Genetic and Root Growth Studies in Cassava (*Manihot esculenta* Crantz): Implications for Breeding

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Abstract

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Abstract
Cassava (*Manihot esculenta* Crantz) is a perennial tropical crop grown for its starch-containing tuberous roots. It is cultivated mainly by small-scale farmers and consumed daily by an estimated 500 million people. Cassava mosaic disease (CMD) has long been recognized as a major limiting factor to cassava production in Africa and severe epidemics hit Uganda in the late 1920's and late 1980's. In spite of its importance as a major food crop it is the least researched major crop and many questions regarding its genetics are still unresolved. This thesis has therefore dealt with studies on: *i)* the effect of CMD on the genetic diversity of cassava in Uganda, *ii)* the composition of varieties and the genetic structure within and between varieties on small farms in Uganda, *iii)* the genetic basis of two agronomic important traits, cyanogenic glucoside potential (CNP) and dry matter content (DMC) in cassava roots, using quantitative trait loci (QTL) mapping and *iv)* the effect of nutrient availability on the growth and tuber formation. The outcome of these studies is relevant for developing strategies for breeding and gene conservation programmes.

CMD did not have a strong selective effect on the genetic diversity of cassava in Uganda in spite of earlier reports on losses of varieties due to the latest CMD epidemic. However, a loss of rare alleles in areas with high CMD incidence in Uganda was found. The composition of varieties differed widely between villages and districts and the genetic variation was surprisingly large within varieties although the variation was larger among varieties. The like-named varieties in different villages were genetically similar, demonstrating farmers' ability to differentiate and maintain the same variety over large areas. We detected two QTL on two different linkage groups controlling CNP and six QTL on four different linkage groups controlling DMC. One QTL for CNP and one QTL for DMC mapped near each other, suggesting pleiotrophy or linkage of QTL. In the root studies, production of storage roots was found to be regulated by nutrient availability and appeared to be positively affected by a gradually increasing limitation of mineral nutrients during the growth of the plants.

Key words: cassava breeding, cassava mosaic disease, cyanogenic glucosides, dry matter content, genetic diversity, QTL mapping, quantitative trait loci, tuber formation

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To

Frank, Jeremy and those yet to come

"Unto the King eternal, immortal, invisible, the only wise God be glory and honour." I Tim.1:17; Rom. 16:27

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Balyejusa Kizito, E., Bua, A., Fregene, M., Egwang, T., Gullberg, U. & Westerbergh, A. 2005. The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda. *Euphytica 146*, 45-54.

II. Balyejusa Kizito, E., Chiwona-Karltun, L., Egwang, T., Fregene, M. & Westerbergh, A. 2006. Genetic diversity and variety composition of cassava on small-scale farms in Uganda: an interdisciplinary study using genetic markers and farmer interviews. *Genetica* (in press).

III. Balyejusa Kizito, E., Rönnberg-Wästljung, A.-C., Egwang, T., Gullberg, U., Fregene, M. & Westerbergh, A. Quantitative trait loci controlling cyanogenic glucoside and dry matter content in cassava roots (*Manihot esculenta* Crantz). (Submitted).

IV. Lundquist, P.-O., Kähr, M., Balyejusa Kizito, E., Gullberg, U. & Westerbergh, A. Growth and nutrient-regulated tuber formation in cassava (*Manihot esculenta* Crantz.). (Submitted).

Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial starchy root crop that is widely grown in the tropical regions of the world. Cassava gives a carbohydrate production per hectare which is about 40% higher than rice and 25% more than maize with the result that cassava is the cheapest source of calories for both human nutrition and animal feeding (Tonukari, 2004). Global production of cassava is around 152 million tons per year. Half of the 16 million hectares of cultivated cassava worldwide is devoted to small-scale cassava cultivation in Africa, 30 percent is grown in Asia and 20 percent in Latin America (CIAT, 2001). Small-scale farming is characterized by cultivation using traditional methods with little or no inputs and frequent intercropping practices. Cassava's role as a traditional human food is changing to an efficient industrial crop in some parts of Africa, for instance in Nigeria (Nweke, 2004) and many parts in Asia and Latin America.

In Uganda, cassava ranks second to bananas in terms of area occupied, total production and per capita consumption, respectively (Otim-Nape & Zziwa, 1990) and it is the leading food security base. Its importance as a food security crop formed the basis of my interest in doing research on the crop. Subsistence farming is faced with many challenges. In Uganda, where 80% of the population depends on subsistence agriculture as a mainstay, cassava production is constrained by factors such as limited adoption of improved varieties in some areas, limited land, lack of good quality planting material, pest and disease incidences and unreliable rainfalls resulting in food insecurity (Kiwanuka & Kintu, 2004). In spite its importance worldwide as a major food crop, cassava is the least researched crop among the major crops of the world. Deliberate effort to improve it through breeding began only recently compared with other major crops. Thus there are many fundamental questions regarding its genetics that are still unresolved. Therefore cassava breeding would largely benefit from increasing the knowledge on the genetic background of agronomic traits and the genetic diversity of cassava. My thesis will present data from a large scale study of the genetic diversity of cassava in Uganda (I), studies of the genetic diversity within and between varieties and how this relates with small-scale farming in Uganda (II), and the mapping of genes controlling the agronomically important root traits cyanogenic glucoside and dry matter content, (III). In addition, I will present data on the growth and tuber formation in cassava (IV).

Cassava: the biology, ecology and origin of the plant

Biology

Cassava (*Manihot esculenta* Crantz) is the only cultigen in the *Euphorbiceae* family (O'Hair, 1995). It is a shrub that grows erect to heights between 1.5-2 m although some varieties are known to reach 4 m. Cassava is monoecious with male and female flowers found on the same plant. The female flowers usually mature one to two weeks earlier than the male flowers (protogyny), a mechanism that enhances outcrossing (Ng & Ng, 2002). Cassava is insect pollinated, mainly by

bees (Hahn *et al.*, 1979). Under natural conditions as well as in plant breeding propagation by seed is common. Seeds normally undergo a dormancy period lasting months and require relatively high temperatures (30-35 °C) for optimum germination (Ellis *et al.*, 1982). Cassava is mainly propagated by stem cuttings. It has thickened, knobby stems resulting from shortened internodes, swollen leaf scars and enlarged stipule bases. The thickening, indicative of increased resource allocation to the stem, is thought to reflect artificial selection for ease of vegetative propagation (Ellis *et al.*, 1982). Cassava forms fibrous roots which can undergo increased tuberization. Due to cambium activity and deposition of starch grains in secondary xylem tissues, a few fibrous roots swell to produce storage roots. A plant can produce between 5 and 20 tubers depending on variety and soil conditions, with fewer roots forming in poorer soils. At maturity tuberous roots can be 15-100 cm in length and weigh up to between 0.5-2.0 kg (Knoth, 1993). Cassava storage roots are not reproductive organs and cannot be used as planting material. The roots start undergoing post-harvest physiological deterioration within a day or two after harvest and this consequently results in their short shelf life (Beeching *et al.*, 1998).

Cassava also produces potentially toxic levels of cyanogenic glucosides (Linamarin [95%] and lotaustralin [5%]) which are synthesized in the leaves (Koch *et al.*, 1992; Conn, 1994) and translocated to all other parts of the plant including the edible tuberous roots (McMahon *et al.*, 1995). The breakdown of cyanogenic glucosides results in hydrogen cyanide (HCN) production when cassava tissues are mechanically damaged. HCN in cassava tissues has been medically proven to be a potential health hazard for consumers if the plant is inadequately processed (Tylleskär *et al.*, 1992; McMahon *et al.*, 1995). There is a considerable variation in the root content of cyanogenic glucosides among genotypes but the level also depends on the growth environment (Mkumbira, 2002; Bokanga, 1994). Environmental factors during the growing season contribute significantly to the variation in CNP among genotypes as well as within genotypes and in various parts of the plant (Dixon *et al.*, 1994). The growth stage of the plant appears to have an effect on the cyanogenic glucoside build up; a high level occurring 120 days after planting (DAP) drops dramatically by 180 DAP coinciding with the beginning of the active root-bulking phase (Bokanga, 1994). Cassava varieties with high cyanogenic glucoside content (>1000 mg Hydrogen cyanide (HCN) equivalent kg^{-1}dry weight) are said to be toxic while cassava with low content of cyanogenic glucosides (<200 mg HCN equivalent kg^{-1}dry weight) are said to be safe for consumption without processing (Iglesias *et al.*, 2002). Traditionally, cassava roots are processed by a variety of methods (Ugwu & Ay, 1992) into many different products and used in diverse ways according to local custom and preference. However, some basic steps are followed. After peeling of the roots processing steps consist of grating, crushing, microbial fermentation, enzymic action or a combination of these. This is usually then followed by either heating or drying to reduce moisture content. The final stage in the processing of the roots is to make cassava flour.

Studies have been conducted on the biochemical pathway of cyanogenic glucosides in cassava. Cassava synthesizes and stores two structurally related cyanoglucosides, linamrin and lotaustralin, which accumulate in the leaves and

roots in the ratio of 93:7 respectively (Nartley, 1969). The cyanoglucosides are synthesized from the amino acids valine and isoleucine. A small family of cytochrome P450 genes (*CYP79D1* and *CYP79D2*) control the first step in the conversion of the amino acids in linamrin and lotaustralin synthesis (Andersen *et al.*, 2000). Multifunctional enzymes convert either valine or isoleucine to their respective oximes which are subsequently converted by another cytochrome P450 (CYP71E) to the respective hydroxynitrile. The hydroxynitrile is then glucosylated by uridine diphosphate glucosyltransferase to make the cyanogenic glucoside. Cyanogenic glucosides are typically stored in cell vacuoles and following tissue damage the glucosides get released allowing them to be de-glucosylated by glucosidase enzymes found in the cell walls (McMahon *et al.*, 1995). The product of the de-glucosylation, acetone cyanohydrin, is then decomposed to yield cyanide and acetone with the action of hydroxynitrile lyase. The decomposition can also occur spontaneously at pH> 5.0 or at elevated temperatures of > 35^{o}C.

Siritunga & Sayre (2003, 2004) developed transgenic acyanogenic plants, in which the expression of the *CYP79D1* and *CYP79D2* genes were selectively inhibited in the leaves, in a bid to eliminate cyanogens from cassava. However, the transgenic plants were unable to grow in the absence of reduced nitrogen (NH_3) suggesting that cyanide derived from linamarin is a major source of reduced nitrogen for cassava root protein synthesis (Siritunga *et al.*, 2004). It should be explained that in this experiment linamarin levels were greatly reduced both in the leaves and roots up to 99% in the transgenic plants whereas in non-transgenic sweet cassava varieties glucosides are produced in the leaves but to a much lesser extent transported to the roots as in bitter varieties. Among other cyanogenic species, for instance white clover, cyanogenesis is polymorphic and both cultivars and natural populations contain both cyanogenic and acyanogenic plants. In clover the polymorphisms are under relatively simple genetic control (Hughes *et al.*, 1984) unlike in cassava where the variation is quantitative and may be polygenically based (Dixon *et al.*, 1994). Cyanogenesis in the white clover protects it from predation by some species of mollusks (Hughes *et al.*, 1997). No acyanogenic cassava plant has yet been found (Bokanga, 1994). So far the only evidence of a differential pest attack on cassava with low versus a high CNP is found with the burrowing bug (*Cyrtomenus bergi*) in South America (Riis *et al.*, 2003). Formal breeding for low CNP in cassava has been fraught with the confounding effect of the environment on its expression and the long growth cycle of cassava. Since the biosynthesis of the cyanogenic glucosides is complex, with different enzymes involved and probably affected by many genes with influence from the environment, mapping regions in the genome affecting the phenotypic variation (quantitative trait loci, QTL) for the trait has the potential to make formal breeding for variety improvement more efficient.

Ecology

Typically the crop is grown between 30° north and 30° south of the equator, in areas where the annual mean temperature is greater than 18 to 20°C. It is traditionally grown in a savanna climate, but can be grown in areas with extreme rainfall. In moist areas it does not tolerate flooding. In droughty areas it looses its

leaves to conserve moisture, producing new leaves when rains resume. It takes 18 months or more to produce a crop under adverse conditions such as cool or dry weather. Under more normal conditions the crop reaches maturity in about 9 months. Cassava does not tolerate freezing conditions. In contrast to other staple crops, it grows well under marginal as well as favourable conditions of soil fertility and rainfall. It tolerates a wide range of soil pH, from 4 to 8 (Howeler, 1978), and is most productive in full sun. Cassava can grow indefinitely, alternating periods of vegetative growth, storage of carbohydrates in the roots and even periods of almost dormancy, brought on by severe climatic conditions such as low temperatures or prolonged water deficit (Alves, 2002). The implication is not only that the crop is found in an array of environments across the continent, but also that it can adapt to variations in relief, soils and cropping systems within the same agroecological zone.

Origin

Cassava is originally from South America, probably domesticated in the Amazon region (Olsen & Schaal, 2001; Olsen, 2004). The process of cassava domestication involved selection for root size, growth habit, number of stems and the ability of clonal propagation through stem cuttings (Jennings, 1979). About 98 species of *Manihot* are known. (Rogers & Appan, 1973). Sexual barriers within the genus appear to be weak, indicating a recent evolution of the genus (Nassar, 1982). Most traditional domestication hypotheses have envisioned the crop to be a "compilospecies" derived from one or more species complexes, either in Mexico and Central America (Rogers & Appan, 1973) or throughout the Neotropics (Sauer, 1993; Rogers, 1963; Ugent *et al.*, 1986). However, evidence provided from recent findings studying the glyceraldehyd 3-phosphate dehydrogenase (*G3pdh*) gene showed that the crop does not seem to be derived from several progenitor species, and cassava does not share haplotypes with *Manihot pruinosa*, a closely related, potentially hybridizing species (Olsen & Schaal, 1999). The *G3pdh* locus provides high levels of noncoding sequence variation in cassava and its wild relatives. The studies showed that cassava was likely domesticated from wild *M. esculenta* populations along the southern border of the Amazon basin. Furthermore, studies using single nucleotide polymorphisms (SNPs) and simple sequence repeat (SSR) markers showed that cassava was likely domesticated from the wild *Manihot* species, *Manihot esculenta* ssp. *flabellifolia* (Olsen & Schaal, 2001; Olsen, 2004). These findings have provided the clearest insight to date on cassava's origin.

Cassava use

The starchy roots are mainly used as human food, fresh when low in cyanogens, or in many processed forms and products, mostly starch, flour, and for animal feed. Cassava utilization patterns differ depending on the region of the world. In Africa the majority of cassava produced is used for food consumption with 50% in processed form and 38% in the fresh and boil form. Twelve per cent is used for

animal feed. In Latin America between 35 and 40 percent of the cassava production is used for food consumption and a third of the cassava produced is for animal feed, the remaining percentage being used in industry or for export. Starch represents an important use in Latin America; however, this use is more significant in Asia where cassava chips are exported to Europe for use in animal feed (Westby, 2002). Although cassava is primarily grown for its starchy tuberous roots, in some cultures in Africa young leaves are used as vegetables or as a constituent in a form of sauce eaten along with main staple meals (Lancaster & Brooks, 1983; Lutaladio & Ezumah, 1981). Cassava, however, produces cyanogenic glucosides, which is often rightly or wrongly seen as a health hazard to consumers, particularly the very poor of the population that depend on cassava as a staple crop. In many cassava farming and food systems, cassava varieties are classified based on their perceived toxicity determined by taste (Chiwona-Karltun *et al.*, 2004). Farmers' varieties with high levels of cyanogenic glucosides have in general bitter taste and are referred to as bitter while those with low levels of cyanogenic glucosides are called sweet or cool varieties. The cyanogens have been observed to protect the plant from herbivory (Riis *et al.*, 1994) as well as theft (Nweke *et al.*, 2002; Chiwona-Karltun *et al.*, 1998) improving food security for the small-scale farmers.

Introduction and spread of cassava in Africa and Asia

Cassava was first taken to Africa from Latin America as early as the 15th century by European traders as a potentially useful food crop. Cassava spread through Africa by a number of mechanisms. The most important appear to have been initial contacts with the Portuguese-Brazilian culture at the African coasts where the crop infiltrated inland through riverine trade routes. Cassava arrived and diffused first along the West African coast, while it arrived at the East African coast during the 18th century (Figure 1; Jones, 1959; Carter *et al.*, 1992). It was much consumed in Zanzibar also by the end of the eighteenth century (Jones, 1959). Both bitter and non-bitter varieties were grown at an early state after cassava was introduced to Africa. Distinction between early maturing and late-maturing varieties was important and the latter were used in areas where famine was a frequent threat (Jones, 1959). Many, if not all, late-maturing varieties were bitter (Jones, 1959). Cassava was a major crop in the areas bordering Uganda where bitter varieties predominated Central Congo ‚Jones 1959). Cassava may have reached Lake Victoria along trade routes from the east (Jameson & Thomas, 1970) or from the west (Purseglove, 1968). Langlands (1966) indicates that cassava could have arrived before 1920 to the Acholi (northern Uganda) from Sudan. It is also believed that Arab traders who followed Speke in 1862 brought the cassava to Uganda (Jones, 1959). It is claimed that cassava was spread from Buganda to the north-west (Bunyoro) around 1870, to the east (Busoga) in the late 19th century and to the north even later (Langlands, 1966). In most of the areas away from the coast and riverside trading posts in Africa cassava spread took place during the 20th century due to colonial powers encouraging its cultivation as a reserve against famine and the ability of the crop to survive locust attack.

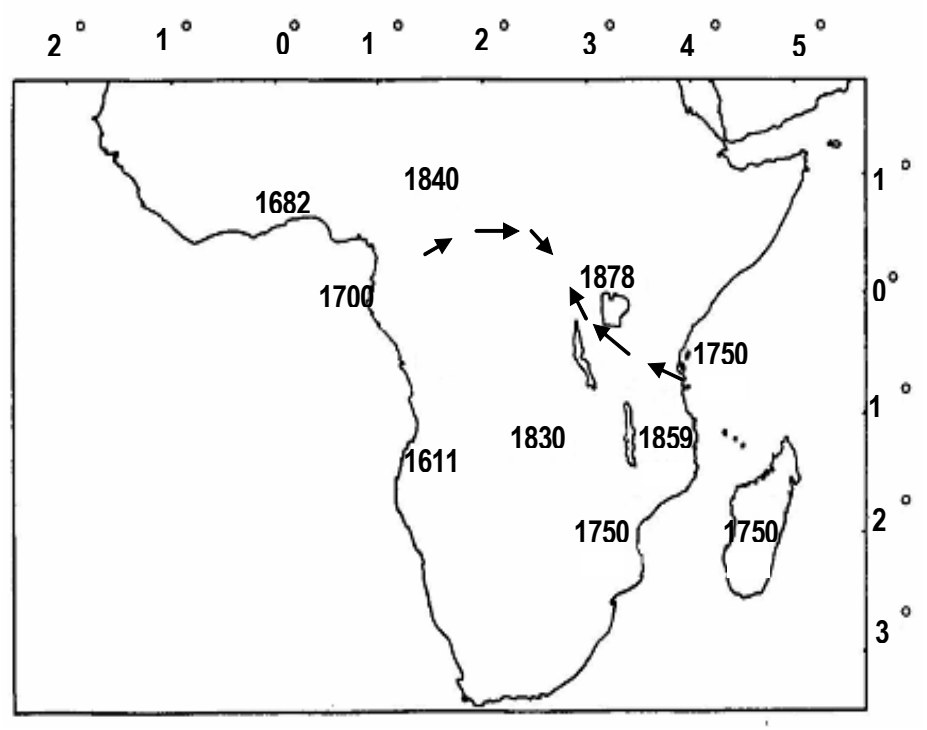

Figure 1. Year of cassava arrival and spread in Africa (Source: Carter *et al.*, 1992). Arrows show arrival routes into Uganda.

Also, the successful introduction in Africa and its cultivation in a great variety of African farming systems can be explained by its adaptability to a wide variety of ecologic and agronomic conditions. Due to its inherent tolerance to stressful environments, where other food crops would fail, it is often considered a food-security source against famine. It is a crop that requires minimal care for instance it is less labour intensive during the growth period compared to other crops and can be left for seven months to two years in the ground after maturity and harvested as needed.

Cassava was taken to Asia by European traders in the late 18th and 19th centuries. It was cultivated mainly on marginal lands by poor farmers as an emergency crop. By the 19th century cassava was an important food crop in southern India, Java islands in Indonesia, the Philippines and in China. It was introduced from Malaysia to Thailand during the period 1786-1840 (Sriroth *et al.*, 2000). In Malaysia and parts of Indonesia it was used mainly for the extraction of starch. Cassava products from Asia began to compete on world markets with similar products from Latin America by the end of the first half of the 20th century (Onwueme, 2002). Today, major cassava production in Asia is found in Indonesia, Thailand, India, China, Philippines and Vietnam.

Cassava in Africa

Total production of cassava in Africa increased from approximately 35 million tonnes in 1965 to over 80 million tonnes in 1995 (FAO, 1998). According to FAO (1999), the rate of increase of cassava production has been higher than any other crop in Africa since the 1980s (Hillocks *et al*., 2001). It is now one of Africa's major starchy staples second to maize, feeding 200 million people (Nweke, 2004). Most of the increase in cassava production has been due to increase in area under cultivation rather than increases in yield per hectare (Hillocks *et al.*, 2001). Increase in the cultivation of cassava during the 1990s occurred at least partly in response to declining soil fertility and increased costs of fertilizers for example in countries like Malawi, resulting in replacement of maize cultivation with cassava (Teri *et al.*, 1999). On the whole, the replacement of maize and other major crops with cassava in Africa have been fostered by the interplay of a wide range of agroecological and socio-economic factors which have resulted in a diversity of conditions under which crops are grown, marketed and consumed. The socio-economic factors include declining fallow periods due to population pressure, infrastructure and market access and the ecological factors include different abiotic and biotic stresses (Hillocks *et al.*, 2001). The interplay of these factors causes farmers to replace more demanding crops with cassava. In the Collaborative Study of Cassava in Africa (COSCA) survey, comprising 80% of the cassava cultivation in Africa, it was found that farmers are also continually abandoning old cassava cultivars and introducing new ones (Nweke, 1994) reflecting a need for better varieties.

Cassava mosaic disease

Most important among the biotic factors affecting cassava production are viral diseases, specifically cassava mosaic disease (CMD), which has long been recognized as a major limiting factor to cassava production in Africa. CMD is prevalent and causes severe losses in all the important cassava growing areas of Sub Saharan Africa. The disease is caused by viruses of the genus *Begomivirus* in the family Geminiviridae that are transmitted by the whitefly *Bemisia tabaci* and disseminated in the stem cuttings used routinely for propagation (Pita *et al.*, 2001). In Africa CMD was first described in 1894 under the name *Krauselkrankheit* (Fauquet & Fargette, 1990). In Uganda it was first recorded in 1928 (Martin, 1928) and in West Africa it was first recorded in the coastal areas of Nigeria, Sierra Leone and Ghana in 1929 (Fauquet & Fargette, 1990). After the second pandemic in Uganda in the 1990s, the disease has spread to other countries of eastern and central Africa and become a regional pandemic (Otim-Nape *et al.*, 1998; Legg, 1999; Otim-Nape *et al.*, 2000; Legg & Thresh, 2000; Legg & Fauquet, 2004). No record of CMD has been reported in Latin America. Conservative estimates of the production loss due to this pandemic in Africa amount to 12-23 million tonnes (Thresh *et al.,* 1997). The most visible symptom of the disease is the expression of the characteristic leaf mosaic and young plants are more severely affected than old ones. Two terms are commonly used to describe the effect of CMD in cassava (Thresh & Mbwana, 1998, Otim-Nape *et al.*, 1997, 1998, 2001). One is "disease incidence" which is the proportion of plants affected by the cassava mosaic virus in

a field and the second is "CMD severity" which is based on the score of the symptoms observed on single plants (Sseruwagi *et al.*, 2004). High disease incidences (disease occurrence in > 50% of the plants) in various parts of Africa have been associated with high inoculum availability from infected fields, population outburst of the vector and cultural practices of the farmers (Fauquet & Fargette, 1990). However, it is unclear whether the low incidence (disease incidence < 50%) of disease recorded in some areas is due to the inherent resistance of varieties or to the lack of inoculum (Thresh & Mbwana, 1998). The virus can infect all cultivars although disease susceptibility varies greatly (Fauquet & Fargette, 1990). CMD could cause a total devastation of some varieties with stunted growth and no yields while other varieties show only a few symptoms and may produce some yield. Some plants may also recover from CMD symptoms, showing no symptoms at all at later stages of growth- known, this is known as reversion (Calvert & Thresh, 2002; Otim-Nape *et al*., 2001). During the last CMD outbreak in Uganda 1989 many varieties were lost due to their high susceptibility (Otim-Nape *et al.*, 2001). Susceptibility refers to a condition in the host plant that allows the replication of the virus. Resistance relates to the degree to which the plant host can limit the effects of an infection, ranging from tolerance, in which symptoms are suppressed, to hypersensitivity, in which only a few cells surrounding the infected cells are affected, to immunity, in which the virus does not multiply due to lack of susceptible cells (*Definition*, Academic Press Dictionary of Science and Technology, 1992). Since the major pandemic in Uganda in the 1990s, the disease has spread to other countries of eastern and central Africa and become a regional pandemic (Otim-Nape *et al.*, 1998; Legg, 1999; Otim-Nape *et al.*, 2000; Legg & Thresh, 2000; Legg & Fauquet, 2004). No record of CMD has been reported in Latin America.

Farmers' management

Apart from its ecological adaptability, cassava also displays certain characteristics that make it adaptable to a variety of socio-economic conditions. Cassava offers the advantage of a flexible harvesting date, allowing farmers to keep the roots in the ground until needed. It's tolerance of low field labour inputs and variability in planting and harvesting dates makes it much less tightly constrained by seasonality compared with other staple crops. The majority of small-scale cassava farmers worldwide are self-reliant and self-provisioning for their planting materials with a few having access to planting material from breeding programmes (Manu-Aduening *et al.*, 2005). In managing their crops to meet their needs for food or cash the small-scale farmers involve various activities which include choosing which variety to grow, the size of the cultivated population allotted to a given variety, procurement of the planting material, the percentage to buy or exchange from various sources. Planting material procurement strategies include both saving from previous harvest and replacing it with cuttings of obtained from other farmers. This can lead to high turnover of varieties. For instance such turnovers of varieties occurred in Uganda especially during the CMD epidemics of the 1920s and late 1980s as farmers sought to ensure their food security when their varieties were being wiped out by the epidemic. Also there was a lot of germplasm

movement between districts with interventions from governmental and non governmental organisations to supply planting material in districts that had especially been hit by the epidemic. During sample collection for the genetic diversity studies we found that many farmers sought their planting material from distant relatives, some sourced planting material from forested fields that had been left in fallow while others actively propagated cassava seedlings (Figure 2).

Figure 2. Sources of planting material. (a) A volunteer seedling picked in the middle of a cassava garden (note tap root) (b) Farmers in a forest from where they obtained planting material (c) A farmer showing cuttings he had prepared for planting.

Farmers select and name their cassava according to complex values based on morphology, food, social and commercial interests. Their preferences determine the degree to which varieties will be adopted or change between human generations, evolve over generations or stay the same. Research on variety choice has revealed that farmers maintain local crop varieties in part because they perform better than other varieties in marginal environments (Brush, 1995). The observed performance of a cassava variety in the local environment and farming system with respect to the farmers preferences determines whether it will continued to be cultivated. In the process of acquiring and adopting new planting material the name of a variety may change as the needs and selection may be specific to different farmers. Thus variety names may differ among regions, among communities and even among families. In this thesis a farmer's variety is a group of plants identified by farmers under a single name.

Cassava genetic diversity

A primary concern of population geneticists and conservation biologists is to gain insight into the level and distribution of genetic diversity as well as the evolutionary processes shaping this diversity. Various evolutionary processes such as mutation, recombination, genetic drift, migration and natural selection influence genetic composition and genetic diversity in populations (Hartl & Clark, 1997). Factors such as population bottlenecks brought about by diseases, founder effects such as occurred with introgression of cassava into Africa may decrease genetic diversity. Factors such as the gene flow between cassava populations that occurs through seeds, pollen or actual movement of plants between localities under farmers management may increase genetic diversity in populations.

Molecular markers are recognized as significant tools to orient plant genetic resource conservation management, providing a means to accurately estimate the

genetic diversity and genetic structure for a species of interest (Hamrick & Godt, 1997). The genetics of cassava as a crop is interesting to study because it is a crop that has hardly undergone extensive breeding. Currently the pattern of genetic diversity within large cassava collections at the International Centre for Tropical Agriculture (CIAT), Colombia and the International Institute for Tropical Agriculture (IITA), Nigeria is well characterized but this does not necessarily reflect the extant genetic structure of varieties under cultivating conditions. Using simple sequence repeat (SSR) markers we found that the farmers' varieties consisted of high genetic diversity (II).

Molecular diversity has been studied in plants for over three decades now. Variation in allele frequency at many unlinked loci is the preferred way to assess genetic diversity and differentiation and to estimate the strengths of the various forces shaping the diversity (Nei, 1973; Wu & Tansley, 1993). A lot of work has been published on genetic diversity with the use of DNA markers in cassava. The genetic diversity of African cassava was evaluated using restriction fragment length polymorphisms (RFLP) (Beeching *et al.*, 1993), random amplified polymorphic DNA (RAPD) markers (Marmey *et al.*, 1994) and simple sequence repeat markers (SSR) markers (Fregene *et al.*, 2003; I, II). Molecular markers have been used in a number of studies and crops to see how farmers generally select and exchange planting material: in pearl millet (Djè *et al.*, 2000), in maize (Bellon & Brush 1994; in beans (Sperling & Loevinsohn, 1993), in cassava in Latin America (Elias *et al.*, 2000, 2001, 2004) and also in Africa (Mkumbira *et al.*, 2003; Fregene *et al.*, 2003, II). Studies on the genetic diversity of farmers' varieties are complex and may sometimes require an anthropological approach to better understand farmers' cultivation methods and how it affects the diversity of cassava in their fields. An amazing finding in one of the genetic diversity studies was that the diversity in a single field of a Makushi Amerindian community in southern Guyana was shown to be equal that of the core collection of 38 accessions representing the world's cassava collection held at CIAT (Elias *et al.*, 2000). This may show the underestimation of genetic diversity found on farmers' fields and that there might be a lot more than is known within research stations. Nevertheless, most of the diversity work done on cassava has elucidated the organisation of genetic diversity and differentiation of cassava from its centre of origin in Latin America to areas where it has been introduced, for example in Africa. The analysis of geographic pattern of variation for the crop helps in the choice of areas for plant exploration and germplasm assembly for breeding programmes. Molecular characterization of farmers' varieties can also help researchers better target any future support towards the farmers in terms of meeting their needs and preferences.

Farmers' management influences the genetic diversity of cassava. For instance, exchange of varieties is not uncommon among the farming communities in Uganda encouraging migration and thus effecting gene flow into and out of their farms. In South America exchange of germplasm has been documented for distances as large as over 300 kilometres (Chernela, 1987). Also, we found incidences where farmers during periods of scarcity of planting material were generating their own material from cuttings of cassava plants in forests or fields in fallow and from volunteer plants derived from sexyally reproduced seedlings or that they collected planting material from forests or fields in fallow (Figure 2). These seedlings are derived

from seeds that germinate after 'slash and burn' procedures as farmers prepare the land for cultivation and are therefore partly a result of natural selection. However, their survival is not entirely left to nature to determine but involves selection by farmers as well. Many are weeded out, however, a few may sometimes get selected if they look vigorous. This incorporation will increase the genetic diversity in the fields and the diversity within the next generation of cassava seeds within the field. The farmers tend to select for vigour among spontaneous seedlings, in so doing, they may indirectly be selecting for heterozygous genotypes. A few farmers had also interacted with government and non-governmental distribution and mitigation programmes of cassava planting material. Thus, in a typical small-scale farmer's field it is common to find a mixture of local varieties (varieties which have been grown continuously for over one farmer generation in the area (Berthaud *et al.*, 2001), and or local and improved varieties, which may be sweet, bitter or both. Like in Ghana (Manu-Aduening *et al.*, 2005) many of the varieties in Uganda that we found on small-scale farmers fields were old or local varieties, which are rather heterogeneous genetically (II) and a result of dynamic evolution involving both natural and human selection (Jarvis & Hodgkin, 1999).

Cassava breeding

Plant breeding, defined as the art and science of changing heredity of plants to improve their economic utility to man, is credited with all the useful changes in crop plants starting from domestication to the current era of modifying plants at molecular level (Chahal & Gosal, 2002). It may have started out merely as an art with selection to improve productivity of desired traits over generations through human perceptions without knowledge of the underlying genetic basis for the observed differences. Such a process of plant improvement continued through subtle modifications reflecting the potential of the plants to increase the food production. Perhaps this process still continues where subsistence or old agricultural practices continue to be used, such as slash and burn agriculture. This forms informal breeding and is conducted by small-scale farmers. With the advent and application of Mendelian and quantitative genetics, desired levels of improvement could be planned and predicted. Plant breeding was introduced into the use of a sound scientific base for crop improvement which is called formal breeding. Today formal breeding is supported by several other branches of science the latest of which is molecular biology.

Most research work involved with cassava began in Amani, Tanzania during the colonial era in 1906. Classical studies in transferring genes for resistance to CMD and cassava brown streak virus disease (CBSD) from related *Manihot* species began in 1937 (Nichols, 1947). Later, the creation of the International Institute of Tropical Agriculture (IITA) with headquarters in Nigeria and the International Centre for Tropical Agriculture (CIAT) in Colombia in the early 1970s brought in a new era of scientific cassava improvement and successful breeding projects (Jennings & Iglesias, 2002). Also National research institutes in most countries today play a key role in conducting research on cassava to meet local needs. Breeding objectives usually centre on increased yield, multiple pest and disease

resistance, desirable agronomic and consumer preference traits such as early bulking of storage roots, combined with high dry matter content and low cyanide content. Given the recent start in cassava improvement, local accessions still play a more relevant role in cassava breeding than in other major crop breeding schemes (Ceballos *et al.*, 2004). Even so, significant achievements of cassava breeding in Africa has been in the development of a range of elite genotypes such as TMS 30572 that combine high stable yields, agronomy and consumer quality with acceptable levels of resistance to CMD and cassava bacterial blight (CBB), the major threats to cassava production in Africa (Hahn *et al.*, 1989). The adoption of some of these genotypes in Uganda for instance averted the devastating effect of the CMD on cassava production (Otim-Nape *et al.*, 1994).

The definition of a farmer's variety involves a diverse genetic component and is complex as opposed to the definition of what a breeder's variety is as derived from a conventional breeding programme. According to the International Union for the protection of varieties Act of 1991 (articles 7, 8 and 9) the requirements of a bred variety usually are that it should fulfil the characteristics of distinctness, uniformity and stability over crop cycles. The essence of plant breeding is the discovery or creation of genetic variation in a plant species. Selection follows from within this variation for plants with desirable traits that can be inherited in a stable fashion. For this reason the breeders keep specified populations of characteristic traits from which selection for specific traits is made. These are the breeding populations. Plant breeders use all available technology both to create genetic variation and to select from within that variation. The plant breeders' final selections of superior plants will form the basis of one or more plant varieties which are released to farmers and also a portion maintained on the research station. This is the production population.

The selection cycle

Table 1 illustrates a typical selection cycle in a cassava breeding programme. A typical breeding programme begins with creation of recombination by crossing of elite clones which are selected based on their performance per se and ends with a few clones in the regional trials (RT) that have survived the selection process. The first selection is done in the second year on the nurseries with plants derived from botanical seed. At this stage, depending on the breeding programme selection for resistance to specific diseases such as cassava bacterial blight (CBB) or cassava mosaic disease (CMD) usually begins, resulting in about 3000 genotypes from an initial 100, 000 genotypes. At the following stage about 6-12 (Table 1) vegetative cuttings from each of the selected seedlings are planted in a clonal evaluation trial (CET). Selection at this stage is on highly heritable traits such as harvest index (Kawano, 2003) and plant types whose branching starts from 1m are favoured (Kawano *et al.*, 1978; Hahn *et al.*, 1979). Other traits of interest looked at are high dry matter content (DMC) and low cyanogenic glucoside potential (CNP) (Iglesias & Hershey, 1994). The preliminary yield trial (PYT) follows where 20-80 (Table 1) plants/clone are evaluated either in plots or replicated trials. Here low heritability traits such as yield start being evaluated. In the advanced yield trial (AYT) and the regional yield trials (RT) which follow, greater emphasis is put on

yield and stability across locations. Also, cooking quality characteristics are evaluated in these trials. Clones showing outstanding performance in RT are released as new varieties (production population) and often incorporated as parents in crossing nurseries (breeding population).

Table 1. A typical selection cycle in cassava, beginning with crossing of elite clones, through the different stages of the selection process (adapted from Jennings & Iglesias, 2002).

Year	Activity	Number of genotypes	Plants per Genotype
1	Crosses among elite clones	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 evaluation trial (CET)	2000- 3000[a, b]; 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], 20-18[c]	100- 500
6	Regional trials (RT)	5- 30 [a,b,c]	500- 5000

Figures for cassava breeding at [a] IITA (Ibadan, Nigeria); [b] CIAT (Cali, Colombia) and CIAT-Rayong Field Crops Research Centre (Thailand). Averages and data in Kawano (2003).

Selection for CNP and DMC

Selection for genetically complex traits such as CNP and high DMC in cassava is usually done after the crop has matured after 9 months. CNP has been reported to be negatively correlated with DMC in cassava and current breeding strategies involve breeding for high DMC, and in so doing, a low CNP may be bred (Dixon *et al.*, 1994). We have, however, not found any strong evidence for this correlation (III). Also, in other studies no clear correlation between CNP and most of the agronomic traits including DMC have been found as the correlation between the traits depended, not only on the population evaluated, but also on the influence of other environmental factors (Mahungu *et al.*, 1994).

Diallel studies, which are genetic analysis studies representing all possible F_1 crosses among a set of individuals, made up of 9-10 families in three different environments were designed in CIAT to study DMC between 2000 and 2002. It was observed that there are significant epistatic effects and that additive variance was considerably larger than dominance variance (Cach *et al.*, 2005; Perez *et al.*, 2005). Diallel studies are useful in identifying the presence of additive, dominance and epistatic gene actions in many genetic backgrounds. The studies can thus be used to estimate the degree of dominance and classify parents according to their breeding values (Chahal & Gosal, 2002). Further experiments with 12 different clones planted in two different sites in Malawi have shown that the genotype has a larger influence than the environment on DMC. For cassava farmers in Uganda it seems to be important that the varieties are sweet (II) but there is also a part of the population that prefer the bitter genotypes (Essers, 1995). While in other parts of Africa, for instance Malawi and Nigeria, bitter varieties are mostly grown (Westby,

2002). Our present knowledge about the pros and cons for bitter and non-bitter cultivars among small-scale farmers suggests that breeders should be prepared to develop genotypes with different CNP levels. This is because of increasing demand among small-scale farming communities in Africa with different cassava taste preferences, the demand for adapting the crop to new urban or industrial markets so that cassava can contribute more to improving their livelihoods from sales.

Selection for the small-scale farmer

Since resource-poor farmers operate under a wide range of environmental, social and economical conditions, it is unlikely that a single technical solution can be developed to suit all of them (Ashby & Sperling, 1995; Ceccarelli, 1994). For small-scale farmers the diversity within a variety is much more central for the food security of the small-scale farmer since it gives them the flexibility not only to cope with an unreliable, resource-poor environment, but also to manage their environmental variability to their best advantage. Therefore it might be more cost effective to giving them a range of products for instance genotypes from the breeding population, from which to choose and tailor according to their specific circumstances. To create a 'useful basket' of options, researchers must have a relatively good idea of the broad range of the farmers' needs and constraints and this can best be met through participatory research involving both farmers and researchers (Thro & Spillane, 2000). Also, in order to be able to use farmers' varieties in the development of breeding populations in cassava breeding programmes and for conservation of local varieties, it is of great interest to understand the genetic diversity and structure of farmers' cassava varieties.

Limitations of formal breeding

Formal breeding has certain distinguishing characteristics. It tends to be strongly linked to a formal variety release and seed dissemination systems. This has implications for the mechanisms needed to enable better diffusion and adoption of improved varieties from the research stations. For instance, although several improved cassava varieties show great potential in yield or disease resistance, studies in Africa have found that the adoption or diffusion of improved varieties among small-scale farmers is not widespread (Nweke, 1994; Manu-Aduening *et al.*, 2005). During interviews of farmers in eleven villages (II) we observed that varietal characteristics, specifically taste was important for adoption of improved cassava varieties. Also that the extent to which small-scale farmers in the studied areas readily adopted new cassava varieties depended on presence of disease pressures, access to market and an efficient extension system. It is often thought that the failure to deliver improved varieties to the farmers can be attributed to the communication gap between breeders and farmers (Thro & Spillane, 2000, Almekinders *et al.*, 2001). For a long time the development of many improved varieties has been carried out largely on research stations which may be far from farmers' settings geographically and environmentally. Obtaining farmers' participation in the selection process at various stages in the breeding cycle can take full advantage of their knowledge and preferences, speeding up the transfer

and adoption process without the involvement of complex procedures of variety release and inefficient extension systems (Hecht, 1999). However, government policy has a larger role to play than participatory breeding in ensuring that new technologies and in this case, new improved varieties, reach the people that most need them.

Progress in genetic improvement of cassava has also considerably been hindered by biological characteristics of the crop and the fact that there is still limited knowledge on the inheritance of traits of agronomic relevance in cassava. The biological constraints among others include a heterozygous genetic background making difficult consolidation of genetic gain in the breeding process due to inherent instability of the heterozygous status (CIAT, 2004), a long growth cycle and a poor knowledge of the organization of crop diversity (Fregene *et al.*, 2001). However, the heterozygous status creates variation within the crop and facilitates a directional selection of additive genes towards desirable traits. Most studied traits in cassava as well as in most crops are polygenic (Hahn *et al.*, 1989). Detection of polygenes for a trait by doing a genetic analysis in a specific cross alone and a single environment is not sufficient because of differences due to genotype by environment interaction. One of the most difficult problems in plant breeding is identifying plants or progenies with the desired traits. Besides, the effectiveness of selection may be more limited by the reliability of the screening method for the trait than by any other factor. The matter is further complicated because breeders usually evaluate, simultaneously in many populations, four or more quantitatively inherited traits. The success of a crop improvement program thus is highly reliant on the power and efficiency with which the genetic variability in the different populations can be manipulated. The ideal situation using formal breeding methods is that the trait has high heritability and that the phenotype can be observed easily before maturity. However this does not hold for cassava root quality characters since they are evaluated late in the growing season. Thus the breeding of a new variety can take between eight and twelve years and even then the release and adoption of an improved variety cannot be guaranteed. Hence breeders are extremely interested in new methodologies and/or technologies that can enhance efficiency to select for traits with cost effectiveness. Molecular marker technology offers such a possibility.

Molecular genetics in cassava

The advent of molecular DNA markers, genome studies and plant genetic transformation holds promise of providing ways around breeding obstacles of long growth cycle because selection can be made earlier in the growth cycle, even at the seedling stage. The detection of naturally occurring DNA sequence polymorphisms in different individuals within a species or group forms the basis for an application of DNA markers. In contrast to formal breeding methods that rely on the direct selection by phenotypic effect only, DNA markers use indirect selection by identification of desirable genotypes for quantitative traits like DMC earlier than the time such traits may be assessed phenotypically. DNA markers are detectable in all tissues and they are unaffected by environmental conditions.

Today, molecular techniques are at the forefront of much biological research — basic, adaptive and applied. Genetic markers have become fundamental tools for understanding the inheritance and diversity of natural variation. The earliest genetic markers in cassava were morphological (Graner, 1942; Hershey & Ocampo, 1989) followed by biochemical markers, such as isozymes (Hussain *et al.*, 1987; Ocampo *et al.*, 1992; Lefevre & Charrier, 1993a, 1993b). Over the last decade, a number of DNA markers have been developed and used in the study of genes, the cassava genome and genetic diversity in cassava. Marker systems such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Expressed Sequence Tags (EST), Amplified Fragment Length Polymorhpisms (AFLPs), Single nucleotide polymorphisms (SNPs), Simple Sequence Repeats (SSRs) also called microsatellites and others have been developed and applied. However, RFLP AFLP and SSR markers stand out as most effective in detecting polymorphism in cassava (Weising *et al.*, 2005). However, given the large amount of DNA required for RFLP detection and the difficulties in automating RFLP analysis and the dominance of AFLP markers and their requirement of high quality DNA to ensure complete restriction (Weising *et al.*, 2005) makes SSRs the markers of choice. DNA markers have greatly contributed to cassava breeding and genetics in the understanding of the phylogenetic relationships in the genus (Fregene *et al.*, 1994; Roa *et al.*, 1997; Olsen & Schaal, 1999; 2001; Olsen, 2004), assessment of genetic diversity (Beeching *et al.*, 1993; Mkumbira *et al.*, 2003; Fregene *et al.*, 2003; Elias *et al.*, 2000, 2001; I, II), development of genetic maps and identification of quantitative trait loci (QTL) for some traits of importance (Fregene *et al.*, 1997; Jorge *et al.*, 2001; Okogbenin & Fregene, 2002, 2003; Cortes *et al.*, 2002; III).

QTL mapping

One of the great uses of DNA markers has been in the construction of genetic linkage maps. Linkage maps indicate the position and relative genetic distance between markers along chromosomes, which is analogous to signs or landmarks along a highway (Collard *et al.*, 2005). They have been used for identifying chromosomal regions that contain genes controlling quantitative traits by QTL analysis. QTL analysis is based on the principle of detecting statistically an association between phenotype and genotype of the markers. The process of constructing linkage maps and conducting a QTL analysis is called QTL mapping or genome mapping (Paterson, 1996a, b; McCouch & Doerge, 1995). The simplest method for QTL mapping is analysis of variance (ANOVA) at the marker loci, sometimes called marker regression or single-marker analysis. Other methods such as interval mapping (Lander and Botstein, 1989), composite interval mapping (Jansen, 1993; Jansen and Stann, 1994; Zeng 1993, 1994) and most recently multiple interval mapping (Kao *et al*., 1999) have been developed. The first step in a QTL mapping experiment is usually to construct a mapping population (often at the F_2 level) that originates from parents that differentiate as far as possible for the specific trait of interest. The second step is to look for associations between genotypes and phenotypes in the (F_2) mapping population. If the parents carry different alleles for the QTL controlling a given trait the trait values in the segregating mapping population will be associated with the alleles of the markers

that are closely linked to the QTL. By scanning the markers on a linkage map for association with trait values, likely map positions for QTL can be detected, which is an important step towards understanding the inheritance and genetic basis of the traits. The accuracy of locating QTL is limited by a number of factors. Among the most important is the experimental design which includes factors such as having a small sample size. This affects the number of recombinants one can obtain and therefore a large sample size would greatly increase the chances of detecting QTL. Other factors that may influence the detection of QTL are environmental effects. Replicated phenotypic measurements or the use of clones can be used to improve accuracy of QTL mapping by reducing environmental effects.

Marker assisted selection (MAS)

Marker assisted selection (MAS) is the use of DNA markers for selection of desired individuals within a population, by linking the molecular marker to a desired trait. To be of maximal use, the markers should be closely linked to one or more target loci, which may often be quantitative trait loci (Goff & Salmeron, 2004). In the past decade many research institutes and breeding companies have began using molecular markers and MAS to increase the effectiveness of selection in breeding to shorten the development time of varieties (Ribaut & Hoisington, 1998). The markers used to probe the progeny of a cross may not be the QTL themselves but they are close to the QTL on the genetic map. Successful examples in MAS include marker assisted back cross (MABC) programmes. Using formal breeding programmes it typically takes 6-8 backcrosses to recover the recurrent parent genome. If tightly-linked markers flanking a QTL and evenly spaced markers from other chromosomes of the recurrent parent are used for selection, the recovery of the recurrent parent may be accelerated. This has been applied in maize reducing the number of generations needed for recovery of the recurrent genome in shorter time from eight to three generations (Frisch *et al.*, 1999). In maize breeding it has been used to transfer quality maize phenotype (QMP), controlled by a mutant allele of a gene called opaque2 from one elite maize inbred line to another elite maize inbred line (CIMMYT, 1999). While in rice, MAS has centered on pyramiding disease resistance genes, particularly to blight and blast (Koebner, 2003). In cassava, the application of MAS has been developed more recently compared to other major crops, with the construction of genetic linkage maps using RFLP, isoenzymes, SSR markers at CIAT (Fregene *et al.*, 1997; Mba *et al.*, 2001). Despite the low saturation of loci in the genetic maps of cassava, the marker loci are randomly distributed over linkage groups and the information from these maps has been utilised in cassava genetics. The use of these maps have led to the identification of several QTL for cassava bacterial blight (Jorge *et al.*, 2000, 2001), QTL for early root bulking, productivity and plant architecture (Okogbenin & Fregene, 2002, 2003) and QTL for DMC and CNP (III). In addition, genes for resistance to CMD have been mapped including a major one (CMD2) (Akano *et al.*, 2002). MAS for breeding CMD resistance has successfully been applied for introducing resistance into elite genepools at CIAT (Fregene & Mba, 2004; CIAT, 2003) and also to introgress resistance to cassava green mite (CGM) and CMD in local Tanzanian varieties (Kullaya *et al.*, 2004). The potential use of the identified QTL in cassava will be in pyramiding the disease resistance genes together with

those for agronomic traits in one genotype. Assembly of many genes and alleles from different loci is practically difficult through phenotypic analysis only. The QTL dispersed in different parental lines can be recombined through screening for molecular markers tightly linked to each of such genes.

Currently the cost of utilizing MAS is possibly the most important factor limiting the use in breeding programmes especially in the developing countries. It is thus envisaged that the use of MAS may remain restricted to large breeding programmes (Chahal & Gosal, 2002) such as are found in the Consultative Group for International Agricultural Research (CGIAR) centres like the International Centre for Tropical Agriculture (CIAT) and the International Institute for tropical Agriculture (IITA). Several other factors will also influence the efficiency of use of MAS research in the future: new developments and improvements in marker technology such as the use of single nucleotide polymorphisms (SNPs) (Collard *et al.*, 2005), the availability of high density maps and the integration of functional genomics with QTL mapping such as microarray analysis and expressed sequence tags (ESTs) (Morgante & Salamini, 2003; Anderson *et al.*, 2004). To enhance the efficiency of MAS, knowledge of DNA sequence of the gene enables the designing of the 'perfect marker' which is actually located within the actual gene sequence (Collard *et al.*, 2005). However knowledge of gene sequences for important traits especially in cassava is still an uphill task and may remain unknown for a long while. In the meantime scientists will continue to use QTL maps and markers that tag genes of agronomic importance.

Aims of the thesis

Not much is known about the genetics of cassava compared to other major crops in the world. Because of the increased importance of cassava in agricultural and economic development and in food security, particularly in Africa, it is imperative that more research should be conducted that could be helpful in its genetic improvement. With respect to this, the aims of this thesis were to:

- Assess the genetic diversity in different agroecological zones and areas with high and low incidence of CMD in Uganda
- To study the effect of the introgression history on the gene pool of cassava and the genetic differentiation between Latin America, East and West Africa
- Assess the composition of small-scale farmers' cassava varieties and the genetic variation within and between them in different villages in Uganda
- Study how the Ugandan small-scale farmers maintain and differentiate their varieties.
- Identify QTL for two important agronomic traits in cassava, CNP and DMC in cassava roots, using an F_2 population and elucidate the inheritance of these traits.
- Evaluate cultivation methods for cassava and study the importance of nutrients for tuber formation under controlled growth chamber conditions.

Study areas

The studied areas in the genetic diversity studies are shown in figure 3. Collections in paper I covered five agroecological zones in Uganda. CMD affected these zones differently. The areas are designated either as those with high CMD incidence (disease occurrence in >50% of the plants) or low CMD incidence (disease occurrence in <50% of the plants). Collections in paper II which involved more in-depth studies into the genetic composition of farmers' varieties covered three districts, Kumi, Luwero and Hoima.

Kumi district lies in the northern agroecological zone characterized by pastoral and crop farming with millet and cassava as the main crops. It lies at an altitude of between 760 and 900 meter above sea level and has an average annual rainfall of between 1,000 and 1,200 mm. Cotton used to be the major cash crop, however it suffered a drastic decline over the last two decades due to the collapse of government input supply and extension programmes. Cassava is important as a major food crop in the farming system and Kumi is also major dried cassava supplier to market (Otim-Nape *et al.*, 1997). The collapse of the cotton industry in

the 1970s due to the decline of administrative systems and efficiency in Government input supplies and extension programs led to the dependence on cassava as a cash source for farmers, particularly in Kumi. This dependence became emphasised in the late 80s with the outbreak of CMD where the most widely grown cultivars succumbed to the disease causing a loss of local varieties (Otim-Nape *et al.,* 1997). This was coupled with failure of the grain cereals due to drought and political unrest, which led to food insecurity. Consequently cassava mitigation programmes by government and non-governmental organisations (NGOs) have had a major impact on the varieties grown by the farmers. The villages visited were Atiira, Apama-Oteteen, Kachaboi and Omolokony. The major language spoken is Teso.

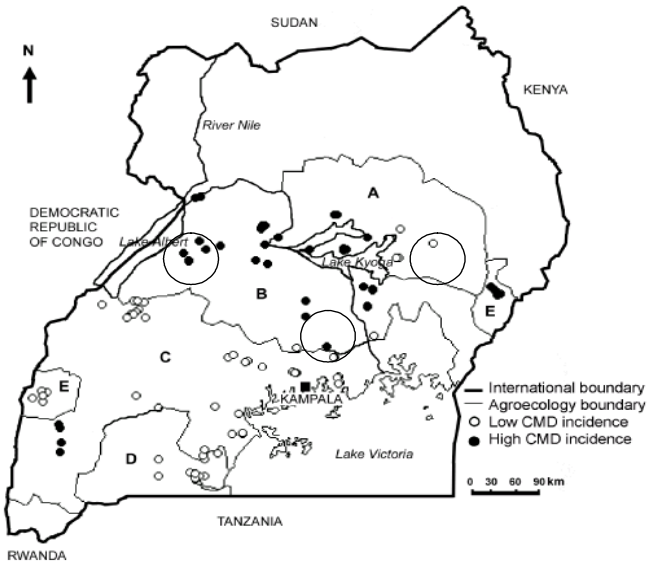

Figure 3. Map of Uganda showing the areas of cassava collection. Paper I: cassava collected in the high and low CMD incidence zones and also in agroecological zones; A- northern agroecosystem, B-banana/ millet/ cotton agroecosystem, C-Banana/ coffee agroecosystem, D- Pastoral ecosystem and E- mountain ecosystem. Paper II: collection sites circled; Kumi- eastern, Luwero- central and Hoima- western.

Luwero lies at an altitude of between 1,082m - 1,372m above sea level in the Southern and Western tall-grassland agro-ecological zone (tall-grassland area producing perennial and annual crops in mixed farming) with banana-coffee mixed farming systems. It was in the past scheduled for the valuable cash crop of robusta coffee and individuals have also taken advantage of other income generating opportunities, of which the most recent is horticultural farming favoured by the nearness of the markets in Kampala. Luwero also supplies fresh cassava roots to the Kampala markets. Due to their proximity to the Namulonge Agricultural Research Institute (NAARI) the farmers have been quite exposed to the varieties

developed by the cassava program especially after the recent CMD outbreak through various on-farm trials carried out in the area by the breeding program. The villages visited were Kabembe, Kibanga, Nattyole and Vvumba. Luwero district has many people of different origins and ethnic backgrounds. Among them include the Baganda, the original inhabitants of the district. Other ethnic groups include Banyarwanda, the Banyankole from Western Uganda, the Luo and Nubians of Sudanese origin.

Hoima on the other hand is in western Uganda bordering Lake Albert to the east. It has a population of about 341,700 people. It gets an average rainfall of 1,000mm with two heavy rain seasons running from March to May and from August to November. It lies at an altitude of 600m to 1000m above sea level and falls under the category of banana-millet-cotton agroecology. Hoima district has had the least influence from the government breeding programs or NGO activities. Cassava is a major food crop in Hoima. It supplies lower volumes of cassava to the Kampala market compared to Luwero or Kumi though the flour has higher quality (Graffham *et al.*, 2003). CMD has also affected the district but to a lesser extent than the either Luwero or Kumi (Otim-Nape *et al.*, 1997).The major language spoken is Runyoro and other tribes which include Rutoro, Rukiga, Alur and Rugungu.

Results and discussion

Genetic diversity of cassava

A primary concern of population geneticists and conservation biologists is to gain insight into the level and distribution of genetic diversity. To estimate the genetic diversity and the genetic structure within and between cassava varieties in Uganda as well as to understand the forces that have influenced the diversity and structure, molecular markers have been used

Genetic diversity between eastern and western Africa

It is known that there were two independent arrivals of cassava from Latin America (LA) to Africa. Cassava arrived and diffused earlier at the West African coast by the15th century compared with the 18th century in East Africa (Jones, 1959; Carter *et al*., 1992). The greatest genetic diversity for cassava exists in LA; although there is substantial diversity in Africa some factors have reduced it. Being mainly a vegetatively propagated crop, a reduction in genetic diversity due to the accumulation of systemic pathogens and the spread of a few, vigorous, adapted landraces could be expected. This together with possible founder effect during the crop's spread to Africa further influence the diversity and the genetic differentiation from the source.

In a dendrogram based on the unweighted paired group method with the arithmetic mean (UPGMA), illustrating the genetic relationship among cassava

variety groups, the varieties in Uganda and Tanzania in East Africa and Ghana in West Africa group closely together (Figure 2, I). Interestingly, the Nigerian varieties grouped closer to the LA varieties than to the varieties in the other African countries. Also pairwise calculations of the genetic differentiation between country variety groups (F_{st}) showed small differentiation between the varieties in Uganda and Tanzania in East Africa and Ghana in West Africa, and between varieties in Ghana and Nigeria in West Africa (average F_{st}=0.048). Interestingly, larger differentiation was found between Ugandan and Nigerian cassava varieties, and between Tanzanian and Nigerian varieties, than between varieties in Latin America and the African countries (average F_{st}=0.067). There being closer relationship between Uganda and Tanzania and Ghana, we conclude that there is no evidence for a separation into eastern and western African lineage according to routes of independent introduction when cassava first came to Africa. A possible difference in the genetic constitution of the introduced cassava material into East and West Africa may have been diminished by movement of germplasm between countries during the Amani breeding programme era. During this programme open pollinated seed of progenies from crosses that had been made between *M. glaziovii* and different cassava varieties were sent to many parts of Africa notably Uganda (Jameson, 1964), Kenya, Nigeria (Jennings, 1994) and Ghana (Doku, 1969). Uganda and Ghana may have got more closely related germplasm than what was received in Nigeria. Nigerian farmers tend to prefer bitter varieties that require processing as opposed to Ghana and Uganda where sweet, fresh and boil varieties tend to predominate (Nweke & Bokanga, 1994; Westby, 2002). A wider survey involving comparison of varieties from a larger number of east and west African countries, especially countries that have not interacted in germplasm exchanges would throw more light on the differentiation between East and west African cassava varieties.

Genetic diversity among agroecological zones in Uganda

In Uganda, the severe outbreaks of CMD were followed by large-scale governmental multiplication schemes of some cassava varieties and release of CMD resistant varieties and great loss of local varieties. Therefore one might expect to find a reduction of genetic diversity in the Ugandan cassava gene pool. Surprisingly high genetic diversity estimates were found for the Ugandan cassava genepool (Table 2, I). The smallest values for the average expected heterozygosity (corrected for small sample sizes, Nei 1978) occurred in the northern agroecosystem (0.487) while the montane agroecosystem had the highest value (0.594). The average observed heterozygosity estimates within populations were all above 0.5. This high genetic diversity is indicative of different alleles present in the population representing the existence of variation. This may suggest a high number of local varieties and that the farmers have had a major impact on the cassava gene pool by either maintaining the high genetic diversity by cloning or encouraging its creation by incorporating cuttings from voluntary seedlings. The partitioning of the diversity in the agroecological zones as indicated by the heterozygosity within populations averaged over the entire data set (H_s) was high at 0.545 while the average gene diversity between agroecological zones (D_{st})

however, was small (0.019). This indicates a high gene flow among agroecological zones. This gene flow is likely to be mediated by exchange of varieties among agroecological zones as well as the extensive distribution of varieties.

Difference in genetic diversity between Africa and Latin America

Twenty-seven alleles were found to be unique in Africa compared to 24 alleles unique to LA (I). Some of these alleles might have been introduced when the wild cassava species *Manihot glaziovii* was used as a donor of genes conferring resistance to CMD in the Amani breeding program, Tanzania, in the 30s and 40s. This might also indicate a response of selection to African conditions in the genepool. An interesting result was that there were 19 of the 38 rare alleles (occurred in <1%) found in Uganda that were unique to Uganda and Latin America only; five non-rare alleles were unique to both Uganda and LA, two in considerably high frequencies in Uganda of 16.1% and 10.2%. These two loci do not map near each other on the cassava genetic linkage map. We speculate that these might be a result of the offspring of one of the dominant varieties that were multiplied in the 1920s or a remnant of cassava that originally entered Uganda at its initial introduction in the late 19th century.

Effect of cassava mosaic disease on the genetic diversity

In spite of the repeated CMD outbreaks and large-scale multiplication schemes in Uganda's history the genetic diversity is high (Table 2, I). The values for the average expected heterozygosity (corrected for small sample sizes, Nei 1978) were not significantly different between the high and low CMD incidence areas (0.538 and 0.550, respectively). The average observed heterozygosity estimates within populations were all above 0.5 as well. However, a significant difference ($P<0.05$) was found with rare alleles - 33 rare alleles were found in areas with low CMD incidence compared to 13 in the high CMD incidence areas. This shows CMD has had an effect on the cassava gene pool in Uganda even though it could not be detected by using the heterozygosity estimates. When the population size is drastically reduced, as in the high CMD incidence areas, rare alleles are most susceptible to loss (Nei, 1975). Knowledge about the structure of genetic diversity within and among cassava areas is important for developing strategies for germplasm conservation and in participatory breeding programs.

Genetic diversity within and among farmers' varieties

The most grown cassava varieties were identified by the farmers in eleven villages in Uganda during interviews and cuttings of each identified plant were collected for genetic marker analysis. Each plant were also classified into four categories: (1) local – varieties which have been grown continuously for over one farmer generation in the area (Berthaud et al., 2001), (2) newly introduced – varieties acquired from outside the village (relatively recently within the last farmer generation), (3) improved – varieties from the Bukalasa or NASE cassava breeding programmes and (4) unknown – plants for which the farmers did not have a name.

We found considerable genetic variation both within and between cassava varieties though the variation was larger between varieties. All local, newly introduced and improved varieties consisted of several multilocus genotypes (I, Table 4) and surprisingly, in general no clones (accessions with identical multilocus genotype) were found. There was greater variation generally within recently introduced varieties compared with improved or local categories, and the unknown category had the most variation within it. However, most local and improved varieties showed predominating genotypes at many loci. Accessions of commonly grown varieties meeting farmers' preferences could therefore be selected and implemented in future breeding programmes involving development, dissemination and adoption. A difference in genetic structure within each of the variety categories was found. An excess of heterozygotes was found within the local varieties as indicated by the negative F_{is} value (-0.138) while the positive F_{is} values showed a deficiency of heterozygotes within the improved (0.051) and introduced varieties (0.130). The large genetic variation within the varieties and the structure observed in these varieties may be consequences of farmers' selection of volunteer seedlings produced by sexual recombination. The differences observed, however, between variety categories may be due to a combination of factors among which could be the history of the variety, its source and the farmer's management.

Farmers' knowledge and role in cassava genetic diversity in Uganda

We have found that varieties given the same name by the farmers in different villages showed relatively small genetic distance (Table 6, II) suggesting that the Ugandan farmers are quite knowledgeable, distinguish well their varieties and maintain the same variety over large areas. These findings concurred with farmers' knowledge on the occurrence of the two major breeding efforts that have taken place in Uganda, represented by the Bukalasa- and the NASE-series. The local varieties, where the Bukalasa varieties grouped, are most distinguished from NASE varieties while the unknown category largely clustered between the NASE and Bukalasa categories (Figure 4). The unknowns might represent recombination events between different varieties hence lacking the distinct features of known varieties to the farmers. Some farmers we found had incorporated cuttings obtained from volunteer seedlings they had found growing in their fields. We also noted that all unknown varieties were sweet varieties. This might suggest that giving a name to a plant may not be as important to Ugandan farmers as distinguishing its toxicity levels.

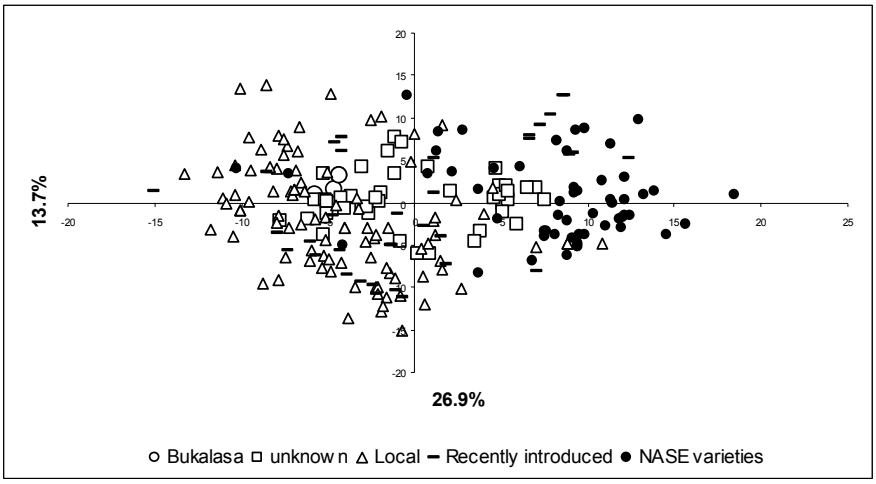

Figure 4. Distribution of cassava varieties as categorized by farmers in local, NASE, Bukalasa, recently introduced and unknown varieties based on 1- proportion of shared alleles and analyzed by principal component analysis (PCA).

Farmers are invariably keen to try out new varieties, these might be varieties they have seen in formal breeders' demonstration plots, or a local selection or a sample carried home from a trip to a distant relative. Farmers are no strangers to experimentation, but their perceptions and approaches to their experiments are often very different from formal breeding methods. An important difference between formal and informal (i.e. farmers) plant breeders lies in their management of spatial phenotypic variation. Among the characteristics of formal breeding programmes is that from a wide variation available to them they generally do selection exclusively for yield and pest or disease resistance and they promote varieties which can be grown over large areas or widely adapted in a geographical sense (Ceccarelli, 1994; Ceballos *et al*., 2004). Considering the diverse the area is over which cassava is grown; it is often harder to find varieties that are reasonably good everywhere. Whereas a farmer whose target is one small farm or even one field will seek the varieties that do best in that site, regardless of their performance elsewhere. Farmers thus influence the levels of genetic diversity as they collect different genotypes from different sources.

The relative importance of the Bukalasa-series to today's cassava smallholder farms does not considerably differ between the villages studied neither among different areas in the countrywide survey (unpublished data) but Bukalasa seems not to be of much importance over all based on the collection and farmers' naming. A possible explanation would be that the farmers' exchange of planting material during the half-century since the release of Bukalasa varieties has evened out the distribution. This may indicate that the Bukalasa-series may be of less importance in today's breeding population. The relative importance of the NASE-series on the other hand differs significantly over districts. A low estimate in Hoima district was expected considering there was no emphasis on this area in the multiplication schemes after the CMD outbreak *(*Otim-Nape *et al*., 1997*)*. This may, however,

indicate the farmer-to-farmer exchange of varieties from this series is slow considering that the first NASE varieties were released in the early 90s.

Composition of varieties based on farmers' naming

Going by the names given by the farmers in study II farmers fairly rapidly create new names on varieties that are released from breeding programs as observed for some NASE varieties having been given new names hardly a decade after their release e.g. Omongole for NASE1 . Five decades after the introduction of the Bukalasa-series nearly none of the plants had maintained names alluding to the Bukalasa name. The largest proportion (40%) of cassava varieties growing in Hoima were local varieties while in Kumi and Luwero the largest proportion (40% and 54%, respectively) of their varieties were from the NASE breeding programme. The difference in variety composition may reflect farmers' decisions made under their respective circumstances.

We observed that the choices made by the farmers over which varieties to grow were largely due to economic, for instance nearness to a market, and epidemiological reasons, like susceptibility of their varieties to CMD. The Ugandan farmers in our study tend to adopt improved varieties to a greater extent when there is a nearby market, a disease susceptibility problem (CMD epidemic) and good extension service.

The farmers especially in Hoima district (Figure 1) where there was apparent lack of extension work did not grow any of the most recently released improved NASE varieties and a very low frequency of the older Bukalasa varieties. In comparison with the farmers in the two other districts Luwero and Kumi, the Hoima farmers grew a larger frequency of introduced varieties, suggesting that the Hoima farmers have relied more on other farming communities for sources of planting material than the breeding programmes. Farmers in Kumi and Luwero on the other hand have had more access to planting material from the cassava breeding programme and therefore seem to rely more on planting material from there than from other farming communities. Access to urban markets by both Luwero and Kumi farmers has largely influenced the production choices made by the farmers in that they may have opted to use more material from the breeding programme since improved varieties are usually higher yielding than landraces. Kumi is a major dried cassava supplier to market (Otim-Nape *et al*., 1997) while Luwero supplies fresh cassava. Additionally, the recent CMD epidemic in Kumi and Luwero led to interventions by government and NGOs to restore cassava production and counter the heavy variety losses that were caused by the disease with improved varieties. Accordingly, the recently released NASE varieties have had high adoption rates in areas with high CMD incidence and strong influence by the breeding programme.

QTL for CNP and DMC

The study in paper III has been a first step towards identifying QTL for CNP and DMC in cassava. We used a mapping population (AM320) which is an S_1 population made from the variety Rayong 1 and Mcol1684 that was developed in CIAT. This mapping population was studied for CNP and DMC at five months after planting (MAP). A linkage map was earlier constructed using 95 SSR markers and 104 individuals of the AM320 population (CIAT, 2003). We re-constructed the linkage map based on an additional 95 individuals of the AM320 population and 15 new SSR markers using the MAPMAKER linkage analysis software, version 2.0 (Lander *et al*., 1987). The cassava genome was scanned for the presence of a QTL at 2.0 cM intervals using composite interval mapping (CIM) in the computer package QTL Cartographer version 1.15 (Basten *et al.*, 1997). We found two QTL on two different linkage groups (LG) controlling CNP and six QTL on four different LG controlling DMC (Figure 2, Table 2, III). One QTL for CNP and one QTL for DMC mapped near each other. The maximum likelihood positions of the different QTL for CNP and DMC varied in distance to their nearest flanking molecular marker locus. One of the QTL for DMC on LG3 mapped at the marker locus SSRY9 and the QTL for CNP on LG23 mapped close to marker locus NS119. The rest of the QTL for these two traits mapped in between their flanking marker loci. All the QTL for DMC and CNP reported showed a LOD score above 2.5 (Lander and Botstein, 1989). However, only the QTL on LG 10 for CNP near marker locus SSRY105 and the QTL on LG 3 at marker locus SSRY9 and the QTL on LG 6 closest to marker locus SSRY32 for DMC were significant according to the permutation test.

The QTL for CNP near marker locus SSRY105 had the larger additive effect (162 mg HCN equivalent kg^{-1} dry weight) while the other CNP QTL at locus SSRY242 also contributed considerably to the additive effect (99 mg HCN equivalent kg^{-1} dry weight). The DMC QTL on LG6 closest to marker locus SSRY45 showed the largest additive effect (2.4%) while the other QTL on LG6 for DMC showed the largest dominance effect (2.9%). The two QTL controlling CNP showed high additive effects while most QTL for DMC showed dominance or overdominance (Table 2, III). Overdominance may indicate a heterozygote advantage. The large dominance effect that we observed for DMC is in contrast to the diallel study by Cach *et al.* (2005) where additive effect plays a more important role than the dominance effect for DMC in cassava. This may be due to the specificity of the cross, differences in the environmental conditions and the age of the cassava plants at harvest. In addition, we may not have a complete picture of the genetic background of DMC since it is likely that we have not been able to detect all QTL. This may also be true for CNP.

The study, conducted in a single environment, had broad sense heritability for DMC of 0.42, comparable to the highest value found in studies on bred cassava clones in Nigeria conducted by Dixon *et al*. (1994). Broad sense heritabilities ranging between 0.50 and 0.97 for DMC were estimated by Kawano *et al*. (1998) at different evaluation stages in a breeding program for cassava in Asia. In an experiment with bred and local cassava clones in Malawi Benesi *et al*. (2004) found that a large part of the total phenotypic variation in DMC was due to genetic

differences. In our study a broad sense heritability of 0.43 was found for CNP in the AM320 population. The moderate to large heritabilities found for DMC and CNP indicate that the phenotypic variation of these traits is explained by a genetic component. However, it may be difficult to compare the heritability estimates of the different DMC studies discussed as different cassava material and plant age have been used.

Weak phenotypic correlation between CNP and DMC

We have found a weak negative correlation between CNP and DMC (III). Only one genomic region where a QTL for CNP and a QTL for DMC mapped together was detected. This may partly account for the weak phenotypic correlation found between these traits. The clustering of the two QTL could either be a result of pleiotropic effect where a single QTL affects the expression of both CNP and DMC. Alternatively, it could be two closely linked QTL, each controlling one of the traits. The finding that most QTL for DMC did not map near QTL for CNP shows that these traits are at least partly controlled by different genetic backgrounds.

Implications of the QTL found on cassava breeding

Formal breeding has so far contributed to some varieties with improved DMC as well as reduced CNP both in Africa and Latin America (CIAT, 1999; IITA, 1999, 2000). The identification of QTL for CNP and DMC would provide a tool to enhance the efficiency of selection in cassava breeding. Our study provides a preliminary insight into the control of the above traits especially at a pivot point in the growth and development of the roots five MAP. During the growth and development of the cassava roots cyanogenic glucosides build up; a high level occurring four MAP dropping dramatically by five MAP coinciding with the beginning of the active root-bulking phase or the accumulation of dry matter in the roots (Bokanga, 1994). The QTL that we have been detected for CNP and DMC at 5 MAP may therefore not be found for cassava plants in later developmental stages, plants grown in other environments or in crosses between other varieties. The result of this study is therefore limited to this particular cross and at a relatively early plant age, and cannot be directly used in cassava breeding programs.

The effective use of molecular markers linked to the QTL identified however, will require first a validation of the markers through testing them in a larger population, in many environments and thereby achieving a more accurate performance regarding the stability of the QTL across environments. For purposes of allocation of resources this same population can be used to study several other traits. One of the useful applications, with the new knowledge of the markers for CNP and DMC in a cassava breeding programme would be genotype building. There are QTL known for resistance to CMD and cassava green mite and favourable alleles are present in different breeding populations. Genotype building strategies can be applied to design new genotypes that combine favourable alleles

at all loci. When the desired populations have been put together selection is then based on the molecular score alone which is determined by the genotypes at those loci estimated indirectly by the markers linked to those loci. Starting from a cross between two parents the simplest genotype building involves screening a population with individuals with the desired markers. The best of these selections are then crossed to elite lines of the appropriate pool to capture more genes of adaptation or yield for instance. The resulting hybrids are selected with markers to eliminate those progeny that do not have the desired alleles leaving a smaller number of progeny to be thoroughly evaluated in the regular breeding programme.

Root tuber formation and growth

In efforts to enhance cassava crop yield it is important to understand physiological and nutritional factors regulating tuberous root formation. Also, for studies of genetic diversity and content of the toxic cyanogenic glucosides in the storage roots it is essential to use uniform and stable growth conditions. In paper IV we investigated the role of nutrient availability in the control of storage root production in two different growth systems. The first experimental treatment was cultivation in pots with mineral wool as support and with regular addition of nutrients. In pots the volume and amount of substrate was fixed, the capacity to hold nutrients was therefore also fixed. During plant growth, which initially would be at an exponential rate, this method will eventually not be able to meet the increasing nutrient demand by the plants if the growth rate is to be kept. The second experimental treatment was cultivation of plants in a hydroponic system in which addition of nutrients was computer-controlled and continuously monitored by pH and conductivity assays. In the hydroponic computer-controlled cultivation system plants were maintained at steady-state nutrient status at a generally high reproducibility. The experiments were performed under non-limiting and limiting nutrient conditions. Under non-limiting conditions, the plants had free access (FA) to all nutrients. Under limiting nutrient conditions, nutrients were supplied at relative addition rates (RAR) of 0.05 day-1 or 0.10 day-1 maintaining proportions among nutrients and as described in the experimental protocols (Ingestad and Lund, 1986). Cultivation in growth units was as follows. Rooted cuttings were weighed, put into growth units set at FA to nutrients. The units were used in 3 different growth protocols. Protocol 1 was to keep the plants at FA to nutrients for 34 days. In the protocol 2 and 3, the nutrient solution remaining in the growth units was replaced with de-ionized water after 19 days and a RAR of nutrients was set to 0.10 day-1. The starting level was based on calculations of nutrient content from plant weights. In protocol 2, the plants were kept at the RAR of 0.10 day-1 for 34 days until harvest. In protocol 3, the RAR was kept at 0.10 day-1 and after 12 days decreased to 0.05 day-1 that was maintained for 22 days until harvest. At harvest, plants were divided into parts and their fresh weight determined. Plant growth was calculated for the last 34 days as relative growth rate (RGR) as $RGR = \ln (w2/w1)/(t2-t1)$ where weights (w) and time (t) are at harvest and start of experiment. For protocol 2 and 3 the beginning of the RAR of 0.10 day-1 was considered the start of the growth experiment.

The results showed that the allocation of biomass to shoot and root were very similar among plants within treatments although the plants differed in final size. Plant growth in the growth units was affected by gradually decreasing nutrient availability as seen in the FA-0.10 treatment and even more in the FA-0.10-0.05 treatment as the decreased overall growth rate (Figure 2A, IV) and as the decreased growth of shoot, i.e. an increased proportion of biomass allocated to roots (Table 1, Figure 2B, IV). This shows that the plants had adjusted their growth patterns to the nutrient availability.

Storage root formation was highest for plants grown in big pots to about the same size as in the growth units while no storage roots were formed in the FA treatment in the hydroponic system. In the nutrient-limited treatments in the hydroponic system thickened roots appearing as tubers were produced in the FA-0.10 treatment and even more in the FA-0.10-0.05 treatment. In conclusion, the occurrence and proportion of tuber biomass increased with more restricted nutrient availability. This supports the hypothesis that the production of tubers is determined by plant nutrient status. It is proposed that when mineral nutrients gradually becomes limiting during the time-course of growth the plants produce storage roots.

Future perspectives

The work done in this thesis has shed more light on various aspects of cassava from the genetics to the factors influencing tuber formation. However, a lot more needs to be done and understood before the full potential of this crop for the small-scale farmers is realized. For instance more exploration into the informal sources of planting material for the small-scale farmers needs to be understood if they are to benefit from formal cassava breeding. In areas where the extension system is poor they rely on sources from their neighbours, relatives, local markets or even use of material derived from spontaneous seedlings. The small-scale farmers worldwide adopt strategies based on both intraspecific diversity (diversity within a single crop) and interspecific diversity (intercropping different crops within the same field) to provide yield stability and harvest security in the face of pests, diseases, competition and unfavourable environments. Farmers themselves may not value crop genetic resources directly but rather indirectly, by valuing specific attributes (ability to yield well in their field, taste, plant architecture etc.) of the crop populations. Therefore in the conservation of genetic resources farmers should be encouraged to continue to select and manage their local populations. This can be done through the educational or promotional programmes that set out to promote the value of local and diverse crop populations to farmers who might otherwise stop growing them. Also encouraging the increased use of local cassava varieties in the breeding and improvement programmes is another way. More evaluation needs to be done to understand farmers' role, both deliberate and unconscious, in the generation and maintenance of the genetic diversity of their cassava varieties. Evidence of the nature and causation of genetic change is

important for an understanding of on-farm conservation of varieties. Also a wider survey involving comparison of varieties from a larger number of east and west African countries, especially countries that have not interacted in germplasm exchanges would throw more light on the differentiation between East and west African cassava varieties.

More knowledge in the form of identified QTL has been shed on the genetic control of DMC and CNP, important agronomic traits in cassava improvement programmes. However, the classical breeder is far from being able to utilise the QTL identified for routine MAS programme. More testing needs to be done before these can be applied in routine cassava MAS programmes. For instance, would the same QTL be observed even at maturity (after 9 MAP) or in different environments and population backgrounds? This is because markers that are not yet adequately tested before use in MAS programmes may not be reliable for predicting phenotype. Prior to the use of the markers processes such as high-resolution mapping, validation of the markers and possibly marker conversion need to be carried out. A saturated linkage map with markers evenly spaced throughout the whole genome is a prerequisite for a more detailed QTL analysis. This is because even the closest markers flanking the QTL may not be tightly linked to a gene of interest (Michelmore, 1995). To this end the mapping of additional markers on the cassava genetic map needs to be done to saturate the framework map.

Although the experiments described above have not resolved fully the nutrient availability for the production of mature storage roots, possible roles of nutrients on traits investigated in genetic studies should be kept in mind in future research. The hydroponic system used in the present study has advantages in the ability to maintain a steady-state nutrient status at a generally high reproducibility and therefore more information can be obtained especially for controlled genetic studies. But, also other cultivation systems such as pots can be used to add nutrients at varied addition rates and should be evaluated in future research. In field trials the role of nutrients should also be investigated by nutrient additions or at least taken into consideration when interpreting results of general field trials.

References

Akano, A.O., Dixon, A.G.O., Mba, C., Barrera, E. & Fregene, M. 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theoretical and Applied Genetics 105*, 521-525.

Almekinders, C.J.M. & Elings, A. 2001. Collaboration of farmers and breeders: participatory crop improvement in perspective. *Euphytica 122*, 425-438.

Alves, A.A.C. 2002. Cassava botany and physiology. In: *Cassava: Biology, Production and Utilization.* (Eds. A.C. Belloti, J.M. Thresh &.R.J. Hillocks). CAB International, Wallingford, Oxon, UK, pp 67-89.

Andersen, M.D., Busk, P.K., Svendsen, I. & Møller, B.L. 2000. Cytochromes P-450 from Cassava (M. Sculerts Crantz) catalyzing the first steps: the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin cloning, functional expression in *Pichia pastoris*

and substrata specificity of the isolated recombinant enzymes. *Journal of Biological Chemistry 275*, 1966-1975.

Anderson, J.V., Delseny, Fregene, M.A., Jorge, V., Mba, C., Lopez, C., Restrpo, S., Soto, M., Piegu, B., Verdier, V., Cooke, R., Thome, J. & Horvath, P.D. 2004. An EST resource for cassava and other species of Euphorbiacea. *Plant Molecular Breeding 56*, 527-539.

Ashby, J.A. & Sperling, L. 1995. Institutionalizing participatory, client-driven research and technology development in agriculture. *Development and change 26,* 753-770.

Basten, C.J., Weir B.S., Zeng, Z.B. 1997. *QTL cartographer: a reference manual and Tutorial for QTL mapping*. Department of Statistics, North Carolina State Univ., Raleigh, North Carolina.

Beeching, J.R., Ham, Y.H., Gomez-Vasquez, R., Day, R.C. & Cooper, R.M. 1998. Wound and defence responses in cassava as related to post harvest physiological deterioration phytochemical signals and plant microbe interactions. *Recent Advances in Phytochemistry 32*, 231-249.

Beeching, J.R., Marmey, P., Gavalda, M.C., Noirot, M., Haysom, H.R., Hughes, M.A. & Charrier, A. 1993. An assessment of genetic diversity within a collection of cassava (*M. esculenta* Crantz) germplasm using molecular markers. *Annals of Botany 72*, 515-520.

Bellon, M.R. & Brush S.B. 1994. Keepers of maize in Chiapas, Mexico. *Economic Botany 48*, 196-209.

Benesi, I.R.M., Labuschagne, M.T., Dixon, A.G.O. & Mahungu, N.M. 2004. Stability of native starch quality parameters, starch extraction and root dry matter of cassava genotypes in different environments. *Journal of the Science of Food and Agriculture. 84,* 1381-1388.

Berthaud, J., Clement, J.C., Emperaire, L. Louette, D., Pinton, F., Sanou, J. & Second, G. 2001. The role of local-level gene flow in enhancing and maintaining genetic diversity. In: *Broadening the Genetic Base of Crop Production.* (Eds. H.D. Cooper, C. Spillane & T. Hodgkin). CAB International, Wallingford, Oxon, UK, pp 81-103.

Bokanga, M. 1994. Distribution of cyanogenic potential in the cassava germplasm. *Acta Horticulturae 375*, 117-123.

Brush, S.B. 1994. Providing farmers' rights through in situ conservation of crop genetic resources. *A report to the commission on Plant Genetic Resources*. University of California, Davis.

Brush, S.B. 1995. In situ conservation of landraces in centres of crop diversity. *Crop Science 35*, 346-354.

Cach, N.T., Perez, J.C., Lenis, J.I., Calle, F., Morante, N. & Ceballos, H. 2005. Epistasis in the expression of relevant traits in cassava (*Manihot esculenta* Crantz) for subhumid tropics. *Journal of Heredity 96*, 586-592.

Calvert, L.A. & Thresh, J.M. 2002. The viruses and virus diseases of cassava. In: *Cassava: Biology, Production and Utilization.* (Eds. A.C. Belloti, J.M. Thresh & R.J. Hillocks). CAB International, Wallingford, Oxon, UK, pp. 281-300.

Carter, S.E., Fresco, L.O. & Jones, P.G. & Fairbairn, J.N. 1992. *An atlas of cassava in Africa: historical, agroecological and demographic aspects of crop distribution*. CIAT, Cali, Colombia.

Ceballos, H., Iglesias, C.A., Perez, J.C. & Dixon, A.G.O. 2004. Cassava breeding: opportunities and challenges. *Plant Molecular Biology 56*, 503-516.

Ceccarelli, R. 1994. Specific adaptation and breeding for marginal conditions. *Euphytica 77*, 205- 219.

Chahal, G.S. & Gosal, S.S. 2002. *Principles and Procedures of Plant Breeding. Biotechnological and Conventional Approaches*. Alpha Science International Ltd. Pangbourne England. 585 pp.

Chernela, J.M. 1987. Os cultivares de mandioca na area do Uaupés (Tukano). In: *Suma: Etnologia Brasiliera, I, Etnobiologia.* (Ed. B. Ribeiro), FINEP, Petrópolis, Brazil. pp. 151-158.

Chiwona-Karltun, L., Brimer, L., Saka, J.D.S., Mhone, A.R., Mkumbira, J., Johansson, L., Bokanga, M., Mahungu, N.M. & Rosling H. 2004. Bitter taste in cassava roots correlates with cyanogenic glucoside levels. *Journal of the Science of Food and Agriculture 84*, 581-590.

Chiwona-Karltun, L., Mkumbira, J., Saka, J., Bovin, M., Mahungu, N. and Rosling, R. 1998. The importance of being bitter - a qualitative study on cassava cultivar preference in Malawi. *Ecology of Food Nutrition 37*, 219-245.

CIAT, 1999. Project IP3, Improved cassava for the developing World. *Annual Report 1999.* Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT, 2001. Project IP3, Improved cassava for the developing World. *Annual Report 2001.* Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT 2003. Project IP3, Improved cassava for the developing World. *Annual Report. 2003*. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT 2004. Project IP3, Improved cassava for the developing world. *Annual Report. 2004*. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIMMYT. 1999. Maize production in drought-stressed environments: Technical options and research resource allocation. *CIMMYT 1997/98 world maize facts and trends.* Mexico D.F.: CIMMYT.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B. & Pary, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica 142*, 169-196

Conn E. 1994. Cyanogenesis – a personal perspective. *Acta Horticulturae 375*, 31-43.

Cortes, D.F., Reily, K., Okogbenin, E., Beeching, J.R., Iglesias, C. & Tohme, J. 2002. Mapping wound-response genes involved in post-harvest physiological deterioration (PPD) of cassava (*Manihot esculenta* Crantz). *Euphytica 128*, 47-53.

Dixon, A.G.O., Asiedu, R. & Bokanga, M. 1994. Breeding of cassava for low cyanogenic potential: problems, progress and perspectives. *Acta Horticulturae 375*, 153-161.

Djè, Y., Heuertz, M., Lefebvre, C. & Vekemans, X. 2000. Assessment of the genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellites. *Theoretical and Applied Genetics 100*, 918-925.

Doku, E.V. 1969. *Cassava in Ghana*. Ghana University Press, Accra. 44 pp.

Elias, M., Mühlen, G.S., McKey, D., Roa, A.C. & Tohme, J. 2004. Genetic diversity of traditional South American landraces of cassava (*Manihot esculenta* Crantz): an analysis using microsatellites. *Economic Botany 58*, 242-256.

Elias, M., Panaud, O. & Robert, T. 2000. Assessment of genetic variability in a traditional cassava (*Manihot esculenta* Crantz) farming system, using AFLP markers. *Heredity 85*, 219-230.

Elias, M., Penet, L., Vindry, P., MacKey, D., Panaud, O. & Robert, T. 2001. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. *Molecular Ecolology 10*, 1895-907.

Ellis, R.H., Hong, T.D. & Roberts, E.H. 1982. An investigation of the Influence of Constant and alternating temperature on the germination of cassava seed using a two-dimensional temperature gradient plate. *Annals of Botany 49*, 241-246.

Essers, A.J.A 1995. Removal of cyanogens from cassava roots: studies on domestic sun-drying and solid substrate fermentation in rural Africa. PhD Thesis, Wageningen Agricultural University, The Netherlands.

FAO production Yearbook. 1998. FAOSTAT database. http://apps.fao.org/cgi-bin/nph_db.pl

FAO. 1999. FAOSTAT database. www.fao.org.

Fauquet, C. & Fargette, D. 1990. African cassava mosaic-virus-etiology, epidemiology and control. *Plant Disease 74*, 404-411.

Fregene, M., Angel, F., Gomez, R., Rodriguez, F., Chavarriago P., Roca, W., Tohme, J. & Bonierbale M. 1997. A Molecular genetic Map of cassava (*Manihot esculenta* Crantz) *Theoretical and Applied Genetics 95*, 431-441.

Fregene M. & Mba C. 2004. Marker-Assisted Selection (MAS) In: *Cassava Breeding*. (Ed. Hershey C.). FAO, Rome Italy.

Fregene, M., Okogbenin, E., Mba, C., Angel, F., Suarez, M.C., Guiterez, J., Chavarriaga, P., Roca W., Bonierbale, M. & Tohme, J. 2001. Genome mapping in Cassava improvement: challenges, improvements and opportunities. *Euphytica 120*, 159-165

Fregene, M.A., Suarez, M., Mkumbira, J., Kulembeka, H., Ndedya, E., Kullaya, A., Mitchel, S., Gullberg, U., Rosling, H., Dixon, A.G.O., Dean, R. & Kresovich, S. 2003. Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. *Theoretical Applied Genetics 107*, 1083-1093.

Fregene, M. A., Vargas, J., Ikea, J., Angel, F., Tohme, J., Asiedu, R.A., Akorada, M. O. & Roca, W.M. 1994. Variability of chloroplast DNA and nuclear ribosomal DNA: cassava (*Manihot esculenta* Crantz) and its wild relatives. *Theoretical and Applied Genetics 89*, 719-727

Frisch, M., Bohn, M. & Melchinger, A.E. 1999. Comparison of selection strategies for marker-assisted backcrossing of a gene. *Crop Science 39*, 1295-1301.

Graffham, A., Kleih, U., Jagwe, J., Nabawanuka, J., Wanda, K., Kalunda, P., Ntibarikure, G. & Ferris, S. 2003. A market opportunities survey for value-added utilization of cassava-based products in Uganda. Part 1: Demand analysis for industrial utilization. *ASARECA/ IITA Monograph 3*. IITA, Ibadan, Nigeria.

Graner, E.A. 1942. Genetica de Manihot. I. Heriteriada de ca formafotha e da coloracao da pelicula externa das raices en *Manihot utilissima* Pohl. *Bragantia 2*, 13-22.

Goff, S.A. & Salmeron, J.M. 2004. Back to the future of cereals. *Scientific American 291*, 42-49.

Hahn, S.K., John, C., Isoba, G., & Ikoun, T. 1989. Resistance breeding in root and tuber crops at the International Institute for Tropical Agriculture (IITA), Ibadan Nigeria. *Crop Protection 8*, 147-168.

Hahn, S.K., Terry, E.R., Leuschner, K., Akobundu, I.O., Okali, C. & Lal, R. 1979. Cassava improvement in Africa. *Field Crops Resources 2*, 193-226.

Hamrick, J.L. & Godt, M.J.W. 1997. Allozyme diversity in cultivated crops. *Crop Science 37*, 26-30.

Hartl, D. & Clark, A. 1997. *Principles of Population Genetics*, 3rd edition. Sinauer. ISBN 0-87893-306-9.

Hecht, S. 1999. Social issues in participatory plant breeding: a report to the CGIAR gender working group. *Working document no. 5*. Program on participatory research and gender analysis, Cali, Colombia.

Hershey, C.H., & Ocampo, C. 1989. New marker genes found in Cassava. *Cassava Newsletter 13*, 1-5.

Hillocks, R.J., Thresh, J.M., Tomas, J., Batao, M., Macia, R. & Zavier R. 2001. Cassava brown streak disease in northern Mozambique. *International Journal of Pest Management 48*, 179-182.

Howeler, R.H. 1978. The mineral nutrition and fertilization of cassava. In: *Cassava Production Course*. CIAT, Cali, Colombia pp 247-292

Hughes, M.A, Hughes, J., Brown, R. & Liddle, S. 1997. Recent advances in molecular and biochemical studies of cyanogenesis in cassava: complexity of the cassava genome. *African Journal of Root and Tuber Crops 2,* 77-80.

Hughes, M.A., Stirling, J.D. & Collinge, D.B. 1984. The inheritance of cyanoglucoside content in *Trifolium repens* L. *Biochemical Genetics, 22*, 139-151.

Hussain, A., Bushuk, W., Ramirez, H. & Roca, W.M. 1987. Identification of cassava (*Manihot esculena* Crantz) cultivars by electrophorectic patterns of esterase isozyme. *Seed Science Technology 155*, 19-22.

IITA, 1999. *Annual Report Project 14*. International Institute of Tropical Agriculture, Ibadan, Nigeria.

IITA, 2000. Enhancement of income generation potential through the development and transfer of improved cassava genetic material and adoption of post-harvest technologies in Africa. *A report by IITA to the International Fund for Agricultural Development (IFAD)*. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Iglesias, C.A. & Hershey, C. 1994. Cassava breeding at CIAT: Heritability estimates and genetic progress in the 1980's. In: *Tropical Root Crops in a Developing Economy.* (Eds. F. Ofori & S.K. Hahn). Wageningen, Netherlands, pp. 149-163.

Iglesias, C., Sanchez, T. & Yeoh, H.H. 2002. Cyanogens and linamarase activities in storage roots of cassava plants from breeding programme. *Journal of food composition and analysis 15*, 379-387.

Ingestad, T. & Lund, A.-B. 1986. Theory and techniques for steady state mineral nutrition and growth of plants. *Scandinavian Journal of Forest Research 1,* 439-453

Jameson, J.D. 1964. Cassava mosaic disease in Uganda. *East African Agricultural Journal 29,* 208-213.

Jameson, J.D. & Thomas, D.G. 1970. Cassava. In: *Agriculture in Uganda*. (Ed. Jameson J.D.). Oxford University Press, Oxford, pp 247-251

Jansen, R. 1993. Interval mapping of multiple quantitative trait loci. *Genetics 135*, 205-211.

Jansen, R. & P. Stann. 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics 136*, 1447-1455.

Jarvis, D.I. & Hodgkin, T. 1999. Wild relatives and crop cultivars: detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. *Molecular Ecology 8*, S159-S173.

Jennings, D.L. 1979. Cassava. In: *Evolution of Crop plants*. (Ed. N.W. Simmonds). Longman, London, pp 81-84.

Jennings, D.L. 1994. Breeding for resistance to African cassava mosaic geminivirus in East Africa. *Tropical Science 34*, 110-122.

Jennings, D.L. & Iglesias, C.A. 2002. Breeding for crop improvement. In: *Cassava: Biology, Production and Utilization*. (Eds. A.C. Belloti, J.M. Thresh & R.J. Hillocks). CAB International, Wallingford, Oxon, UK, pp. 149-166.

Jones, W. 1959. *Manioc in Africa*. Stanford University Press. pp 83-84.

Jorge, V., Fregene, M.A., Duque, M.C., Bonierbale, M., Tohme, J. & Verdier, V. 2000. Genetic mapping of resistance to bacterial blight disease. *Theoretical and Applied Genetics 101*, 865-872.

Jorge, V., Fregene, M., Velez, C.M., Duque, M.C., Tohme, J. & Verdier, V. 2001. QTL analysis of field resistance to *Xanthomonas asonopodis* pv. *manihotis* in cassava. *Theoretical and Applied Genetics 102*, 564-571.

Kao, C.-H., Zeng, Z.-B. & Teasdale, R.D. 1999. Multiple interval mapping for quantitative trait loci. *Genetics 152*, 1203-1216.

Kawano, K. 2003. Thirty years of cassava breeding for productivity – biological and social factors for success. *Crop Science 43*, 1325-1335.

Kawano, K., Daza, P., Amaya, A., Rios, M. and Gonçalvez, M.F. 1978. Evaluation of cassava germplasm for productivity. *Crop Science 18*, 377-380.

Kawano, K., Narintaraporn, K., Narintaraporn, P., Sarakarn, S., Limsila, A., Limsila, J., Suparhan, D., Sarawat, V. & Watananonta, W. 1998. Yield improvement in a multistage breeding program for cassava. *Crop Science* 38, 325-332.

Kiwanuka, S. & Kintu, J. 2004. Seed security in Uganda: current status, issues and challenges. *Uganda Journal of Agricultural Sciences 9*, 17-22.

Koch, B., Nielsen, V., Halkier, B., Olsen, C. & Møller, B.L. 1992. The biosynthesis of cyanogenic glycosides in seedlings of cassava (*Manihot esculenta* Crantz). *Archives of Biochemistry and Biophysiology 292*, 141-150.

Koebner, R. 2003. MAS in Cereals: Green for maize, amber for rice, still red for wheat and barley marker assisted selection. A fast track to increase genetic gain in plant and animal breeding. http://www.fao.org/biotech/conf10.htm (accessed 25 May 2006)

Knoth, J. 1993. Traditional storage of yams and cassava and its improvement. *Deutsche Gesellschaft fur Technische Zusammenarbeit* (GTZ) GmbH.

Kullaya, A., Mtunda, K., Kulembeka, H., Ferguson, M., Marin, J., Ospina, C., Barrera, E., Jarvis, A., Morante, N., Ceballos, H., Tohme, J. & Fregene, M. 2004. Molecular marker-assisted and farmer participatory improvement of cassava germplasm for farmer/market preferred traits in Tanzania. In: Adding Value to a Small-Farmer Crop. (Eds. A. Alves & J.Tohme). *Proceedings on the Sixth International Scientific Meeting of the Cassava Biotechnology Network. 8-14 March 2004*. CIAT, Cali Colombia, pp 70.

Lancaster, P.A. & Brooks, J.E. 1983. Cassava as human food. *Economic Botany 37*, 331-348.

Lander, E.S. & Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics 121*, 185-199.
Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daley, M., Lincoln, S. & Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181.
Langlands, B.W. 1966. Cassava in Uganda 1860-1920. *Uganda Journal* 30, 211-218.
Lefevre, F. & Charrier, A. 1993a. Isozyme diversity within African *Manihot* germplasm. Euphytica 66, 73-80.
Lefevre, F. & Charrier, A. 1993b. Heredity of seventeen isozyme loci in cassava (*Manihot esculenta* Crantz). *Euphytica 66*, 73-80.
Legg, J.P. 1999. Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in East and Central Africa. *Crop Protection 18*, 627-637.
Legg, J.P. & Fauquet, C.M. 2004. Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology 56*, 585-599.
Legg, J.P. & Thresh, J.M. 2000. Cassava mosaic virus disease in East Africa: a dynamic disease in a changing environment. *Virus Research 71*, 135-149.
Lutaladio, N.B. & Ezumah, H.C. 1981. Cassava leaf harvesting in Zaire. In: *Tropical Root Crops : Research Strategies for the 1980s*. (Eds. E.R. Tenry, K.A. Oduri & F. Cavaness). IDRC, Ohawa, Canada, pp 134-136.
Mahungu, N.M., Dixon, A.G.O. & Mkumbira, J.M. 1994. Breeding cassava for multiple pest resistance in Africa. *African Crop Science Journal 2*, 539-552.
Manu-Aduening, J.A., Lamboll, R.I., Dankyi, A.A. & Gibson R.W. 2005. Cassava diversity in Ghanaian farming sytems. *Euphytica 144*, 331-340.
Marmey, P., Beeching, J.R., Hamon, S. & Charrier, A. 1994. Evaluation of cassava (*Manihot esculenta* Crantz) germplasm collection using RAPD markers. *Euphytica 14*, 203-209.
Martin, E.F. 1928. Report of the mycologist. In: *Annual Report of the Department of Agriculture.* Government Printer, Entebbe, Uganda pp 31.
Mba, R.E.C., Stephenson, P., Edwards, K., Melzer, S., Mkumbira, J., Gullberg, U., Apel, K., Gale, M., Tohme, J. & Fregene, M.A. 2001. Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. *Theoretical and Applied Genetics 102*, 21-31.
McCouch, S.R. & Doerge, R.W. 1995. QTL mapping in rice. *Trends in Genetics 11*, 483-487.
McMahon, J.M., White, W.L.B. & Sayre, R.T. 1995. Cyanogenesis in cassava (*Manihot esculenta* Crantz). *Journal of Experimental Botany 46*, 731-714.
Michelmore, R. 1995. Molecular approaches to manipulation of disease resistance genes. *Annual Review Phytopathology 33*, 393-427.
Mkumbira, J. 2002. Cassava development for small-scale farmers. PhD thesis. Agraria 365. Swedish University of Agricultural Science.
Mkumbira, J., Chiwona-Karltun, L., Lagercrantz, U., Mahungu, N.M., Saka, J., Mhone, A., Bokanga, M., Brimer, L., Gullberg, U. & Rosling, H. 2003. Classification of cassava into 'bitter' and 'cool' in Malawi: from farmers' perception to characterisation by molecular markers. *Euphytica 132*, 7-22.
Morgante, M. & Salamini, F. 2003. From plant genomics to breeding practice. *Current Opinions in Biotechnology 14*, 214-219.
Nartey F. 1969. Studies on cassava *Manihot utillisima*, biosynthesis of asparagines – ^{14}C from ^{14}C-labelled hydrogen cyanide and its relations with cyanogenesis. *Physiologia. Plantarum 22*, 1085-1096.
Nassar, N.M.A. 1982. Collecting wild cassava in Brazil. *Indian Journal of Genetics 42*, 405-411
Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science USA 70*, 3321-3323.
Nei, M. 1975. *Molecular Population Genetics and Evolution*. Amsterdam, North Holland.
Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics 89*, 583-590.

Ng, N.Q. & Ng, S.Y.C. 2002. Genetic resources and conservation. In: *Cassava: Biology, Production and Utilization*. (Eds. A.C. Belloti, J.M. Thresh & R.J. Hillocks). CAB International, Wallingford, Oxon, UK. pp 167-178.

Nichols, R.F.W. 1947. Breeding cassava for virus resistance. *East African Agricultural Journal 15*, 154-160.

Nweke, F.I. 1994. Cassava distribution in Africa. *COSCA working paper No. 12*. Collaborative study of Cassava in Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Nweke, F., 2004. New challenges in the cassava transformation in Nigeria and Ghana. *Discussion paper No. 118. Environment and Production Technology Division,* International Food Policy Research Institute, IFPRI.

Nweke, F.I. & Bokanga, M. 1994. Importance of cassava processing for production in sub-saharan Africa. *Acta Horticulturae 375*, 401-412.

Nweke, F.I., Dunstan, S.C., Spencer, J. & Lynam, K. 2002. *The cassava transformation. Africa's best-kept secret.* Michigan State University Press, 250 pp.

Ocampo, C., Hershey, C., Iglesias, C. & Iwanaga, M. 1992. Esterase isozyme fingerprinting of the cassava germplasm collection hold at CIAT, In: *Proceedings of the 1st international scientific meeting of the Cassava Biotechnology Network, CIAT.* (Eds. W. Roca & A.M. Thro). Cali, Colombia pp 81-89.

O'Hair, S.K. 1995. Cassava. http://www.hort.purdue.edu/newcrop/CropFactSheets/cassava. (Accessed 11 August 2006).

Okogbenin, E. & Fregene, M. 2002. Genetic analysis and QTL mapping of early root bulking in an F_1 population of non-inbred parents in cassava (*Manihot esculenta* Crantz). *Theoretical and Applied Genetics 106*, 58-66.

Okogbenin, E. & Fregene, M. 2003. Genetic mapping of QTLs affecting productivity and plant architecture in a full-sib cross from non-inbred parents in cassava (*Manihot esculenta* Crantz). *Theoretical and Applied Genetics 107*, 1452-1462.

Olsen, K.M. 2004. SNPs, SSRs and inferences on cassava's origin. *Plant Molecular Biology 56*, 517-526

Olsen, K.M. & Schaal, B.A. 1999. Evidence on the origin of cassava: phylogeography of *Manihot esculenta.* Proceedings *of the National Academy of Sciences, USA 96*, 5586-5591.

Olsen, K. & Schaal, B. 2001. Microsatellite variation in cassava *Manihot esculenta*, Euphorbiaceae and its wild relatives: evidence for a southern Amazonian origin of domestication. *American Journal of Botany 88*, 131-142.

Onwueme, I.C. 2002. Cassava in Asia and the Pacific. In: *Cassava: Biology, Production and Utilization.* (Eds. A.C. Belloti, J.M. Thresh & R.J. Hillocks). CAB International, Wallingford, Oxon, UK. pp 55-65.

Otim-Nape, G.W., Alicai, T. & Thresh, M. 2001. Changes in the incidence and severity of cassava mosaic virus disease, varietal diversity and cassava production in Uganda. *Annals of Applied Biology 138*, 313-327.

Otim-Nape, G.W., Bua, A. & Baguma, Y. 1994. Accelerating the transfer of improved production technologies: Controlling African cassava mosaic virus disease epidemics in Uganda. *African Crop Science Journal 2*, 479-485.

Otim-Nape, G.W., Bua, A., Thresh, J.M., Baguma, Y., Ogwal, S., Ssemakula, G.N., Acola, G., Byabakama, B.A., Colvin, J., Cooter, R.J. & Martin, A. 2000. *The current pandemic of Cassava Mosaic Virus Disease in East Africa and its control.* Chatham, UK: NARO/ NRI/ DFID.

Otim-Nape, G.W., Bua A., Thresh, J.M., Baguma, Y., Ogwal, S., Semakula, G.N., Acola, G., Byabakama, B. & Martin, A. 1997. Cassava mosaic virus disease in Uganda: the current pandemic and approaches to control. *NARO/DFID*. Chatham, UK: Natural Resources Institute, pp 33-38.

Otim-Nape, G.W., Thresh, J.M., Bua, A., Baguma, Y. & Shaw, M.W. 1998. The incidence and severity of cassava mosaic virus disease in Uganda: 1990-92. *Tropical Science 38*, 25-37.

Otim-Nape, G.W. & Zziwa, S. 1990. Cassava as a major staple food crop in Uganda. *Working Paper No. 1*. Collaborative study of Cassava in Africa. Namulonge Research station, Kampala, Uganda.

Paterson, A.H. 1996a. Making genetic maps. In: *Genome Mapping in Plants* (Ed. A.H. Paterson). R.G. Landes Company, San Diego, California; Academic Press, Austin, Texas, pp 23-39.

Paterson, A.H. 1996b. Mapping genes responsible for differences in phenotype, In: *Genome Mapping in Plants.* (E.d. A.H. Paterson). R. G. Landes Company, San Diego, California; Academic Press; Austin, Texas, pp 41-54.

Perez, J.C., Caballos, H., Calle, F., Morante, N., Gaitan, W., Llano G. & Alvarez, E. 2005. Within-family genetic variation and epistasis in cassava (*M. esculenta* Crantz) adapted to the acid-soils environment. *Euphytica 145*, 77-85.

Pita, J.S., Fondong, V.N., Sangare, A., Otim-Nape, G.W., Ogwal, S. & Fauquet, C.M. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology 82*, 655-665.

Purseglove, J.W. 1968. *Tropical Crops: Dicotyledons*: Longman, London.

Ribaut, J. M. & Hoisington, D. 1998. Marker-assisted selection: new tools and strategies. *Trends in Plant Science 3*, 236-239.

Riis, L., Belloti, A.C., Bonierbale, M. & O'Brien, G. 2003. Cyanogenic potential in cassava and its influence on a generalist insect herbivore *Cyrtomenus bergi* (Hemiptera: Cydnidae). *Journal of Economic Entomology 96*, 1915-1921.

Riis, L., Belloti, A.C. & Vargas, O. 1994. The response of a polyphagous pest (*Cyrtomenus bergi* Froeschner) to cassava cultivars with variable HCN content in root parenchyma and peel. CIAT. *Cassava working document No. 150*, Cali, Colombia.

Roa, A.C., Maya, M.M., Duque, M., Allem, C., Tohme, J. & Bonierbale, M.W. 1997. AFLP analysis of relationships among cassava and other manihot species. *Theoretical and Applied Genetics 95*, 741-750.

Rogers, D.J. 1963. Studies of *Manihot esculenta* Crantz and related species. *Bulletin Torrey Botanical Club 90*, 43-54.

Rogers, D.J. & Appan, S.G. 1973. *Manihot, Manihotoides. Flora Neotropica.* Hafner Press, New York.

Sauer, J.D, 1993. *Historical Geography of Crop Plants: a select Roster.* CRC, Boca Raton, Florida, USA.

Siritunga, D., Arias-Garzon, D., White, W. & Sayre, R.T. 2004. Over-expression of hydroxynitrile lyase in transgenic cassava roots accelerates cyanogenesis and food detoxification. *Plant Biotechnology Journal 2*, 37-43.

Siritunga, D. & Sayre, R.T. 2003. Generation of Cyanogen-Free Transgenic Cassava. *Planta 217*, 367-373.

Siritunga, D. & Sayre, R.T. 2004. Engineering cyanogen synthesis and turnover in cassava. *Plant molecular Biology 56*, 661-669.

Sperling, L & Loevinsohn, M.E. 1993. The dynamics of adoption: distribution and mortality of bean varieties among small farmers in Rwanda. *Agricultural Systems 41*, 441- 453.

Sriroth, K., Piyachomkwan, K., Wanlapatit, S. A. & Oates, C. G. 2000. *Starch/Stärke 52*, 439- 449.

Sseruwagi, P., Sserubombwe, W.S., Legg, J.P., Ndunguru, J. & Thresh, J.M. 2004. Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations in Africa: a review. *Virus Research 100*, 129-142.

Teri, J.M., Sandifolo, V.S., Kaeya, E.H., Moyo, C.C., Chipungy, F.P. & Benesi, I.R.M. 1999. Root Crops revolution in Malawi: lessons learned. *Roots 6*, 24-39.

Thresh, J.M. & Mbwana, M.W. 1998. Cassava mosaic and cassava brown streak virus diseases in Zanzibar. *Roots 5*, 6-9.

Thresh, J.M., Otim-Nape, G.W., Legg, J.P. & Fargette, D. 1997. African cassava mosaic virus disease: the magnitude of the problem. *African Journal of Root and Tuber Crops 2*, 13-19.

Thro, A.M. & Spillane, C. 2000. CGIAR systemwide programme on participatory research and gender analysis for technology development and institutional innovation biotechnology-assisted participatory plant breeding: complement or contradiction? *Working document No. 4*.
Tonukari, N.J., 2004. Cassava and the future of starch. *Electronic Journal of Biotechnology 7.* http://www.ejbiotechnology.info/content/vol7/issue1/issues/2/. ISSN: 0717-3458. (accessed 03 March 2005).
Tylleskär, T., Cooke R., Banea, M., Poulter, N., Bokanga, N. & Rosling H. 1992. Cassava cyanogens and konzo, an upper motor neuron disease found in Africa. *Lancet 339*, 208-211
Ugent, D., Pozorski, S. & Pozorski, T. 1986. Archaeological manioc (*Manihot*) from Coastal Peru. *Economic Botany 40*, 78-102
Ugwu, B.O. & Ay, P. 1992. Seasonality of cassava processing in Africa. COSCA *Working Paper No. 9*. Collaborative study of Cassava in Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria.
Weising, K., Nybom, H., Wolff, K. & Kahl, G. 2005. *DNA Fingerprinting in Plants: Principles, Methods, and Applications*, 2nd edition. CRC Press, London.
Westby, A., 2002. Cassava utilization, storage and small-scale processing. In: *Cassava: Biology, Production and Utilization.* (Eds. A.C. Belloti, J.M. Thresh & R.J. Hillocks). CAB International, Wallingford, Oxon, UK, pp 281-300.
Wu, K.S. & Tanksley, S.D. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular Genetics and Genomics 241*, 225-235.
Zeng, Z.B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Science USA 90*, 10972-10976.
Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. *Genetics 136*, 1457-1468.

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The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda

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Summary

Cassava (*Manihot esculenta*) is a tropical crop that is grown in Africa, Latin America and Southeast Asia. Cassava was introduced from Latin America into West and East Africa at two independent events. In Uganda a serious threat to cassava's survival is the cassava mosaic disease (CMD). Uganda has had two notable CMD epidemics since the introduction of cassava in the 1850s causing severe losses. SSR markers were used to study the effect of CMD on the genetic diversity in five agroecologies in Uganda with high and low incidence of CMD. Surprisingly, high gene diversity was detected. Most of the diversity was found within populations, while the diversity was very small among agroecological zones and the high and low CMD incidence areas. The high genetic diversity suggests a mechanism by which diversity is maintained by the active involvement of the Ugandan farmer in continuously testing and adopting new genotypes that will serve their diverse needs. However, in spite of the high genetic diversity we found a loss of rare alleles in areas with high CMD incidence. To study the effect of the introgression history on the gene pool the genetic differentiation between East and West Africa was also studied. Genetic similarities were found between the varieties in Uganda and Tanzania in East Africa and Ghana in West Africa. Thus, there is no evidence for a differentiation of the cassava gene pool into a western and an eastern genetic lineage. However, a possible difference in the genetic constitution of the introduced cassava into East and West Africa may have been diminished by germplasm movement.

Introduction

Cassava (*Manihot esculenta)* is a neotropical crop that is also grown in tropical Africa and south east Asia. It is cross-fertilizing but is vegetatively propagated through stem cuttings. However, in traditional agroecosystems in Latin America small-scale farmers also multiply plants from volunteer seedlings produced by sexual reproduction (Elias et al., 2001). Spontaneous recombination and farmer selection from volunteer seedlings seem also to occur in Africa (Fregene et al., 2003). When visiting farmers in different parts of Uganda, seedlings were seen growing in plots near cassava fields (E. Balyejusa Kizito & U. Gullberg, personal communication). Cassava is believed to have originated from wild *M. esculenta* populations growing along the southern rim of the Amazon Basin in Brazil (Olsen & Schaal, 1999, 2001). Cassava was introduced from Brazil into Africa by Portuguese slave ships and arrived to the western coast in the 17th century and to the eastern coast in the 18th century (Jones, 1959). In Uganda cassava was introduced relatively late and had limited distribution by the 1900s (Jones, 1959; Langlands, 1966). It was most likely introduced from the eastern

introgression route by European and Arab traders (Jones, 1959). It was first grown in Buganda, the central part of Uganda, around 1870 and was soon spread to Bunyoro, the northwestern region. Cassava arrived much later to the eastern (Busoga) and northern Uganda in the early 20th century (Langlands, 1966). Because of its excellent adaptability to erratic rainfall and low fertility soils, it became a major dietary staple, a famine reserve crop and a source of cash to many small-scale farming communities.

A threat to cassava's survival as a dominant crop in subsistence communities in Uganda is its vulnerability to the cassava mosaic disease (CMD). The disease is caused by several viruses belonging to the genus *Begomovirus* in the family *Geminiviridae*, transmitted by whiteflies (*Bemisia tabaci*; Pita et al., 2001). The first outbreak of CMD was in the late 1920s (Martin, 1928; Jameson, 1964). At that time there were also frequent famines caused by drought and locusts, especially in northern Uganda. To alleviate these situations the then colonial government vigorously encouraged cassava multiplication schemes and land area planted to cassava increased greatly in Uganda (Jones, 1959). Also, this led to development of the regional breeding program for CMD resistance in Amani, Tanzania and the distribution of resistant plant material in the 1950s. In Uganda this plant material was released as the Bukalasa series in the early 1960s (Otim-Nape et al., 1994). In this program, *M. glaziovii*, a wild cassava species, was used as a donor of genes conferring resistance to CMD. Open pollinated seed of progenies from crosses that had been made between *M. glaziovii* and different cassava varieties were sent to many parts of Africa notably Uganda (Jameson, 1964), Kenya, Nigeria (Jennings, 1994), and Ghana (Doku, 1969). However, the first breeding activities took place just before the first outbreak of CMD and in 1927 six varieties were introduced to Uganda from the West Indies (Jameson, 1964). Another CMD outbreak that occurred in 1989 has led to development and release of the new varieties in the NASE series in the 1990s. The recent CMD incidence differed significantly between agroecologies with locations in northern parts of Uganda being more affected than those in the southern parts (Otim-Nape et al., 1997; Figure 1). Therefore the impact of governmental and non-governmental mitigation programs to distribute CMD-free or CMD resistant planting material differed accordingly. Studies done on varietal diversity on the basis of the morphology of the cassava varieties showed that there were also marked changes in the predominating varieties being grown, as susceptible ones

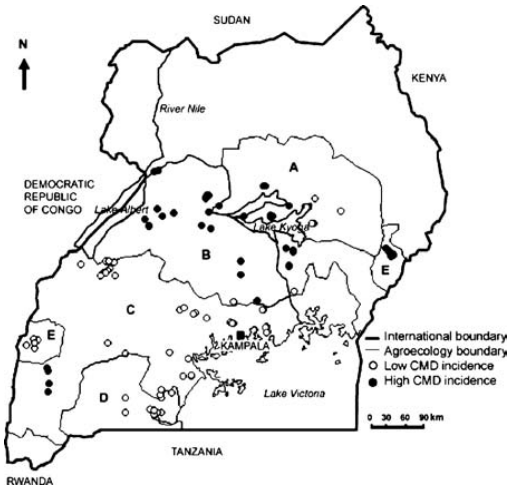

Figure 1. Map of Uganda showing the collection sites with high and low CMD incidence, and the agroecological zones: A: northern agroecosytem, B: banana – millet – cotton agroecosystem, C: banana – coffee agroecosystem, D: pastoral agroecosystem, E: montane agroecosystem.

were being lost and replaced with other CMD-free or CMD tolerant or resistant varieties (Otim-Nape et al., 2001). It is, however, not known how the CMD has affected the genetic diversity and the gene pool of cassava in Uganda. We have therefore studied the genetic diversity in different agroecological zones and areas with high and low incidence of CMD using 35 simple-sequence-repeat (SSR) markers. In addition, because of the two independent introductions of cassava in Africa one might to expect loss of genetic diversity as a result of founder effects and genetic differentiation between East and West Africa. To study the effect of the introgression history on the gene pool of cassava the genetic differentiation between Latin America, East and West Africa was studied. The neighboring countries Uganda and Tanzania was chosen from East Africa, and the neighboring countries Ghana and Nigeria from West Africa.

Materials and methods

Plant materials

The cassava varieties in Uganda were collected in September through to December 2002, from five agroecological zones (Figure 1). These zones lie between

2°12′N and 0°44′S, and 29°56′E and 34°21′E, and on altitudes from 664 to 1484 m above sea. Locations were visited as in Otim-Nape et al. (1998) except the northwestern region of Uganda. These locations were classified as high and low CMD incidence based on Otim-Nape et al. (1998), where a location of high CMD incidence had greater than 50% occurrence of the disease (Figure 1). Based on morphological characteristics plants were collected with assistance from the cassava-breeding program and labeled by the name as given by the farmer. Varieties from the recent breeding work of the 90s, the NASE series, were avoided. These varieties did not exist at the time of the last CMD epidemic in 1989, and therefore would not reflect on the impact of CMD. A total of 245 plants were sampled with 17 from the northern agroecology, 16 from the montane, 63 from the banana–millet–cotton agroecology, 123 from the banana–coffee agroecology and 26 from the pastoral agroecology. The cassava was planted in a screenhouse in Namulonge Agricultural and Animal Research Institute (NAARI) from which leaves for DNA extraction were obtained. A summary of the plant materials and their source can be viewed at http://www.ciat.cgiar.org/molcas/estudios.jsp? Code=6 & pais=Uganda.

Twenty-two Nigerian accessions were included from the international collection at the International Institute of Tropical Agriculture (IITA) with 10 being improved genotypes from the Institute's cassava breeding program or the 1950s breeding efforts at the Moor Plantation Experiment Station, Ibadan, Nigeria. Based on Fregene et al. (2003) 20 Tanzanian accessions and 38 Latin American holdings from the core collection at CIAT; 6 from Colombia, 3 from Brazil, 3 from Peru, 2 from Mexico, 2 from Venezuela, 2 from Argentina and 20 from Guatemala were selected. In addition 20 accessions from Ghana (courtesy of Elizabeth Okai; CIAT, 2003) were included. In order to cover as much of the genetic diversity as possible, these accessions were selected to represent the genetic distribution found by principal component analysis (PCA) based on SSR markers in each study.

DNA analysis

DNA was isolated from young leaf tissue by CTAB method (Doyle & Doyle, 1987). A subset of 35 SSR markers with broad genome coverage and high polymorphism information content (PIC) was selected from 67 markers from an earlier study (Fregene et al., 2003). To obtain a maximum amount of information on allelic diversity in cassava 30 unlinked markers are required (Fregene et al., 2003). PCR was carried out using 10 ng of DNA per reaction following Mba et al. (2001). The PCR product was denatured and electrophoresed on 6% polyacrylamide gels using Bio-Rad sequencing apparatus (Bio-Rad Inc., USA) and visualized by silver staining according to the Promega manufacturer's guide. Allele sizes were then determined based upon an internal gel molecular marker size standard using both manual scoring and the computer software "quantity One" (Bio-Rad Inc.). In addition, a few plants with known genotypes were used as controls on each gel. The genotype data was exported to Microsoft Excel (Microsoft Inc) for further formatting as input files for statistical analysis.

Statistical analysis

Genetic diversity parameters within and among populations (agroecological zones or areas with high and low CMD incidence) were estimated with data from the 35 SSR marker loci using the GEN-SURVEY program (Vekemans & Lefèbvre, 1997). The average expected heterozygosity (H_e) and the distribution of genetic diversity within and among populations were calculated according to Nei (1978). For all loci and populations the total diversity estimate (H_t = total heterozygosity in the entire dataset) was partitioned into within-population diversity (H_s = heterozygosity within populations averaged over the entire dataset) and between-population diversity (D_{st}) estimates, where $H_t = H_s + D_{st}$. Gene diversity between populations was expressed relative to total population diversity as $G_{st} = D_{st}/H_t$. Standard deviations sampled by jackknifing (200 replications) and 95% confidence intervals sampled by bootstrapping (1000 replications; Quenoille, 1956; Efron, 1982) were estimated over loci for the above parameters.

Genetic differentiation between countries was analyzed using Wright's F-statistics (1965) and pairwise calculations of F_{st} overall loci between pairs of country variety groups were estimated using FSTAT 2.9 (Goudet, 1995). The pairwise F_{st} estimates was used to construct a dendrogram on the basis of the unweighted paired group method with the arithmetic mean (UPGMA) using NTSYS-PC (Rohlf, 1993). In addition, pairwise genetic distances between individual cassava plants were calculated from the allele size-data based on the 1-proportion of shared alleles (PSA) using the computer program "microsat" available at http://hpgl.stanford.edu/projects/microsat/microsat.htlm. The distance matrix was analyzed by

principal component analysis (PCA) using the JMP program to deduce multivariate relationships among the cassava genotypes. Several measures of genetic distance have been developed for SSR markers on the basis of the stepwise mutation model (SSM; Kimura and Crow, 1964). SSM assumes that alleles mutate back and forth by adding or subtracting a repeat motif, so that the same allelic states are formed repeatedly over time. An alternative model is the infinite allele model (IAM; Ohta & Kimura, 1973), which assumes that each mutation creates a new allele in the population. Because of the relatively short evolutionary divergence times for the cassava varieties, and thus a smaller number of mutations are expected, we have based the estimates of genetic distance on the IAM. In addition, the difference in number of base pairs between alleles within a SSR locus observed in this study is variable and does not follow any pattern. This may indicate complex patterns of mutation and that the SSR variability in cassava may not fit the stepwise mutation model (SSM; Kimura & Crow, 1964). In fact, a number of studies have shown that many SSR marker loci do not evolve according to the SSM (e.g. Valdes et al., 1993; Matsuoka et al., 2002).

Results

Number of alleles in SSR loci

The observed number of alleles at each locus in the whole dataset (Africa and Latin America) was relatively high and ranged from 2 at SSRY102 and SSRY132, to 12 at SSRY19 (Table 1). Six alleles or more were found in 63% of the 35 studied marker loci and the average number of alleles per locus was 6.1.

Allelic distribution in Uganda

Due to some missing genotype data the number of plants scored per marker locus varied. The average number of plants scored per locus is given in Tables 1 and 2. A total of 183 alleles were found in the Ugandan collection (Table 1). Of the 183 alleles found, 38 alleles occurred in less than 1% within Uganda. These alleles are defined as rare. The number of rare alleles found was highest in the agroecological zone of the banana–coffee agroecosystem (Table 2), which had the largest sample size. The lower number of rare alleles found in the other agroecological zones is likely to be influenced by a smaller sample size. The number of non-rare alleles (occurred in more than 1%) was about the same in the agroecological zones with the exception of the northern agroecosystem. None of the non-rare alleles was unique to any of the five agroecological zones, while some rare alleles were unique to each of the zones: 14 in the banana–coffee agroecosystem, 3 in the banana–millet agroecosystem, 2 in the montane agroecosystem, 2 in the northern agroecosystem and 1 in the pastoral agroecosystem.

When the collection sites were classified with respect to high (>50%) or low (≤50%) CMD incidence according to Otim-Nape et al. (2001) the sample sizes were more equal and the number of scored plants was 89 and 106 from the high and low CMD incidence groups, respectively. Thirty-three rare alleles were found in the areas with low CMD incidence compared to 13 rare alleles in the high CMD incidence areas (Table 2), and a significant difference in the number of rare alleles was found using a Chi-square test ($P < 0.05$). Eight of the rare alleles were common to both the low and high CMD incidence classification groups. The number of non-rare alleles was about the same in the two groups.

Genetic diversity in Uganda

Of the 35 marker loci studied there were on an average 93.7% polymorphic loci across all agroecological zones (Table 2), using the criterion that the frequency of the most common allele does not exceed 0.98. The observed heterozygosities were high in all agroecological zones and ranged from 0.536 to 0.594, at an average of 0.559 (Table 2). The expected heterozygosity (corrected for small sample sizes; Nei, 1978) in the agroecological zones ranged from 0.487 to 0.594, at an average of 0.544. This implies that the probability that two randomly selected alleles in Uganda are different is more than half. The observed and expected heterozygosity was about the same in the high and low CMD incidence groups and close to 0.5. Compared to previous gene diversity studies on cassava where allozyme markers were used (Lefevre & Charrier, 1993; Resende et al., 2000), the observed proportion of heterozygotes is about three-fold greater with SSR markers. Similarly, in domesticated sunflower accessions the observed proportion of heterozygotes was found to be two- to four-fold greater for SSR marker loci than for allozymes (Tang & Knapp, 2003), and in sorghum landraces 20-fold greater for SSR marker loci than for allozymes (Djè et al., 1999). The higher

Table 1. Number of alleles in each SSR locus for cassava varieties in the different countries

	Population					
Locus	Uganda (195)[1]	Tanzania (19)[1]	Ghana (19)[1]	Nigeria (20)[1]	Latin America (34)[1]	Number of alleles
SSRY4	5	5	4	4	6	6
SSRY9	4	4	4	5	5	5
SSRY12	6	4	4	4	4	6
SSRY19	8	7	7	6	10	12
SSRY20	9	6	7	6	7	10
SSRY21	6	5	4	4	7	7
SSRY34	4	3	3	2	3	5
SSRY38	4	2	2	3	4	5
SSRY51	5	5	4	4	6	6
SSRY59	5	4	3	2	6	7
SSRY63	4	2	3	3	3	4
SSRY64	6	4	4	4	5	7
SSRY69	8	7	6	5	7	8
SSRY82	9	7	8	7	8	9
SSRY100	6	5	6	4	7	7
SSRY102	2	2	2	2	2	2
SSRY103	5	5	5	4	5	5
SSRY105	5	4	3	3	5	6
SSRY106	5	5	5	4	5	5
SSRY108	6	3	4	4	6	8
SSRY110	6	4	4	4	5	6
SSRY132	1	1	1	1	2	2
SSRY135	3	3	3	3	3	3
SSRY147	4	2	2	2	3	4
SSRY148	3	3	3	2	5	5
SSRY151	8	6	9	7	8	9
SSRY155	5	2	5	3	5	6
SSRY161	5	4	4	5	4	6
SSRY164	6	3	5	5	6	8
SSRY169	4	2	2	3	4	5
SSRY171	4	2	2	2	5	7
SSRY177	6	4	6	5	6	6
SSRY179	7	6	6	6	6	7
SSRY180	5	3	3	3	6	7
SSRY181	4	3	4	4	4	4
Total alleles	183	137	147	135	183	

[1]Average number of scored plants per locus.

proportion of observed heterozygotes found with SSR marker loci is to be expected because of their high polymorphism.

The total heterozygosity over all loci was high in the agroecological zones ($H_t = 0.564$, Table 2). Only a very small fraction of this was due to differentiation among zones ($G_{st} = 0.035$), while most of the diversity was found within zones ($H_s = 0.545$). The same pattern was found for the CMD incidence groups ($G_{st} = 0.004$).

Table 2. Genetic diversity of cassava varieties in different agroecological zones and areas with high or low CMD incidence in Uganda

Agro-ecology[1]	Mean no. of scored plants/locus	No. of loci	No. of rare alleles[2]	No. of non-rare alleles[3]	Percent of poly-morphic loci	Mean no. of alleles/ locus	Mean no. of alleles/poly-morphic locus	H_o[4]	H_e[5]	H_{e-p}[6]
A	13	35	2	113	88.6	3.3	3.3	0.550	0.467	0.487
B	49	35	6	137	94.3	4.3	4.5	0.536	0.537	0.542
C	99	35	29	145	94.3	4.9	5.1	0.552	0.534	0.537
D	21	35	7	137	94.3	4.1	4.2	0.565	0.547	0.561
E	13	35	8	133	97.1	4.1	4.2	0.594	0.571	0.594
Mean					93.7	4.1	4.3	0.559	0.532	0.544
SD[7]					3.1	0.6	0.6	0.022	0.038	0.039
CMD incidence										
High	89	35	13	139	94.3	4.4	4.6	0.545	0.535	0.538
Low	106	35	33	145	94.3	5.0	5.2	0.557	0.548	0.550
Mean					94.3	4.7	4.9	0.551	0.541	0.544
SD[7]					0.0	0.4	0.4	0.009	0.009	0.009
Agroecology										
	H_t[8]	H_s	D_{st}	G_{st}						
Mean	0.564	0.545	0.019	0.035						
SD[7]	0.192	0.188	0.012	0.020						
95% CI[9]	0.499	0.482	0.015	0.029						
95% CI[9]	0.626	0.606	0.023	0.042						
CMD incidence										
Mean	0.562	0.560	0.002	0.004						
SD[7]	0.197	0.197	0.004	0.007						
95% CI[9]	0.499	0.497	0.001	0.001						
95% CI[9]	0.623	0.621	0.003	0.006						

[1] A: northern agroecosytem, B: banana–millet–cotton agroecosystem, C: banana–coffee agroecosystem, D: pastoral agroecosystem, E: montane agroecosystem.
[2] An allele ocurring in less than 1% in the Ugandan population.
[3] An allele ocurring in more than 1% in the Ugandan population.
[4] Average observed heterozygosity within populations.
[5] Average expected heterozygosity within populations.
[6] Average expected heterozygosity within populations corrected for small sample sizes (Nei, 1978).
[7] Standard deviation estimated by jackknifing over loci (200 replications).
[8] H_t = total heterozygosity in the dataset, H_s = heterozygosity within populations averaged over the entire dataset, D_{st} = average gene diversity between populations, G_{st} = coefficient of genetic differentiation. These parameters are given over loci and over populations.
[9] 95% confidence interval for the mean estimated by bootstrapping over loci.

Allelic distribution in Latin America and the other African countries

Despite the small sample size Latin America had the same number of alleles (183) as Uganda (Table 1). Nineteen of the 38 rare alleles in Uganda occurred in Latin America in frequencies of 2.5–18.8%, 14 in Ghana (2.5–18.8%), 10 in Tanzania (2.5–13.2%) and 8 in Nigeria (2.4–31.8%). Five non-rare alleles found in Uganda occurring in frequencies from 1.1 to 16.7% were not found in any of the other African countries but in Latin America in a range of 4.1 to 16.2%. There were 24 unique alleles to Latin America in frequencies of 1.4 to 28%. Twenty-seven alleles were unique to Africa of which 13 were rare alleles and 3 non-rare alleles only found in Uganda. One of the 27 alleles was unique to Tanzania while 6 occurred in two or three African countries. Four of the 27 alleles were common to all of the African countries.

Table 3. Pairwise estimator of F_{st} between pairs of country groupings of cassava varieties

Population	Uganda	Tanzania	Nigeria	Ghana	Latin America
Uganda	–				
Tanzania	0.051	–			
Nigeria	0.113	0.104	–		
Ghana	0.040	0.050	0.049	–	
Latin America	0.097	0.059	0.057	0.054	–

Genetic differentiation between Latin America and Africa

Pairwise calculations of F_{st} overall loci between country variety groups are shown in Table 3. Small differentiation was found between the varieties in Uganda and Tanzania in East Africa and Ghana in West Africa, and between varieties in Ghana and Nigeria in West Africa (average $F_{st} = 0.048$). Interestingly, larger differentiation was found between Ugandan and Nigerian cassava varieties, and between Tanzanian and Nigerian varieties, than between varieties in Latin America and the African countries (average $F_{st} = 0.067$). Among the African countries Uganda showed the largest differentiation with Latin America.

The genetic relationship between the country variety groups is visualized in a UPGMA dendrogram on the basis of the pairwise F_{st} estimates (Figure 2). The varieties in Uganda and Tanzania in East Africa and Ghana in West Africa clustered closely together.

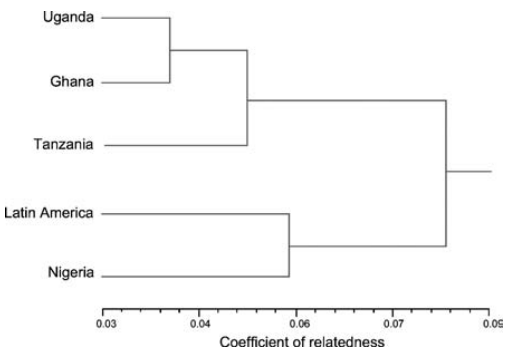

Figure 2. A UPGMA dendrogram illustrating the genetic relationship among cassava variety groups as revealed by pairwise F_{st} estimates between countries.

Interestingly, the Nigerian varieties grouped closer to the Latin American varieties than to the varieties in the other African countries. A principal component analysis was also performed on the genetic distance estimator 1-proportion of shared alleles (1-PSA) and a score plot of the first two principal components (explaining 39 and 10% of the variance, respectively, data not shown) gave essentially the same pattern of relationship among the country variety groups as the dendrogram.

Discussion

Cassava mosaic virus disease (CMD) is one of the most serious cassava diseases in Africa. It is unknown in Latin America and believed to be of African origin. The virus is transmitted by whiteflies and infects all cassava varieties in epidemics, although susceptibility varies greatly among varieties (Fauquet & Fargette, 1990). Due to the severe outbreaks of CMD in Uganda followed by large-scale multiplication schemes of some cassava varieties and release of CMD resistant varieties, one might expect to find a great loss of local varieties and thereby a reduction of genetic diversity in the Ugandan cassava gene pool. In addition, the introductions of cassava from Latin America to West Africa in the 17th century and to East Africa in the 18th century may have resulted in founder effects with loss of genetic diversity and genetic differentiation between the East and West African countries. Being mainly a vegetatively propagated crop a spread of a few vigorous and well-adapted local varieties among farmers would be facilitated and further reduce the genetic diversity of cassava. To study the impact of the history on the genetic diversity of the Ugandan cassava we have used population genetic models. These models are based on a number of assumptions, i.e. sexual reproduction and random mating. This has implications on the appropriateness of using population genetic models on cultivated plants, in particular vegetatively propagated crops, whose diversity is mainly influenced by humans. However, several genetic diversity studies on crops have based their analyses on population genetic models (e.g. Dubreuil & Charcosset, 1998; Djè et al., 2000; Fregene et al., 2003). In cassava local varieties are likely to be a result of spontaneous recombination because of incorporation of sexually produced plants by the Ugandan farmers. Thus, we found it appropriate to use population genetic models to analyze the genetic diversity in cassava.

Genetic diversity in Uganda

In spite of the severe outbreaks of CMD and the large-scale multiplication of cassava in Uganda a surprisingly high genetic diversity ($H_t = 0.567$) of cassava was detected. Most of the genetic diversity was found within populations, while very little diversity was found among agroecological zones and between the high and low CMD incidence groups. This indicates a high gene flow among populations. This gene flow is likely to be mediated by an efficient exchange of varieties among agroecological zones as well as the extensive distribution of varieties in the Amani breeding program from the 1920s to the 1960s throughout Uganda.

The high genetic diversity suggests a high number of local varieties and that the farmers have had a major impact on the composition of the cassava gene pool, while the CMD outbreaks and large-scale multiplication schemes have had a limited effect. Even though varieties were wiped out in certain areas (Otim-Nape et al., 1998) many survived and continue to be used by farmers. In addition, farmers have adopted new genotypes by including volunteer seedlings in their fields and by exchange of new genotypes developed by other farmers. In the Amazonian region of Peru there is a turnover of cassava varieties every 15 years due to pest and disease accumulation in the propagules (Salick, 2001). This dynamic diversity may very well be adaptive and indicative of distinct patterns in the local breeding of cassava and other vegetatively propagated crops.

Although the genetic diversity is high there is a loss of rare alleles in the areas with high CMD incidence. This shows that the CMD has had an effect on the cassava gene pool even though it could not be detected by using the heterozygosity or genetic diversity estimates. When the population size is drastically reduced, as in the high CMD incidence areas, rare alleles are most susceptible to loss (Nei, 1975).

Genetic differentiation between Latin America and Africa

Twenty-four alleles were unique to Latin America and not found in any of the African countries. This may indicate a loss of genetic diversity due to founder effects when cassava was introduced into Africa. However, 27 unique alleles were found in Africa. Some of these alleles might have been introduced when the wild cassava species *M. glaziovii* was used as a donor of genes conferring resistance to CMD in the Amani breeding program, Tanzania, in the 30s and 40s. In spite of the unique alleles the African and Latin American varieties seem not to be separated in two distinct gene pools since the Nigerian varieties showed closer genetic similarity with the Latin American varieties than with the varieties in Uganda and Tanzania (Table 3 and Figure 2). In addition, we found small to moderate genetic differentiation between Latin American and African varieties (average $F_{st} = 0.067$).

Within Africa genetic similarities were found between the varieties in Uganda and Tanzania in East Africa and Ghana in West Africa. Because of the similarity between varieties in these countries there is no evidence for a differentiation of the African cassava gene pool into a western and an eastern genetic lineage. Thus, the two independent introduction events in East and West Africa are not reflected in the African cassava gene pool. However, a larger genetic difference was found between the varieties in Nigeria in West Africa and the varieties in Uganda and Tanzania. A possible difference in the genetic constitution of the introduced cassava material into East and West Africa may have been diminished by movement of germplasm between countries. In the Tanzania Amani breeding program open pollinated seed of progenies from crosses that had been made between *M. glaziovii* and different cassava varieties were sent to many parts of Africa notably Uganda (Jameson, 1964), Kenya, Nigeria (Jennings, 1994) and Ghana (Doku, 1969). Uganda and Ghana may have got more closely related germplasm than what was received in Nigeria. Nigerian farmers tend to prefer bitter varieties that require processing as opposed to Ghana and Uganda where sweet, fresh, and boil varieties tend to predominate (Nweke & Bokanga, 1994; Westby, 2002). Investigation into the pedigree records and movement of germplasm from the Amani breeding programme may bring more clarity on this matter.

In conclusion, one of the important findings in this study is that in spite of severe outbreaks of CMD followed by large-scale multiplication and introduction of bred resistant varieties in Uganda the genetic diversity is high. The mechanism that rapidly compensates the loss of variability is the active involvement of the Ugandan farmer in continuously testing and adopting new genotypes that will serve their diverse needs. However, even though the genetic diversity is high there is a loss of rare alleles in the areas with high CMD incidence. Continuous CMD epidemics and extensive introductions of bred varieties throughout the country with the replacement of local varieties will most likely

further reduce the genetic diversity. For food security a large number of local varieties comprising extensive genetic diversity are important to maintain so that coming generations can cope with unpredictable environmental changes and human needs. Extensive breeding programs should therefore be preceded by investigations of the organization of genetic diversity within cassava that would benefit germplasm conservation.

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References

CIAT, 2003. Annual Report 2003. Project IP-3: Improved Cassava for the developing world, CIAT, Cali, Colombia.

Djè, Y., D. Forcioli, M. Ater, Lefèbvre & X. Vekemans, 1999. Assessing population genetic structure of sorghum landraces from North-western Morocco using allozyme and microsatellite markers. Theor Appl Genet 99: 157–163.

Djè, Y., M. Heuertz, C. Lefèbvre & X. Vekemans, 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. Theor Appl Genet 100: 918–925.

Doku, G.V., 1969. Cassava in Ghana. Ghana University Press, Accra.

Doyle, J.J. & J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11–15.

Dubreuil, P. & A. Charcosset, 1998. Genetic diversity within and among maize populations: A comparison between isozyme and nuclear RFLP loci. Theor Appl Genet 96: 577–587.

Efron, B., 1982. The Jackknife, the Bootstrap and Other Resampling Plans. CBMS-NSF Regional Conference Series in Applied Mathematics, Monograph 38, SIAM, Philadelphia.

Elias, M., L. Penet, P. Vindry, D. MacKey, O. Panaud & T. Robert, 2001. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. Mol Ecol 10: 1895–1907.

Fauquet, C. & D. Fargette, 1990. African cassava mosaic-virus-etiology, epidemiology and control. Plant Dis 74: 404–411.

Fregene, M.A., M. Suarez, J. Mkumbira, H. Kulembeka, E. Ndedya, A. Kullaya, S. Mitchel, U. Gullberg, H. Rosling, A.G.O. Dixon, R. Dean & S. Kresovich, 2003. Simple sequence repeat marker diversity in cassava landraces: Genetic diversity and differentiation in an asexually propagated crop. Theor Appl Genet 107: 1083–1093.

Goudet, J., 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. J Hered 86: 485–486.

Jameson, J.D., 1964. Cassava mosaic disease in Uganda. East Afr Agri J 29: 208–213.

Jennings, D.L., 1994. Breeding for resistance to African cassava mosaic geminivirus in East Africa. Trop Sci 34: 110–122.

Jones, W., 1959. Manioc in Africa. Stanford University Press, Stanford.

Kimura, M. & J.T. Crow, 1964. The number of alleles that can be maintained in a finite population. Genetics 49: 725–738.

Langlands, B.W., 1966. Cassava in Uganda 1860–1920. Uganda J 30: 211–218.

Lefevre, F. & A. Charrier, 1993. Isozyme diversity within African *Manihot* germplasm. Euphytica 66: 73–80.

Martin, E.F., 1928. Report of the mycologist. In: Annual Report of the Department of Agriculture. Government Printer, Entebbe, Uganda, pp. 31.

Matsuoka, Y., S.E. Mitchell, S. Kresovich, M. Goodman & J. Doebley, 2002. Microsatellites in *Zea* – variability, patterns of mutations, and use for evolutionary studies. Theor Appl Genet 104: 436–450.

Mba, R.E.C., P. Stephenson, K. Edwards, S. Melzer, J. Mkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme & M.A. Fregene, 2001. Simple Sequence Repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: Towards an SSR-based molecular genetic map of cassava. Theor Appl Genet 102: 21–31.

Nei, M., 1975. Molecular Population Genetics and Evolution. Amsterdam: North Holland and American Elsevier Pub.

Nei, M., 1978. Estimation of average heterozygosity and genetic distances from a small number of individuals. Genetics 89: 583–590.

Nweke, F.I. & M. Bokanga, 1994. Importance of cassava processing for production in sub-Saharan Africa. Acta Hort 375: 401–412.

Ohta, T. & M. Kimura, 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet Res 22: 201–204.

Olsen, K.M. & B.A. Schaal, 1999. Evidence on the origin of cassava: Phylogeography of *Manihot esculenta*. Proc Natl Acad Sci USA 96: 5586–5591.

Olsen, K. & B. Schaal, 2001. Microsatellite variation in cassava *Manihot esculenta*, Euphorbiaceae and its wild relatives: Evidence for a southern Amazonian origin of domestication. Am J Bot 88: 131–142.

Otim-Nape, G.W., T. Alicai & M. Thresh, 2001. Changes in the incidence and severity of Cassava mosaic virus disease, varietal diversity and cassava production in Uganda. Ann Appl Biol 138: 313–327.

Otim-Nape, G.W., A. Bua & Y. Baguma, 1994. Accelerating the transfer of improved production technologies: Controlling African cassava mosaic virus disease epidemics in Uganda. Afr Crop Sci J 2: 479–485.

Otim-Nape, G.W., A. Bua, J.M. Thresh, Y. Baguma, S. Ogwal, G.N. Semakula, G. Acola, B. Byabakama & A. Martin. 1997. Cassava mosaic virus disease in Uganda: The current pandemic and approaches to control. NARO/DFID, Chatham, UK, Natural Resources Institute.

Otim-Nape, G.W., J.M. Thresh, A. Bua, Y. Baguma & M.W. Shaw, 1998. The incidence and severity of cassava mosaic virus disease in Uganda: 1990–92. Trop Sci 38: 25–37.

Pita, J.S., V.N. Fondong, A. Sangare, G.W. Otim-Nape, S. Ogwal & C.M. Fauquet, 2001. Recombination, pseudorecombination and

synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J Gen Virol 82: 655–665.

Quenoille, M., 1956. Notes on bias in estimation. Biometrika 43: 253–260.

Resende, A.G., P.S.V. Filho & M.P.S. Machado, 2000. Isozyme diversity in cassava cultivars (*Manihot esculenta* Crantz). Biochem Genet 38: 203–216.

Rohlf, F.J., 1993. NTSYS-PC numerical taxonomy and multivariate analysis system. Version 1.8 Exeter Publ., Setauket, NY.

Salick, J., 2001. Diversity, Evolution and Domestication within *Manihot*. In: Abstracts of the Fifth International Scientific Meeting of the Cassava Biotechnology Network (CBN), Danforth Plant Scientific center, St Louis, Missouri, USA, 4–9 November 2001. http://www.danforthcenter.org/iltab/cassavanet/cbnv/abstracts/session1/ps1-03.htm.

Tang, S. & S.J. Knapp, 2003. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. Theor Appl Genet 106: 990–1003.

Valdes, A.M., M. Slatkin & N.B. Freimer, 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics 133: 737–749.

Vekemans, X. & C. Lefèbvre, 1997. On the evolution of heavy-metal tolerant populations in *Armeria maritima*: Evidence from allozyme variation and reproducive barriers. J Evol Biol 10: 175–191.

Westby, A., 2002. Cassava utilization, storage and small-scale processing. In: R.J. Hillocks, J.M. Thresh & A.C. Belloti. (Eds.), Cassava: Biology, Production and Utilization, pp. 281–300. CAB International, Wallingford, Oxon, UK.

Wright, S., 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19: 395–420.

II

Genetic diversity and variety composition of cassava on small-scale farms in Uganda: an interdisciplinary study using genetic markers and farmer interviews

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Abstract

Cassava is a tropical crop and grown for its tuberous starchy roots. In Africa it is mainly cultivated by small-scale farmers who evaluate, select and name their cassava varieties based on morphology, food, social and economic interest. Here we have used an interdisciplinary approach involving farmer interviews, genetic markers and morphological descriptors to study the composition of cassava varieties on small-scale farms in 11 villages located in three districts in Uganda, the genetic structure within and between these varieties and their morphology. The composition of local, newly introduced and improved varieties differed widely between villages and districts. The Ugandan farmers in our study seemed to adopt improved varieties to a greater extent when there was a nearby market, prevalence of disease epidemics and good extension service. We found considerable genetic variation both within and between cassava varieties though the variation was larger between varieties. However, most local and improved varieties showed predominating genotypes at many loci. Accessions of commonly grown varieties meeting farmers' preferences could therefore be selected and implemented in future breeding programmes involving development, dissemination and adoption. The like-named varieties in different villages were genetically similar, demonstrating farmers' ability to differentiate and maintain the same variety over large areas. However, some varieties with different names in different villages showed both genetic and morphological similarity, suggesting that farmers may rename plants when they are introduced into their fields. The large differences found in variety and genetic composition between villages and districts in Uganda may be a result of the diverse needs and growing conditions characteristic for traditional farming system. This suggests that efforts to conserve and increase the genetic diversity in farmers' fields will require policies tailored to each area.

Key words: cassava varieties, crop evolution, farmers' management, genetic differentiation, *Manihot esculenta*, morphological variation, small-scale farmers, SSR markers, traditional farming system

Introduction

Cassava (*Manihot esculenta* Crantz) is one of Africa's major staple crops, feeding about 200 million people (Nweke, 2004). It is mainly grown for its starchy tuberous roots though the leaves can also be used as vegetables. The roots also contain variable levels of cyanogenic glucosides. Varieties with high levels of cyanogenic glucosides (>1000 mg hydrogen cyanide (HCN) equivalent kg^{-1} dry weight) are called bitter and need to be processed for safe consumption, whereas varieties with low levels are called sweet. Cassava is tolerant to drought and low pH. It gives reasonable yields in soils with low nutrient content and does not require high management costs compared to other major food crops. Cassava is therefore an attractive crop for poor resource farmers. Because of cassava's inherent tolerance to stressful environments, where other food crops would fail, cassava is often considered a food-security crop against famine and makes it one of the most useful and important crops in unstable environments in Sub Saharan Africa.

Cassava is mainly grown by small-scale farmers who chose and name their cassava according to complex motivations based on morphology, culinary attributes, social and economic interests. A group of plants identified by farmers under a single name is here referred to as a variety. In Africa the small-scale farmers derive new varieties from farmers of the same or different villages and sometimes from breeding programmes. Farmers mainly propagate cassava vegetatively through stem cuttings obtained from mature plants in their fields. However, small-scale farmers are also known to incorporate cuttings obtained from volunteer seedlings produced by sexual reproduction (Elias et al., 2001b; Fregene et al., 2003). The observed performance of a cassava variety in the local environment and farming system with respect to the farmers preferences determines whether it will continued to be cultivated. In the process of observing new planting material the name of a variety may change as the needs and choices may be specific to different farmers. This may result in the same genotype having different names or different genotypes having the same name in different farmers' fields. In addition, mixing of varieties may arise from farmers' misclassification. Therefore, a variety defined by a farmer may be complex and consist of a diverse genetic component.

In traditional agricultural systems it is common to find different cassava varieties in the farmers' fields, which may be sweet, bitter or both. The proportion of different varieties in the field may, however, be highly dynamic with high turnover. The farmers' decision over which varieties to grow is highly influenced by conditions such as biotic stress. For instance, in the Amazonian region of Peru there is a turnover of cassava varieties every 15 years due to pest and disease accumulation in the propagules (Salick, 2001). Turnovers of varieties also occurred in Uganda as a result of severe epidemics of cassava mosaic disease (CMD) in the 1920s and late 1980s (Otim-Nape et al., 1997; Otim-Nape, Alicai & Thresh, 2001). The earlier breeding programmes of selections from Amani, Tanzania, released in Uganda as the Bukalasa series in the early 1960s (Jennings, 1994) and the

selections from the International Institute of Tropical Agriculture (IITA) starting from the 1980s resulting in the release of the Namulonge–Serere (NASE; Otim-Nape et al., 1997), have contributed to the high turnovers of cassava varieties in Uganda.

Scientific breeding of cassava began only recently compared with other crops and is conducted by international and national research centers. Small-scale farmers have, however, been the engine behind cassava and variety development for thousands of years and continue today to actively select the planting material sourced from other cassava farmers, breeding programs and occasionally from sexually reproduced seedlings. The cultivated cassava on small-scale farmers' fields could therefore be looked upon as both the production and breeding populations of cassava. The local breeding occurring over generations may have resulted in varieties that are adapted to the farmers' needs for consumption, marketing requirements and cultivation conditions. A problem with scientific breeding in general is that the focus is mainly on broad adaptability so that a variety will produce a high average yield over a range of environments and years. Unfortunately, candidate genetic material that produces very good yields in one area, but poor yields in another, tends to be quickly eliminated from the breeder's gene pool. Yet, this may be exactly what the small-scale farmers in some areas need. Traditional farming systems, which are characterized by unstable environments and resource poor farmers with diverse needs, would greatly benefit from maintaining a high genetic diversity within their crops for food security. Professional breeders, often working in relative isolation from farmers, have sometimes been unaware of the multitude of preferences, beyond yield, and resistance to diseases and pests, of their target farmers. This may result in a low adoption rate of improved varieties by the farmers in some areas. As an attempt to solve this problem participatory plant breeding (PPB) projects involving small-scale farmers are developing. In these projects breeders work closely with farmers at different stages of the research process to develop cassava varieties tailored to these farmers' needs and to the requirements of the growing conditions of their cassava. For any successful breeding programme involving development, dissemination and adoption of new varieties it is of great importance to have knowledge about the genetic composition of the varieties grown by the target farmers and also to know which genotypes continue to be of interest to these farmers since their production system may change over time.

The evolution of crops that takes place in farmers' fields in traditional agricultural systems may be underestimated especially in vegetatively propagated crops, and plant varieties are assumed to be conserved without evolution over long periods of time. Our recent results from a broad-scale study on the genetic diversity of cassava in Uganda showed, however, a high genetic diversity both in areas with high and low CMD incidence (Balyejusa Kizito et al., 2005). The genetic differentiation within and among varieties was, however, not investigated in that study. We found it, therefore, interesting to investigate the differentiation of Ugandan farmers' varieties and test whether these varieties are of uniform genetic composition. Here we report the composition of small-scale farmers' varieties in different villages in Uganda, how the genetic diversity of cassava is structured

within and between these varieties and how morphologically distinct they are. In addition, we discuss how the Ugandan small-scale farmers maintain and differentiate their varieties. We have chosen an interdisciplinary approach in which farmer interviews on their naming of varieties was combined with genetic marker and morphological descriptions of cassava varieties. The interdisciplinary approach has been used in a few other cassava studies on small-scale farmers' varieties in Malawi (Mkumbira et al., 2003) and Guyana (Elias, Panaud & Roberts, 2000; Elias et al., 2001a). Our findings will provide a unique basis for the understanding of genetic composition of Ugandan farmers' varieties that can be used for developing strategies for cassava breeding programs with regard to improvement, multiplication and dissemination of cassava varieties.

Materials and methods

Study areas

Three districts where cassava is an important crop in the farming system were chosen for the study: Kumi, Luwero and Hoima (Figure 1). Kumi district is located in the eastern part of Uganda and previous studies in the area have shown that the area experienced high CMD epidemics (Otim-Nape et al., 1997). Consequently there was an increase of activities by different Non Governmental Organisations (NGOs) and the National Cassava Breeding Programme to supply cassava varieties. The villages visited were Atiira, Apama-Oteteen, Kachaboi and Omolokonyo. In Luwero district, central part of Uganda, the villages selected were near the Namulonge Agricultural and Animal Production Research Institute (NAARI). Some of the farmers have participated in on-farm trials of varieties developed by the National Cassava Breeding Programme especially after the recent CMD outbreak. The villages visited were Kabembe, Kibanga, Nattyole and Vvumba. Hoima district, in the western part of Uganda, has been affected by CMD to a lesser extent than Luwero and Kumi districts and has not had many activities from the NASE breeding program (Otim-Nape et al., 1997). The villages studied were Kyeramya, Kyarubanga-A and Kyarubanga-B.

Key informant interviews

Prior to collection of cassava material in the eleven villages, initial information on cassava cultivation in each district was gathered from key officers at governmental and development organizations in individual interviews at the district offices. In each village interviews were held in groups ranging from 3 farmers in Attira, Kumi to 17 farmers in Kyeramya, Hoima to gather information about the preferences, utilization and naming of their most commonly grown varieties. A list of guidelines was developed for the group meetings for purposes of consistency and uniformity. The interviews were carried out in a semi-structured informal manner and were based on methods according to Sperling & Ashby (1997) and Salick, Cellinese & Knapp (1997). In addition, we made direct observation on the growing and use of cassava in the villages. After these interviews one farmer per village, known to be

growing cassava for a long time, was purposely selected for depth interviews. These farmers were asked to give a general description and use of their varieties and how the varieties differed from each other. The source of planting material was classified into four categories: (1) local – varieties which have been grown continuously for over one farmer generation in the area (Berthaud et al., 2001), (2) newly introduced – varieties acquired from outside the village (relatively recently within the last farmer generation), (3) improved – varieties from the Bukalasa or NASE cassava breeding programmes and (4) unknown – plants for which the farmers did not have a name.

Plant material

Each farmer in the group interviews identified their most commonly grown varieties and was asked to give a cutting (of about 1 meter) of the respective variety, here called an accession. All accessions collected from each village and district are listed in Table 1. Three to five accessions were given per farmer. Each accession was divided into four cuttings. Three cuttings of each accession were planted in an experimental field at Namulonge Agricultural and Animal Research Institute (NAARI) for morphological description while the fourth cutting of the same accession was planted in a screenhouse at NAARI for molecular marker analysis.

Molecular marker analysis

All accessions collected from the farmers (Table 1) and an additional accession of each of the improved varieties NASE1, NASE2, NASE3, NASE5, NASE8, NASE10 and NASE12 from NAARI were subjected to molecular marker analysis. DNA was isolated from young leaf tissue following the method by Doyle & Doyle (1987). Eleven microsatellite markers (SSRY4, SSRY9, SSRY19, SSRY51, SSRY64, SSRY82, SSRY103, SSRY148, SSRY 151, SSRY164 and SSRY181) with high polymorphism information content (PIC) were selected from 67 markers from an earlier study (Fregene et al., 2003). PCR (polymerase chain reaction) was carried out as described by Mba et al. (2001) using 10 ng of DNA per reaction. The PCR product was denatured and electrophoresed on 6 % polyacrylamide gels using Bio-Rad Vertical Sequencing System (Bio-Rad Inc., USA) and visualized by silver staining according to the Promega manufacturer's guide. Allele sizes were determined based upon an internal gel molecular marker size standard. Scoring was done manually and with the aid of computer software 'Quantity One' (Bio-Rad Inc.). In addition, a few plants with known genotypes from the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, were used as controls on each gel.

Morphological description

Three cuttings of each accession given by the farmers were randomly planted together in one block consisting of three rows in an experimental field at NAARI. Because of an unusually early beginning of the dry season many accessions did not

sprout or died back. The accessions that survived were studied for 15 morphological traits of which 14 were characterized into different classes as described in Table 6. In order to estimate the trait variation within a variety only the 21 varieties with three or more accessions reaching maturity (9 months after planting) are presented in the table, given a total of 180 accessions. At sprouting the colour of first fully expanded leaf (CEL), the colour of young shoot (CYSH), the colour of leaf vein (CLV) and the pubescence of young leaves (PUYL) were checked. At 6 months after planting leaves on the mid-section part of each plant was scored for the number of leaf lobes per leaf (NLL), the position in which the leaf lobes are held (POLL), the shape of leaf lobes (SHLL), the length of the petiole (LEPE) and the colour of petioles (CPE). In addition, the number of petioles (NPE) on the entire plant was recorded. Finally, at 9 months after planting the stem morphology was checked for the growth habit of stem (GHST), the pubescence of young stem (PUYST), the colour of mature stem (CMST), the prominence of leaf scars (PRLSC) and the height of first apical branch (HFAB). The studied traits are among other traits recommended by the International Plant Genetic Resources Institute (IPGRI) for cassava germplasm characterization and adapted by the National Cassava Programme premised at NAARI.

Statistical analysis

Genetic differentiation within and between varieties was quantified by F-statistic estimators F_{IS}, F_{ST} and F_{IT} (Wright, 1965) as described by Weir & Cockerham (1984) using FSTAT 2.9 (Goudet, 1995). Pairwise distances between the varieties in the different villages based on Nei's genetic distance (Nei, 1972) were calculated using the software package PHYLIP version 3.65 (http://evolution.gs.washington.edu/phylip.html). These pairwise estimates were used to construct a dendrogram based on the Neighbor-Joining method (Saitou and Nei, 1987) and a dendrogram based on the unweighted paired group method with the arithmetic mean (UPGMA) implemented in the PHYLIP package version 3.65. The original data set of allele frequencies were resampled with 1000 bootstraps and a consensus tree was constructed. In order to look for a correlation between genetic and geographic distances between villages a test of isolation by distance (Slatkin, 1993) were performed using a Mantel test (Sokal and Rohlf, 1995) in the GENSURVEY program (Vekemans and Lefèbvre, 1997).

Results

Composition and naming of farmers' varieties

All together 288 accessions were given by the farmers from 11 villages in Luwero, Kumi and Hoima districts. Based on the farmers' interviews these accessions represented as much as 49 different varieties named by the farmers and 44 unnamed accessions (Table 1). In general farmers grew a mixture of their local varieties (varieties that have been grown continuously for over one farmer generation in the area, Berthaud et al., 2001), varieties acquired from outside the

village (relatively recently within the last farmer generation) and improved varieties which they identified as coming from the Bukalasa or NASE cassava breeding programmes, and plants with unknown origin and without a name. The percentage of these categories based on the names given by farmers is plotted in Figure 2. In Hoima district about two thirds were said to be local varieties, while only 4% were said to be from the Bukalasa breeding programme. The Hoima farmers did not identify any accession to come from the NASE programme. In Kumi on the other hand, as much as 40% were identified to be from the NASE programme but no accessions were said to come from the Bukalasa programme. Only 23% of the accessions were identified by the Kumi farmers as local varieties. A similar pattern was found in Luwero where more than half of the accessions were said to be from the NASE programme and only 17% were identified as local varieties. The highest frequency of newly introduced varieties (23%) and the lowest frequency of accessions with unknown origin (7%) were found in Hoima. For example, in Kyeramya village in Hoima none out of 78 accessions were unnamed by the farmers. All unknown and almost all named accessions were identified as sweet by the farmers.

The naming of the varieties, both local and improved, was specific to the districts except the improved Bukalasa and SS4 variety names that were common to both Kumi and Luwero districts (Table 1). Some of the improved varieties such as NASE2 and NASE3 have been renamed in some villages. Many varieties were found in two or more villages, often in the same district, while some were only found in one village. None of the varieties was found in all villages in a district. This resulted in a high number of varieties named by the farmers and a small number of accessions collected for many varieties.

In general there was no indication that farmers purposely used the same variety name for different phenotypes except in Kyeramya village in Hoima where the farmers differentiated two phenotypes of the variety Nyakabiriti. These were labelled as Nyakabiriti-1 and Nyakabiriti-2, respectively, during collection (Table 1). However, no distinct morphological differences were found between cuttings of these two varieties when grown in an experimental field (Table 7).

Number of alleles and percentage of polymorphic loci

The number of alleles found at each locus ranged from 3 at SSRY148 and SSRY181 to 7 alleles at SSRY9, SSRY82 and SSRY 151 with a mean of 4.9 alleles per locus (Table 2). Percentage of polymorphic loci and mean number of alleles per locus are presented in Table 3 for varieties with 4 or more accessions given by the farmers. The small number of accessions given by the farmers of some varieties may represent a limited part of the genetic variation existing in these varieties. The genetic analysis of these varieties is therefore intended to be heuristic rather than definitive. The lowest frequency of polymorphic loci (45.5%) and the lowest mean number of alleles per locus (2.0) were found in the local variety Bamunaanika, while the three newly introduced varieties Emusugut, Nyakakwa and Nyalanda, and the local variety Kidimo had 100% polymorphic loci

and on average 3.4 to 3.7 alleles per locus (Table 3). Both the local and improved varieties showed an average of 2.6 alleles per locus while the three introduced varieties showed an average of 3.5.

Genetic differentiation within and between varieties

Genetic differentiation within and between varieties was quantified by F-statistics as described by Weir & Cockerham (1984) for varieties that had 4 or more accessions (Table 3). Of the total diversity (F_{IT} = 0.236) most diversity was found among varieties (F_{ST}=0.250) compared to the within-variety diversity (F_{IS}= −0.021) showing that the varieties were genetically differentiated from each other. The local varieties were more greatly differentiated (F_{ST} = 0.275) than either the newly introduced (F_{ST} = 0.172) or improved (F_{ST} = 0.164) varieties. However, all the variety categories showed large confidence intervals for the F_{ST} values indicating that some varieties are genetically more similar than others. The within-variety diversity differed among the local, newly introduced and improved varieties (F_{IS} = −0.138, 0.130 and 0.051, respectively). There was an excess of heterozygotes within the local varieties as indicated by the negative F_{IS} value while the positive F_{IS} values showed a deficiency of heterozygotes within the newly introduced and improved varieties. However, the confidence intervals of the within-variety diversity ranged from negative to positive F_{IS} values for all three variety-categories indicating a difference in the genetic structure of varieties within each category.

All local, newly introduced and improved varieties consisted of several multilocus genotypes (Table 4) and in general no clones (accessions with identical multilocus genotype) were found. For most varieties all accessions within a variety differed in at least one marker locus. Accessions within a variety with an identical multilocus genotype were only found in 2 of the 21 varieties studied, namely Nigeria and Nyakabiriti-2. Interestingly, none of the farmers' NASE accessions showed identical multilocus genotype with any of the single accessions of the NASE varieties collected from NAARI. The newly introduced varieties Emusugut and Nyakakwa showed a high number of different genotypes with no predominating genotype at almost all loci. However, most of the other varieties had predominating genotypes at many loci. The highest number of predominating genotypes was found in the local varieties Nyakabiriti-1, Nyakunyaku (10 out of the 11 loci) and Nyakabirit-2 (9 out of the 11 loci). About two thirds of the varieties had five or more loci with predominating genotypes while no predominating genotype was found in the newly introduced variety Emusugut.

The pattern of variation found differed between varieties and marker loci (Table 4). For example, the local varieties Mulyandongo and Nyakabiriti-2 showed little variation at the marker loci SSRY82 and SSRY164, while great variation was found in the local variety Kidimo within the same loci. The marker loci SSRY9 and SSRY181 showed the same predominating genotype within ten and nine different varieties, respectively, while SSRY4 showed no predominating genotypes in all but two varieties.

The 44 unknown accessions given by the farmers showed all different multilocus genotypes. Twenty-nine of these showed unique multilocus genotypes not similar to any of the varieties named by the farmers, while 6 accessions showed similar multilocus genotypes to the introduced varieties (3 to Emusugut, 2 to Nyakakwa and 1 to Nyalanda), 5 accessions showed similarity to some of the varieties in the NASE breeding program (3 to TME14 and NASE 10, 1 to Nigeria and 1 to No. 00057) and 4 accessions had similar multilocus genotypes to some of the local varieties (2 to Bamunaanika, 1 to Kidimao and 1 to Mulyandongo).

Genetic distances between farmers' varieties

Because missing genotype data is not allowed in analysis with the phylogeny software package PHYLIP version 3.65 (http://evolution.gs.washington.edu/phylip.html), handling both allele frequencies and bootstrap resampling, only 165 out of 244 named accessions by the farmers and one additional NAARI accession were analyzed. These varieties were divided into 32 groups depending on variety and village. Nei's genetic distances (Nei, 1972) between varieties in the different villages are presented in Table 5. Most varieties given the same name in different villages showed among the closest genetic similarity, for example the local varieties Kidimo and Nyakunyaku in two villages in Hoima district and the improved variety Nigeria in three villages in Kumi district. The like-named local accessions differentiated into two phenotypes by the farmers, here called Nyakabiriti-1 and Nyakabiriti-2 in Kyeramya village, Hoima, showed relatively small genetic distance. On the other hand, some accessions given different names by the farmers such as the local varieties Ebwanateraka and Emulai in different villages in Kumi district were also genetically similar. Also Nyakabiriti-1 and Nyakabiriti-2 showed similarity with the relatively newly introduced Nyalanda variety within the same village. As expected, relatively close genetic similarity was also found between some of the improved varieties in the NASE breeding programme such as SS4 in Apama-Oteteen village in Kumi district, released as NASE4 and the Nigerian variety, released as NASE3 in several villages in Kumi. Interestingly, SS4 in Apama-Oteteen village and NASE3 from NAARI showed no genetic distance. NASE3 from NAARI showed also genetic similarity between NASE3 in Apama-Oteteen village. In addition, Vumba in Nattyole village in Luwero, relased as NASE12, showed relatively small genetic distances with Nigeria and 00057 from among the on-farm trials in the NASE breeding programmes.

Relatively close genetic similarity was found between the improved Bukalasa and the local variety Bamunaanika in the village Nattyole in Luwero district. Other local varieties in different villages showed larger genetic distances to Bukalasa. This may suggest that Bamunaanika has a Bukalasa origin and is younger than the other local varieties or that the farmers in Nattyole misclassified the Bukalasa and Bamunaanika accessions. Due to missing genotype data only Bukalasa and Bamunaanika from one village, Nattyole, could be analyzed and it is therefore difficult to further discuss the genetic similarity between the two varieties.

When comparing the varieties in the NASE breeding program with non-improved varieties, NASE3 and Nigeria showed the smallest genetic distance with the newly introduced variety Emusugut in the village Apama-Oteteen in the Kumi district. Most other non-improved varieties showed considerable distances between the varieties in the NASE breeding program. The largest genetic distances were found between the improved variety in the Bukalasa breeding program, developed in Amani, Tanzania and in Uganda, released in the 1960s, and the improved varieties in the NASE breeding program (NASE, Nigeria, SS4, TC1), starting from the 1980s at IITA. This suggests that the Bukalasa and the NASE varieties have different origin. However, additional Bukalasa accessions from other villages need to be analyzed to prove this. Even though most non-improved varieties showed large genetic distances to the NASE-varieties (average D=0.67) and many of the NASE varieties showed relatively small genetic distances (average D=0.40) no clustering of larger groups of varieties were supported by bootstrap resampling with the Neighbor-Joining (Saitou and Nei, 1987) or the UPGMA methods based on Nei's genetic distance (1972) implemented in PHYLIP version 3.65 (data not shown). The average genetic distance between varieties within villages ranged from 0.40 in Kyeramya, Hoima district to 0.96 in Kachaboi, Kumi district. The relatively large genetic distances suggest that many varieties grown by farmers within the same village were genetically distinct. When comparing the genetic variability among villages the accessions in nearby villages seemed in general to be more genetically similar than accessions in villages far apart since there was a positive significant correlation between genetic and geographic distances among villages (r=0.195, P=0.002) found by the Mantel test (Sokal and Rohlf, 1995) in the GENSURVEY program (Vekemans and Lefèbvre, 1997).

Morphological description

Table 7 presents the morphological class with the highest frequency for the respective trait in each variety. In addition, the average of all cuttings and accessions of each variety for the trait HFAB is given. Plants given the same name by the farmers were grouped together irrespective of the village they came from. In order to estimate the trait variation within a variety only the varieties with three or more accessions surviving in the field are presented here. Cuttings of the same accession showed the same morphology class in respective traits. Seven of the traits (CEL, CYSH, CLV, LEPE, CPE, CMST and HFAB) showed more variation within and among varieties than the other traits (PYL, NLL, POLL, SHLL, NPE, GHST, PUYST and PRLSC). Four of the traits (CEL, CPE, CMST and HFAB) showing more variation within and among varieties and two of the traits (SHLL and GHST) showing less variation were said to be important by the farmers in differentiating their varieties (Table 7).

The genetically similar local varieties Ebwanateraka and Emulai showed similar morphology. Also the like-named and genetic similar accessions differentiated into two phenotypes by the farmers, here called Nyakabiriti-1 and Nyakabiriti-2, had similar morphology. However, the morphology class which showed the highest

frequency in several traits important by the farmers in differentiating their varieties differed between the genetically similar local variety Bamunaanika and the improved Bukalasa variety. Most of the improved varieties in the NASE breeding program, showing genetic similarity, differed also in at least two traits. The relatively newly introduced variety Nyalanda showed a distinct morphology in CEL and CPE compared to all other varieties.

The morphological variation found within and between varieties could be an effect of both genotype differences and differences in the growing conditions in the experimental field, and the phenotypic expression may differ in the farmers' fields. The individual effect of these parameters could, however, not be estimated without a randomized complete block design with several blocks and replicates of each accession in each block (Chahal & Gosal, 2002). Since this design was not used here we believe it would be inappropriate to further analyse the morphological variation with multivariate statistical methods.

Discussion

Evolution of crops that takes place in farmers' fields in traditional agricultural systems is often underestimated especially in vegetatively propagated crops, and plant varieties are assumed to be conserved without evolution over long periods of time. Knowledge related to farmer management in influencing the genetic diversity of their crops is limited even though it is of major importance for developing strategies for breeding programs with regard to improvement, multiplication and dissemination of varieties. In this study we have focused on the composition and genetic structure of cassava varieties in farmers' fields in Uganda and test the hypothesis that very little variation is found within these varieties.

Genetic diversity

We found considerable genetic variation both within and between cassava varieties though the variation was larger between varieties. Very few clones were found among the accessions collected from different farmers and villages. This may be surprising considering that cassava is mainly vegetatively propagated. The large genetic variation within varieties may be a result of farmers' selection of volunteer seedlings produced by spontaneous sexual recombination. Most unnamed accessions showed multilocus genotypes which were very different from the multilocus genotypes found in any of the varieties identified by the farmers. This may further illustrate farmers' selection of spontaneous seedlings with unknown origin. The extent to which the African farmers incorporate seedlings into the planting population may therefore be underestimated. However, a small number of accessions and only one accession of each variety were collected per farmer in this study. This study has therefore not investigated whether there are clones within farmers' fields. Large genetic variation within varieties has also been found in the

small-scale farming communities in Guyana (Elias et al., 2001a) and Brazil (Second et al., 1997; Sambatti, Martins & Ando, 2001).

We found that the pattern of variation differed between varieties so that some varieties showed extensive variation within almost all marker loci such as the newly introduced varieties Nyakakwa and Emusugut, while many local varieties showed predominating genotypes in most loci (Table 4). The finding that some loci showed extensive variation within varieties may indicate that these loci are not linked to any trait of interest for the farmers whereas loci that showed less variation and predominating loci within varieties may be linked to traits of interest. Also varieties grown by small-scale farmers in a village in Guyana showed differing levels of variation within them, some were monomorphic while others had high intra-varietal variability (Elias et al., 2001b).

Differences in levels of variation in the Ugandan farmers' varieties may be a result of a combination of their history, the source of each variety and farmers' management. At some point varieties are brought into farmers' villages from neighbouring communities and sometimes from breeding programmes. Before being given to farmers improved varieties usually have undergone cycles of selection based on the respective breeding objectives. Ultimately improved varieties go through selection cycles for morphological uniformity, which would most likely result in more genetically homogenous varieties. Among the few Bukalasa accessions identified by the farmers no identical multilocus genotypes were found. In Uganda the bred Bukalasa varieties were released in the early 1960s (Otim-Nape, Bua & Baguma, 1994) and have since been subject to various evolutionary forces such as gene exchange between farmers, gene flow and human and natural selection within farmers' fields. One might therefore not expect to find high genetic homogeneity within the Bukalasa varieties.

Even though less variation was found in the improved NASE varieties than in the introduced varieties surprisingly no clones were found in the NASE varieties given by the farmers. Furthermore, none of the farmers' NASE accessions showed identical multilocus genotype with any of the single accessions of the NASE varieties collected from NAARI. For further studies it would be interesting to more thoroughly compare the genetic constitution of the NASE varieties within the breeding programme with the genetic constitution of farmers' NASE varieties in both within and outside target areas to shed light on the short-term effect of farmers' management on the genetic changes within varieties.

In contrast to varieties from breeding programmes, varieties introduced from farmers outside the village may come from various sources and a variety consisting of plants given the same name may therefore consist of more variability as was seen for Emusugut and Nyakakwa varieties (Table 4). Local varieties not related to the improved varieties probably start as varieties introduced from different areas outside the village. All varieties, local, improved or newly introduced undergo selection on farmers' fields. Phenotypes that do not meet the farmers' preferences get eventually eliminated and the genetic variation within a variety may reduce with time. The varieties that are grown in the Ugandan farmers' fields may

therefore be at different stages of selection with the old local varieties being at more advanced levels of selection than the newly introduced varieties.

Our findings on the intra-varietal diversity differ from cassava studies in Malawi where a single multilocus genotype predominated within each of the ten most grown local varieties and showed a wide distribution in the cassava growing areas in the country, although varieties went by different names in different areas (Mkumbira, 2002; Mkumbira et al., 2003). The clonality of cassava varieties in Malawi may be explained by governmental intervention efforts in the late 1980s in response to mealy bug infestation that focused on the identification, recommendation of specific genotypes of some local varieties and the rapid distribution of cleaned and improved cuttings thereof (Mkumbira, 2002; Haggblade & Zulu, 2003). The use of tissue culture in the multiplication scheme may also have helped to perpetuate the selected clones from each variety together with an efficient distribution system. Varieties identified by the Malawian farmers that were not among the ten most grown varieties showed extensive genetic variation within varieties (Mkumbira et al., 2003).

Farmers' maintenance and differentiation of varieties

In spite of intra-varietal variation, the large genetic differentiation (Table 3) and genetic distances (Table 5) found in general among Ugandan farmers' varieties show farmers ability to maintain and differentiate their varieties. A closer genetic similarity among accessions in nearby villages suggests occurrence of exchange of planting material between farmers in nearby villages. The exchange of planting material may lead to misclassification of accessions. However, varieties given the same name by farmers in different villages were genetic similar (Table 5), showing that the farmers are able to maintain the same variety over larger areas. Based on our interviews with the Ugandan farmers, certain morphological traits such as HFAB and CPE and culinary attributes such as taste of a plant seemed to be of most importance to differentiate and name varieties. Thus, plants that showed similar morphology would most likely be given the same name in the same village. Plants with unfamiliar morphology, which was found to have multilocus genotypes different from any of the accessions identified by the farmers, were unnamed but continued to be grown if they met other farmer's demands such as taste. In fact, all unknown varieties were sweet. However, when cuttings obtained from farmers were grown in an experimental field, morphological variation in several traits was found within varieties. If the morphological heterogeneity within varieties is maintained within farmers' fields the identification of plants by the farmers may be difficult and may lead to misclassification in naming and increased heterogeneity within varieties. Some varieties with different names were genetically similar. Based on the morphological description of cuttings obtained from farmers some of these varieties showed the same morphology while others differed in several morphological traits considered to be important by the farmers in differentiating varieties. Varieties which were genetically and morphologically similar but had different names in different villages may suggest that farmers rename plant material when it is introduced to the farmers' fields.

Composition of varieties

We found a difference in the composition of local, newly introduced and improved varieties in the Ugandan villages and districts and many varieties had names specific to the district and some even to the village. The difference in variety composition may reflect farmers' decisions made under different circumstances such as the access to urban markets, influence from government extension programmes, biotic stress such as pest and diseases in the farmers' fields and innovativeness of farmers in acquiring new planting material.

The farmers in Hoima district did not grow any of the most recently released NASE varieties and a very low frequency of the older bred Bukalasa varieties. In comparison with the farmers in the two other districts Luwero and Kumi, the Hoima farmers grew a larger frequency of newly introduced varieties, suggesting that the Hoima farmers have relied more on other farming communities for sources of planting material than the breeding programmes. Farmers in Kumi and Luwero on the other hand have had more access to planting material from the cassava breeding programme and therefore seem to rely more on planting material from there than from other farming communities. Access to urban markets by both Luwero and Kumi farmers has also largely influenced their production choices in that they may have chosen to use more material from the breeding programme since improved varieties are usually higher yielding than local varieties. Kumi is a major dried cassava supplier to market (Otime-Nape et al., 1997) and Luwero supplies fresh cassava. Additionally, the recent CMD epidemic in Kumi and Luwero led to interventions by government and NGOs to restore cassava production and counter the heavy variety losses that were caused by the disease with improved varieties. The recently released NASE varieties seem to have had high adoption rates in these areas, showing farmers motivation to adopt new varieties from the breeding programme. The dissemination policy and time are important factors in the adoption rate therefore further studies in time would give a more conclusive reflection of the adoption of improved varieties in areas near and distant from urban markets.

In conclusion, our results showed large genetic differentiation among varieties. However, genetic and morphological variation was also found within local, newly introduced and improved varieties and almost no clones were found within varieties. This shows the complexity of defining a farmer's variety and the importance of combining genetic analysis with farmers' naming in determining the composition of farmers' varieties and understanding the role of farmers management on the cassava genetic diversity. However, most local and improved varieties had predominating genotypes at many loci. Accessions of commonly grown varieties meeting farmers' preferences could therefore be selected with the same interdisciplinary approach used in this study and implement in future breeding programmes in the country. We also found that the composition of local, newly introduced and improved varieties differed between villages and districts. The Ugandan farmers in our study seemed to adopt improved varieties to a greater

extent when there was a nearby market, high CMD incidence and good extension service. Accordingly, breeders can strongly affect the composition of farmers' varieties by controlling the number and dissemination of improved varieties, which may lead to a loss of local varieties and a reduction of genetic diversity. However, as traditional farming systems, which are characterized by unstable environments and diverse needs of farmers, would greatly benefit from maintaining a high genetic diversity within their crops for food security it is of great importance to prevent loss of genetic diversity in the farmers' fields. Therefore the large differences found in variety and genetic composition between villages and districts in Uganda suggest that efforts to conserve and increase genetic diversity in farmers' fields will require policies tailored to each area. In addition, as the genetic constitution within a variety and the composition of varieties on small-scale farms may change over time the national programs for gene conservation and breeding need to consider the dynamics of genetic change.

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References

Balyejusa-Kizito, E., A. Bua, M. Fregene, T. Egwang, U. Gullberg, & A. Westerbergh, 2005. The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda. Euphytica 146: 45-54.

Berthaud, J., J.C. Clement, L. Emperaire, D. Louette, F. Pinton, J. Sanou, & G. Second, 2001. The role of local-level gene flow in enhancing and maintaining genetic diversity, pp. 81-103 in Broadening the Genetic Base of Crop Production, edited by H.D. Cooper, C. Spillane & T. Hodgkin. CABI, Wallingford, Oxon, UK.

Chahal, G.S. & S.S. Gosal, 2002. Principles and procedures of plant breeding: Biotechnological and conventional approaches. Alpha Science International Ltd. Pangbourne, UK

Doyle, J.J. & J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

Elias, M., O. Panaud,. & T. Robert, 2000. Assessment of genetic variability in a traditional cassava (Manihot esculenta Crantz) farming system, using AFLP markers. Hered. 85: 219-230.

Elias, M., L. Penet, P. Vindry, D. MacKey, O. Panaud, & T. Robert, 2001a. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. Mol. Ecol. 10: 1895-907.

Elias, M., D. McKey, O. Panaud, M.C. Anstett, & T. Robert, 2001b. Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians

(Guyana, South America): Perspectives for on-farm conservation of crop genetic resources. Euphytica 120: 143-157.
Fregene, M.A., M. Suarez, J. Mkumbira, H. Kulembeka, E. Ndedya, A. Kullaya, S. Mitchel, U. Gullberg, H. Rosling, A.G.O. Dixon, R. Dean, & S. Kresovich, 2003. Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. Theor. Appl. Genet. 107: 1083-93.
Goudet, J., 1995. FSTAT (vers. 1.2): a computer program to calculate *F*-statistics. J. Hered. 86: 485-486.
Haggblade, S. & B. Zulu, 2003. The recent cassava surge in Zambia and Malawi. Paper 9. InWent/IFPRI/NEPAD/CTA conference: Successes in African Agriculture, pp. 7-12.
Jennings, D.L., 1994. Breeding for resistance to African cassava mosaic geminivirus in East Africa. Trop. Sci. 34: 110-122.
Mba, R.E.C., P. Stephenson, K. Edwards, S. Melzer, J. Mkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme, & M.A. Fregene, 2001. Simple Sequence Repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theor. Appl. Genet. 102: 21-31.
Mkumbira, J., 2002. Cassava development for small-scale farmers. PhD thesis. Agraria 365. Swedish University of Agricultural Science.
Mkumbira, J., L. Chiwona-Karltun, U. Lagercrantz, N.M. Mahungu, J. Saka, A. Mhone, M. Bokanga, L. Brimer, U. Gullberg, & H. Rosling, 2003. Classification of cassava into 'bitter' and 'cool' in Malawi: from farmers' perception to characterisation by molecular markers. Euphytica 132: 7-22.
Nei, M., 1972. Genetic distance between populations. Am. Nat. 106: 283-292.
Nweke, F., 2004. New challenges in the cassava transformation in Nigeria and Ghana. Discussion paper No. 118. Environment and Production Technology Division, International Food Policy Research Institute.
Otim-Nape, G.W., A. Bua & Y. Baguma, 1994. Accelerating the transfer of improved production technologies: Controlling African cassava mosaic virus disease epidemics in Uganda. Afr. Crop Sci. J. 2: 479-485.
Otim-Nape, G.W., A. Bua, J.M. Thresh, Y. Baguma, S. Ogwal, G.N. Semakula, G. Acola, B. Byabakama & A. Martin, 1997. Cassava mosaic virus disease in Uganda: the current pandemic and approaches to control. NARO/DFID. Chatham, UK: Natural Resources Institute, pp. 33-38.
Otim-Nape, G.W., T. Alicai,. & J.M. Thresh, 2001. Changes in the incidence and severity of cassava mosaic virus disease, varietal diversity and cassava production in Uganda. Ann. Appl. Biol. 138: 313-327.
Saitou, N. & M. Nei, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.
Salick, J., N. Cellinese, & S. Knapp,. 1997. Indegineous diversity of cassava: generation, maintenance,use and loss among the Amuesha, Peruvian upper Amazon. Econ. Bot. 51: 6-19.
Salick J., 2001. Diversity, evolution and domestication within Manihot. Fifth International Scientific Meeting ofthe Cassava Biotechnology Network November 4 - 9, 2001, St. Louis, Missouri USA, PS1-03.
Sambatti, J.B.M., P.S. Martins, & A. Ando, 2001. Folk taxonomy and evolutionary dynamics of cassava: a case study in Ubatuba- Brazil. Econ. Bot. 55: 93-105.
Second, G., A.C. Allem, R.A. Mendes, L.C.J.B. Carvalho, L. Emperaire, & C. Ingram, 1997. Molecular marker (AFLP)-based *Manihot* and cassava numerical taxonomy and genetic structureanalysis in progress: implications for their dynamic conservation and genetic mapping. Afr. J. Root Tuber Crops. 2: 140-14.
Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47: 264-279.
Sokal, R.R. & F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition, Freeman and Co., New York. 887 pp. ISBN: 0-7167-2411-1.

Sperling, L. & J. Ashby, 1997. Participatory plant breeding: emerging models and future development. In: R. Tripp (ed.) New seeds and Old Laws, pp 198-213. Regulatory reform and the diversification of national seed systems. IT publications, London.
Vekemans, X. & C. Lefèbvre, 1997. On the evolution of heavy-metal tolerant populations in *Armeria maritima*: evidence from allozyme variation and reproducive barriers. J Evol Biol 10: 175-191.
Weir, B.S. & C.C Cockerham, 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370.
Wright, S., 1965. The interpretation of population structure by F-statistics with special regards to systems of mating. Evolution 19: 395-420.

Table 1. Collected cassava accessions named by the farmers (abbreviations in parentheses used in Table 5) in 11 villages in Hoima, Kumi and Luwero districts, and farmers' remarks

District	Village	No. of plants	Variety	Remarks given by the farmers
Hoima	Kyarubanga-A (1)	12	Kidimo (KI-1)	Local, sweet variety
		3	Bukalasa	Bukalasa breeding programme, sweet
		3	Nyakunyaku (NU-1)	Local, bitter
		2	Nyapamitu	Local, sweet
		3	Sibampale	Local, sweet, roots last up to 4 years in the ground
		1	Asianju	Local, sweet
		1	America	,,
		1	Misi	,,
		1	Tanzania	,,
		1	Nyakakwa	Relatively newly introduced, bitter
		1	Kakyakyari (KA-1)	,,
		5	Unknown	All sweet
	Kyarubanga-B (2)	7	Kidimo (KI-2)	Local, sweet
		1	Lyaholole	,,
		1	Kitika	,,
		2	Sibampale	,,
		3	Nyakunyaku	Local, bitter
		2	Bukalasa	Bukalasa breeding programme, sweet
		4	Unknown	All sweet
	Kyeramya (3)	14	Mulyandongo (MU-3)	Local, roots last up to 3 years in the ground, sweet
		16	Nyakabiriti-1 (NB1-3)	Local, yields well even with CMD,
		11	Nyakabiriti-2 (NB2-3)	Local, yields well even with CMD,
		13	Nyakakwa (NW-3)	Foreign, bitter
		7	Nyakunyaku (NU-3)	Local, bitter
		17	Nyalanda (NL-3)	Relatively newly introduced, sweet
		0	Unknown	
Kumi	Apama-Oteteen (4)	1	Omotoka (OT-4)	Local variety, literally meaning 'belonging to cars,' that is it sells quick, sweet
		5	Emulai (EL-4)	Local variety, sweet
		6	Emusugut (ES-4)	Relatively newly introduced by the NGO called Oxfam in the late 80s and 90s; literally meaning man', sweet
		6	NASE3 (N3-4)	From the NASE breeding programme of the 90's, roots are bitter before 9 months after planting
		3	Nigeria	,, released as NASE3

Table 1. Cont.

		1	NASE10	NASE breeding programme, sweet
		1	SS4 (S4-4)	,, released as NASE4
		2	Oxfam (OX-4)	Relatively newly introduced by the NGO called Oxfam in the late 80s and 90s, sweet
		1	2327	NASE breeding programme; from lines on on-farm trials, sweet
		1	TME414	,,
		10	Unknown	All sweet
	Attira (5)	1	TME414	NASE breeding programme; From among the lines on on-farm trials, sweet
		1	NASE10	NASE breeding programme, sweet
		2	Nigeria (NI-5)	NASE breeding programme; released as NASE3, roots are bitter before 9 months after planting
		3	Ebwanateraka	Local variety, meaning 'bachelor's crop,' sweet
		0	Unknown	
	Kachaboi (6)	4	Ebwanateraka (EB-6)	Local variety, meaning 'bachelor's crop,' sweet
		4	Nigeria (NI-6)	NASE breeding programme; released as NASE3, roots are bitter before 9 months after planting
		1	NASE3	NASE breeding programme; roots are bitter before 9 months after planting
		3	Unknown	All sweet
	Omolokonyo (7)	2	Ebwanateraka	Local variety, meaning 'bachelor's crop,' sweet
		2	Fumbachai	Relatively newly introduced, good for snacks, literally meaning 'boil some tea', sweet
		1	Oxfam	Relatively newly introduced by the NGO called Oxfam in the late 80s and 90s, sweet variety
		4	Nigeria (NI-7)	NASE breeding programme, released as NASE3, roots are bitter before 9 months after planting
		1	Unknown	Sweet
Luwero	Nattyole (8)	2	Bamunaanika (BA-8)	Local, sweet
		3	Mbwa (MB-8)	Local, sweet

Table 1. Cont.

	1	Njule	Local, sweet
	2	SS4	NASE breeding programme, released as NASE4, sweet
	1	TME204	NASE breeding programme, from among the lines on on-farm trials, high yielding, preferred for commercial purposes, sweet
	4	TME14 (T14-8)	NASE breeding programme, from among the lines on on-farm trials, sweet
	1	TME5	,,
	1	TC1 (T1-8)	,,
	3	Vumba (VU-8)	NASE breeding programme; released as NASE12, sweet
	1	NASE12	NASE breeding programme, sweet
	1	NASE10	,,
	1	NASE 2	,, also called Okumu
	1	Bukalasa (BU-8)	Bukalasa breeding programme, sweet variety
	1	Kisamba	Relatively newly introduced, named after the member of parliament of the area then, sweet
	1	Unknown	Sweet variety
Vvumba (9)	2	Bamunaanika	Local, sweet
	2	Mbwa	,,
	1	Masaka	,,
	1	Njule	
	1	Kitengi	Relatively newly introduced, sweet
	1	Tongolo	,,
	1	TME14	NASE breeding programme; from among the lines on on-farm trials; high yielding, preferred it for commercial purposes, sweet
	1	00063	NASE breeding programme, from among the lines on on-farm trials, sweet
	4	00057 (57-9)	,,
	1	00036 (36-9)	,,
	1	00087	,,
	1	4363	,,
	1	TME	,,
	1	TME204	,,
	1	Omongole (ON-9)	NASE breeding programme; also called NASE1, sweet
	1	NASE3	NASE breeding programme, roots are bitter before 9 months after planting
	3	SS4 (S4-9)	NASE breeding programme; released as NASE4, sweet

Table 1. Cont.

		1	NASE10	NASE breeding programme, sweet
		1	NASE12	,,
		4	Vumba	NASE breeding; released as NASE12, sweet
		8	Unknown	All sweet
	Kibanga (10)	1	Nankinga	NASE breeding programme, sweet
		4	TME14	,,
		2	Vumba	NASE breeding programme; released as NASE12, sweet
		1	Mbwa	Local variety, sweet
		1	Unknown	Sweet
	Kabembe (11)	1	Bamunnanika	Local, sweet
		1	Njule	,,
		1	Mwogo omweru	,,
		2	SS4 (S4-11)	NASE breeding programme; released as NASE4, sweet
		1	Vumba	,,
		1	Omongole	,,
		1	Okumu	,, also called NASE 2
		11	Unknown	All sweet
Total		288		

Table 2. Number of alleles in the studied SSR loci

Locus	No. of alleles	Locus	No. of alleles
SSRY4	5	SSRY103	4
SSRY9	7	SSRY148	3
SSRY19	6	SSRY151	7
SSRY51	4	SSRY164	4
SSRY64	4	SSRY181	3
SSRY82	7		

Table 3. Number and percentage of polymorphic loci, and mean number of allele per locus for each variety, *F*-statistics estimates for the local, relatively newly introduced and improved varieties (Wright, 1965; Weir & Cockerham, 1984)

Category	Variety	No. of plants	No. of poly-morphic loci	Percentage of polymorphic loci	Mean no. of alleles per locus
Local	Bamunaanika	5	5	45.5	2.0
	Ebwanateraka	6	8	72.7	2.7
	Emulai	5	8	72.7	2.6
	Kidimo	19	11	100.0	3.5
	Mbwa	6	10	90.9	2.6
	Mulyandongo	14	9	81.8	2.4
	Nyakabiriti-1	16	10	90.9	2.7
	Nyakabiriti-2	11	10	90.9	2.5
	Nyakunyaku	13	10	90.9	3.1
	Sibampale	5	7	63.6	2.6
Introduced	Emusugut	6	11	100.0	3.4
	Nyakakwa	14	11	100.0	3.7
	Nyalanda	17	11	100.0	3.4
Improved Bred	Bukalasa	4	8	72.7	2.3
	NASE10	4	8	72.7	2.1
	NASE3	8	8	72.7	2.8
	Nigeria	10	10	90.9	3.1
	No.00057	4	9	81.8	2.5
	SS4	8	10	90.9	2.5
	TME14	6	11	100.0	2.9
	Vumba	6	9	81.8	2.5
	All varieties	**Local**		**Introduced**	**Improved**
F_{IT}	0.236	0.178		0.281	0.207
SE	0.087	0.117		0.103	0.085
95% CI	0.085	-0.028		0.088	0.075
	0.406	0.402		0.469	0.380
F_{ST}	0.250	0.275		0.172	0.164
SE	0.032	0.050		0.030	0.043
95% CI	0.185	0.183		0.114	0.081
	0.302	0.367		0.223	0.239
F_{IS}	-0.021	-0.138		0.130	0.051
SE	0.092	0.105		0.113	0.079
95% CI	-0.180	-0.318		-0.069	-0.076
	0.168	0.082		0.344	0.222

Table 4. Genotypes observed at 11 SSR loci for each variety. Genotypes found in 60% or more of the plants (n) in a variety is given in bold type

Category	Variety (n)	Genotypes at SSR loci										
		4	**9**	**19**	**51**	**64**	**82**	**103**	**148**	**151**	**164**	**181**
Local	Bamunaanika (5)	12 22 24 33	**22**	**33**	**13**	12 22 44	16 27	22*	**12** 22	**22**	**22**	22 33
	Ebwanateraka (6)	**12** 24	**22**	**13** 24	34*	12 22 44	25 **77**	11 22 33	13 33	16 26 57	**23**	**22** 23
	Emulai (5)	12 23 24 33	**22**	**13** 36	**34**	**12** 24 44	22 **77**	11 22	**13** 22	12 26 57 66	23 33 44	**22**
	Kidimo (19)	12 13 22 23 24 33 34	12 **22**	13 36 **45**	14 **34**	**12** 14 24	17 22 27 55 57 77	**22** 23 33	12 **33**	**12** 26 66	11 22 23 33 34 44	13 22 **33**
	Mbwa (6)	11 12	12 24	22 44	11 13 14	**12** 23 44	24 26 66	22 24	12 22	13 22 66 77	22*	22 33
	Mulyandongo (14)	11 12 13 22 23 24	**22** 26	**33**	11 **14**	**24** 44	**22**	11 24	12 22 23	22 **25** 45 55 57	22 **23**	12 **22**
	Nyakabiriti-1 (16)	11 **12** 13 22 23	**12** 22	33 **36**	11 **12** 34	11 12 **13** 24	11 **27** 77	**11**	12 22 **33**	22 **24**	22 23 44	12 **22** 33
	Nyakabiriti-2 (11)	11 12 22 44	12 **22**	**16** 36 66	11 **12**	**12** 13 24	**27** 57	**11** 22	22 23 33	**12** 24	22 **33** 34	**22**
	Nyakunyaku (13)	11 12 24 33	**12** 14 22	**13** 33 36 45	**14** 33 34	12 23 **24**	**22** 77	13 22 **33**	**12** 22 33	**12**	**22** 23 33	**13** 22 33
	Sibampale (5)	11 12 24	**12** 22	14 15 34	11 34	12 13 23 24	26*	23*	22 33	26 57 66	23*	33*
Introduced	Emusugut (6)	11 12 22 44	12 14 22	24 25 33 34	11 14 33	14 24 34 44	22 23 25 57 67	11 22 33	22 23 33	12 22 25 57	11 23 44	22 23 33
	Nyakakwa (14)	12 22 24 33 44	12 22 23 24 25 26 36	15 16 33 36 66	11 12 13 23 33	14 24 33 34 44	22 26 27 55	11 12 22 23 33	22 **33**	12 26 57 **66**	22 23 33 44	13 22 23 33
	Nyalanda (17)	11 12 13 24 44	12 **22** 24	16 24 **36**	12 14 22 33 34	12 **24** 34	**22** 27	**11** 14 22	12 22 23 **33**	**12** 46 66	13 22 23 24 33	**22** 33
Improved	Bukalasa (4)	12 22	12 23	13 33 34	13 14	12 22	16 **26**	22*	12 23	**22**	**22**	**13** 22
	NASE10 (4)	12 34	22 23 33	**33**	34*	33 **34**	25 55	22 24	22 23 33	13 35	11*	12 22
	NASE3 (8)	11 12 13 22 24	**22**	13 **24**	11 13 34	12 14 44	25 27 55 77	23 24	22 **33**	11 26 **57**	33 44	**22**

Table 4. Cont.

Nigeria (10)	11	**22**	13	**11**	14	22	22	12	16	23	**22**
	12		**24**	13	24	25	23	13	26	**44**	33
	13		33	34	**44**	55	33	22	35		
	23					77		23	57		
	24							33			
	33										
	34										
No. 00057 (4)	12	22	23	14	14	23	22*	**22**	11	22	12
	24	24	33	23	44	55			66	33	22
	25		36							44	
	33										
SS4 (8)	11	22	22	11	**24**	25	11	22	**11**	23	**22**
	12	36	23	**14**	44	56	**22**	23	57	**44**	
	22	37	24			**57**	23	33			
	33	44	33								
TME14 (6)	12	22	14	11	24	**25**	22	**22**	13	11	12
	23	**23**	**33**	13	34	55	23	23	35	33	22
	34			**34**			**24**		45	44	
									57		
Vvumba (6)	12	**22**	**33**	11	**44**	25	13	12	**35**	23	12
	13	26		13		55	22	**22**	45	**33**	22
	24			34						44	33

*predominating genotype is not indicated due to missing data for several accessions

Table 5. Nei's genetic distance (Nei, 1972) between pairs of varieties in the different villages (abbreviations see Table 1).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
BA-8	(1)																															
BU-8	(2)	0.17																														
EB-6	(3)	0.60	0.56																													
EL-4	(4)	0.58	0.62	0.10																												
ES-4	(5)	0.59	0.50	0.49	0.41																											
KA-1	(6)	0.89	0.87	0.70	0.61	0.62																										
KI-1	(7)	0.38	0.41	0.34	0.39	0.31	0.73																									
KI-2	(8)	0.53	0.55	0.30	0.29	0.45	0.71	0.14																								
MB-8	(9)	0.68	0.52	0.79	0.65	0.46	0.93	0.74	0.91																							
MU-3	(10)	0.60	0.58	0.49	0.35	0.30	0.50	0.80	0.88	0.41																						
N3-4	(11)	0.96	0.87	0.39	0.24	0.29	0.69	0.48	0.39	0.42	0.44																					
N3-NA	(12)	1.19	1.09	0.84	0.62	0.31	0.96	0.66	0.72	0.70	0.62	0.22																				
NI-5	(13)	1.44	1.18	0.83	0.69	0.28	0.95	0.73	0.80	0.54	0.60	0.20	0.07																			
NI-6	(14)	0.78	0.78	0.96	0.58	0.28	0.82	0.46	0.55	0.52	0.62	0.22	0.13	0.17																		
NI-7	(15)	0.87	0.90	0.46	0.20	0.29	0.66	0.51	0.38	0.56	0.44	0.13	0.22	0.23	0.22																	
36-9	(16)	0.71	0.78	0.68	0.59	0.34	0.89	0.80	0.97	0.42	0.26	0.49	0.84	0.63	0.54	0.61																
57-9	(17)	0.70	0.75	0.73	0.35	0.39	0.96	0.62	0.59	0.53	0.43	0.33	0.50	0.59	0.30	0.28	0.32															
NB1-3	(18)	0.69	0.58	0.31	0.38	0.34	0.43	0.64	0.62	0.54	0.27	0.52	0.73	0.70	0.87	0.59	0.56	0.76														
NB2-3	(19)	0.86	0.72	0.34	0.29	0.38	0.28	0.58	0.59	0.51	0.31	0.36	0.71	0.68	0.66	0.50	0.56	0.51	0.22													
NW-3	(20)	0.66	0.59	0.40	0.37	0.29	0.72	0.52	0.44	0.69	0.46	0.35	0.38	0.40	0.42	0.35	0.46	0.42	0.35	0.54												
NU-1	(21)	0.48	0.41	0.56	0.57	0.32	0.71	0.36	0.69	0.65	0.36	0.83	0.73	0.80	0.72	0.76	0.55	0.65	0.57	0.57	0.50											
NU-3	(22)	0.40	0.32	0.64	0.66	0.37	0.86	0.43	0.80	0.53	0.42	1.03	1.14	1.00	0.90	0.79	0.43	0.62	0.60	0.75	0.57	0.12										
NL-3	(23)	0.86	0.69	0.33	0.31	0.36	0.40	0.56	0.53	0.58	0.24	0.40	0.64	0.58	0.68	0.50	0.50	0.57	0.18	0.14	0.34	0.48	0.66									
ON-9	(24)	0.40	0.47	0.83	0.75	0.60	0.86	0.67	0.85	0.38	0.41	0.87	1.37	1.32	0.86	0.91	0.32	0.52	0.66	0.83	0.71	0.51	0.33	0.82								
OT-4	(25)	0.84	0.75	0.45	0.37	0.47	0.56	0.79	0.62	0.95	0.32	0.59	0.84	0.82	1.02	0.50	0.76	0.77	0.29	0.50	0.42	0.64	0.78	0.28	1.03							
OX-4	(26)	0.50	0.50	0.36	0.44	0.29	0.65	0.51	0.56	0.51	0.21	0.57	0.80	0.69	0.77	0.61	0.31	0.65	0.16	0.42	0.38	0.41	0.43	0.29	0.48	0.28						
S4-11	(27)	0.61	0.58	0.49	0.39	0.40	0.82	0.53	0.50	0.33	0.42	0.25	0.40	0.43	0.34	0.30	0.53	0.28	0.54	0.40	0.41	0.75	0.70	0.51	0.53	0.90	0.59					
S4-4	(28)	1.19	1.09	0.84	0.62	0.31	0.96	0.66	0.72	0.70	0.62	0.22	0.00	0.07	0.13	0.22	0.84	0.50	0.73	0.71	0.38	0.73	1.14	0.64	1.37	0.84	0.80	0.40				
S4-9	(29)	1.16	1.08	0.66	0.52	0.42	1.07	0.76	0.79	0.74	0.57	0.48	0.62	0.57	0.53	0.46	0.38	0.36	0.66	0.59	0.62	1.00	0.84	0.62	0.80	0.95	0.66	0.37	0.62			
T1-8	(30)	1.16	0.98	0.56	0.36	0.34	0.93	0.66	0.60	0.79	0.42	0.33	0.30	0.30	0.37	0.31	0.50	0.42	0.50	0.56	0.34	0.67	0.88	0.30	1.02	0.40	0.46	0.61	0.30	0.44		
T14-8	(31)	0.81	0.79	0.82	0.50	0.50	0.96	0.89	0.71	0.79	0.42	0.45	0.82	0.89	0.68	0.42	0.44	0.29	0.83	0.78	0.54	0.79	0.69	0.71	0.48	0.70	0.78	0.49	0.82	0.59	0.64	
VU-8	(32)	0.53	0.68	0.97	0.51	0.35	1.01	0.66	0.74	0.82	0.45	0.41	0.42	0.49	0.22	0.27	0.38	0.22	0.89	0.86	0.47	0.70	0.62	0.82	0.56	0.79	0.69	0.50	0.42	0.57	0.41	0.30

Table 6. Morphological descriptors used in the study and the importance of each trait for farmers in differentiating varieties

Trait	Abbreviation	Classes	Remarks
Colour of first fully expanded leaves	CEL	1 (light green), 2 (dark green), 3 (green purple), 4 (purple)	Important trait used by farmers
Colour of young shoot	CYSH	,,	Mainly used by the breeding programme
Colour of leaf vein	CLV	,,	Mainly used by the breeding programme
Pubescence of young leaves	PUYL	1 (absent), 2 (moderate), 3 (high)	Mainly used by the breeding programme
Number of leaf lobes	NLL		Mainly used by the breeding programme
Position in which leaf lobes are held	POLL	1 (erect), 2 (horizontal), 3 (deflexed), 4 (retorse)	Mainly used by the breeding programme
Shape of leaf lobes	SHLL	1 (broad), 2 (narrow)	Important trait used by farmers
Number of petioles	NPE	1 (5-10), 2 (45-60), 3 (75-90)	Mainly used by the breeding programme
Length of petiole	LEPE	1 (5-10cm), 2 (15-20cm), 3 (25-30cm)	Mainly used by the breeding programme
Colour of petiole	CPE	1 (light green), 2 (dark green), 3 (green purple), 4 (purple), 5 (pink), 6 (red)	Important trait used by farmers
Growth habit of stem	GHST	1 (straight), 2 (zigzag)	Trait used by farmers
Pubescence of young stem	PUYST	1 (absent), 2 (moderate), 3 (high)	Mainly used by the breeding programme
Colour of mature stem	CMST	1 (silver green), 2 (light brown), 3 (dark brown)	Important trait used by farmers
Prominence of leaf scars	PRLSC	1 (little), 2 (moderate), 3 (prominent)	Mainly used by the breeding programme
Height of first apical branch	HFAB	cm	Important trait used by farmers

Table 7. The most common morphological trait classes and their frequencies given in parentheses for 14 traits and the average and standard deviation (std) of the height of first apical branch (HFAB) for each variety. For abbreviation of traits see Table 6.

Variety	No. of plants	Trait														
		CEL	CYSH	CLV	PUYL	NLL	POLL	SHLL	NPE	LEPE	CPE	GHST	PUYST	CMST	PRLSC	HFAB cm (std)
Bamunaanika	5	3(0.60)	4(1.00)	1(1.,00)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	3(1.00)	5(1.00)	1(1.00)	1(1.00)	1(1.00)	3(1.00)	160.5 (19.9)
Bukalasa	5	3(0.60)	4(1.00)	1/4 (0.40	2(1.00)	7(1.00)	2(1.00)	2(0.60)	3(1.00)	2/3(0.50)	4(0.60)	1(1.00)	1(1.00)	1/2(0.50)	3(1.00)	125.2 (31.9)
Ebwanateraka	3	2(1.00)	3(1.00)	1(1.00)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	2(0.67)	4(1.00)	1(1.00)	1(1.00)	3(1.00)	3(1.00)	153.3 (23.1)
Emulai	5	2(1.00)	3(1.00)	1 (0.80)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	2(0.80)	4(0.80)	1(1.00)	1(1.00)	3(0.80)	3(1.00)	138.6 (48.1)
Emusugut	6	2(1.00)	3(1.00)	1 (0.67)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	2(1.00)	4(0.83)	1(1.00)	1(1.00)	1(1.00)	3(1.00)	140.7 (24.1)
Kidimo	18	2(0.78)	3(0.89)	1(0.72)	2(1.00)	7(0.88)	2(0.94)	1(1.00)	3(1.00)	2(0.76)	4(0.89)	1(1.00)	1(0.94)	1(1.00)	3(0.94)	147.7 (22.2)
Mbwa	6	2/3(0.50)	4(0.67)	1/3(0.50)	2(1.00)	7(1.00)	2(1.00)	2(0.83)	3(0.83)	3(0.83)	4(0.83)	1(1.00)	1(1.00)	1(0.83)	3(1.00)	152.8 (32.1)
Mulyandongo	14	2(1.00)	3(0.93)	3(0.64)	2(0.56)	7(0.93)	2(0.93)	1(1.00)	3(1.00)	3(0.64)	4(0.86)	1(1.00)	1(1.00)	1(1.00)	3(1.00)	127.5 (44.8)
NASE10	4	3(0.75)	3(0.75)	1(0.75)	2/3(0.50)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	3(0.75)	4(1.00)	1(1.00)	1(1.00)	1(0.75)	3(1.00)	84.8 (28.5)
NASE3	8	2(1.00)	3(1.00)	1(0.88)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	2(0.88)	1(0.50)	1(1.00)	1(1.00)	1(1.00)	3(1.00)	74.4 (15.7)
NO.0057	4	2(0.75)	4(0.50)	1(0.67)	2(0.57)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	3(1.00)	4(0.50)	1(1.00)	1(1.00)	3(0.75)	3(1.00)	115.5 (41.1)
Nigeria	10	2(1.00)	3(1.00)	1(1.00)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(0.89)	3(0.56)	1(1.00)	1(1.00)	1(1.00)	1(0.89)	3(1.00)	71.9 (11.4)
Nyakabiriti-1	16	2(1.00)	3(0.50)	1(0.94)	2(0.88)	7(0.64)	2(0.94)	1(0.94)	3(0.94)	2(0.75)	5(0.56)	1(1.00)	1(1.00)	2(0.94)	3(1.00)	132.0 (22.3)
Nyakabiriti-2	10	2(1.00)	3/4 (0.50)	1(1.00)	2(1.00)	7(1.00)	2(1.00)	1(0.77)	3(1.00)	2(0.76)	5(0.80)	1(1.00)	1(1.00)	2(0.62)	3(1.00)	128.9 (33.3)
Nyakakwa	14	2(1.00)	4(0.64)	1(0.93)	2(0.86)	7(0.64)	2(1.00)	1(0.77)	3(1.00)	2(0.64)	1(0.86)	1(1.00)	1(0.93)	1(1.00)	3(1.00)	96.1 (44.3)
Nyakunyaku	12	3(1.00)	4(1.00)	4(0.91)	2(1.00)	7(0.72)	2(0.92)	1(1.00)	3(1.00)	2(0.67)	4(1.00)	1(1.00)	1(1.00)	1(1.00)	3(1.00)	121.6 (21.7)
Nyalanda	16	1(0.75)	1(0.75)	1(0.75)	2(1.00)	7(0.73)	2(0.88)	1(1.00)	3(1.00)	2(0.93)	6(0.81)	1(1.00)	1(1.00)	1(0.69)	3(1.00)	109.0 (24.2)
SŠ4	5	2(0.80)	1/3 (0.50)	1(0.60)	2(1.00)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	2(0.60)	4(0.60)	1(1.00)	1(1.00)	1(0.80)	3(1.00)	61.2 (17.2)
Sibampale	5	3(0.60)	4(0.80)	4(0.60)	2(0.60)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	3(1.00)	4(0.60)	1(0.80)	1(1.00)	2(0.80)	3(1.00)	134.1 (67.0)
TME14	8	2(1.00)	1(0.75)	1(0.50)	2(0.62)	7(1.00)	2(0.88)	1(1.00)	3(1.00)	3(0.75)	4(0.62)	1(1.00)	1(1.00)	1(1.00)	3(1.00)	85.2 (8.6)
Vumba	6	2(0.83)	1(0.83)	1(0.67)	2(0.83)	7(1.00)	2(1.00)	1(1.00)	3(1.00)	2/3(0.50)	4(0.83)	1(1.00)	1(1.00)	2(0.83)	3(1.00)	48.8 (9.8)

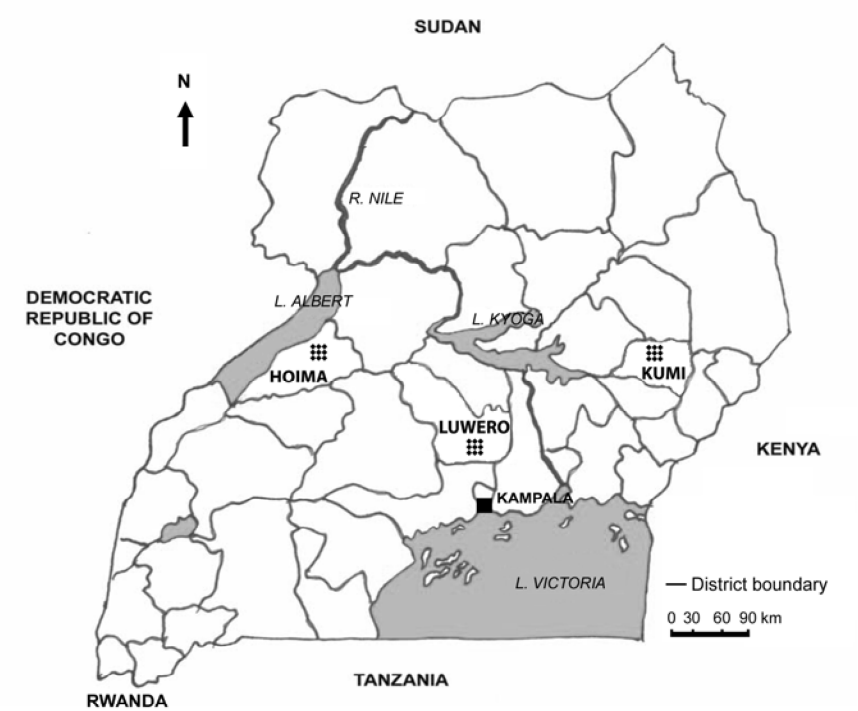

Figure 1. Map of Uganda showing the study areas (▒) in Hoima, Kumi and Luwero districts.

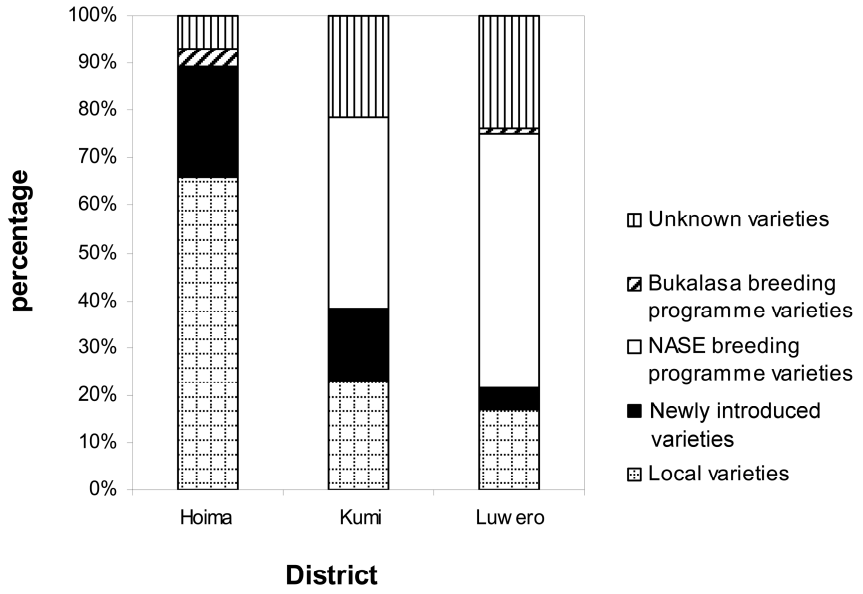

Figure 2. Percentage of local, newly introduced and improved varieties in the Bukalasa and NASE breeding programmes, and unknown plants in Hoima, Kumi and Luwero districts.

III

Quantitative trait loci controlling cyanogenic glucoside and dry matter content in cassava roots (*Manihot esculenta* Crantz)

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Abstract

Cassava (*Manihot esculenta* Crantz) is a starchy root crop grown in the tropics mainly by small-scale farmers even though agro-industrial processing is rapidly increasing. For this processing market improved varieties with high dry matter root content (DMC) is required. Potentially toxic cyanogenic glucosides are synthesized in the leaves and translocated to the roots. Selection for varieties with low cyanogenic glucoside potential (CNP) and high DMC is among the principal objectives in cassava breeding programs. However, these traits are highly influenced by the environmental conditions and the genetic control of these traits is not well understood. An S_1 population derived from a cross between two bred cassava varieties (Mcol1684 and Rayong 1) that differ in CNP and DMC was used to study the heritability and genetic basis of these traits. A broad sense heritability of 0.43 and 0.42 was found for CNP and DMC, respectively. The moderate heritabilities for DMC and CNP indicate that the phenotypic variation of these traits is explained by a genetic component. We found two quantitative trait loci (QTL) on two different linkage groups controlling CNP and six QTL on four different linkage groups controlling DMC. One QTL for CNP and one QTL for DMC mapped near each other, suggesting pleiotrophy and/or linkage of QTL. The two QTL for CNP showed additive effects while the six QTL for DMC showed additive effect, dominance or overdominance. This study is a first step towards developing molecular marker tools for efficient breeding of CNP and DMC in cassava.

Key words: cassava, cyanogenic glucoside content, dry matter content, heritability, molecular markers, quantitative trait locus mapping

Introduction

Cassava (*Manihot esculenta* Crantz) is a tropical root crop that is widely grown as a staple food and animal feed in countries of tropical and subtropical Africa, Asia and Latin America. It ranks fourth in production among all tropical crops, standing at 192 million tons per year in the world (FAO 2004). More than 70% of this production is in Africa and Asia from small-scale farmers by virtue of its remarkable tolerance to abiotic stresses and adverse environments. Its main value is in its storage roots though in some areas, particularly in Africa, young leaves are also harvested and processed for human consumption as a vegetable (Lancaster and Brooks 1983). The storage roots can be harvested from 6-24 months after planting depending on cultivar and growing conditions (Cock 1985). Fresh roots of cassava may differ in dry matter content (DMC, 10% - 50%) depending on genotype, age and environmental condition (Kawano et al. 1987; Chavez et al. 2005). On average, about 90% of DMC is carbohydrates (Kawano et al. 1987). Cassava also produces cyanogenic glucosides, which are synthesized in the leaves and translocated to the roots (Koch et al. 1992; Siritunga and Sayre 2003; Jørgensen et al. 2005). Cassava varieties with high cyanogenic glucoside levels (>1000 mg hydrogen cyanide (HCN) equivalent kg^{-1} dry weight) are said to be toxic while cassava with low levels of cyanogenic glucosides (<200 mg HCN equivalent kg^{-1} dry weight) are considered to be safe for consumption without processing (Iglesias et al. 2002). Hydrogen cyanide (HCN) in cassava tissues has been medically proven to be a potential health hazard for consumers if the plant is inadequately processed (Mlingi et al.1992; Tylleskär et al. 1992). Genotypes with high levels of cyanogenic glucosides must be processed, for example by fermentation, to remove HCN and its toxic precursors (Essers et al. 1995). Farmers' varieties with high levels of cyanogenic glucosides have in general bitter taste and are referred to as bitter while those with low levels of cyanogenic glucosides are called sweet or cool varieties (Chiwona-Karltun et al. 2004). Although cyanogenesis in cassava has been attributed to cyanogenic glucosides in the roots, other bitter tasting compounds have been detected both in the parenchyma and cortex and the correlation between bitterness and cyanogenic capacity does not always hold (Bokanga 1994; King and Bradbury 1995). Small-scale farmers grow both sweet and bitter varieties. They are known to be able to taste and predict the levels of cyanogenic glucosides in their varieties and classify them into sweet or bitter varieties (Chiwona-Karltun et al. 2004). The preference and proportions of the bitter and sweet varieties grown differ in different areas (Salick et al. 1997; Elias et al. 2001; Balyejusa Kizito et al. 2006) depending on their cultures (Westby 2002). Since most of cassava production in Africa is for human consumption the farmers tend to emphasize cooking quality or starch characteristics (Ceballos et al. 2004). It has been observed that bitter cassava improves the food security for many small-scale farmers because they are less prone to predation and theft (Essers et al. 1995; Chiwona-Karltun et al. 1998).

During the past 30 years significant progress has been made in cassava breeding and selection for the major traits such as improved yield, improved plant architecture and resistance or tolerance to pests and diseases (Kawano 2003). In

Africa, due to repeated cassava mosaic disease (CMD) pandemics, breeding has tended towards development of varieties with CMD resistance (Thresh and Cooter 2005). Even though cassava is mainly grown by small-scale farmers its use for agro-industrial processing is increasing in Asia (Kawano et al. 1998). For this processing market improved varieties with higher root yield and DMC is required (Kawano et al. 1998). Selection for low levels of cyanogenic glucosides and high DMC is among principal objectives in cassava improvement programmes (Dixon et al. 1994). However, cyanogenic glucoside content, also known as cyanogenic glucoside potential (CNP), is one of the least understood agronomic traits in cassava due to its phenotypic plasticity (Bokanga et al. 1994). In addition, DMC in roots is significantly influenced by age at harvest, genotype, location and season of harvest (Kawano et al. 1987). In Dixon et al. (1994) CNP was found to be negatively correlated with DMC in cassava ranging between -0.73 and -0.55 depending on genotype and location. Given the long growth cycle of cassava and that CNP and DMC traits are probably controlled by several genes with influence from the environment, marker-assisted selection (MAS) for these traits would be more efficient. The identification and mapping of quantitative trait loci (QTL) controlling these traits and the identification of nearby marker loci to the QTL can help to improve the efficiency of the selection process. QTL analysis also gives additional information on the inheritance of the traits that could be of use to the breeding.

We have here used a QTL mapping approach to study the genetic basis of the differences of CNP and DMC of roots at a relatively early developmental stage, harvested 5 months after planting (MAP), between two cassava cultivars, MCol1684 (high in cyanogenic glucosides) and Rayong1 (relatively low in cyanogenic glucosides).

Materials and methods

Plant materials and field experiment

The cassava variety Rayong 60 (accession MTAI8 at the Germplasm Bank at CIAT), an F_1 hybrid between the Colombian variety Mcol1684 and the Thai variety Rayong 1, was selfed to produce the S_1 mapping population AM320 of 199 individuals used in this study. The population was developed at the Centro Internacional de Agricultura Tropical (CIAT), Cali Colombia. The parents of accession MTAI8 differed in CNP, an average CNP of 1318 and 900 mg HCN equivalent kg^{-1} dry weight respectively, was obtained from two different field experiments. Segregation of CNP was also found within the S_1 population, where CNP ranged from 260 to 1569 mg HCN equivalent kg^{-1} dry weight (Fig 1). DMC among other traits also differed in the parents of MTAI8 and segregated in the S_1 population.

The germination of the S_1 seeds from embryo axes *in vitro* was according to standard procedures (Fregene et al. 1998) for safe keeping of the QTL mapping

population. The plantlets were then micro-propagated *in vitro*, hardened in a screen house and thereafter transferred to the field for further cloning to obtain sufficient cuttings for the experimental set up (see below).

The field experiment was established in August 2004 at CIAT headquarters in Palmira, Valle del Cauca department (latitude 3^o 31' N and longitude 76^o21'W), situated in the mid-altitude tropics of Colombia (at 1000 m above sea level). The mean temperature is 25 $\pm$ 1 ^{0}C monthly and the annual rainfall is about 1000 mm. The soil has a mollisol texture (Lian and Cock 1979a; Lian and Cock 1979b) and the water holding capacity of the soil is such that cassava rarely suffers from water stress at this site. Selected mature stem cuttings from each of the 199 S_1 clones were used in the experiment. The experimental layout was a randomized complete block design (Chahal and Gosal 2002) with 8 blocks, where each block contained one plant per genotype. Borderline plants from a different variety were included. Cuttings of 199 genotypes were planted vertically on ridges at a spacing of 1 x 1 m. The experiment was weeded regularly and no fertilizers were applied.

Phenotypic measurements

The experiment was harvested in January 2005 at 5 MAP and the roots were immediately taken to the laboratory for CNP and DMC analyses. All tuberous roots on a plant were considered. Calculations of DMC were made by measuring specific gravity by weighing roots in air and then in water (weight in air / weight in water – weight in water). This method is based on the correlation which exists between root specific gravity, DMC and starch content (CIAT 1976). The DMC (%) was determined using the formula: DMC% = [158.3 x (weight in air / weight in water – weight in water)] (Okogbenin and Fregene 2002). CNP was measured using the enzymic assay developed by Cooke (1978) and modified by O'Brien et al. (1991).

Marker analysis

DNA was isolated from young leaf tissue by CTAB method (Doyle and Doyle 1987). The procedures for marker analysis followed Mba et al. (2001).

Data analyses

The phenotype data was submitted to analyses of variance (ANOVA) using the JMP programme version 3 (SAS Institute 1994). Thirty-nine different genotypes had poor vigour resulting in few or none replicates in the field experiment and were therefore not included in the ANOVA and the QTL analysis. The final phenotypic data included 160 genotypes of the S_1 population with the genotype effect considered random. The ANOVA procedure was performed according to the model:

$Y_{ij} = \mu + B_i + G_j + e_{ij}$

where Y_{ij} is the phenotypic value for the *j*th genotype in the *i*th block, μ is the overall mean, B_i is the fixed effect of the block, G_j is the random effect of the *j*th

genotype and e_{ij} is the residual error. Broad sense heritability (H^2) was estimated using variance components from our analysis of variance in the formula:
$H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$
where $\sigma_g^2$and σ_e^2 are the variance components for the genotype effect and the residual error, respectively. Pearson correlation coefficient were estimated for the two traits using mean values for each genotype and tested for significance (P<0.05).

A linkage map of cassava was earlier drawn using 95 simple sequence repeat (SSR) markers and 104 individuals of the AM320 population (CIAT 2003). We re-constructed the linkage map based on an additional 95 individuals of the AM320 population and 15 new SSR markers using the MAPMAKER linkage analysis software, version 2.0 (Lander et al. 1987). However, during the reconstruction of the map 12 of the previous SSR markers were excluded since they did not map to any of the linkage groups (LG) in the new map. We tested each of the markers for normal Mendelian segregation using χ^2- tests with a significance level of 0.05 corrected according to the Bonferroni-Holm sequential method (Rice 1989). The cassava genome was scanned for the presence of a QTL effect at 2.0 cM intervals using composite interval mapping (CIM) in the computer package QTL Cartographer version 1.15 (Basten et al. 1997). CIM combines interval mapping (which calculates the ratio of the likelihood that there is a QTL to that there is not a QTL at any position in the interval between two markers or at the markers themselves) with multiple regression so that the most significant markers outside the test interval will be included in the model (Zeng 1993; 1994). We used model 6 of Basten et al. (1997) with the five most significant markers as genetic background parameters and a window size of 10 cM on either side of the markers flanking the test site. All QTL above a LOD score (the strength of the data supporting a QTL) of 2.5 were presented according to Lander and Botstein (1989). Empirical experiment-wise threshold values for significance (P= 0.05) were estimated from 1 000 permutations of the data for each trait (Churchill and Doerge 1994). Using the dominance (d) and additive (a) values given for each QTL by the programme the ratio of d/a was calculated.

Results

Phenotypic analyses

We found a significant difference between S_1 genotypes for both CNP and DMC (Table 1). The distribution of the phenotypic traits in the S_1 population revealed continuous variation, typical of quantitative traits (Figure 1). The mean CNP for the S_1 plants was 903.0 mg HCN equivalent kg^{-1} dry weight with a confidence interval of 864.3-941.7 and the mean DMC was 37.7% with a confidence interval of 37.3-38.1. The broad sense heritability was 0.43 for CNP and 0.42 for DMC. The moderate heritability observed for CNP and DMC indicates that the phenotypic variation in these traits has a genetic component thus making the

population suitable for QTL mapping. CNP and DMC showed a significant (P<0.001), although weak negative correlation (-0.24).

Marker Segregation

After checking for normal Mendelian segregation (1:2:1 for co-dominant loci, P<0.05), we found distorted segregation for 17 of the 98 marker loci (17.3%) at a significance level of 0.05 corrected according to the Bonferroni-Holm sequential method (Rice 1989).

QTL for CNP and DMC

Two QTL for CNP were found on linkage groups (LG) 10 and 23, respectively (Table 2, Figure 2). For DMC six QTL were detected, two on LG 3, two on LG 6, one on LG 10 and one on LG 17 (Table 2, Figure 2). The QTL on LG 10 for DMC mapped close to the QTL found for CNP on the same LG. The maximum likelihood positions of the QTL for CNP and DMC varied in distance to their nearest flanking molecular marker locus (Figure 2). One of the QTL for DMC on LG3 mapped at the marker locus SSRY9 and the QTL for CNP on LG23 mapped close to marker locus NS119. The rest of the QTL for these two traits mapped in between their flanking marker loci. All the QTL for DMC and CNP reported showed a LOD score above 2.5 (Lander and Botstein 1989). However, only the QTL on LG 10 for CNP near marker locus SSRY105 and the QTL on LG 3 at marker locus SSRY9 and the QTL on LG 6 closest to marker locus SSRY32 for DMC were significant according to the permutation test (Table 2). The significant LOD threshold for CNP was 3.9 while for DMC it was 5.2. In some of the permuted data sets for DMC, the ECM algorithm 'bailed out' between pairs of markers due to numerical problems. This may influence the estimation of the significant threshold based on the permutation test.

Gene action and magnitudes of effect

The QTL for CNP near marker locus SSRY105 had the larger additive effect (162 mg HCN equivalent kg^{-1} dry weight) while the other CNP QTL at locus SSRY242 also contributed considerably to the additive effect (99 mg HCN equivalent kg^{-1} dry weight). The DMC QTL on LG6 closest to marker locus SSRY45 showed the largest additive effect (2.38%) while the other QTL on LG6 for DMC showed the largest dominance effect (2.90%). Five of the six QTL for DMC showed dominance or overdominance and one QTL showed additive gene action. The two QTL for CNP were both additive (Table 2). The two QTL found for CNP explained 7% and 20%, respectively of the phenotypic variation (R^2) in the S_1 population (Table 2). Individual QTL for DMC explained 14% to 37% of the variance. The relatively high R^2 values found for some QTL with lower LOD scores for DMC may be influenced by large distances between flanking markers.

Discussion

Until recently the selection programmes in cassava have been conducted without much knowledge of the genetic architecture of the selected traits. A lot of effort has been put into breeding for major traits important for productivity such as root yield, DMC and resistance or tolerance to diseases and pests in cassava (Kawano 2003). Breeding for CNP in cassava is complicated because evaluation is considered time-consuming and is highly influenced by the environment (Dixon et al. 1994). DMC in roots is also significantly influenced by growing conditions and season of harvest (Kawano et al. 1987). MAS has the potential to make field-based breeding for CNP and DMC improvement more efficient. We have used a QTL mapping approach to study the genetic control of CNP and DMC in cassava. We have found two QTL on two different LG controlling CNP and six QTL on four different LG controlling DMC. One QTL for CNP and one QTL for DMC mapped near each other (Figure 2, Table 2).

The translocation of carbohydrates to the roots changes with age of the cassava plant (Alves 2002) and the onset of the root bulking differs among genotypes (Okobenin and Fregene 2002). Because of the correlation which exists between DMC and starch content (CIAT 1976) DMC is also influenced by the age and genotype of the plant. In our study, conducted in a single environment, DMC had a broad sense heritability of 0.42, comparable to the highest value found in studies on bred cassava clones in Nigeria conducted by Dixon et al. (1994). Broad sense heritabilities ranging between 0.50 and 0.97 for DMC were estimated by Kawano et al. (1998) at different evaluation stages in a breeding program for cassava in Asia. Benesi et al. (2004) found that a large part of the total phenotypic variation in DMC was due to genetic differences in an experiment with bred and local cassava clones in Malawi. In our study a broad sense heritability of 0.43 was found for CNP in the AM320 population. The moderate to large heritabilities found for DMC and CNP indicate that the phenotypic variation of these traits is explained by a genetic component. However, it may be difficult to compare the heritability estimates of the different DMC studies discussed as different cassava material and plant age have been used. They have also been conducted in different number of locations, seasons and years.

The two QTL controlling CNP showed high additive effects while most QTL for DMC showed dominance or overdominance. Overdominance may indicate a heterozygote advantage. The large dominance effect that we observed for DMC is in contrast to the diallel study by Cach et al. (2005) where additive effect plays a more important role than the dominance effect for DMC in cassava. This may be due to the specificity of the cross, differences in the environmental conditions and the age of the cassava plants at harvest. In addition, we may not have a complete picture of the genetic background of DMC since it is likely that we have not been able to detect all QTL. This may also be true for CNP.

In our study we have found a weak negative correlation between CNP and DMC. We detected only one genomic region where a QTL for CNP and a QTL for DMC

mapped together. This may partly account for the weak phenotypic correlation found between these traits. The clustering of the two QTL could either be a result of pleiotropic effect where a single QTL affects the expression of both CNP and DMC. Alternatively, it could be two closely linked QTL, each controlling one of the traits. The finding that most QTL for DMC did not map near QTL for CNP shows that these traits are at least partly controlled by different genetic backgrounds.

Since DMC is controlled by QTL showing additive effect, dominance or overdominance a simple recurrent selection program is not sufficient to capture the potential gain. A reciprocal recurrent selection program that takes into account both additive and dominance effects would therefore be a better strategy in cassava breeding. Dominance has been found in many other traits in cassava and this strategy has also been suggested by Cash et al. 2005. The fact that some improved cassava varieties are not adopted by small-scale farmers in some areas is a clear indication of differences in selection criteria between small-scale farmers and breeders. This therefore shows a need for the breeders to relate farmers' criteria to the researchers' tools. The fact that taste (which in farmers' varieties correlates with levels of CNP, Chiwona-Karltun et al. 2004) is important for small-scale farmers and a variety can be rejected on the basis of taste suggests that breeders should prioritize selection for this trait as early as possible in the breeding cycle. In addition, considering the different preferences of the small-scale farmers it would be important to breed towards different levels of CNP for the small-scale farmers. However, if the genetic background for CNP and the taste is different (Bokanga 1994; King and Bradbury 1995) the breeders need to consider both traits in the breeding of cassava.

It is important to have in mind that the QTLs that we have detected for CNP and DMC at 5 MAP may not be found for cassava plants in later developmental stages, plants grown in other environments or in crosses between other varieties. The result of this study is therefore limited to this particular cross and at a relatively early plant age, and cannot be directly used in cassava breeding programs. However, this study has been a first step towards identifying QTL for CNP and DMC and contributes to the understanding of the genetic basis of two cassava important traits. For future QTL studies of these traits a saturated linkage map is needed. This will help us to find a closer linkage between the molecular marker loci and the responsible genes that will improve breeding based on MAS. It is important to verify if the same QTL for CNP and DMC will be detected at other developmental stages of cassava and in other environments using the AM320 population. Also studies are needed to investigate the genetic correlation between the level of cyanogenic glucosides and the level of bitter tasting compounds in cassava. Furthermore, it would also be important to study the genetic basis of DMC and CNP in other populations.

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References

Alves A.A.C. 2002. Cassava botany and physiology. In: Hillocks R.J., Thresh J.M. and Bellotti A.C. (eds), Cassava: Biology, Production and Utilization, CAB International, Wallingford, Oxon, UK, pp. 67-89.

Balyejusa Kizito E., Chiwona-Karltun L., Egwang T., Fregene M. and Westerbergh A. 2006. Genetic diversity and variety composition of cassava on small-scale farms in Uganda: an interdisciplinary study using genetic markers and farmer interviews. Genetica (in press).

Benesi I.R.M., Labuschagne M.T., Dixon A.G.O. and Mahungu N.M. 2004. Stability of native starch quality parameters, starch extraction and root dry matter of cassava genotypes in different environments. J. Sci. Food Agric. 84: 1381-1388.

Bokanga M. 1994. Distribution of cyanogenic potential in cassava germplasm. Acta Hort. 375: 117-123.

Bokanga M., Ekanayake I.J. and Dixon A.G.O. 1994. Genotype- environment interactions for cyanogenic potential in cassava. Acta Hort. 375: 131-139.

Basten C.J., Weir B.S. and Zeng Z.B. 1997. QTL cartographer: a reference manual and tutorial for QTL mapping. Dept. of Statistics, North Carolina State Univ., Raleigh, NC.

Cach N.T., Perez J.C., Lenis J.I., Calle F., Morante N. and Ceballos H. 2005. Epistasis in the expression of relevant traits in Cassava (*Manihot esculenta* Crantz) for subhumid tropics. J. Hered. 96: 586-592.

Ceballos H., Iglesias C.A., Perez J.C. and Dixon A.G.O. 2004. Cassava breeding: opportunities and challenges. Plant Mol. Biol. 56: 503-516.

Chahal G.S. and Gosal S.S. 2002. Principles and procedures of plant breeding: Biotechnological and conventional approaches. Alpha Science International Ltd. Pangbourne, UK.

Chávez A.L., Sánchez T., Jaramillo G., Bedoya JM., Echeverry J., Bolaños E.A., Ceballos H. and Iglesias C.A. 2005. Variation of quality traits in cassava roots evaluated in landraces and improved clones. Euphytica 143: 125-133.

Chiwona-Karltun L., Mkumbira J., Saka J., Bovin M., Mahungu N.M. and Rosling H. 1998. The importance of being bitter- a qualitative study on cassava cultivar preference in Malawi. Ecol. Food Nut.r 37: 219-245.

Chiwona-Karltun L., Brimer L., Saka J.D.K., Mhone A.R., Mkumbira J., Johansson L., Bokanga M., Mahungu N.M. and Rosling H. 2004. Bitter taste in cassava roots correlates with cyanogenic glucoside levels. J. Sci. Food Agric. 84: 581-590.

Churchill G.A., Doerge R.W. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971.

CIAT (CentroInternacional de Agricultura Tropical) 1976. Annual report 1975. Cali, Colombia.

CIAT (Centro Internacional de Agricultura Tropical) 2003. Annual report 2003 Project IP3: Improved cassava for the developing world, CIAT, Cali, Colombia.

Cock J.H. 1985. Cassava: new potential for a neglected crop. West-view Press, Boulder, Colorado.

Cooke, R.D. 1978. An enzymatic assay for the total cyanide content of cassava. J. Sci. Food Agric. 29: 345-352.

Dixon A.G.O., Asiedu R. and Bokanga M. 1994. Breeding of cassava for low cyanogenic potential: problems, progress and perspectives. Acta Hort. 375:153-161.
Doyle J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.
Elias M., Penet L., Vindry P., MacKey D., Panaud O. and Robert T. 2001. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. Mol. Ecol. 10: 1895-907.
Essers A.J.A., Ebong C., Grift R.M., Nout M.J.R., Otim-Nape W. and Rosling H. 1995. Reducing cassava toxicity by heap-fermentation in Uganda. Int. J. Food Sci. Nutr. 46: 125-136.
FAO 2004. Food Outlook - No. 2 June 2004.
Fregene M., Ospina J.A. and Roca W. 1998. Recovery of cassava (*Manihot esculenta* Crantz) plants from culture of immature zygotic embryos. Plant Cell Tissue Organ Cult. 55: 39-43.
Iglesias C.A., Sánchez T. and Yeoh H.H. 2002. Cyanogens and linamarase activities in storage roots of cassava plants from breeding program. J. Food Comp. Anal. 15: 379-387.
Jørgensen K., Bak S., Kamp Busk P., Sørensen C., Olsen C.-E., Puonti-Kaerlas J. and Lindberg Møller B. 2005. Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of biosynthesis by RNA interference technology. Plant Physiol. 139: 363-374.
Kawano K. 2003. Thirty years of cassava breeding for productivity-biological and social factors for success. Crop Sci. 43: 1325-1335.
Kawano K., Narintaraporn K., Narintaraporn P., Sarakarn S., Limsila A., Limsila J., Suparhan D., Sarawat V. and Watananonta W. 1998. Yield improvement in a multistage breeding program for cassava. Crop Sci. 38: 325-332.
Kawano K., Fukuda Goncalves W.M. and Cenpukdee U. 1987. Genetic and environmental effects on dry matter content of cassava root. Crop Sci. 27: 69-74.
King N.L.R. and Bradbury J.H. 1995. Bitterness of cassava: Identification of a new apiosyl glucoside and other compounds that affect its bitter taste. J. Sci. Food Agric. 68: 223-230.
Koch B., Nielsen V.S., Halkier B.A., Olsen C.E. and Møller B.L. 1992. The biosynthesis of cyanogenic glucosides in seedlings of cassava (*Manihot esculenta* Crantz) Arch. Biochem. Biophys. 292: 141-150.
Lancaster P.A. and Brooks J.E. 1983. Cassava leaves as human food. Econ. Bot. 37: 331-348.
Lander E.S., Green P., Abrahamson J., Barlow A., Daley M., Lincoln S. and Newburg L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.
Lander E.S and Botstein D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199.
Lian T.S. and Cock J.H. 1979a. Branching habit as yield determinant in cassava. Field crops Res. 2: 281 289.
Lian T.S. and Cock J.H. 1979b. Cassava plant forms and their associated morpho-physiological characters. MARDI Res. Bull. 7: 55-69.
Mba R.E.C., Stephenson P., Edwards K., Melzer S., Mkumbira J., Gullberg U., Apel K., Gale M., Tohme J. and Fregene M.A., 2001. Simple Sequence Repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theor. Appl. Genet. 102: 21-31.
Mlingi N., Poulter N.H. and Rosling H. 1992. An outbreak of acute intoxication from insufficiently processed cassava in Tanzania. Nat. Res. 12: 677-687.
O'Brien G.M., Taylor A.J. and Poulter N.H. 1991. Improved enzymatic assay for cyanogens in fresh and processed cassava. J. Sci. Food Agric. 56: 277-289.
Okogbenin E. and Fregene M. 2002. Genetic analysis and QTL mapping of early root bulking in an F_1 population of non-inbred parents in cassva (*Manihot esculenta* Crantz). Theor. Appl. Genet. 106: 58-66.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.
Salick J., Cellinese N. and Knapp S. 1997. Indigenous diversity of Cassava: generation, maintenance, use and loss among the Amuesha, Peruvian Upper Amazon. Econ. Bot. 51: 6-19.
Siritunga D. and Sayre R.T. 2003. Generation of Cyanogen-Free Transgenic Cassava. Planta 217: 367-373.
Thresh J.M. and Cooter R.J. 2005. Strategies for controlling cassava mosaic disease in Africa. Plant. Path. 54: 587-614.
Tylleskär T., Banea M., Bikangi N., Cooke R.D., Poulter N.H. and Rosling H. 1992. Cassava cyanogenesis and Konzo, an upper motor neuron disease found in Africa. Lancet 339: 208-211.
Westby A. 2002. Cassava utilization, storage and small-scale processing. In: Hillocks R.J., Thresh J.M. and Belloti A.C. (eds), Cassava: Biology, Production and Utilization, CAB International, Wallingford, Oxon, UK, pp. 281-300.
Zeng Z.-B. 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA 90: 10972-10976.
Zeng Z.-B. 1994. Precision mapping of quantitative trait loci. Genetics 136: 1457-1468.

Table 1. Analysis of variance performed for cyanogenic glucoside potential (CNP) and dry matter content (DMC) in the S_1 population.

	Source of variation	Degrees of Freedom (DF)	F ratio	Probability
CNP				
	Genotype	159	5.93	<0.001
	Block	7	9.45	<0.001
	Error	942		
DMC				
	Genotype	159	5.80	<0.001
	Block	7	11.23	<0.001
	Error	942		

Table 2. Quantitative trait loci (QTL) for cyanogenic glucoside potential (CNP) and dry matter content (DMC), their locations reported by linkage group (LG), nearest flanking molecular marker locus, LOD score, additive effect (a) listed as a trait unit contribution, dominance (d) effect, dominance and additive ratio (d/a), gene action and proportion of phenotypic variance explained by a QTL (R^2).

Trait	**LG**	**Marker**	**LOD**	**a**	**d**	**d/a**	**Gene action**	R^2
CNP	10	SSRY105	8.4***	161.96	4.46	0.03	additive	0.20
	23	SSRY242	3.2	98.96	10.35	0.10	additive	0.07
DMC	3	SSRY9	5.3*	1.72	0.81	0.47	additive	0.14
	3	SSRY313	3.0	0.33	2.31	7.00	overdominance	0.20
	6	SSRY32	5.9*	1.32	2.90	2.20	overdominance	0.40
	6	SSRY45	3.0	2.38	1.39	0.58	dominance	0.34
	10	SSRY223	2.8	1.86	1.45	0.78	dominance	0.21
	17	SSRY41	2.5	1.15	2.81	2.44	overdominance	0.37

*** significant at $p<0.001$, * significant at $p<0.05$ with the permutation test

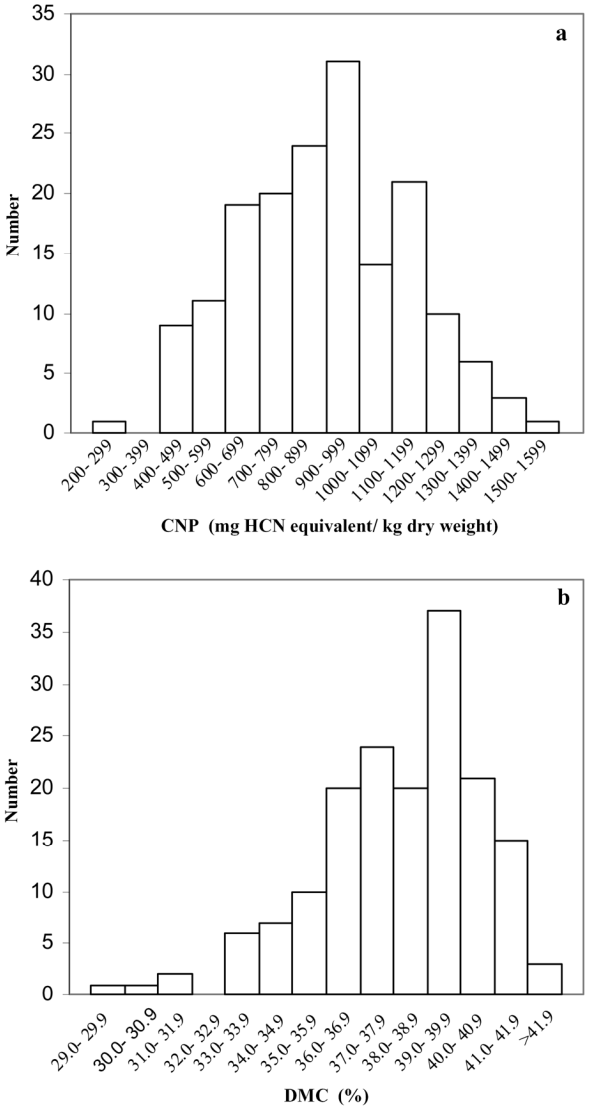

Figure 1. Phenotypic distribution of the genotype mean (a) cyanogenic glucoside potential (CNP) and (b) dry matter content (DMC) in the S_1 population.

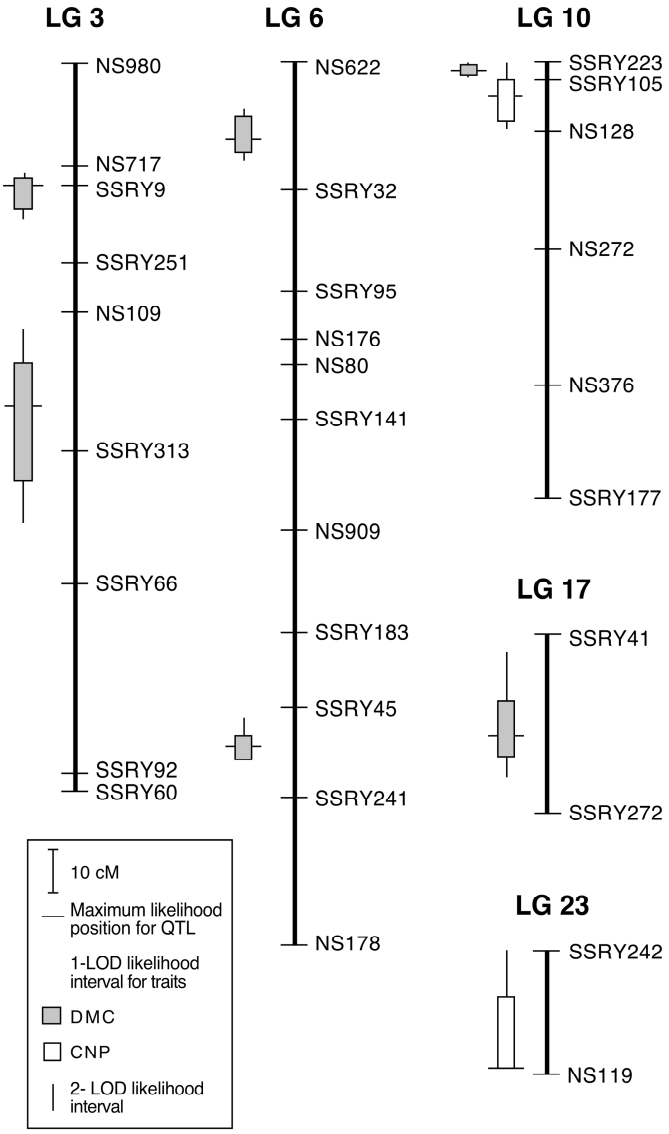

Figure 2. Linkage groups for the S_1 population showing the positions of quantitative trait loci for cyanogenic glucoside potential (CNP) and dry matter content (DMC).

IV

Growth and nutrient-regulated tuber formation in cassava (*Manihot esculenta* Crantz).

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Abstract

Cassava produces starch-containing tuberous storage roots of world-wide importance as food. In efforts to enhance cassava crop yield it is important to understand physiological and nutritional factors regulating tuberous root formation. Also, for studies of genetic diversity and content of the toxic cyanogenic glucosides in the storage roots it is essential to use uniform and stable growth conditions. Our goal was to (i) evaluate cultivation methods for cassava in controlled growth chamber conditions that would result in tuber formation and (ii) conduct preliminary experiments that would develop our hypothesis that storage root formation is negatively affected by high availability of plant nutrients. We cultivated plants in pots with regular constant additions of nutrients and in hydroponic growth units where plants had either free access to nutrients or received nutrients in an exponentially increasing relative addition rate (RAR). Since plants grow exponentially when nutrients are available and the cultivation in pots and growth units are two fundamentally different ways to make nutrient available we also tried to, in a controlled way in growth units, mimic the decreasing availability of nutrients as it may appear in pots by letting plants grow at gradually lower RAR. We found that whole plant growth rate was highest in the free access treatment and the allocation of biomass to roots was higher in the more nutrient-limited treatments with RAR of 0.10 day^{-1} or 0.10 day^{-1} followed by 0.05 day^{-1} in growth units. Plants grown in big pots to about the same size as in the growth units produced the highest amount of storage roots while no storage roots were formed in the free access treatment. The nutrient-limited treatments in growth units had intermediate production of storage roots. We conclude that the production of storage roots on cassava is regulated by nutrient availability and propose that it occurs in such a way that as mineral nutrients gradually become limiting during the time-course of growth the plants produce storage roots.

Key words: biomass allocation, cassava, *Manihot esculenta*, nutrition, plant growth, storage root, tuber

Introduction

Cassava produces starch-containing tuberous storage roots of world-wide importance as food. In efforts to enhance production or carry out studies to understand genetic control mechanisms it is important to know physiological and nutritional factors regulating tuberous root formation. Also, for studies of genetic diversity in content of the toxic cyanogenic glucosides in the storage roots it is essential to use uniform growth conditions since the amount of cyanogenic glucosides are also dependent on environmental factors (Bokanga 1994, Dixon *et al.* 1994). With regards to nutrient requirements, cassava is considered to be well adapted to poor soils and tolerate low pH (Howeler 2002). Cassava responds well to fertilizer with increased yields but over-fertilization should be avoided (Howeler 2002). Although field studies are informative and relevant, factors related to plant nutrient status are in such conditions also confounded in hydrological, edaphic and biotic factors. So far, detailed physiological studies of a role of nutrient availability for cassava tuber formation have not yet been conducted.

Cultivation of plants requires addition of mineral nutrients. However, to maintain a cellular steady-state level of nutrients the addition rate needs to be at a rate that corresponds to the exponential rate of plant growth according to the theories developed by Ingestad and co-workers (Ingestad 1982, Ingestad and Ågren 1995). Several studies have used exponentially increasing additions of nutrients to control plant growth at a certain growth rate and in such situations studied effects of specific nutrients that were kept as the limiting nutrient (e.g. Ingestad 1982, Ingestad and Kähr 1985, Ericsson and Ingestad 1988, Ericsson and Kähr 1995, Göransson 1998). It has been shown that allocation of biomass between shoot and root, i.e. the shoot:root ratio is generally affected by availability of many nutrients and internal nutrient status (e.g. Ågren and Ingestad 1987, Mattsson *et al*. 1991, Ericsson 1995, Ericsson *et al*. 1996).

Traditional cultivation of plants in pots meaning regular additions of a nutrient solution corresponds to a constant addition rate of nutrients. This may not be sufficient for maximal growth and maintenance of a cellular steady-state level of nutrients as plants become larger. Allocation of nutrients to storage organs e.g. excess carbon to storage roots is conceivably not consistent with plant growth under conditions with free access to nutrients. In this study we hypothesized that development of cassava storage roots occur when factors other then photosynthetic carbon are limiting growth in these plants that have the genetic predisposition to form such storage organs. The goal was to (i) evaluate cultivation methods for cassava in controlled growth chamber conditions that would result in tuber formation and (ii) conduct preliminary experiments that would develop our hypothesis on how tuber formation is regulated. We therefore investigated effects on storage root formation when addition of nutrients deviated from an exponentially increasing addition rate either in pots or in a computer-controlled hydroponic system.

Material and methods

Plant material, nutrient solutions and growth conditions

Offspring of the selfed cassava (*Manihot esculenta* Crantz; Euphorbiaceae) variety MTAI8, accession Rayong 60 at the germplasm bank at CIAT (Cali, Colombia), an F_1 hybrid between the Colombian variety Mcol1684 and the Thai variety Rayong 1, were converted into tissue culture and subsequently into plants. The plants were cultivated in a growth chamber and multiplied by making cuttings. In our experiments, 1-internode cuttings were taken from plants grown in growth units at free access to nutrients for about 35 days. Rooting took place in a highly dilute complete nutrient solution with nutrient proportions of 20:13:65 for N, P and K and with N as nitrate at 1 mg N L^{-1} during 9 to 14 days. The conditions in the growth chamber were set at 14/10 h day/night length, 70 % relative humidity, a temperature of 26 °C and a photosynthetic photon flux density of about 300 μmol m^{-2} s^{-1} (metal halogen OSRAM HQIE 250 W).

For cultivation in growth units (Fig 1; constructed in 2002 by Biotronic, Uppsala, Sweden), rooted cuttings were put into the closed root compartment of the growth unit in which the nutrient solution was continuously sprayed onto the roots (Ingestad and Lund 1986). The nutrient additions were computer-controlled according to principles described by Ingestad and Ågren (1992, 1995). For plants to have free access (FA) to nutrients, the growth units analyzed pH and conductivity and at every 10 min automatically added nutrient solution in relation to a set value and due to nutrient uptake. In two treatments of our experiments the units were set to add nutrients at pre-determined relative addition rate (RAR) as described by Ingestad (1982). These exponentially increasing rate of nutrient addition were set to either 0.05 day^{-1} or 0.1 day^{-1}. The nutrient proportions were 100:13:65:7:8.5:9:0.7:0.4:0.2:0.03:0.03:0.03:0.007:0.003 for N, P, K, Ca, Mg, S, Fe, Mn, B, Cu Zn, Cl, Mo, Na on a weight basis with nitrate as the sole nitrogen source. In the FA treatment the conductivity never exceeded 500 μS.

For cultivation in pots, rooted cuttings were planted into 2 sizes (0.250 L and 9.5 L) of pots with mineral wool as supporting inert substrate. Nutrient solution given to potted plants was a stock solution (Cederroth International AB, Sweden) diluted to the following final concentrations expressed as mg L^{-1}: N, 102 (NH_4^+-N, 40, NO_3^--N, 62), P 20, K 86, S 8.0, Ca 6.0, Mg 8.0, Fe 0.34, Mn 0.4, B 0.2, Zn 0.06, Cu 0.03, Mo 0.0008.

Experiments to investigate plant growth and tuber formation

To test the hypothesis that the production of storage roots is determined by availability of plant nutrient we cultivated plants in 3 types of treatments ranging from free access to nutrients and nutrient-limited exponentially increasing availability to regular constant additions of nutrients in pots. We also tried to, in a controlled way in growth units, mimic the decreasing availability of nutrients as it may appear in pots by letting plants grow at gradually lower RAR.

Cultivation in growth units was as follows. Rooted cuttings were weighed, put into growth units set at free access to nutrients. The units were used in 3 different growth protocols. Protocol 1 (FA) was to keep the plants at non-limiting nutrient conditions with free access (FA) to nutrients for 34 days. In the protocol 2 and 3, plants were after a period with FA exposed to nutrient-limiting conditions. The nutrient solution remaining in the growth units was replaced with de-ionized water after 19 days and a RAR of nutrients was set to 0.10 day^{-1} maintaining proportions among nutrients. The starting level was based on calculations of nutrient content from plant weights. In protocol 2 (FA-0.10), the plants were kept at the RAR of 0.10 day^{-1} for 34 days until harvest. In protocol 3 (FA-0.10-0.05), after 12 days at a RAR of 0.10 day^{-1}, the RAR was decreased to 0.05 day^{-1} which was maintained for 22 days until harvest. At harvest, plants were divided into parts and their fresh weight determined. Whole plant growth was calculated for the last 34 days as relative growth rate (RGR) as $RGR = \ln(w_2/w_1)/(t_2-t_1)$ where weights (w) and time (t) are at harvest and start of experiment. For protocol 2 and 3 the beginning of RAR at 0.10 day^{-1} was considered the start of the growth experiment. The data were tested for statistical differences among cultivation protocols for each tissue type using a t-test.

During cultivation in pots nutrient solution was added to the water-holding capacity of the substrate by soaking them for 20 min followed by draining. Big pots received nutrients twice per week and small pots three times per week during in total 49 days.

Results and Discussion

In preliminary studies we found that cassava plants produced significant amounts of tubers when grown in pots while plants grown at a free access to nutrients lacked tubers. This made us ask whether tuber formation is controlled by plant nutrient status and our results from the present study made it possible to partly answer this question.

Cassava plants showed good growth in big pots (Fig 1, Tab 1). The potted experiment also showed that growth depended on pot size since the plants in the small pots were several-fold smaller than those in big pots, which illustrated the lower holding capacity of nutrient solution in the small pot. Compared to growth in the growth units, the final shoot biomass of the plants in the big pots were in the same range although the total growth time was longer in the pots (Tab 1).

In the growth units, cassava plant growth, as well as allocation of biomass between shoot and root, was clearly affected by the gradually decreasing nutrient availability (Fig 2, Tab 1). Although the total growth time was shorter for the plants having free access (FA) to nutrients, shoot weights were in the same range as for the FA-0.10 and the FA-0.10-0.05 treatments (Tab 1). The overall relative growth rate was lower in the FA-0.10 treatment and even more in the FA-0.10-0.05

treatment compared to the relative growth rate of the FA plants (Fig 2A). Also, an increased proportion of biomass was allocated to roots (Fig 2B), essentially due to lower shoot growth in the nutrient-limited treatments (Tab 1).

The plants grown in big pots had a high production of tubers making up as much as 39 g FW for these plants with a shoot FW of 114 g (Tab 1, Fig 3). The higher tuber production in big pots compared to in small pots was most likely due to overall better growth. In growth units we found that production of tubers occurred as thickened roots to various degree (Fig 3) and they were produced in the treatments FA-0.10 and more so in the FA-0.10-0.05 treatment (Tab 1) with tuber percentages of whole-plant biomass of 1.2 % and 5.1 %, respectively as calculated for the plants that clearly showed the thickened root phenotype. To facilitate comparisons and reduce possible errors due to incomplete recovery of roots from the growth substrate in the pots, the tuber biomass was also expressed as a ratio of tuber to shoot biomass (Fig 4). This clearly showed that the plants cultivated in pots had a higher production of tubers than plants grown in the growth units.

The results are in accordance with the production of tubers being regulated by a gradually nutrient-limited condition. The low growth in the small pots clearly suggests that nutrients are required to sustain growth and that more frequent additions of nutrients would have resulted in higher growth. It is conceivable that if plants are exposed to nutrient-limitation at larger plant size, as found in the growth units, there will be already sufficient photosynthetically active biomass to sustain storage of carbon in tubers and somehow induce tuber formation. In relation to a situation in the field, where production of tubers occurs, the conditions may resemble that in pots although other factors such as drought and competition also limit the availability of nutrients.

The two cultivation systems, pots and growth units, used in the present study represent to fundamentally different systems of adding nutrients to plants. In growth units the nutrient solution is sprayed onto the roots thus making very thin boundary layers for nutrient uptake. In contrast, in the pots the uptake of nutrients is more dependent on diffusion and also root growth into new unexplored volumes. In addition, in a pot where total volume and amount of substrate is fixed the capacity to hold nutrients is therefore also fixed. With regular additions of nutrients, and with the same concentration of nutrients as usually is the case, the addition of nutrients will therefore be constant. During plant growth, which initially is at an exponential rate, this method will eventually not be able to meet the increasing nutrient demand by the plants if the growth rate is to be kept. To meet the demand of an exponentially growing plant at growth rate determined by the genetic potential of the plant under the prevailing conditions, plant nutrients have to be added at an exponential rate to maintain a steady-state of the internal plant cellular nutrient status. This can be achieved in the growth units used in this study and several other studies on plant growth (e.g., Ingestad 1982, Ingestad and Kähr 1985, Ericsson and Ingestad 1988, Ericsson and Kähr 1995, Göransson 1998), but also in potted experiments when the regular addition of nutrients is increasing at an exponential rate (e.g. von Fircks 2001).

The strength of using this hydroponic computer-controlled cultivation system is that plants can be cultivated at a generally high reproducibility. This can be seen in our results in that the allocation of biomass to shoot and root was very similar among plants within a treatment although the plants had a large range in final size. The per cent root biomass of total biomass was independent of plant size (data not shown). Also, the standard error of root biomass to total was low, 8 % compared to 29 % for the total plant biomass data (Tab 1) when the standard error is expressed as per cent of the average, indicating low variability.

Further studies on cassava physiology should investigate uptake and internal concentrations of nutrients to verify that plant nutrient status is affected by the treatments. The relative importance of various nutrients in the control of the development of tubers should also be investigate and possible candidates for important nutrients are likely to be found among N, P and S, which are known to affect the ratio of shoot to root growth (Ericsson 1995). In recent studies of effects of N-supply to cassava grown in pots for 90 days Cruz *et al*. (2003, 2004) demonstrated a trend with decreasing percentage of tuber biomass with increasing amounts of nitrate added whether expressed per total biomass or per shoot biomass. Also, tubers of these plants accumulated less starch with increasing addition of nitrate (Cruz *et al*. 2003).

This study describes a process in which a steady-state situation with growth balanced by mineral nutrient uptake and products of photosynthesis maintaining a stable internal nutrient status changes into a situation with a non-stable internal nutrient status and nutrient limitation. This results in the development of a new root organ or clear differentiation of storage roots. An analogous situation is the development of N_2-fixing root nodules which are modified roots or new organs formed by certain plants during conditions of low availability to N and in response to infection by compatible bacteria. These root nodules are also strong sinks for carbon and there could be similarities between tuberous roots and root nodules in some parts of the regulation of their formation.

We conclude that the production of tubers on cassava is regulated by nutrient availability. The use of growth units gave informative results about the role of nutrients and should be useful in further studies of nutrient regulation in cassava and other crops with carbon storage organs. However, cultivation in pots would also be sufficient for some studies if nutrients are added in an exponentially increasing way or at other desired rates.

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Literature

Bokanga M (1994) Distribution of cyanogenic potential in the Cassava Germplasm. In: International workshop on Cassava Safety. Working Group on Cassava Safety WOCAS, (Eds. M. Bokanga, S.A.J.A. Essers, N. Poulter, H. Rosling & O. Tewe). Acta Horticulturae 375: 117-123

Cruz J L, Mosquim, P R, Pelacani C R, Araújo W L and DaMatta F M (2003) Carbon partitioning and assimilation as affected by nitrogen deficiency in cassava. Photosynthetica 41: 201-207

Cruz J L, Mosquim, P R, Pelacani C R, Araújo W L and DaMatta F M (2004) Effects of nitrate nutrition on nitrogen metabolism in cassava. Biologia Plantarum 48: 67-72

Dixon A G O, Asiedu R and Bokanga M (1994) Breeding of cassava for low cyanogenic potential: problems, progress and perspectives. In: International workshop on Cassava Safety. Working Group on Cassava Safety WOCAS, (Eds. M. Bokanga, S.A.J.A. Essers, N. Poulter, H. Rosling & O. Tewe). Acta Horticulturae 375: 153-161.

Ericsson T (1995) Growth and shoot:root ratio of seedlings in relation to nutrient availability. Plant and Soil 168-169: 205-214

Ericsson T and Ingestad T (1988) Nutrition and growth of birch seedlings at varied relative phosphorus addition rates. Physiol Plant 72: 227-235

Ericsson T and Kähr M (1995) Growth and nutrition of birch seedlings at varied relative addition rates of magnesium. Tree Physiol 15: 85-93

Ericsson T, Rytter L and Vapaavuori E (1996) Physiology of carbon allocation in trees. Biomass and Bioenergy 11: 115-127

Göransson A (1998) Steady-state nutrition and growth responses of *Betula pendula* to different relative supply rates of copper. Plant Cell Environ 21: 937-944

Howeler RH (2002) Cassava minteral nutrition and fertilization. In: Cassava: Biology, production and utilization. CAB International, pp 115-147

Ingestad T (1982) Relative addition and external concentration; Driving variables used in plant nutrition research. Plant Cell Environ 5: 443-453

Ingestad T and Ågren GI (1992) Theories and methods on plant nutrition and growth. Physiol. Plant 84: 177-184

Ingestad T and Ågren GI (1995) Plant nutrition and growth: basic principles. Plant and Soil 168-169: 15-20

Ingestad T and Kähr M (1985) Nutrition and growth of coniferous seedlings at varied relative nitrogen addition rate. Physiol Plant 65: 109-116

Ingestad T and Lund A-B (1986) Theory and techniques for steady state mineral nutrition and growth of plants. Scand J For Res 1: 439-453

Mattsson M, Johansson E, Lundborg T, Larsson M and Larsson C-M. (1991) Nitrogen utilization in N-limited barley during vegetative and generative growth: I. Growth and nitrate uptake kinetics in vegetative cultures grown at different relative addition rates of nitrate-N. J Exp Bot 42: 197-205

von Fircks Y, Tom Ericsson T and Sennerby-Forsse L (2001) Seasonal variation of macronutrients in leaves, stems and roots of *Salix dasyclados* Wimm. grown at two nutrient levels. Biomass and Bioenergy 21: 321–334

Table 1. Biomass allocation of cassava plants cultivated in growth units with different nutrient addition rates or cultivated in pots with constant nutrient addition rates.

Tissue type	Pots		Growth units		
	Big pots	**Small pots**	**Free access (FA)**	**FA-0.10**	**FA-0.10-0.05**
Start					
Rooted cutting (g)	-	-	4.4 ±0.36 a	5.3 0.44 a	4.2 ±0.89 a
Plant at start of treatment (g)	-	-	4.4 ±0.36	13.4 ±2.07 a	12.8 ±2.92 a
Harvest					
Shoot (g)	114 ±3.0 a	17.3 ±1.3 b	165 ±37.6 a	133 ±32.8 a	65.7 ±22.5 a
Cutting (g)	9.4 ±0.9 a	4.2 ±0.72 a	7.6 ±1.3 a	12.4 ± 1.3 a	8.7 ±1.64 a
Root + tuber (g)	* a	* a	52.8 ** a	54.9 ±17.9 a	57.5 ±20.2 a
Tuber (g)	38.9 ±8.5 ab	3.2 ±0.40 a	-	3.9 ab	12.7 ±6.8 *** ab
No. of plants	4	5	5	5	5
No. of plants having tubers	4	3	0	1	2
No. of tubers per plant	6.8 ±0.63 a	1.7 ±0.33 b	-	1	2.5

The treatments in growth units were: (FA) free access to nutrients for 34 days, (FA-0.10) FA for 19 days followed by 34 days at 0.10 day^{-1} relative addition rate of nutrients, or (FA-0.10-0.05) FA for 19 days followed by 0.10 day^{-1} relative addition rate for 12 days followed by 22 days at 0.05 day^{-1} relative addition rate. The cultivation in pots lasted 49 days. Values are mean values ± SE. *root biomass could not be quantitatively harvested, **root systems of individual plants could not be separated, ***calculated for the plants having tubers. Data followed by different letters (a, b) were significantly different among each tissue type at the $P<0.05$ level.

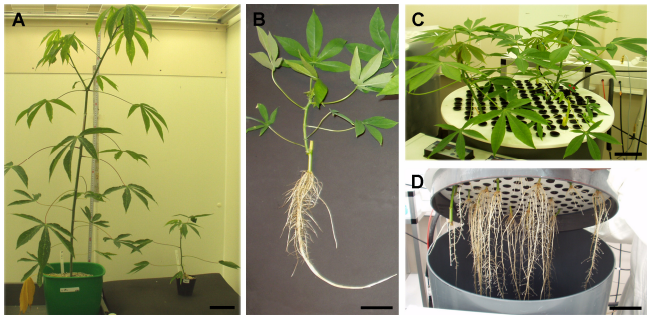

Figure 1. Photographs of (A) cassava plants growing in big and small pots, (B) cassava cutting rooted and grown for 12 days in free access to nutrients, and cassava plants mounted in a growth unit showing (C) shoot and (D) root. Bar is 10 cm.

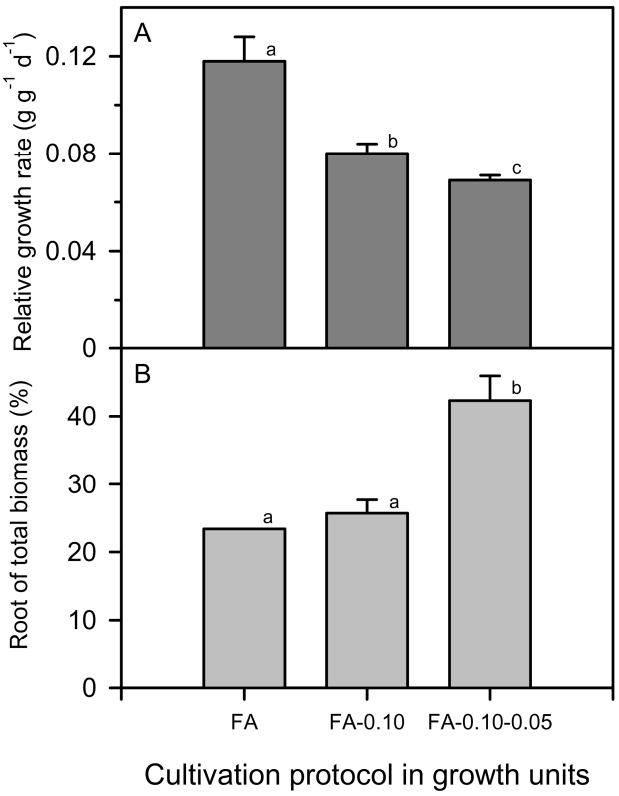

Figure 2. Whole plant relative growth rates (A) and allocation of biomass to roots (B) of cassava plants grown at three different nutrient addition protocols in hydroponic computer-controlled growth units. FA, free access to nutrients; 0.05 and 0.10 indicates 0.05 and 0.10 day^{-1} relative addition rates of nutrients; see Material and Methods for explanations of protocols. Values are means ± SE. Means followed by the same letter are not significantly different at the $P < 0.05$ level (t-test), n=5.

Figure 3. Root systems of cassava grown a (A) big or (B) small pot or in growth units with (C) free access to nutrients for 34 days, or (D) with free access for 19 days followed by a nutrient relative addition rate of 0.10 day^{-1} for 12 days and 0.05 day^{-1} for 22 days. Root systems of plants in A, B and D had tubers, which are indicated by arrows. All photos are in the same scale and bar is 1 cm.

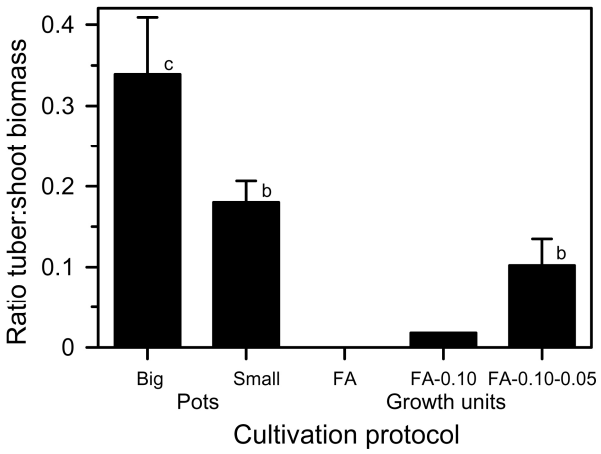

Figure 4. Tuber production by cassava plants expressed as ratio of tuber to shoot biomass. Plants were grown in big and small pots or at three different nutrient addition protocols in hydroponic computer-controlled growth units; see Figure 2 and Material and Methods for explanations.