2: A) Cassava male flowers collected from the field and incubated at room temperature. Note placement of flowers on a film of water to aid in opening of the flower buds; B) Tagged pollinated female flower; C) Mature fruits covered with mosquito netting bag

One male flower was used to pollinate two female flowers. To improve efficiency, pollination was done between 13h00 and 17h00 (Kawano, 1980). Hand pollination was carried out by rubbing the stigma of the female flower with pollen from male flowers collected in the morning. After pollination, each flower was tagged and labelled. The pollinated flowers were then left to grow freely for three weeks and later covered by mosquito net. The unpollinated female and male flowers were removed to minimize nutrient competition with hand pollinated flowers. A thin cotton thread was used to rub the pollen on to the female flower of cultivars that had shown low receptivity.

The pollinated flowers were monitored soon after crossing (three days later) to check for any abortions. Further monitoring on a weekly basis was done to check for any presence of insect pests or sign of diseases on the young developing fruits.

5.2.4 Cassava seed propagation

The botanical seeds produced from the crosses were planted at the end of December 2009, approximately four months after storage at room temperature. Planting was done according to family in plastic bags of approximately 11 cm diameter and 20 cm depth (Figure 5.3). To determine seed viability, seeds were immersed in water and those that floated were discarded. Pine-bark was used as a growth media and nutrient status and pH of the growing medium was measured before planting (Table 5.4). Water was applied when necessary.

Due to the low night temperatures in the field, a controlled environment growth room was used for germination of the seed. The room was kept dark and temperature was held constant at approximately 36^{o}C using a thermal heater (Einhell, NHK 1500 D). The room temperature was monitored on a daily basis using a minimum and maximum thermometer (G.H. Zeal, England). Air circulation between the plastic bags, was maintained by leaving 15 cm spaces between the bags. Relative humidity was maintained by placing water in open containers on the floor.

Figure 5. 3: A) Plastic bags filled with pine bark in the germination room; and B) Seedlings placed on an open area and grouped according to family at Mount Makulu Research Station, December/January, 2010

After germination the seeds were transferred in their bags to an open field where they were monitored regularly. An insecticide (Orizon, active ingredient: Acetamiprid) was applied to all the seedlings three weeks after germination to prevent sap-sucking insects from feeding on the plants.

Table 5.4: Mineral composition of pine bark (growth media)

Element	Unit	Value
pH	-	5.09
N	%	0.27
Organic content	%	12.62
P	mg kg^{-1}	1125
Mg	mg kg^{-1}	3000
Ca	mg kg^{-1}	8500
K	mg kg^{-1}	975

Source: Zambia Agriculture Research Institute (ZARI) soil advisory unit, Chilanga

5.2.5 Seedling trial

When the seedlings were 20-28 cm high, watering was reduced in order to harden them off in preparation for transplanting. Transplanting was done during the first week of February 2010. Tanganyika did not produce enough flowers and only 20 F_1 crosses could therefore be made instead of 24 crosses. The 20 F_1 crosses were randomly allocated to the plots using a 4 x 5 α lattice design. Each cross comprising 56 progenies was equally divided across the two replications. Therefore, each plot within a replication consisted of 28 plants spaced at 1.5 m within rows and 2 m between the rows.

Systemic insecticide (Orizon; active ingredient, Acetamiprid) was sprayed regularly to ensure that the cassava plants were free from insects. Basal dressing fertiliser (N: 10, P: 20, K: 10) was applied to the plants at 25 g per plant. The plants were irrigated where necessary and weeding was done regularly.

5.2.6 Data collection

Agronomic traits for the seedling trial were recorded on individual plant basis in December 2010, 11 MAP. However, leaf retention was scored 6 MAP. The plants were harvested and bulked per genotype for various measurements. The number and mass (kg) of all the storage roots (fresh root yield) per plant was recorded. Root dry mass content (RDMC) was determined using a specific gravity procedure (Okogbenin et al., 2003). Approximately 1-3 kg roots were weighed in the air using a hanging balance and then submerged into water and weighed again. The formula used to determine RDMC was

$$\text{RDMC}\,(\%) = \left(\frac{\text{MA}}{\text{MA} - \text{MW}} \text{ X } 158.3 \right) - 142$$

Where, MA is mass in air and MW is mass in water

Storage roots were classed: size 3 (small sized roots); size 5 (medium sized roots); and size 7 (large sized roots). Harvest index was determined as storage root mass expressed as a proportion of total plant mass. Leaf retention was assessed on a scale of 1-5 (Lenis et al., 2006), where: 1 is very poor retention; 2 is less than average retention; 3 is average leaf retention; 4 is better than average retention; and 5 is outstanding leaf retention. Plant height and branching height were measured for each plant.

5.2.7 Data analysis

The residual maximum likelihood (REML) in Genstat version 14 (Payne et al., 2011) was used to analyse the data. The families and progenies were considered as fixed effects, while replications were treated as random effects. Means were separated by least significant difference (LSD) and phenotypic correlations were determined using Genstat.

5.3 Results

5.3.1 Seed set and seed germination

For each cross the target was to produce 130 seeds in order to compensate for potential losses during seed germination and transplanting. The seeds started germinating after nine days. A number of the germinated seeds expressed slow expansion of the growth point and as a result plant development was retarded (Figure 5.4).

Figure 5. 4: A) Seedling with reduced growth point two weeks after germination; B) Seedling with normal vigorous growth point two weeks after germination

The highest (201) number of seeds were obtained from Chila x Nulumino (Table 5.5) while the lowest (82) was from Bangweulu x TME2 (Table 5.5). The highest (84.6%) seed germination was from Chila x Nulumino and the lowest (57.0%) was from Kampolombo x Nalumino.

Table 5.5: Seed set and seed germination at Mt Makulu Research Station, January 2010

No	Cross	Seed set[1]	Number of seeds planted cross^{-1}	Germination (%)
1	Chikula x TMS190	130	93	71.0
2	Bangweulu x TMS190	120	84	70.0
3	Chila x TMS190	134	102	76.0
4	Kampolombo x TMS190	90	70	77.8
5	Chikula x TMS30001	140	92	65.0
6	Bangweulu x TMS30001	150	97	64.0
7	Chila x TMS30001	140	90	64.0
8	Kampolombo x TMS30001	90	65	72.0
9	Chikula x Nalumino	150	93	62.0
10	Bangweulu x Nalumino	190	115	60.5
11	Chila x Nalumino	201	170	84.6
12	Kampolombo x Nalumino	140	80	57.0
13	Chikula x TME2	160	95	59.3
14	Bangweulu x TME2	82	60	73.2
15	Chila x TME2	170	103	60.5
16	Kampolombo x TME2	120	92	76.6
17	Chikula x Mweru	100	80	80.0
18	Bangweulu x Mweru	91	70	76.9
19	Chila x Mweru	130	103	79.0
20	Kampolombo x Mweru	103	83	80.5
Mean		131.6	92.3	70.5
Minimum		82	60	57.0
Maximum		201	170	84.6
Standard deviation		33.3	22.7	8.3
Standard error of mean		7.4	5.1	1.9
Skewness		0.35	1.89	-0.07

[1]Based on total number of seeds harvested per cross

5.3.2 Yield and agronomic components of individual progeny

The yield and agronomic related traits of 800 individual progeny were evaluated at Mt. Makulu Research Station. Significant (P<0.001) differences were observed among the crosses for the agronomic traits (leaf retention, fresh root yield, root dry matter content, harvest index, plant height and branching height). Leaf retention ranged from 1 to 5. Fresh root yield was as low as 0.1 to as high as 5 kg plant^{-1}. Root dry matter content ranged from 10.3 to 69.6 % with an overall mean of 39.4 % (Table 5.6).

Table 5.6: Residual maximum likelihood Wald's F statistic, minimum and maximum and mean values for yield and yield components of 800 individual progenies evaluated at the seedling trial stage, Mount Makulu, 2010

	Cross					
Variable	DF	F statistic	Min	Max	Mean	SEM
LR	19	4.94***	1.0	5.0	3.3	0.03
FRY	19	10.25***	0.1	5.0	1.6	0.04
RDMC	19	5.35***	10.3	69.6	39.4	0.68
HI	19	5.73***	0.01	0.8	0.3	0.01
PH	19	12.94***	40.0	300.0	160.2	1.37
BH	19	11.69***	5.0	205.0	81.5	1.10

LR (leaf retention, scale: 1 – very poor and 5 – outstanding retention); FRY (fresh root yield, kg plant^{-1}); RDMC (root dry matter content %); HI (harvest index); PH (plant height, cm); BH (branching height, cm); Min (minimum); Max (maximum); SEM (standard error of the mean); ***, significant at 0.001

5.3.3 Leaf retention

Significant (P<0.001) variation were observed for leaf retention (Table 5.7). Observations in the field showed most plants retaining leaves. Leaf retention was as high as 3.8 (Bangweulu x TMS190) to as low as 2.9 (Chikula x TMS 3001, Chila x Nalumino, Chikula x Mweru and Chila x Mweru).

Table 5.7: Minimum, maximum and means for leaf retention evaluated in the seedling trial, Mount Makulu, 2010

No	Cross	Min	Max	Mean	SD	SEM	Skew
1	Chikula x TMS190	2	5	3.4	0.7	0.11	-0.29
2	Bangweulu x TMS190	3	5	3.8	0.7	0.10	0.21
3	Chila x TMS190	2	4	3.5	0.6	0.09	-0.53
4	Kampolombo x TMS190	2	4	3.3	0.6	0.09	-0.11
5	Chikula x TMS3001	1	4	2.9	0.7	0.11	-1.21
6	Bangweulu x TMS3001	3	4	3.7	0.5	0.07	-1.01
7	Chila x TMS3001	1	4	3.2	0.7	0.11	-0.70
8	Kampolombo x TMS3001	2	4	3.4	0.7	0.11	-0.58
9	Chikula x Nalumino	2	4	3.3	0.7	0.08	-0.03
10	Bangweulu x Nalumino	2	5	3.3	0.7	0.11	-0.17
11	Chila x Nalumino	1	5	2.9	0.9	0.13	0.19
12	Kampolombo x Nalumino	1	4	3.1	0.8	0.13	-0.79
13	Chikula x TME2	1	4	3.1	0.8	0.12	-0.44
14	Bangweulu x TME2	1	4	3.2	0.9	0.14	-0.85
15	Chila x TME2	1	5	3.3	0.8	0.12	-0.51
16	Kampolombo x TME2	1	4	3.3	0.8	0.12	-0.96
17	Chikula x Mweru	1	4	2.9	0.9	0.13	-0.59
18	Bangweulu x Mweru	2	4	3.2	0.5	0.08	0.44
19	Chila x Mweru	1	4	2.9	0.9	0.18	-0.62
20	Kampolombo x Mweru	3	4	3.4	0.5	0.08	0.25
Overall mean	3.24						
SEM	0.11						
LSD (0.05)	0.32						
F probability	0.001						

Min (minimum); Max (maximum); SD (standard deviation); SEM (standard error of the mean); Skew (skewness); leaf retention, scale: 1 – very poor and 5 – outstanding retention; Skew (skewness); LSD (least significant difference)

5.3.4 Fresh root yield

The results for the fresh root yield are presented in Table 5.8. Significant (P<0.001). differences for fresh root yield were observed among the F_1 crosses. Bangweulu x Nalumino out-performed all the other crosses with a mean fresh root yield of 2.5 kg plant^{-1}(Table 5.8). This was followed by Chila x TME2 with a yield of 2.1 kg plant^{-1}. Nalumino and Bangweulu are local landraces grown in many areas of Zambia.

Table 5.8: Minimum, maximum and means for fresh root yield (kg plant^{-1}) evaluated at seedling evaluation trial, Mount Makulu, 2010

No	Cross	Min	Max	Mean	SD	SEM	Skew
1	Chikula x TMS190	0.5	3.5	1.7	0.80	0.12	0.48
2	Bangweulu x TMS190	0.3	2.5	0.9	0.60	0.12	0.67
3	Chila x TMS190	0.1	2.0	0.7	0.50	0.09	1.10
4	Kampolombo x TMS190	0.2	2.5	1.1	0.70	0.12	0.46
5	Chikula x TMS3001	0.5	4.0	1.7	0.80	0.12	0.65
6	Bangweulu x TMS3001	0.2	3.5	1.7	0.90	0.14	0.09
7	Chila x TMS3001	0.1	5.0	1.8	1.00	0.17	0.83
8	Kampolombo x TMS3001	0.3	4.5	1.9	1.00	0.17	0.52
9	Chikula x Nalumino	0.2	4.0	2.0	0.90	0.14	0.15
10	Bangweulu x Nalumino	0.5	5.0	2.5	0.90	0.15	0.56
11	Chila x Nalumino	0.2	3.5	1.9	0.90	0.15	-0.09
12	Kampolombo x Nalumino	0.2	4.0	1.4	1.00	0.17	1.02
13	Chikula x TME2	0.2	3.0	1.4	0.60	0.13	0.42
14	Bangweulu x TME2	0.1	3.0	1.4	0.90	0.13	0.29
15	Chila x TME2	0.3	4.0	2.1	1.00	0.16	0.28
16	Kampolombo x TME2	0.2	3.0	1.4	0.80	0.12	0.23
17	Chikula x Mweru	0.2	3.5	1.3	0.90	0.14	0.49
18	Bangweulu x Mweru	0.3	3.5	1.6	0.70	0.12	0.59
19	Chila x Mweru	0.2	3.0	1.4	0.80	0.13	0.42
20	Kampolombo x Mweru	0.2	4.5	1.2	0.90	0.15	1.60
Overall mean	1.52						
SEM	0.14						
LSD (0.05)	0.38						
F-probability	0.001						

Min (minimum); Max (maximum); SD (standard deviation); SEM (standard error of the mean); Skew (skewness); LSD (least significant difference)

1.3.5 Root dry matter content and harvest index

The results for the root dry matter content (RDMC) are presented in Table 5.9. There were significant (P<0.001) variations for both root dry matter content and harvest index. Root dry matter content was highest (45.6%) in Bangweulu x TME2 and lowest in Chila x TMS190 (29.9%). For the harvest index, Kampolombo x TMS 190 and Bangweulu x Mweru recorded the highest (0.5) and Chila x TMS 3001 and Chikula x TME2 had the lowest (0.19).

Table 5.9: Cross means of root dry matter content and harvest index evaluated at seedling trial, Mount Makulu, 2010,

No	Cross	RDMC (%)	HI
1	Chikula x TMS190	43.8	0.3
2	Bangweulu x TMS190	34.9	0.4
3	Chila x TMS190	29.9	0.4
4	Kampolombo x TMS190	34.9	0.5
5	Chikula x TMS3001	44.4	0.3
6	Bangweulu x TMS3001	34.6	0.3
7	Chila x TMS3001	39.2	0.2
8	Kampolombo x TMS3001	38.8	0.3
9	Chikula x Nalumino	44.7	0.3
10	Bangweulu x Nalumino	45.6	0.3
11	Chila x Nalumino	37.5	0.4
12	Kampolombo x Nalumino	40.8	0.3
13	Chikula x TME2	39.6	0.2
14	Bangweulu x TME2	45.6	0.3
15	Chila x TME2	41.1	0.4
16	Kampolombo x TME2	36.2	0.4
17	Chikula x Mweru	36.3	0.3
18	Bangweulu x Mweru	44.5	0.5
19	Chila x Mweru	38.8	0.2
20	Kampolombo x Mweru	32.8	0.3
Overall mean		39.07	0.33
SEM		2.83	0.03
LSD (0.05)		7.86	0.09
F-probability		0.001	0.001

RDMC (root dry matter); HI (harvest index); SEM (standard error of the mean); LSD (least significant difference)

5.3.6 Plant height

Plant height of the F_1 progeny varied significantly ($P<0.001$) (Table 5.10). The highest (202.8 cm) mean plant height was recorded by Bangweulu x TMS3001 and the lowest (125.3 cm) by Bangweulu x TMS190.

Table 5.10: Minimum, maximum and means for plant height in cm evaluated at seedling evaluation trial, Mount Makulu, 2010

No	Cross	Min	Max	Mean	SD	SEM	Skew
1	Chikula x TMS190	60	186	132.1	31.4	4.96	-0.32
2	Bangweulu x TMS190	60	169	125.3	28.8	4.55	-0.52
3	Chila x TMS190	52	195	142.6	37.6	5.94	-1.08
4	Kampolombo x TMS190	66	205	152.2	34.7	5.49	-1.01
5	Chikula x TMS3001	60	229	167.7	31.7	5.00	-0.92
6	Bangweulu x TMS3001	119	300	202.8	45.7	7.22	0.24
7	Chila x TMS3001	80	233	156.5	38.6	6.26	-0.11
8	Kampolombo x TMS3001	94	182	141.8	20.9	3.30	-0.50
9	Chikula x Nalumino	120	224	170.8	22.3	3.52	-0.10
10	Bangweulu x Nalumino	120	240	179.6	28.8	4.55	-0.17
11	Chila x Nalumino	67	240	159.0	33.0	5.21	-0.32
12	Kampolombo x Nalumino	107	203	167.9	27.0	4.26	-0.59
13	Chikula x TME2	89	206	149.4	29.9	4.72	-0.11
14	Bangweulu x TME2	71	202	166.8	30.4	4.81	-1.11
15	Chila x TME2	70	205	158.6	32.6	5.14	-0.85
16	Kampolombo x TME2	60	207	149.0	34.7	5.47	-1.04
17	Chikula x Mweru	107	217	172.8	28.1	4.43	-0.39
18	Bangweulu x Mweru	105	260	198.6	37.6	5.94	-0.92
19	Chila x Mweru	56	260	163.7	48.9	7.72	-0.37
20	Kampolombo x Mweru	40	209	146.1	41.8	6.60	-0.94
	Overall mean	160.2					
	SEM	5.36					
	LSD (0.05)	14.9					
	F-probability	0.001					

Min (minimum); Max (maximum); SD (standard deviation); SEM (standard error of the mean); Skew (skewness); SEM (standard error of the mean); LSD (least significant difference)

5.3.7 Branch height

Significant (P<0.001) differences were observed among the F_1 crosses (Table 5.11). The highest mean branch height was from cross Bangweulu x TMS30001 (110.5 cm) followed by Bangweulu x Nalumino (110.5 cm) and Bangweulu x Mweru (105.1 cm). The lowest (52.9 cm) mean branch height was recorded by Chikula x TMS190.

Table 5.11: Minimum, maximum and means for branching height (cm) evaluated at seedling evaluation trial, Mount Makulu, 2010

No	Cross	Min	Max	Mean	SD	SEM	Skew
1	Chikula x TMS190	8	118	52.9	28.63	4.52	0.16
2	Bangweulu x TMS190	40	110	67.6	16.65	2.63	0.57
3	Chila x TMS190	30	180	90.7	33.97	5.37	0.26
4	Kampolombo x TMS190	30	122	82.1	20.98	3.31	-0.43
5	Chikula x TMS3001	37	130	81.1	24.04	3.80	-0.15
6	Bangweulu x TMS3001	40	205	110.5	37.86	5.98	0.52
7	Chila x TMS3001	16	146	79.6	34.09	5.53	-0.10
8	Kampolombo x TMS3001	40	110	69.2	15.36	2.42	0.22
9	Chikula x Nalumino	10	150	69.8	35.57	5.62	-0.14
10	Bangweulu x Nalumino	50	160	110.4	26.35	4.16	-0.30
11	Chila x Nalumino	5	160	63.2	42.18	6.66	0.16
12	Kampolombo x Nalumino	33	110	81.1	20.97	3.31	-0.66
13	Chikula x TME2	30	120	73.5	22.54	3.56	0.04
14	Bangweulu x TME2	34	94	76.2	13.92	2.20	-1.18
15	Chila x TME2	10	120	81.0	25.27	3.99	-0.78
16	Kampolombo x TME2	33	120	78.4	20.99	3.31	-0.11
17	Chikula x Mweru	24	140	85.8	27.19	4.29	-0.14
18	Bangweulu x Mweru	37	156	105.1	30.39	4.80	-0.67
19	Chila x Mweru	25	140	94.3	31.64	5.00	-0.55
20	Kampolombo x Mweru	30	150	77.8	27.58	4.36	0.77
Overall mean	81.5						
SEM	4.37						
LSD (0.05)	12.2						
F-probability	0.001						

Min (minimum); Max (maximum); SD (standard deviation); SEM (standard error of the mean); Skew (skewness); SEM (standard error of the mean); LSD (least significant difference)

5.3.8 Agronomic related trait correlations

A positive, significant (P<0.001) correlation was observed between plant height and branch height (Table 5.13). Similarly a positive significant (P<0.001) correlation was observed between fresh root yield and root dry matter content. Plant height and root dry matter content were weakly and positively correlated. The fresh root yield was also significantly (P<0.001) and positively correlated with plant height. There was weak correlation between fresh root yield and harvest index. Similarly, there was weak correlation was observed between harvest index and root dry matter content.

Table 5.12: Correlation coefficients for agronomic related traits on 800 genotype of a seedling evaluation trial, 2011

BH	-					
RDMC	-0.048	-				
HI	0.038	-0.012	-			
LR	-0.037	-0.0013	0.0009	-		
FRY	0.0059	0.37***	0.051	0.023	-	
PH	0.21***	0.086*	0.0078	-0.058	0.16***	-
	BH	RDMC	HI	LR	FRY	PH

BH (branching height, cm); RDMC (root dry matter content); HI (harvest index); LR (leaf retention); FRY (fresh root yield, kg plant-^{1}); PH (plant height, cm)*, *** significant at P<0.05, P<0.001, two-sided test of correlations different from zero

5.4 Discussion

Twenty families were produced from the original 4x6 NCII crossing block. The number of seeds obtained from each cross was sufficient for field evaluation. The crossing success rate to that obtained at locations with lower altitudes and higher temperatures (Kamau et al., 2010; Mtunda, 2010; Ojulong, 2006). The study also demonstrated successful F_1 seedling with sufficient cuttings at a site which experienced cold temperatures during winter months. Temperatures at Mount Makulu Research Station (trial site) were low between May and July. On some days, minimum temperatures were as low as 5^{o}C (Figure 5.1 and Appendix 3). With low temperatures cassava growth is retarded. Although growth for most of the plants was generally good, plants in some crosses experienced slow growth. The favourable plant growth might also have been due to sufficient moisture in the soil as the trial was irrigated from planting to harvest. Supplementary irrigation also facilitated in obtaining sufficient cuttings for the clonal trial.

A high seed germination was achieved (mean, 70.5%), which could be attributed to favourable environmental conditions in the growth room. Cassava seed germination is sensitive to

temperature fluctuations (Ellis and Roberts, 1979). Temperatures were kept constant at 36^{o}C and relative humidity at 80%. High temperatures (35^{o}C) have been reported to enhance seed germination (Pujol et al., 2002). Ellis and Roberts (1979) observed high germination rates at constant temperature of 35^{o}C.

Tall plants are required by the farmers (Chapter 2) as they are able to obtain more cuttings for planting. Noteworthy is that some plants had reduced growth points (Figure 5.4); however, it was not possible in this study to determine whether the cause was genetic or environmental.

Significant variation in leaf retention was observed among the crosses. Although, leaf longevity has been reported to contribute to high yields (El-Sharkawy, 2003), there was no correlation between leaf retention and fresh root yield in this study. Some clones within families retained most of their leaves at seedling stage. Lenis et al. (2006) has suggested selecting for stay green trait as an alternative to harvest index; however, in this study it was not possible to select for stay green as irrigation might have caused the plants to retain leaves longer.

Fresh root yield varied significantly across families. In some crosses the root yield was low and some clones were without storage roots. The differences could also be due to differences in genetic make-up and seedling growth rates. Since the seeds germinated at different times this could have contributed to differences in storage root mass for individual progenies. The overall mean yield (1.6 kg plant^{-1}) recorded in this study 11 MAP was similar to that reported by Mtunda (2010). At seedling stage, the tap root tends to dominate other roots, creating variability in root mass. Rajendran et al. (2004) reported an increase in the mass of storage roots from cassava seedlings from which tap roots were removed. Supplementary irrigation might have also played a part in improving storage root mass and other yield components as the crop was watered during most of the growing period. The relatively high root yield in some families (11 MAP) indicates the potential of the developed clones to bulk early and suggests that selection can be made at the seedling stage. Early bulking is an important trait as indicated by most small scale farmers in Zambia (Chapter 2), Kenya (Kamau, 2006), and Nigeria (Nweke et al., 1996).

Root dry matter content was also significantly different among the crosses. For individual clones the mean dry matter ranged from 10.3 to 69.6%. In some clones, the tap root below the soil line bulged and was considered as part of the root. This could also mean that some clones had high

dry matter content and low yields and can be used to develop cultivars with high dry matter content.

Harvest index ranged from 0.01 to 0.80 with an overall mean of 0.33. These values are in agreement with that (0.05 to 0.9) obtained by Ojulong et al. (2006) at seedling trial stage using a diallel cross. Selecting clones with high harvest index has been reported to be more effective in identifying high yielding genotypes than using root yield (Kawano et al., 1978). Harvest index is a high heritable and consistent trait at all stages of selection (Kawano et al., 1987; Kawano et al., 1998; Kawano 2003). Selection based on fresh root yield is affected by the environment, whereas harvest index is not (Jaramillo et al., 2005).

To conclude, selection for yield and other yield related traits can be made at the seedling stage. Bangweulu x Nalumino performed better in terms of fresh root yield than all the other crosses. Bangweulu in combination with TMS3001 also performed consistently better for plant height and branch height. The results also demonstrate that F_1 clones can be produced with sufficient planting materials in areas experiencing short cold winters at high elevation and at high altitude. The variation observed in the segregating populations can be exploited to improve cassava.

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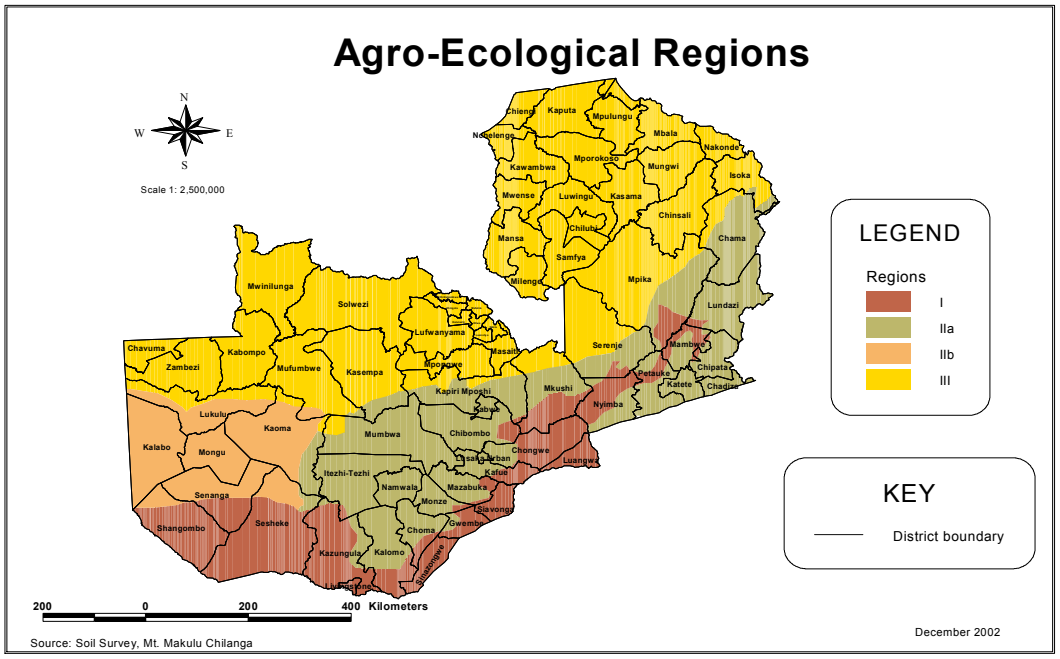
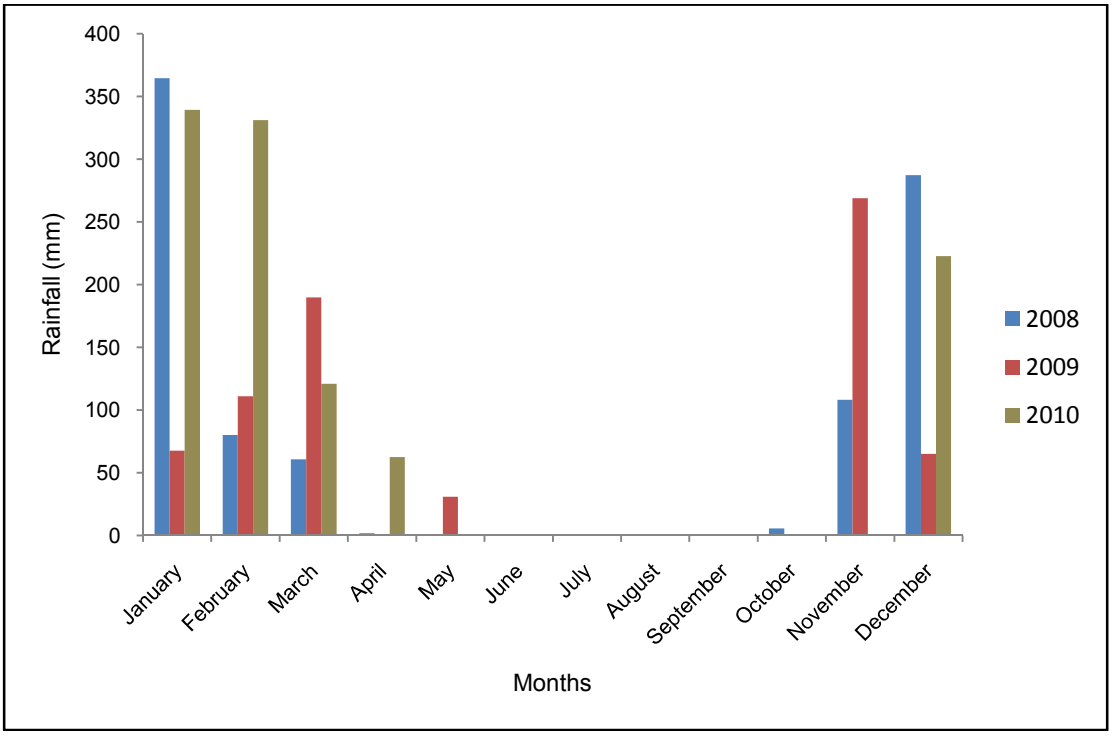
Appendices

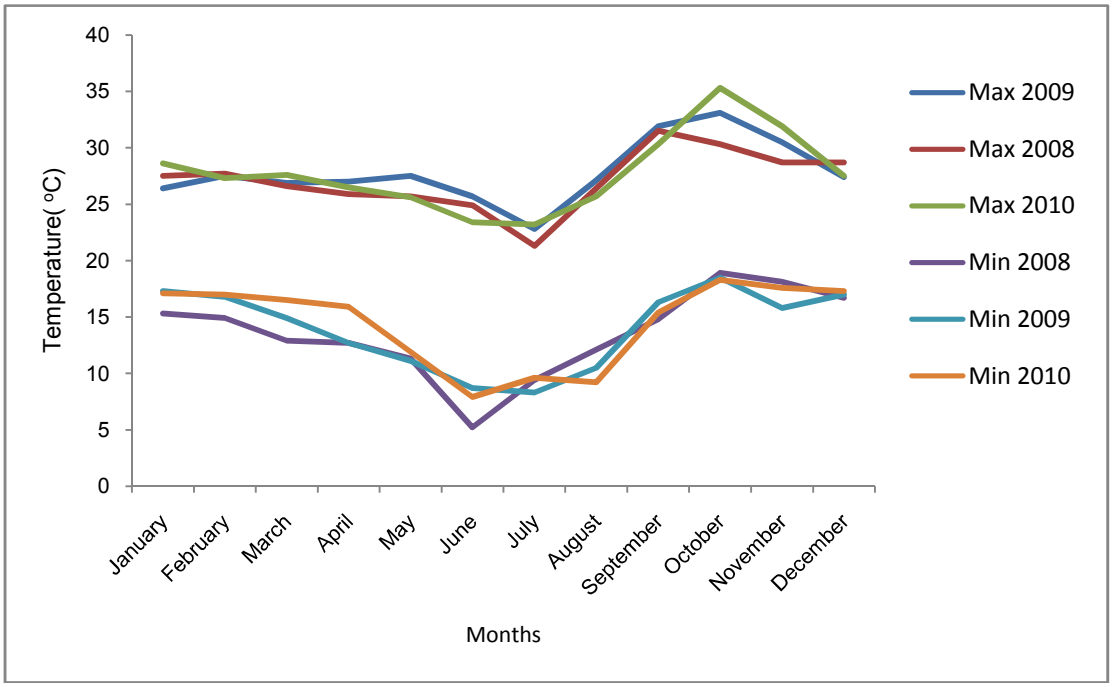
Appendix1: Agro-ecological regions of Zambia



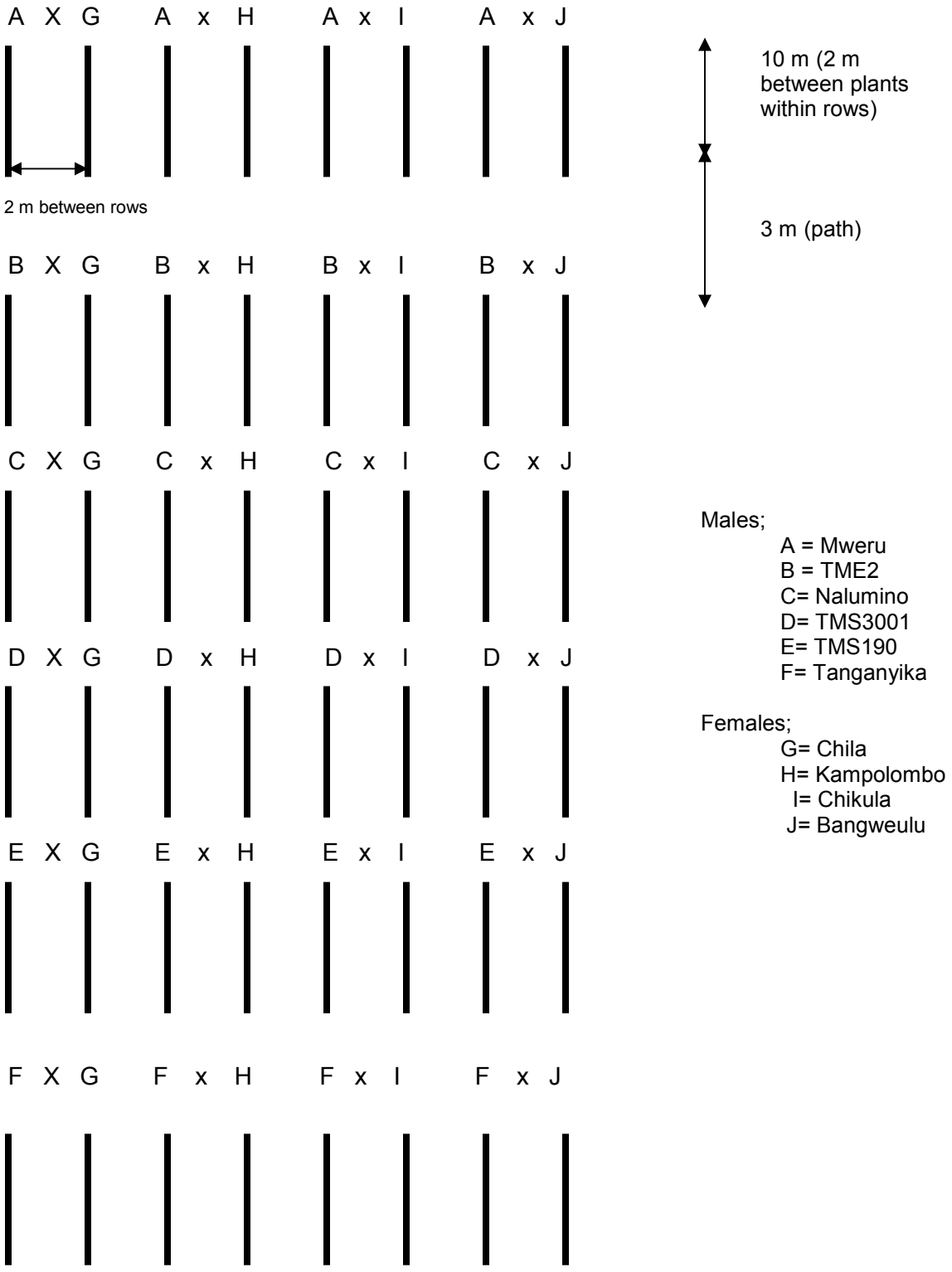
Appendix 2: Monthly rainfall distribution for 2008, 2009 and 2010 seasons, Mount Makulu



Appendix 3: Average maximum and minimum temperature for 2008, 2009 and 2010 seasons, Mount Makulu



Appendix 4: Cassava crossing block based on a 4 x 6, reduced to 4 x 5 NCII design



Males;

A = Mweru
B = TME2
C= Nalumino
D= TMS3001
E= TMS190
F= Tanganyika

Females;

G= Chila
H= Kampolombo
I= Chikula
J= Bangweulu

CHAPTER 6: COMBINING ABILITY ANALYSIS OF CASSAVA GERMPLASM FOR RESISTANCE TO CASSAVA MOSAIC DISEASE

Abstract

Despite the importance of cassava as a staple crop in Luapula province and other regions of Zambia, there is a paucity of information on the resistance to diseases and associated combining ability of the locally cultivated cassava cultivars. Therefore, this study was carried out to: i) identify progeny with resistance to cassava mosaic disease (CMD); ii) evaluate the performance of F_1 progeny for agronomic traits; and iii) determine general and specific combining abilities (GCA and SCA) for resistance to CMD. A total of 809 genotypes comprising parents and progeny were evaluated using a 4 x 5 α-lattice design. There were highly significant differences among the F_1 crosses for CMD, fresh root yield, root size, plant height, total fresh biomass and harvest index. The GCA and SCA mean squares (MS) were highly significant ($P<0.001$) for CMD. The sum of squares (SS) for CMD was mainly accounted for by SCA effects (67.9%). Bangweulu a local highly susceptible cultivar had the most negative, significant ($P<0.001$) GCA effect among the landraces. Among the introductions from the International Institute of Tropical Agriculture (IITA), TMS190 had high negative significant ($P<0.001$) GCA effects for CMD. For most of the traits GCA MS were significant, while SCA MS for fresh root yield, harvest index, total fresh biomass and root size were not. Significant GCA MS were recorded more for the female parents than for the male parents in most of the traits. No correlation was observed between CMD and all the traits studied. All other correlations among the various traits were positive and significant. In summary the results indicated that the local landraces used as parents may be used as sources of CMD resistance.

6.1 Introduction

Pest and diseases are among the most important biotic constraints to cassava production. Cassava mosaic disease (CMD) caused by ACMV, EACMV and SACMV is a particularly devastating disease and in Africa, yield losses to CMD may be as high as 100% (Thresh *et al*., 1994). In Zambia yield losses to CMD of between 50 to 70% have been recorded in farmer's fields (Muimba-Kankolongo *et al*., 1997). In Africa CMD occurs wherever cassava is grown and consequently in Zambia it is found in most farmers' fields (Muimba-Kankolongo *et al*., 1997). The disease is transmitted by the whitefly vector (*Bemisia tabaci* Gennadius) from plant to plant within the field (Calvert and Thresh, 2002) and also spread from one field to another through infected cuttings (J. Ndunguru, *personal communication*). Cassava mosaic disease symptoms range from mild to severe depending on the cultivar and environmental factors (Calvert and Thresh, 2002).

Yield reduction due to CMD varies from agroecological zone to zone and so do the management strategies. The strategies, which are not mutually exclusive, include: phytosanitation, cultural methods (cultivar mixtures) (Thresh and Cooter, 2005), vector management through use of insecticides (Calvert and Thresh, 2002), and resistance breeding (Jennings and Iglesias, 2002). For longer term cost-effective management, breeding for resistance is essential. Genetic based resistance has been sought since the 1930s through intra-specific and inter-specific crosses with *Manihot glaziovii* Muell.-Arg. to produce progeny with high levels of disease or insect pest resistance (Legg and Fauquet, 2004). Host plant resistance is cost effective in managing cassava viruses and is common in most of the research institutions in Africa such as the International Institute of Tropical Agriculture (IITA) and National Agriculture Research Stations (NARS). In view of yield reduction caused by CMD, plant breeding has the potential to develop resistant cultivars to sustainably manage the disease.

To produce new genetic combinations and generate genetic information, mating designs have been used in breeding cassava, among them the diallel (designs I, II, and III) and factorial or North Carolina (designs I, II, and III). The information generated from these designs is used to determine the general combining ability (GCA) and specific combining ability (SCA) of the parents. In this study the NCII mating design was used to generate progeny from crosses involving a set of male and female parents. The NCII mating design has been used in several crop species, for example maize (Eberhart and Gardner, 1966), sugarcane (Hogarth et al., 1981), pearl millet (Angarawai et al., 2008), and wheat (Virk et al., 1985). Kamau et al. (2010)

have also used the design to study the inheritance of yield ability and secondary traits in cassava.

Generation of genetic information involves managing a large number of genotypes, particularly the progeny of crosses. Therefore, selection (of plant type and reaction to certain diseases) during the seedling trial and clonal evaluation trial was previously done visually without recording data (Ceballos et al., 2004). In addition, no replication was made during early stage selection. In this study, to overcome some of the shortcomings of previous strategies, progeny of each cross were randomly divided into four equal groups and each group was allocated to a plot in one of the four replications.

The objectives of the study were to:

i) identify parents and progeny with resistance to CMD;
ii) evaluate the performance of F_1 progeny for agronomic traits and earliness;
iii) determine general combing and specific combing ability for resistance to CMD;

6.2 Materials and methods

6.2.1 Site description

The study was carried out during the 2010/11 rainy season at Mansa research station, Luapula Province, Zambia. The research station is located in agroecological zone III and receives more than 1 000 mm of rainfall per annum. Site and soil descriptions were recorded (Table 6.1). The soils at Mansa research station are acidic and have low soil fertility.

Table 6.1: Site and soil descriptions for the Mansa research site, 2010/11

Site description	
Altitude (masl)	1199
Latitude (S)	11°14.416'
Longitude (E)	28°56.456'
Annual rainfall (mm)	1155.6
Mean max temperature (°C)	29.3
Mean min temperature (°C)	14.5
Soil description	
Soil classification	Acrisols
Soil type	sandy loam
pH	4.8
N%	0.05
Org C%	0.56
P (mg kg^{-1})	13
K (mg kg^{-1})	141
Ca (mg kg^{-1})	200
Mg (mg kg^{-1})	50

Source: Zambia Agriculture Research Institute (ZARI) soil advisory unit, Chilanga

6.2.2 Trial layout and management

Cuttings were taken from the middle part of each selected plant in the seedling trial (Chapter 5) to establish the clonal evaluation trial. The cuttings were planted on 10 December 2010 in a 4 x 5 α-lattice design with four replications. Replication was done according to the crosses. The 40 progeny from each cross were divided into four equal groups. Each group was allocated to a plot in one of the four replication. Each sibling within a cross was represented by a single plant in a plot. Planting was done at a standard 1 x 1 m inter and intra-row spacing providing a plant population of 10 000 plants ha^{-1}. The replications were separated by 2 m wide alleys. Planting was done on ridges (standard farm practice of planting cassava in Luapula Province, Zambia). Weeding of the trial was done as necessary and no fertiliser was applied.

6.2.3 African cassava mosaic virus and East African cassava mosaic virus inoculum source and maintenance

Virus and satellite infected cassava cuttings were collected from different parts of Luapula Province (Chapter 3). The cuttings were then planted in 4 L pots in the screenhouse and watered regularly (Figure 6.1). Total DNA was extracted from the leaves, amplified by PCR and fragments separated by gel electrophoresis (Chapter 3). Plants for which the extracted DNA tested positive for the ACMV, EACMV and satellite were retained for use in the clonal evaluation trial.

Figure 6.1: Cassava landraces in the screenhouse showing CMD and satellite symptoms

6.2.4 Virus inoculation technique

Although Mansa has high CMD prevalence, the whitefly population is too low to ensure efficient and rapid virus transmission. To breed for CMD resistance, it is important to consider the infection method and ensure an even distribution of the viruses. Therefore, grafting was employed to transmit the viruses (ACMV and EACMV) and satellites (satellite II and III) to the test plants (Chapter 4) in addition to planting the diseased spreaders in all four replications. Grafting was done at 3 MAP in the field by cutting the scions of the infected plants in a tapered fashion and the rootstocks of the test plants in a wedge. The scion and the rootstock were then held firmly together by wrapping a strip of plastic around the graft union.

6.2.5 Data collection

Plants were scored for CMD on rootstocks at 5, 6, and 7 MAP using a scale of 1-5 (Hahn et al., 1980) where: 1, no symptoms observed; 2, mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only, with the remainder of the leaflets appearing green and healthy; 3, moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets; 4, severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size; 5, severe mosaic distortion of the entire leaf. The CMD symptoms were assessed visually and recorded using the above scale. Plants were harvested by hand at 7 MAP for yield and yield components. The number and fresh mass (kg) of all the storage roots (fresh root yield) per plant were counted and recorded. Root size was categorized into three classes: size 3 (small sized roots); size 5 (medium sized roots); and size 7 (large sized roots). Harvest index was determined as a percentage of fresh root mass relative to total fresh

biomass. Leaf retention was assessed on a 1-5 scale (Lenis et al., 2006), where: 1, very poor retention; 2, less than average retention; 3, average leaf retention; 4, better than average retention; and 5, outstanding leaf retention.

6.2.6 Data analysis

The residual maximum likelihood procedure (REML) in Genstat version 14 (Payne et al., 2011) statistical package was used to analyse the data. The relative contributions of the various traits was carried out based on Jollife's (2002) approach using principal component analysis (PCA) procedure in Genstat. Mid-parent heterosis (relative to mid-parent value) was determined for all the variables. The performance of the genotypes within each of the crosses was expressed on a cross i.e. family mean basis for all the traits. The general combining ability (GCA), effects and specific combining ability (SCA) effects (genetic components) were estimated from the expected mean squares. The mean squares of GCA and SCA were used to determine GCA:SCA ratios (Haussmann et al., 1999). The parental cultivars and progeny were regarded as fixed effects while the replications were considered as random effects. Therefore, inferences drawn from this study cannot be generalised and extended to other populations. The GCA and SCA effects were estimated using the following model (Hallauer and Miranda, 1988):

$Y_{ijk} = \mu + GCA_i + GCA_j + SCA_{ij} + R_k + \varepsilon_{ijk}$

Y_{ijk} is the observed family mean performance of a cross between the ith and jth parents in the kth replication

μ is the population mean

GCA_i is the GCA effect of the ith female parent

GCA_j is the GCA effect of the jth male parent

SCA_{ij} is the SCA effect for the cross between the ith and jth parent

R_k is the replication effect

ε_{ijk} is the error effect associated with each observation.

6.3 Results

The F_1 progeny expressed different reactions to CMD. Three weeks after grafting, CMD symptoms were first observed on the rootstock of the grafted branch and later on other branches of the plant. Mild and severe symptoms were expressed in several clones with some plants presenting deformed leaves with green and yellow patches. In some clones no symptoms

were observed (Figures 6.2A and 6.2B), and in some clones symptom reversion occurred. Symptoms characteristic of satellites (filiform and curled leaves) were observed on some clones (Figure 6.2D).

Figure 6. 2: A) clone F6-1-R3 from family Chikula x TMS3001 without symptoms; B) Close-up of the same plant with scion having CMD symptoms; C) Susceptible plant with CMD symptoms on most plant parts; D) Plant leaf with symptoms characteristic of satellites, note curling of the leaf blade

6.3.1 Performance of the 800 F_1 genotypes

The full range of scores from 1 to 5 was recorded for CMD with a mean of 1.31. Fresh root yield ranged from 0.01 to 3.35 kg plant^{-1} with a mean of 0.64 kg plant^{-1}. Harvest index ranged from a low of 0.05 to a high of 0.91 with a mean of 0.54. Plant height varied from 25 to 190 cm with a mean of 79.5 cm. Total biomass ranged from 0.10 to 5.55 kg plant^{-1} with a mean of 1.07 kg plant^{-1}. Leaf retention varied from 1 to 5 with a mean of 2.2.

Table 6.2: Minima, maxima and means for cassava mosaic disease and agronomic traits of 800 cassava genotypes at the clonal evaluation stage, Mansa, 2011

Variables	Min	Max	Mean	SD	SEM
CMD	1	5	1.31	0.68	0.012
FRY	0.01	3.35	0.64	0.43	0.009
HI	0.05	0.91	0.54	0.12	0.002
PH	25	190	79.5	24.88	0.460
TB	0.10	5.55	1.07	0.67	0.013
LR	1	5	2.22	0.54	0.010
RN	1	13	5.42	2.65	0.054
RS	3	7	3.42	0.83	0.016

CMD (Cassava mosaic disease (scale 1-5)); FRY (fresh root yield, kg plant^{-1}); HI (harvest index); PH (plant height, cm); TB (total fresh biomass, kg plant^{-1}); LR (leaf retention 1-5); RN (root number); RS (root size, 3-7); Min (minima); max (maxima); SD (standard deviation); SEM (standard error of mean)

6.3.2 Performance of the F_1 crosses

The CMD scores ranged from 1.09 (Bangweulu x TMS3001) to 1.55 (Chikula x TMS190), respectively (Table 6.3). Mean fresh root yield ranged from 0.51 (Bangweulu x Mweru) to 0.74 kg plant^{-1} (Bangweulu x TME2). The majority of the clones had developed storage roots (Figure 6.3), however, some clones within crosses had none. Mean harvest index ranged from a low of 0.51 (Chikula x TMS190 and Chila x Nalumino) to a high of 0.59 (Kampolombo x Nalumino). Plant height across the families varied from 69.32 (Bangweulu x TMS190) to 85.94 cm (Chila x Nalumino). The lowest mean fresh biomass of 0.90 kg plant^{-1} was recorded in crosses Chikula x TME2 and Chikula x Nalumino and the highest of 1.26 kg plant^{-1} in Bangweulu x Mweru. Mean leaf retention ranged from 2.03 (Chila x Mweru) to 2.39 (Bangweulu x TME2). The lowest mean root number of 4.46 was recorded in Bangweulu x Nalumino and the highest of 6.22 in Bangweulu x TME2. Root size ranged from 3.15 (Chikula x TMS190) to 3.73 (Kampolombo x TMS190).

Figure 6.3: Roots of clonal stage plants harvested at 7 MAP

6.3.3 Combining ability mean squares for cassava mosaic disease and agronomic traits

The CMD general combining ability (GCA) and specific combining ability mean squares (MS) were highly significant (P<0.001) (Table 6.4). The GCA SS for male parents accounted for less of the CMD crosses sums of squares (SS) at 12.5% than the GCA SS for female parents at 19.6%. The SCA SS accounted for 67.9% of the CMD crosses SS. The GCA MS for the fresh root yield for the female parents was highly significant (P<0.001), while the GCA MS for male parents and the SCA MS were not. The GCA effects for harvest index for the female parents were significant (P<0.05). The SCA MS for plant height was highly significant (P<0.001) and non-significant for fresh root yield, harvest index, total biomass and root size. The GCA SS % (male and female) was higher than the SCA SS % for fresh root yield (70.2%), total biomass (69.7%) and root size (60.3%). For cassava mosaic disease, harvest index (0.63) and plant height (0.45), GCA:SCA ratio was lower than a unit.

Table 6.3: Cross means for cassava mosaic disease, fresh root yield, harvest index, plant height, total fresh biomass, leaf retention, root number and root size at the clonal evaluation stage, Mansa, 2011

	Variables							
Cross	CMD	FRY	HI	PH	TB	LR	RN	RS
Bangweulu xTMS190	1.17	0.71	0.56	69.3	1.16	2.28	5.58	3.38
Chikula x TMS190	1.55	0.51	0.51	79.1	0.94	2.21	5.51	3.15
Chila x TMS190	1.37	0.66	0.55	81.9	1.12	2.03	5.53	3.32
Kampolombo x TMS190	1.47	0.71	0.56	84.3	1.22	2.24	5.43	3.73
Bangweulu x TMS3001	1.09	0.72	0.57	78.2	1.09	2.27	5.71	3.61
Chikula x TMS3001	1.41	0.56	0.52	77.2	0.98	2.16	5.14	3.28
Chila x TMS3001	1.51	0.62	0.54	82.0	1.01	2.26	4.90	3.47
Kampolombo x TMS3001	1.32	0.72	0.56	81.6	1.16	2.35	5.53	3.61
Bangweulu x Mweru	1.37	0.74	0.55	80.8	1.26	2.17	5.75	3.68
Chikula x Mweru	1.19	0.62	0.52	78.0	1.05	2.12	5.73	3.48
Mweru x Chila	1.42	0.65	0.55	73.3	1.08	2.03	5.50	3.21
Kampolombo x Mweru	1.23	0.66	0.53	81.5	1.10	2.14	5.64	3.52
Bangweulu x Nalumino	1.36	0.56	0.52	82.3	0.95	2.18	4.46	3.50
Chikula x Nalumino	1.40	0.55	0.53	84.8	0.9	2.19	4.86	3.29
Chila x Nalumino	1.14	0.56	0.51	85.9	0.99	2.29	5.05	3.30
Kampolombo x Nalumino	1.14	0.70	0.60	79.6	1.08	2.25	5.59	3.47
Bangweulu x TME2	1.19	0.74	0.57	84.1	1.17	2.39	6.20	3.27
Chikula x TME2	1.31	0.55	0.53	70.4	0.90	2.15	5.34	3.21
Chila x TME2	1.39	0.60	0.52	78.5	0.97	2.25	5.15	3.36
Kampolombo x TME2	1.21	0.61	0.52	79.1	1.05	2.28	5.25	3.31
Mean	1.31	0.64	0.54	79.4	1.06	2.21	5.39	3.40
SEM	0.04	0.06	0.02	2.68	0.09	0.09	0.32	0.14
CV%	9.1	21.2	3.1	6.9	20.4	5.1	11.9	4.3
LSD (0.05)	0.011	0.161	0.047	7.60	0.243	0.24	0.91	0.39
F-probability	0.001	0.648	0,060	0.002	0.802	0.80	0.31	0.51

CMD (Cassava mosaic disease); FRY (fresh root yield, kg plant^{-1}); HI (harvest index); PH (plant height, cm); TB (total fresh biomass, kg plant^{-1}); LR (leaf retention); RN (root number); RS (root size); SEM (standard error)

Table 6.4: Mean squares for cassava mosaic disease and agronomic traits, proportion of general combining ability and specific combining ability effects relative to the sums of squares for the crosses and general combining ability:specific combining ability ratios

		Mean square value					
Source	df	CMD	FRY	HI	PH	TB	RS
Rep	3	0.282	0.3676	0.00613	596.15	0.955	0.437
Crosses	19	0.0711***	0.0219ns	0.00214*	82.38***	0.0391ns	0.113ns
GCA (Male)	4	0.042***	0.0141ns	0.000449ns	77.46*	0.0457ns	0.107ns
GCA (Female)	3	0.088***	0.0783***	0.004677*	58.82ns	0.1117*	0.289*
SCA	12	0.076***	0.0103ns	0.002076ns	89.91**	0.0188ns	0.0711ns
Error	57	0.00604	0.01288	0.001118	28.78	0.0295	0.0748
		Proportion of crosses SS (%) and GCA:SCA ratio					
Crosses SS							
GCA (Male)		12.5	13.6	4.4	19.8	24.6	19.9
GCA (Female)		19.6	56.6	34.4	11.3	45.1	40.4
SCA		67.9	29.8	61.2	68.9	30.3	39.7
GCA:SCA ratio		0.47	2.36	0.63	0.45	2.3	1.52

CMD (Cassava mosaic disease); FRY (fresh root yield, kg plant^{-1}); HI (harvest index); PH (plant height, cm); TB (total fresh biomass, kg plant^{-1}); LR (leaf retention); RN (root number); RS (root size); GCA (general combining ability); SCA (specific combining ability); SS (sums of squares); *,**,*** Significant at P<0.5, P< 0.01 and P < 0.001 probability, respectively

6.3.4 General combining ability effects

In the female parents, Bangweulu had the most significant (P<0.001), negative GCA effect for CMD (Table 6.5). Kampolombo also had a significant (P<0.01), negative GCA effect. Positive significant GCA effects were recorded in Chikula (P<0.001) and Chila (P<0.01). In the male parents, significant (P<0.01), negative GCA effects were recorded for TME2 and Nalumino. TMS190 had a significant (P<0.001) positive GCA effect. The GCA effect for fresh root yield for Bangweulu was positive and significant (P<0.01), while Chikula had a significant (P<0.001), negative GCA effect. For plant height, Nalumino had a significant (P<0.01), positive GCA effect (Table 6.6). Chikula had a significant (P<0.01), negative GCA effect for total fresh biomass and harvest index (Tables 6.6 and 6.7). Kampolombo also had a significant (P<0.01), positive GCA effect for root size while Chikula had a significant (P<0.01), negative effect.

Table 6.5: General combining ability effects for cassava mosaic disease and fresh root yield of nine cassava parents

	Cassava mosaic disease scores (1 to 5)			Fresh root yield (kg plant^{-1})		
Genotype	Mean	GCA	GCA (SE)	Mean	GCA	GCA (SE)
Bangweulu	1.23	-0.07***	0.02	0.69	0.06**	0.03
Chikula	1.37	0.06***	0.02	0.56	-0.08***	0.03
Chila	1.36	0.05**	0.02	0.62	-0.02	0.03
Kampolombo	1.27	-0.04**	0.02	0.68	0.04	0.03
TMS190	1.39	0.08***	0.02	0.65	0.01	0.03
TMS3001	1.33	0.02	0.02	0.65	0.02	0.03
Mweru	1.30	-0.01	0.02	0.67	0.03	0.03
Nalumino	1.26	-0.05**	0.02	0.59	-0.05	0.03
TME2	1.28	-0.04**	0.02	0.63	-0.01	0.03

GCA (general combining ability); SE (standard error); *, **, *** significant at P<0.05, P<0.01, P<0.001

Table 6.6 General combining ability effects for plant height and total biomass

	Plant height (cm)			Total fresh biomass (kg plant^{-1})		
Genotype	Mean	GCA	GCA (SE)	Mean	GCA	GCA (SE)
Bangweulu	78.9	-0.50	1.20	1.12	0.062	0.04
Chikula	77.3	-2.15	1.20	0.97	-0.094**	0.04
Chila	80.3	0.87	1.20	1.04	-0.026	0.04
Kampolombo	81.2	1.78	1.20	1.12	0.058	0.04
TMS190	77.9	-1.54	1.34	1.11	0.048	0.04
TMS3001	79.7	0.30	1.34	1.06	-0.003	0.04
Mweru	78.4	-1.03	1.34	1.12	0.059	0.04
Nalumino	83.1	3.71**	1.34	0.10	-0.063	0.04
TME2	78.0	-1.44	1.34	1.02	-0.041	0.04

GCA (general combining ability); SE (standard error); *, **, *** significant at P<0.05, P< 0.01, P<0.001

Table 6. 7: General combining ability effects for harvest index and root size

	Harvest index			Root size		
Genotype	Mean	GCA	GCA (SE)	Mean	GCA	GCA (SE)
Bangweulu	0.56	0.0118	0.01	3.49	0.080	0.06
Chikula	0.53	-0.0181**	0.01	3.27	-0.128**	0.06
Chila	0.53	-0.0072	0.01	3.33	-0.075	0.06
Kampolombo	0.56	0.0135	0.01	3.52	0.122**	0.06
TMS190	0.54	0.0040	0.01	3.39	-0.011	0.07
TMS3001	0.55	0.0071	0.01	3.49	0.085	0.07
Mweru	0.54	-0.0023	0.01	3.46	0.065	0.07
Nalumino	0.54	-0.0042	0.01	3.39	-0.018	0.07
TME2	0.54	-0.0047	0.01	3.28	-0.121	0.07

GCA (general combining ability); SE (standard error); *, **, *** significant at P<0.05, P<0.01, P<0.001

6.3.5 Specific combining ability effects

Eleven crosses had significant SCA effects for CMD, five of which had negative effects, namely: Bangweulu x TMS190 (P<0.001), Bangweulu x TMS3001 (P<0.001), Chikula x Mweru (P<0.001), Chila x Nalumino (P<0.001) and Kampolombo x Nalumino (P<0.01), and the other six had significant, positive effects, namely: Chikula x TMS190 (P<0.01), Kampolombo x TMS190 (P<0.01), Chila x TMS 3001 (P<0.001), Bangweulu x Mweru (P<0.001), Bangweulu x Nalumino (P<0.001) and Chikula x Nalumino (P<0.01) (Table 6.8).

Table 6.8: Mean performance and specific combining ability effects for cassava mosaic disease scores of 20 crosses

Cross	Cassava mosaic disease score		
	Mean	SCA effects	SCA(SE)
Bangweulu xTMS190	1.17	-0.14***	0.04
Chikula x TMS190	1.55	0.10**	0.04
Chila x TMS190	1.37	-0.07	0.04
Kampolombo x TMS190	1.47	0.12**	0.04
Bangweulu x TMS3001	1.09	-0.17***	0.04
Chikula x TMS3001	1.41	0.02	0.04
Chila x TMS3001	1.51	0.12***	0.04
Kampolombo x TMS3001	1.32	0.02	0.04
Bangweulu x Mweru	1.37	0.14***	0.04
Chikula x Mweru	1.19	-0.17***	0.04
Mweru x Chila	1.42	0.06	0.04
Kampolombo x Mweru	1.23	-0.03	0.04
Bangweulu x Nalumino	1.36	0.17***	0.04
Chikula x Nalumino	1.40	0.08**	0.04
Chila x Nalumino	1.14	-0.18***	0.04
Kampolombo x Nalumino	1.14	-0.08**	0.04
Bangweulu x TME2	1.19	-0.01	0.04
Chikula x TME2	1.31	-0.02	0.04
Chila x TME2	1.39	0.06	0.04
Kampolombo x TME2	1.21	-0.02	0.04
Grand mean	1.31		

SCA (specific combing ability); SE (standard error); *, **, *** significant at P< 0.05, P<0.01, P<0.001, respectively

Bangweulu x TME2 had significant (P<0.01), positive effects for plant height (Table 6.9). Significant negative effects were recorded in Bangweulu x TMS190 (P<0.001), Mweru x Chila (P<0.01) and Chikula x TME2 (P<0.01).

Table 6.9: Mean performance and specific combining ability effects for plant height of 20 crosses

	Plant height (cm)		
Cross	Mean	SCA	SCA(SE)
Bangweulu xTMS190	69.32	-8.07***	2.81
Chikula x TMS190	79.07	0.34	2.81
Chila x TMS190	81.87	3.11	2.81
Kampolombo x TMS190	84.31	4.63	2.81
Bangweulu x TMS3001	78.17	-1.06	2.81
Chikula x TMS3001	7716	-0.41	2.81
Chila x TMS3001	82.02	1.42	2.81
Kampolombo x TMS3001	81.56	0.05	2.81
Bangweulu x Mweru	80.82	2.91	2.81
Chikula x Mweru	77.97	1.72	2.81
Mweru x Chila	73.27	-6.00**	2.81
Kampolombo x Mweru	81.54	1.36	2.81
Bangweulu x Nalumino	82.29	-0.36	2.81
Chikula x Nalumino	84.83	3.84	2.81
Chila x Nalumino	85.86	1.85	2.81
Kampolombo x Nalumino	79.60	-5.33	2.81
Bangweulu x TME2	84.08	6.58**	2.81
Chikula x TME2	70.35	-5.49**	2.81
Chila x TME2	78.48	-0.38	2.81
Kampolombo x TME2	79.07	-0.71	2.81
Grand mean	79.43		

SCA (specific combing ability); SE (standard error); *, **, *** significant at 0.05, 0.01, 0.001, respectively

6.3.6 Phenotypic correlations between traits

Most of the traits were significantly correlated with one another. However, there was no significant correlation between CMD and fresh root yield, harvest index, total biomass, leaf retention, root number and root size. Similarly no significant correlation was recorded between leaf retention and fresh root yield. A significant (P<0.001) and high positive correlation was recorded between total biomass and fresh root yield. Significant (P<0.001), positive correlations was recorded between harvest index and fresh root yield. A positive and significant (P<0.001) correlation was also recorded between plant height and fresh root yield. Correlation between root number and fresh root yield; harvest index and total biomass; leaf retention and root number were also positive and significant (P<0.001). Positive and significant (P<0.01) correlation was also recorded between plant height and total biomass, leaf retention, root number and root size. Total biomass was also significantly (P<0.001) positively correlated with root number and root size. Leaf retention was also significantly (P<0.001), positively correlated with root size.

Table 6.10: Phenotypic correlation coefficients for CMD and agronomic traits for 800 genotypes at the clonal evaluation stage, 2011

CMD	-							
FRY	0.199	-						
HI	0.039	0.624***	-					
LR	0.055	0.218	0.007	-				
TB	0.268	0.947***	0.436***	0.318**	-			
PH	0.240	0.551***	0.118	0.427***	0.680***	-		
RN	0.133	0.819***	0.463***	0.160	0.771***	0.352**	-	
RS	0.112	0.522***	0.300**	0.294**	0.553***	0.581***	0.296**	-
	CMD	FRY	HI	PH	TB	LR	RN	RS

CMD (Cassava mosaic disease); FRY (fresh root yield, kg plant^{-1}); HI (harvest index); PH (plant height, cm); TB (total biomass, kg plant^{-1}); LR (Leaf retention); RN (root number); RS (root size); *, **,*** Significantly different from zero at the 0.05, 0.1 and 0.01 probability levels, respectively (two-tailed test)

6.3.7 Trait contribution to genotype performance

Most of the total variation (74.5%) was accounted for by the first three principal components (PCs) (Table 6.11). The PC1 accounted for 41.4% of the total variation with an eigenvalue of 2.49. The traits that contributed the most to the PC1 were harvest index, biomass, fresh root yield and root size. The PC2 accounted for 17.7% of the total variation with harvest index, plant height and leaf retention being the major contributors. The PC3 accounted for 15.4% of the total variation with harvest index, leaf retention and root size as the main contributors.

Table 6. 11: Principal component coefficients of the various traits with loadings of the various yield and yield components

Traits	PC1	PC2	PC3	PC4
HI	0.372	-0.413	0.187	0.356
PH	0.054	0.790	0.538	0.229
TB	0.572	0.045	0.026	0.053
FRY	0.607	-0.079	0.068	0.141
LR	0.213	0.429	-0.817	0.185
RS	0.343	0.120	0.056	-0.874
Eigenvalue	2.485	1.060	0.924	0.811
% Total variation	41.4	17.7	15.4	13.5

PC (principal component); PH (plant height, cm); HI (harvest index); TB (total biomass, kg plant^{-1}); FRY (fresh root yield, kg plant^{-1}); LR (leaf retention); RS (root size)

6.3.8 Estimates of heterosis

The best five crosses with desired negative CMD heterosis (relative to mid-parent value) were Bangweulu x TMS3001 (-113.9%), Chikula x TMS3001 (-113.9%), Chikula x Nalumino (-107.9%), Bangweulu x Nalumino (-107.8%), Chikula x TMS190 (-104.6%) (Table 6.12). All these crosses involved a resistant and susceptible parent. The best five crosses with positive heterosis for plant height were Kampolombo x TMS190 (2589.5%), Kampolombo x TMS3001 (2547.5%), Kampolombo x Mweru (1782.0%), Bangweulu x TME2 (1691.5%), and Kampolombo x TME2 (1518.5%). For total biomass the best five crosses with positive heterosis were Chikula x Nalumino (21.9%), Kampolombo x TMS3001 (18.4%), Kampolombo x TME2 (16.8%), Bangweulu x TME2 (15.4%) and Kampolombo x TMS190 (11.4%). For fresh root yield most of the crosses had positive heterosis and the best five were Bangweulu x TME2 (24.2%), Bangweulu x TMS3001 (20.9%), Kampolombo x Nalumino (19.1%), Kampolombo x TMS3001 (17.1%) and Bangweulu x Mweru (16.6%). Positive heterosis for harvest index was also recorded in most crosses. The best five crosses with positive heterosis for harvest index were Bangweulu x TME2 (11.5%), Kampolombo x Nalumino (10.4%), Chikula x Nalumino (9.2%), Bangweulu x TMS3001 (8.4%), and Cikula x TME2 (8.3%).

The mid parent heterosis for the F1 progeny is presented in Table 6.13. The best two clones with negative heterosis for CMD were 7737 (-128.3%) and 1813 (-126.5%). For the fresh root yield, clones with high positive heterosis were 8444 (284.2%) and 4979 (239.5%). Clone 4979 had also the highest positive heterosis for harvest index. Clones 7585 (85.0%) and 8444 (63.4%) had high positive heterosis for leaf retention. Clones with the highest positive heterosis for plant height were 1813 (4858.5%), 2904 and 2734 (4208.5%).

Table 6.12: Mean performance and mid-parent heterosis (%) for traits evaluated at the clonal evaluation stage, Mansa, 2011

Parents and crosses	CMD		PH		TB		FRY		HI		LR		RS	
	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH
P1	3.23		83.8		1.01		0.54		0.46		2.43		3.38	
P2	2.86		68.0		0.88		0.45		0.45		2.55		3.15	
P3	1.67		96.0		1.37		0.75		0.53		2.00		3.32	
P4	1.67		61.0		1.06		0.62		0.56		3.63		3.74	
P5	1.20		55.8		1.16		0.63		0.52		2.87		4.40	
P6	1.23		51.2		0.90		0.48		0.51		2.30		4.00	
P7	1.67		74.8		1.07		0.61		0.53		2.43		3.20	
P8	1.33		67.8		0.76		0.39		0.43		2.53		3.00	
P9	1.27		61.5		0.97		0.47		0.45		2.57		3.55	
1	1.17	-104.6	69.3	-51.0	1.16	7.65	0.71	13.15	0.56	7.60	2.28	-37.00	3.38	-65.75
2	1.55	-48.6	76.1	1416.5	0.94	-8.15	0.51	-2.90	0.51	3.00	2.21	-49.95	3.15	-58.75
3	1.37	-6.5	81.9	596.5	1.12	-14.05	0.66	-2.65	0.55	2.80	2.03	-40.25	3.32	-37.70
4	1.47	3.4	84.3	2589.5	1.22	11.40	0.71	8.35	0.56	2.20	2.24	-100.60	3.74	-79.45
5	1.09	-113.9	78.2	1067.0	1.09	0.25	0.72	20.85	0.57	8.35	2.27	-9.35	3.61	-22.35
6	1.41	-63.5	77.2	1757.5	0.98	8.60	0.56	9.10	0.52	4.55	2.16	-26.50	3.28	-25.85
7	1.51	6.1	82.0	843.5	1.01	-12.20	0.62	0.05	0.54	2.45	2.26	10.60	3.47	-2.60
8	1.32	-13.0	81.6	2547.5	1.16	18.35	0.72	17.05	0.57	3.35	2.35	-61.55	3.61	-72.15
9	1.37	-107.9	80.8	149.0	1.26	21.90	0.74	16.60	0.55	5.95	2.17	-26.40	3.68	24.95
10	1.19	-107.8	78.0	655.5	1.05	6.80	0.62	8.15	0.52	3.15	2.12	-37.75	3.48	34.75
11	1.42	-24.9	73.3	-1214.5	1.08	-14.30	0.65	-3.20	0.55	2.05	2.03	-18.35	3.21	10.50
12	1.23	-43.8	81.5	1362.5	1.10	3.15	0.66	4.10	0.53	-0.95	2.14	-89.00	3.52	-41.15
13	1.36	-92.1	82.3	646.0	0.95	6.10	0.56	9.05	0.52	7.55	2.18	-29.90	3.50	16.95
14	1.40	-70.1	84.8	1691.5	0.98	15.40	0.55	13.00	0.53	9.15	2.26	-28.65	3.29	25.05
15	1.14	-36.5	85.9	394.5	0.99	-7.00	0.56	-1.55	0.51	2.75	2.20	-6.75	3.30	29.80
16	1.14	-36.2	79.6	1518.5	1.08	16.75	0.70	19.05	0.60	10.35	2.29	-79.70	3.47	-36.15
17	1.19	-105.7	84.1	1141.5	1.17	17.75	0.74	24.15	0.57	11.45	2.39	-10.60	3.27	-34.15
18	1.32	-75.0	70.4	560.0	0.90	-2.95	0.55	8.50	0.53	8.25	2.15	-40.85	3.21	-9.95
19	1.39	-7.9	78.5	-27.0	0.97	-19.35	0.60	-0.65	0.52	3.45	2.25	-3.65	3.36	8.60
20	1.21	-25.8	79.1	1782.0	1.05	3.40	0.61	7.05	0.52	2.05	2.28	-81.60	3.31	-80.45

CMD (cassava mosaic disease); PH (plant height, cm); TB (total fresh biomass, kg plant^{-1}); FRY (fresh root yield, kg plant^{-1}); HI (harvest index); LR (leaf retention); RS (root size); MPH (mid-parent heterosis) P1 (Bangweulu); P2 (Chikula); P3 (Chila); P4 (Kampolombo); P5 (TMS190); P6 (TMS3001); P7 (Mweru); P8 (Nalumino); P9 (TME2); 1 (Bangweulu x TMS190); 2 (Chikula x TMS190); 3 (Chila x TMS190); 4 (Kampolombo x TMS190); 5 (Bangweulu x TMS3001); 6 (Chikula x TMS3001); 7 (Chila x TMS3001); 8 (Kampolombo x TMS3001); 9 (Bangweulu x Mweru); 10 (Chikula x Mweru); 11 (Chila x Mweru); 12 (Kampolombo x Mweru); 13 (Bangweulu x Nalumino); 14 (Chikula x Nalumino); 15 (Chila x Nalumino); 16 (Kampolombo x Nalumino); 17(Bangweulu x TME2); 18 (Chikula x TME2); 19 (Chila x TME2)

Table 6.13: Mean performance and mid-parent heterosis (%) for best 20 F_1 progeny evaluated for the traits at the clonal evaluation stage, Mansa, 2011

Parents	CMD		FRY		LR		TB		PH		HI	
and clones	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH
P1	1.2		0.6		2.9		1.2		55.8		0.5	
P2	1.2		0.5		2.3		0.9		51.2		0.5	
P3	1.3		0.4		2.5		0.8		67.8		0.4	
P4	1.3		0.5		2.6		1.0		61.5		0.5	
P5	1.7		0.6		2.4		1.1		74.8		0.5	
P6	3.2		0.5		2.4		1.0		83.8		0.5	
P7	1.7		0.7		2.0		1.4		96.0		0.5	
P8	1.7		0.6		3.6		1.1		61.0		0.6	
P9	2.9		0.5		2.4		0.9		68.0		0.4	
3101	1.0	-121.7	2.9	226.8	3.0	35.0	4.4	331.8	95.0	2517.0	0.6	16.2
3121	1.0	-121.7	1.5	86.8	3.0	35.0	2.6	146.8	60.0	-983.0	0.6	8.3
4202	1.0	-43.4	1.7	96.2	2.0	-43.4	2.4	108.9	70.0	-591.5	0.7	18.0
6313	1.0	-43.4	2.5	182.7	3.0	-25.0	3.4	224.5	55.0	-341.5	0.7	19.5
8444	1.0	-123.3	3.4	284.2	3.0	63.4	5.6	459.6	60.0	-750.0	0.6	12.2
7585	3.0	155.0	1.2	53.6	3.0	85.0	1.6	46.7	50.0	-2358.5	0.7	20.1
4906	4.0	255.0	1.5	95.1	3.0	3.4	2.0	102.4	70.0	1391.5	0.8	21.9
7737	1.0	-128.3	1.9	138.6	3.0	51.7	3.3	236.5	80.0	417.0	0.6	12.7
6848	1.0	-50.0	1.7	113.0	2.0	-26.7	3.3	223.6	95.0	1308.5	0.5	3.7
4979	1.0	-50.0	2.9	239.5	2.0	-108.3	4.0	304.3	70.0	585.0	0.7	24.2
2010	1.0	-106.5	1.3	79.0	3.0	44.1	2.3	137.5	55.0	-975.0	0.5	9.4
3211	1.0	-125.0	1.9	135.0	3.0	50.0	2.8	181.2	80.0	733.5	0.7	20.7
5512	1.0	-46.7	2.0	139.4	2.0	-28.4	3.4	223.3	70.0	-875.0	0.6	9.9
1813	1.0	-126.5	2.1	151.7	3.0	50.8	3.2	222.2	120.0	4858.5	0.6	15.1
2014	1.0	-66.7	1.6	98.5	3.0	-3.3	2.5	138.7	105.0	3708.5	0.7	11.0
2904	1.0	-66.7	1.6	98.5	3.0	-3.3	2.8	168.7	110.0	4208.5	0.6	3.8
2734	1.0	-66.7	1.4	73.5	2.0	-103.3	2.2	108.7	110.0	4208.5	0.6	8.4
2994	1.0	-66.7	1.4	78.5	3.0	-3.3	2.3	118.7	100.0	3208.5	0.6	7.9
2914	1.0	-66.7	1.3	68.5	3.0	-3.3	2.2	113.7	85.0	1708.5	0.6	4.7
5215	2.0	33.3	1.3	57.0	2.0	-21.7	1.8	58.0	50.0	-3541.5	0.7	16.5

CMD (cassava mosaic disease); FRY (fresh root yield, kg plant^{-1}); LR (leaf retention); TB (total fresh biomass, kg plant^{-1}); PH (plant height, cm); HI (harvest index); MPH (Mid-parent heterosis); P1 (TMS190); P2 (TMS3001); P3 (Nalumino); P4 (TME2); P5 (Mweru); P6 (Bangweulu); P7 (Chila); P8 (Kampolombo); P9 (Chikula); 3101 and 3121 (TMS190xBangweulu); 4202 (TMS190xChila); 6313 (TMS190xKampolombo); 8444 (TMS3001xBangweulu); 7585 (TMS3001xChila); 4906 (TMS3001xKampolombo); 7737 (Nalumino x Bangweulu); 6848 (Nalumino x Chila); 4979 (Nalumino x Kampolombo); 2010 (TME2 x Chikula); 3211 (TME2 x Bangweulu); 5512 (TME2 x Chila); 1813 (Mweru x Chikula); 2014, 2904, 2734, 2994 and 2914 (Mweru x Kampolombo); 5215 (Mweru x Chila)

6.4 Discussion

Progeny with increased resistance to CMD were produced from crosses between the selected parents. For the individual clones, the full range of CMD scores from 1 to 5 with a mean of 1.31 was recorded in the 800 F_1 progeny. Significant differences between the F_1 progeny and parents were observed for CMD resistance. Low mean CMD scores were recorded in crosses of Bangweulu x TMS3001, Bangweulu x TMS190, and Chila x Nalumino indicating high tolerance to CMD.

The overall cross mean for fresh root yield was 0.64 kg plant^{-1} (6.4 t ha^{-1}) at 7 MAP. This apparently low yield may be explained by the early harvesting at 7 MAP. The mean yield is comparable to that recorded by Munga (2008), Mtunda (2010) and Kamua (2010). It has been documented (Ngeve, 1999) that cassava undergoes root bulking from 4 MAP and during the initial stages root growth is slow (Hahn et al., 1979). For individual progeny plants, fresh root yield ranged from 0.10 kg plant^{-1} (1.0 t ha^{-1}) to 3.35 kg plant^{-1} (33.5 t ha^{-1}), while for the parents, it ranged from 0.1 kg plant^{-1} (1.0 t ha^{-1}) to 1.9 kg plant^{-1} (19 t ha^{-1}) with a mean of 0.56 kg plant^{-1}. This is indicative of the progress being made in developing high yielding, early bulking cassava genotypes. Cassava cultivars currently cultivated in Zambia are usually harvested 2 to 3 years after planting. Understandably, early bulking is one of the traits desired by farmers (Chapter 2). The PCA helped to explain the relative contribution of the various traits to genotype performance and as expected fresh root yield made the greatest contribution.

Significant hybrid vigour for yield and yield components was recorded for most of the traits. Most of the crosses recorded positive heterosis for fresh root yield, total fresh biomass, plant height and root size. Negative heterosis for CMD was recorded for most of the crosses. A number of promising genotypes combined high positive heterosis for yield at 7 MAP with negative heterosis for CMD.

Genetic information was generated at the clonal evaluation stage in order to estimate general combining abilities or breeding values of the parental lines for the traits of interest (Ceballos et al., 2004). The GCA and SCA MS were highly significant implying that additive and non-additive gene effects were both important. For CMD, 67.9% of the variation was explained by SCA indicating that non-additive gene action predominated over additive gene action for this trait. This was also reflected in GCA:SCA ratio which was lower than a unity for the CMD trait.

Kamua (2010) also reported higher SCA effects for CMD. However, Lokko et al. (2006) reported the reverse. The significant GCA and SCA MS recorded for the traits of interest indicate sufficient genetic variance in the population to enable effective selection for the traits (Ragsdale and Smith, 2007). However, the breeding strategy adopted will depend on the prevalent gene action.

The significance of parents' MS for CMD was indicative of the diversity of the parents suggesting that both landraces and introductions could be sources of resistance to CMD. Significant negative SCA effects for CMD were recorded in five crosses, namely: Bangweulu x TMS190, Bangweulu x TMS3001, Chikula x Mweru, Chila x Nalumino and Kampolombo x Nalumino. A significant, negative SCA effect for a cross signifies that the observed resistance of the cross is greater than that predicted by the grand mean of all the crosses plus the GCA (additive) effect of the male and female parents. A cross with significant, positive SCA means that the observed susceptibility of the cross is greater (higher CMD score) than that predicted by the grand mean plus the GCA (additive) effects of the parents. For most of the crosses negative mid-parent heterosis was observed for CMD emphasizing the progress made in breeding for resistance.

The GCA SS comprised 70.2% of the crosses SS for fresh root yield indicated the strong influence of additive gene action in the expression of this trait. The GCA and SCA MS for plant height were also significant meaning that both additive and non-additive gene action were important in the determination of this trait. However, since the SCA SS comprised 68.9% of crosses SS, non-additive gene action predominated over additive gene action for this trait. Bangweulu had a significant positive GCA effect for fresh root yield implying that this landrace which was used as a female parent made an above average contribution to increased fresh root yield in all of its crosses. TMS190 also had a significant positive GCA effect for total biomass and therefore made an above average contribution to increased biomass accumulation in all of its crosses. Similarly for Kampolombo, which made a significant above average positive contribution to root size in all of its crosses.

Bangweulu had the most significant, negative GCA effect for CMD which is an indication of its important contribution to CMD resistance. Kampolombo also had a significant, negative GCA effect for CMD. The significant negative GCA effects for CMD exhibited by these two parents implicates additive gene action in determining improved resistance to CMD in the crosses

involving these two parents. These parents were consequently considered to be good general combiners for improved resistance to CMD. Chikula, TMS190 and Chila exhibited significant positive effects for CMD suggesting that the three parents determined increased susceptibility of their progeny relative to the mean performance of all progeny.

The GCA MS for fresh root yield was significant, however, the SCA MS was not. This implies that additive gene action was predominant over non-additive gene action for this trait. Bangweulu x TME2 had significant positive SCA effects for plant height meaning that non-additive gene action was important for the trait.

Bangweulu recorded a significant, negative GCA effect for CMD and a high positive GCA effect for fresh root yield suggesting that it would be a valuable parent for high yield in association with high CMD resistance. Based on its significant positive GCA effect for total biomass, TMS190 may be categorised as a good general combiner for this trait. In the same vein, Nalumino was a good general combiner for plant height. By way of contrast, Chikula had significant large negative GCA effects for biomass and root size which indicated that it was a poor general combiner for these two traits.

Despite grafting the F_1 cassava progenies with CMD infected scions, it is interesting to note that CMD score was not significantly correlated with any of the other traits. This is probably due to the low virus titre as the clones were virus free when planted and harvested early (7 MAP). The non-significant correlation suggests the need for caution in the selection of progeny for improved resistance to CMD in association with improved performance in the other traits. Zacarias (2008) also recorded non-significant correlations between CBSD and yield. Fresh root yield was positively correlated ($P<0.001$) with harvest index, root size, root number, leaf retention and total fresh biomass. The significant and high positive correlation between fresh root yield and harvest index has been reported by other workers (Cach et al., 2006; Kawano, 2003; Kawano et al., 1998). The relevance of significant correlations between harvest index and other traits is that harvest index can be used as a selection criterion.

In final conclusion: GCA and SCA MS for CMD were recorded as significant in this study. Bangweulu, Kampolombo, TME2 and Nalumino had significant, high negative GCA effects which means they contributed to an above average improvement in CMD resistance in the crosses of which they were parents. The good general combining ability for CMD of Bangweulu

and Kampolombo is in direct contrast to their own *per se* performance as they are both susceptible to CMD. That they both have the genetic potential to contribute to breeding for CMD resistance would not have been revealed without conducting a combining ability analysis. Having produced progeny with resistance to CMD, there is a need to conduct extensive GXE evaluation of the stability of the resistance in association with expression of the important agronomic traits. Further studies to elucidate the extent of interaction between viruses and associated satellites are necessary to correlate resistance levels with actual virus titre. The adoption of appropriate breeding strategies to exploit the additive and non-additive gene action determining CMD resistance and the other traits is now possible. The prospects for substantial improvements in the productivity of cassava cultivars grown in Zambia are very real and exciting indeed.

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CHAPTER 7: OVERVIEW OF THE RESEARCH FINDINGS AND IMPLICATIONS FOR CASSAVA BREEDING

7.1 Introduction

Cassava is one of the most important root crops in Zambia making a significant contribution to the national economy. However, production of cassava is constrained by CMD, a viral disease to which the available local cultivars are susceptible. This study was aimed at improving local cassava cultivars with resistance to the viruses and associated satellites which cause CMD and to contribute to national and household food security. The research study included a PRA, a CMD survey, and an evaluation of local and introduced cultivars for CMD resistance, root qualities, and combining ability for resistance to CMD.

7.2 Farmer's perception of cassava mosaic disease

A PRA was conducted in Samfya, Mansa and Mwense districts with the aim of understanding farmers' perception of CMD and cassava cultivar preferences. The involvement of farmers was essential in the early stages of breeding process as it facilitated directing the research focus to the needs of the farmers. Major findings were:

- The farmers had insufficient knowledge of CMD, its spread and effects on yield.
- The disease was prevalent in most of the cassava fields; however no control strategies were in place.
- The farmers could not distinguish between the symptoms due to diseases, viruses and insect pests
- The reasons given from a cross section of respondents on the cause of CMD were numerous such as mealybug, harvesting of leaves, old age, cold and lack of rain, etc
- Apart from lack of knowledge on the effects and cause of CMD, the respondents mentioned early bulking and high yield as some of the most important attributes preferred.
- Re-use of planting materials was common in the districts visited. Few farmers occasionally use clean materials from established nurseries and NGOs supporting agriculture.
- Farmers obtain planting materials mostly from their fellow farmers. The materials are recycled and have high levels of CMD.

- Other constraints pointed out by the farmers included distance to markets and lack of transport to ferry the produce, lack of capital and labour to work their fields and inadequate planting materials.
- Most of the farmers grew local landraces on small fields of less than 1 ha.
- Farmers intercrop cassava with either bean or maize. Cassava is also grown as a sole crop in some districts.

7.3 Cassava geminiviruses and satellites occurring in Luapula province

The comprehensive cassava virus and satellite survey conducted in this study was the first to be carried out in the five districts (Samfya, Mansa, Mwense, Kawambwa and Nchelenge) of Luapula province in Zambia. The outcome of the survey formed the basis of the research study. The following were the findings of the survey:

- The two viral strains, EACMV, ACMV and associated satellites (II and III) were present in all the five districts surveyed signifying the potential to cause severe yield losses. This is the first report of CMD associated satellites in Zambia.
- Cassava brown streak virus and Ugandan variant (UgV) strain of EACMV was not detected in the surveyed areas.
- East Africa cassava mosaic virus was more predominant than ACMV in the districts surveyed.
- The ACMV and EACMV were found in single and mixed infections. In plants with mixed infection, the plants were characterised by severe symptoms with yellow and green patches.
- Leaf distortion with reduced leaf blade characteristic of satellites was observed on a number of infected plants.
- Variation of CMD on cassava cultivars was observed implying that the genotypes have an influence on disease expression. To select plants with CMD resistance the two virus strains have to be taken into account.
- Generally CMD incidence was high in all the districts surveyed with an average of 59%. CMD incidence ranged from 13.3 to 93.3% while disease severity ranged from 1.9 to 2.6 on a 1-5 scale

7.4 Evaluation of genotypes to CMD

The reaction of selected cultivars to CMD was evaluated at Mansa Research Station in Luapula province. The findings were:

- Genotypes significantly ($P<0.001$) varied in reaction to CMD.
- Bangweulu, Mwakamoya, Chila7, Kalaba, Namuyongo and Chila11 were the most susceptible cultivars.
- Mweru, Nalumino, and Kampolombo were some of the local cultivars which exhibited tolerance to CMD
- All the traits evaluated were significantly ($P<0.001$) different

7.5 Evaluation of F_1 progeny

F_1 seedling evaluation trial was conducted at Mount Makulu Research Station. The main findings were:

- Seed germination averaged 70.5%.
- Variation in the F_1 progeny was observed in all the traits measured
- Significant correlations between branching height, dry mater content and leaf retention were recorded. However, there was significant correlation between fresh root yield and dry matter content
- The overall mean root dry matter content was high at 39.4%.
- The fresh root yield ranged from 0.1 to 5 kg plant^{-1} with a mean of 1.57 kg plant^{-1}.

7.6 Combining ability analysis

The trial to determine combining ability for CMD analysis of cassava to cassava mosaic disease was conducted at Mansa Research Station. Eight hundred genotypes were evaluated for CMD using spreader rows and wedge grafting. Wedge grafting was performed to ensure that susceptible genotypes did not present as false resistant genotypes. The following were the findings:

- General combining ability (GCA) and specific combining ability (SCA) MS were significant for CMD
- GCA SS for male parents accounted for 12.5% and, GCA SS for female parents accounted for 19.6%, while SCA SS accounted for 67.9% of the crosses for CMD

- The high SCA SS for resistance to CMD signified predominance of non-additive gene action over additive gene action for this trait.
- Female parents, Bangweulu and Kampolombo had desired negative GCA effects for CMD. In terms of male parents, TMS190, Nalumino and TME2 were good combiners with negative GCA effects.
- The GCA SS at 70.2% of the crosses SS predominated over SS for fresh root yield.
- Bangweulu and Chikula had significant GCA effects for fresh root yield and are considered as good general combiners for the trait.

7.7 Progress in breeding for CMD resistance

Major strides were made in terms of developing CMD resistant progeny arising from different cross combinations using local cultivars and introductions as parents. The progeny on average had a lower disease severity than the parents an indication of a major improvement as reflected in the general negative heterosis for CMD. Similarly there was noticeable fresh root yield improvement in some of the families and specific clones. Among the best performing clones were genotype 8444 (3.35 kg plant^{-1}) from TMS3001 x Bangweulu and 3101 (2.85 kg plant^{-1}) from Bangweulu x TMS190. Mid parent heterosis for the above clones was more than 220%. These clones did not only perform better for fresh root yield but were also resistant to CMD. Furthermore, the progeny exhibited earliness at 7 MAP.

7.8 Further research

Further research is required to understand the implication of using the grafting technique on movement of the viruses within the plant. In addition, there is need to understand why the buds immediately below the graft showed symptoms (in susceptible genotypes) unlike buds further away from the grafted area of the same branch. There is need to evaluate the promising clones in other ecological regions for all the agronomic traits.

7.9 Implications of the research findings for cassava breeding and the way forward

Since there have never been formal efforts to develop cassava genotypes with CMD resistance and associated satellites in Zambia, it was important to develop a local breeding programme with specific emphasis on breeding for resistance to this disease. Breeding for resistance

breeding to CMD should be priotised if higher yields and more stable production are to be achieved by resource poor farmers in Zambia. As established through the CMD survey and PRA, incidence of the disease is high. Host or cultivar resistance offers the most cost effective strategy to managing cassava viruses compared to other control methods. The genotypes developed in this study when certified to be used by the farmers can be moved between districts and to regions free of CMD with reduced probability of introducing the disease. The results also revealed that most of the farmers recycle cuttings. Some of the recycled planting materials are often diseased thereby contributing to the spread of CMD. Extension support should be provided to farmers to inculcate good agricultural practices i.e. field sanitation and use of disease resistant planting materials.

High yield and early bulking are some of the most preferred attributes suggested by the farmers (Chapter 2). Most of the existing cultivars are harvested 2 to 3 years after planting. In this study some genotypes yielded substantially 7 MAP. It is recommended that the genotypes be evaluated further in different ecological regions of Zambia.

Farmers should be involved in the evaluation and selection stages of all future preliminary and regional yield trial. Selection of genotypes should be done together with farmers to achieve desirable targets (Gibson, 2005). Attention should be given to CMD resistance in association with farmer preferred traits. Farmer participation will ensure quick and successful adoption of the improved cultivars when eventually released.

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