



Classification of cassava into ‘bitter’ and ‘cool’ in Malawi: From farmers’ perception to characterisation by molecular markers

Jonathan Mkumbira^{1,2}, Linley Chiwona-Karlton^{3,4}, Ulf Lagercrantz², Nzola Meso Mahungu⁵, John Saka⁶, Albert Mhone⁵, Mpoko Bokanga⁷, Leon Brimer⁸, Urban Gullberg^{2,*} & Hans Rosling³

¹Bvumbwe Agricultural Research Station, P.O. Box 5748, Limbe, Malawi; ²Department of Plant Biology, Swedish University of Agricultural Sciences, Box 7080, SE-750 05 Uppsala, Sweden; ³Division of International Health, Department of Public Health Sciences, Karolinska Institute, SE-171 76 Stockholm, Sweden; ⁴Nutrition Unit, Department of Medical Sciences, Uppsala University, Dag Hammarskjölds väg 21, SE-752 37 Uppsala, Sweden; ⁵IITA/SARRNET, Chitedze Research Station, Box 30258, Lilongwe 3, Malawi; ⁶Department of Chemistry, Chancellor College, University of Malawi, Box 280, Zomba, Malawi; ⁷International Institute for Tropical Agriculture, Oyo Road, P.M.B. 5320, Ibadan, Nigeria; ⁸Department of Pharmacology and Pathobiology, The Royal Veterinary and Agricultural University, 13 Bülowssvej, DK-1870 Frederiksberg C., Denmark; (*author for correspondence)

Received 16 October 2001; accepted 31 August 2002

Key words: cassava cultivars, cyanogenic glucosides, molecular markers, farmers’ perception, genotype, *Manihot esculenta*

Summary

Cassava roots, a major food in Africa, contain cyanogenic glucosides that may cause toxic effects. Malawian women farmers considered fields of seemingly similar cassava plants to be mixes of both ‘cool’ and ‘bitter’ cultivars. They regard roots from ‘cool’ cultivars as non-toxic. Roots of ‘bitter’ were considered to require extensive traditional processing done by women to be safe for consumption. But curiously, these women farmers preferred ‘bitter’ cultivars since toxicity confers protection against theft, which was a serious threat to the food security of their families. We studied how well these farmers comprehend the effects of genetic variations in cassava when dealing with cyanogenesis in this complex system. Using molecular markers we show that most plants farmers identified as belonging to a particular named cultivar had a genotype typical of that cultivar. Farmers’ ethno-classification into ‘cool’ and ‘bitter’ cultivars corresponded to a genetic sub-division of the typical genotypes of the most common cultivars, with four-fold higher cyanogenic glucoside levels in the bitter cultivars. Examining morphology, farmers distinguished genotypes better than did the investigators when using a standard botanical key. Undoubtedly, these women farmers grasp sufficiently the genetic diversity of cassava with regard to cyanogenesis to simultaneously benefit from it and avoid its dangers. Consequently, acyanogenic cassava – the breeding of which is an announced good of some cassava genetic improvement programmes – is not a priority to these farmers. Advances in molecular genetics can help improve food supply in Africa by rapid micropropagation, marker assisted breeding and introduction of transgenic varieties, but can also help to elucidate tropical small-scale farmers’ needs and skills.

Introduction

The starchy roots of cassava, *Manihot esculenta* Crantz, have become the most important source of dietary energy in Sub-Saharan Africa (FAO, 2000; Scott et al., 2000). This is due to the high and stable

yield (Akoroda, 1995), especially in areas with arable land shortage and declining soil fertility (Romanoff & Lynam, 1992). Other advantages of cassava are its flexible planting and harvesting time, its suitability for inter-cropping and the fact that it is vegetatively propagated. Most small-scale cassava farmers grow a

number of cultivars, each with locally preferred qualities such as good taste, early maturation or good processing characteristics (Salick et al., 1997; Chiwona-Karlton et al., 2000). African small-scale farmers mainly acquire new cultivars from their neighbours, during travels or by collecting seedlings of sexually propagated cassava (*volunteers*) found in fields left in fallow for several years (Chiwona-Karlton et al., 1998). The performance of a cultivar within the local environment and farming system determines whether it will be adopted, and continue to be cultivated. There is evidence from several parts of Africa that few cassava cultivars originate from breeding programmes (Spencer, 1994; Nweke et al., 1994; Chiwona-Karlton et al., 1998).

An earlier study in one area of northern Malawi showed that farmers have descriptive names for up to 50 cultivars just in one village (Chiwona-Karlton et al., 2000). The number of plants grown of each cultivar varies considerably and it changes over time. Cultivars may be 'killed' when their yield becomes unsatisfactory. The local cultivar names denote phenotypic attributes, place of origin, the person that introduced it or the fact that it originates from a volunteer seedling (Chiwona-Karlton et al., 1998). As in many other areas the Malawian small-scale farmers grow a mix of cultivars with seemingly similar plants and claim that some cultivars yield roots that can be eaten raw whereas others yield 'bitter' roots that must be subjected to elaborate processing before consumption (Dufour, 1988; Chiwona-Karlton et al., 2000). 'Bitter' taste of the roots is associated with a high risk of poisoning if roots are not processed prior to consumption.

Previous studies in Malawi revealed an ethno-classification of cassava cultivars into two groups based on whether the roots could be eaten raw without prior processing (or simply boiled, or roasted) or if they needed to be processed before consumption. In the local language these groups were called 'cool' and 'bitter' (Chiwona-Karlton et al., 1998; Chiwona-Karlton et al., 2000). A similar division of cassava cultivars into two groups, mostly referred to in English as 'sweet' and 'bitter', has been reported from many areas (Nordenskiöld, 1924; Schery, 1947; Sauer, 1963; Ugent et al., 1986; Dufour, 1988; Nweke, 1995). Exclusive cultivation of 'sweet' cultivars is mostly found where cassava only comprises a small part of a diverse crop system (Cousins, 1903; Nordenskiöld, 1924; Renvoize, 1972; Allem, 1994; Dufour, 1995). In almost all areas where cassava is

the main crop the 'bitter' cassava cultivars dominate and 'sweet' cultivars are grown in less amount or not at all (Purseglove, 1968; Lathrap, 1973; Dufour, 1993; Dufour, 1995; Nweke et al., 1994; Chiwona-Karlton et al., 2000; Nweke et al., 2001). The division of cassava cultivars into 'sweet' and 'bitter' has not been found to correlate with phenotypic features (Rogers & Appan, 1973; Rogers & Fleming, 1973) and to our knowledge the character of this division has not been studied in Africa using modern molecular genetic markers.

Advances in molecular genetics lend promise in the development of acyanogenic cassava. This would undoubtedly improve the understanding of the biological role of cyanogenesis in cassava. However, caution is needed before considering acyanogenesis as a means of preventing possible poisoning from cassava consumption. The reason being that the small-scale farmers at risk of dietary cyanide exposure from cassava seem to prefer bitter cultivars, because the bitter taste and toxicity of the roots confer protection against theft and attacks by vermin (McKay & Beckerman, 1993; Chiwona-Karlton et al., 1998). We therefore found it prudent to also apply the advances in molecular plant genetics to elucidate how small scale farmers presently understand, handle or fail to handle the genetic diversity of cassava with respect to cyanogenesis.

This study investigated the local ethno-classification of cassava using Short Sequence Repeat (SSR) markers on material obtained by a survey in northern Malawi. The first aim was to verify to what degree each of the 10 most grown cultivars constituted a single genotype or a mixture of genotypes. The second aim was to determine the accuracy with which farmers are able to identify plants as belonging to the named cultivars. The third aim was to ascertain to what degree the classification of 'bitter' or 'cool' cultivars comprised two different genetic pools. In this paper the term cultivar denotes the different types of cassava that are recognised within a local farming system by a specific local name and the term genotype implies a specific allele pattern found in eight SSR loci.

Methods

Study area

The study was conducted in Nkhata-Bay district, with a predominantly rural population of approximately 165,000 (Malawi government, 2000). It is situated

along the shore of lake Malawi in the northern part of the country. Cassava is the major staple crop, in this district, with about 70% of the farmed land allocated to cassava (Pelletier & Msukwa, 1990). The district comprises four agro-ecological zones: the islands of Likoma and Chizumlo, the lakeshore zone between 475–600 m above sea level, the escarpment between 600–900 m and the less densely populated mountainous plateau zone above 900 m. The district is divided into 53 agricultural extension sections. Each section is further divided into 8 blocks, each with about 100 farming households. An agricultural extension worker, known as a Field Assistant (FA) is responsible for each section. Two adjacent sections, Lweya and Mgodji, were selected for this study on the basis of being regarded as representative for the lakeshore zone (Chiwona-Karlton et al., 1998). The dominant ethnic groups in both sections were the Tonga. One block from each section ‘Thowolo-B’, and ‘Matyenda-1’ were selected on the basis of being ‘typical’ of the agricultural and social variation within each section. ‘Thowolo-B’, and ‘Matyenda-1’ comprised 98 and 102 households, respectively, and they were located about 3 km apart (Chiwona-Karlton et al., 2000).

The farming communities in both blocks have a long tradition of growing cassava and many of the named cultivars have been grown for more than fifty years (Berry & Petty 1992; Chiwona-Karlton et al., 1998). Roots from ‘bitter’ cassava cultivars are preferentially soaked, fermented, dried and pounded into flour. The flour is used to make the staple food *kondowole*, a dumpling-like dish that is eaten with the fingers together with a sauce. The farmers actively select cultivars through informal exchange of stem cuttings or from collecting *volunteer* plants from re-grown fallow fields. Cassava is mainly grown by women in small fields as a mix of plants of up to 15 cultivars that are largely maintained by vegetative propagation of stem cuttings. Since everyone in the area is essentially a cassava farmer and since there are hardly any market opportunities, the harvesting of cassava for household consumption is done on a piecemeal basis throughout the year. Most of the planting is done in direct relation to the piecemeal harvesting. This results in fields with a mix of plants of different cultivars and at different ages.

The study was approved by the Departments of Agricultural Research and Extension Service of the Ministry of Agriculture, the District Health Commis-

sioner in Nkhata-Bay, and by oral consent from the community leaders and farmers.

Main survey in August/September, 1996

A study in the same study area in July 1996 started with a house-to-house survey of all the 200 households. The 176 eligible women being in charge of cooking and farming in these households were interviewed and reported growing a total of 45 name-given cassava cultivars (Chiwona-Karlton et al., 2000). Plants of the 10 cultivars reportedly grown by the largest proportion were sampled during the main survey in August and September, 1996. Three cultivars were designated as ‘cool’ (c) and seven as ‘bitter’ (b). Out of the 176 women farmers interviewed, 92 reported growing ‘mbundumali’ (c), 31 ‘chimpuno’ (c), 31 ‘nyachikundi’ (c), 122 ‘gomani’ (b), 113 ‘depwete’ (b), 76 ‘koloweki’ (b), 52 ‘nyaharawa’ (b), 37 ‘nyankhata’ (b), 27 ‘ng’wenyani’, and 23 (b) ‘nyamakozo’ (b).

From the household census list compiled in July, 1996 (Chiwona-Karlton et al., 2000) we consecutively sampled 30 households for this study in September, 1996. One household from ‘Matyenda 1’ was excluded because the woman interviewed in July was not available in September when this study was conducted, and one from ‘Thowolo B’ due to later failure of extracting DNA from the plants collected. Thus a total of 28 farmers from these households were finally included, 13 in ‘Thowolo-B’ and 15 in ‘Matyenda-1’. The criteria for inclusion were (i) the presence of the woman farmer previously interviewed to indicate the plants of each cultivar in her own field, (ii) the cultivation of one or more of the ten most frequently grown cultivars, and (iii) having at least two plants of each cultivar in the same field with roots ready for harvest.

Identification of plants by farmer was done under observation (LC-K). Each of the 28 farmers walked through their own fields to identify pairs of plants that they claimed belonged to one of the ten cultivars. Farmers were asked to only identify plants regarded as being ready for harvest. They did this by carefully examining the various plants including parts of the roots that could be observed by unearthing with their hands. The number of cultivars with plants ready for harvest that could be identified by each farmer ranged from one to eight and the survey continued until about, 20 plants of each of the 10 cultivars had been collected. All 246 plants collected were independently identified by the farmers, immediately labelled (HR) and

Table 1. Distribution of morphological categories within each character of the plants classified by farmers as belonging to the ten most grown cultivars

Cultivar	No. of plants	Morphological characters with 2–3 categories ^a														
		Skin colour		Stem colour		Shoot colour		Shoot pubesc		Lamina colour		Petiole colour		Root neck constriction		
		1	2	1	2	1	2	1	2	1	2	1	2	3		
Mbundumali	24	24	0	24	0	17	4	<u>0^b</u>	<u>21</u>	23	0	<u>20</u>	<u>3</u>	8	12	4
<i>Non-typical^c</i>	2	2	0	2	0	1	1	0	2	2	0	1	1	1	1	0
Chimpuno	18	9	9	17	1	4	14	2	16	<u>6</u>	<u>11</u>	4	14	10	7	1
<i>Non-typical</i>	5	1	4	4	1	0	5	2	3	3	2	3	2	2	2	0
NyaChikundi	20	0	20	<u>10</u>	<u>8</u>	<u>7</u>	<u>11</u>	<u>12</u>	<u>6</u>	<u>12</u>	<u>6</u>	0	20	5	13	2
<i>Non-typical</i>	5	0	5	3	2	<u>2</u>	<u>2</u>	<u>1</u>	<u>3</u>	5	0	0	5	1	4	0
Gomani	22	0	22	8	14	1	21	18	4	<u>16</u>	<u>5</u>	<u>9</u>	<u>11</u>	8	13	1
Depweti	26	<u>21</u>	<u>4</u>	<u>25</u>	<u>0</u>	<u>4</u>	<u>19</u>	<u>21</u>	<u>2</u>	24	2	<u>4</u>	<u>20</u>	13	9	2
<i>Non-typical</i>	6	<u>2</u>	<u>3</u>	6	0	1	5	5	1	6	0	2	4	2	4	0
<i>typ-Ng</i>	1	0	1	<u>0</u>	<u>0</u>	1	0	1	0	1	0	1	0	0	1	0
Koloweki	24	23	1	24	0	<u>1</u>	<u>22</u>	<u>15</u>	<u>8</u>	<u>20</u>	<u>2</u>	<u>14</u>	<u>8</u>	7	10	7
<i>Non-typical</i>	5	5	0	5	0	<u>0</u>	<u>4</u>	<u>3</u>	<u>1</u>	5	0	4	1	0	5	0
<i>typ-De</i>	5	4	1	5	0	1	4	4	1	5	0	2	3	5	0	0
NyaHarawa	24	2	22	11	13	4	20	10	14	22	2	9	15	13	9	2
<i>Non-typical</i>	8	0	8	1	7	1	7	2	6	8	0	5	3	6	1	1
<i>typ-De</i>	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	0
<i>typ-Go</i>	1	0	1	1	0	0	1	0	1	1	0	0	1	0	0	1
<i>typ-Nk</i>	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	0
NyaNkhata	26	19	7	21	5	0	26	18	8	18	8	9	<u>16</u>	8	18	0
<i>Non typical</i>	8	7	1	8	0	0	8	4	4	8	0	4	4	4	4	0
<i>typ-De</i>	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
<i>typ-Go</i>	1	0	1	1	0	0	1	0	1	0	1	0	1	0	1	0
<i>typ-Nm</i>	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1	0
Ng'wenyani	24	7	18	22	2	<u>0</u>	<u>23</u>	<u>14</u>	<u>9</u>	<u>19</u>	<u>4</u>	13	11	9	10	5
<i>Non typical</i>	9	3	6	7	2	<u>0</u>	<u>8</u>	<u>3</u>	<u>5</u>	7	2	5	6	4	2	3
<i>typ-De</i>	2	2	0	2	0	0	2	2	0	1	1	1	1	2	0	0
Nyamakozo	24	0	24	<u>4</u>	<u>18</u>	<u>0</u>	<u>23</u>	<u>23</u>	<u>0</u>	23	1	<u>16</u>	<u>7</u>	3	12	9
<i>Non-typical</i>	3	0	3	1	2	0	3	3	0	3	0	2	1	0	3	0

^a skin colour = outer root skin colour (1 = white/cream, 2 = brown); stem colour = mature stem colour (1 = white/grey, 2 = brown); shoot colour (1 = green, 2 = green-purple or purple); Shoot pubesc = shoot pubescence (1 = absent, 2 = present); Lamina colour = leaf lamina colour (1 = green, 2 = green-purple or purple); petiole colour (1 = green, 2 = green-purple or purple); root neck constriction (1 = short, 2 = intermediate, 3 = long).

^b Underlined number indicates that data are missing for this variable for 1 to 3 plants.

^c Numbers in italics are for those of the plants identified as belonging to each cultivar found to have a typical genotype of another cultivar or a non-typical genotype.

within minutes examined and collected (JM, NM) by the investigators.

Morphological characterisation of above and below ground parts of each plant was conducted (JM and NM) using a modified cassava morphological descriptor (Nweke et al., 1994). The eleven morphological characters were: shoot pubescence, shoot colour, leaf colour, leaf shape, petiole colour, leaf lamina colour, mature stem colour, root neck length, outer root skin colour, inner root skin colour and root pulp col-

our. The categories of each character are shown in the footnote in Table 1.

Two mature roots from each plant were collected for chemical analysis immediately after the morphological characterisation. The roots were transported to the chemical laboratory of the Mkondezi Agricultural Research Station (approximately 70 km north of the study area) in the early hours of the afternoon on each day. In the laboratory, the roots were peeled, washed and split longitudinally. One longitudinal half was cut

(with a sharp stainless steel knife) into approximately one cm sized cubes on plastic chopping boards. An amount of 49.5–50.5 g was weighed into a plastic cup and mixed with 160 ml 0.1 M orthophosphoric acid and homogenised. Cyanogenic glucoside levels were determined using the methods of Brimer et al. (1997) and Saka et al. (1998). The mean value for the two roots of each plant was calculated.

Labelled stem cuttings were collected and planted in a nursery at Mkondezi Agricultural Research Station. At the end of the four week survey and with due permission from the Ministry of Agriculture in Malawi, the cuttings were transported to Sweden and re-planted in a greenhouse at the Swedish University of Agricultural Sciences, Uppsala. Following leaf development after about one month, DNA was extracted from two fresh unexpanded young leaves of each plant (Edwards et al., 1991). All DNA samples were treated with RNase. Out of 123 collected pairs of plants, sufficient quantities of DNA were extracted from both plants of 116 pairs and these 232 plants were included in the study.

Complementary survey, 1997

In June, 1997, a complementary survey was carried out in the same area. Eligible farmers stating that they had plants of cultivars besides the ten most grown cultivars were included. Stem cuttings were collected from 45 plants identified by farmers as belonging to a cultivar that were not among the ten most grown studied in 1996. The stem-cuttings were transported to Sweden for molecular marker analysis as described above.

Molecular marker analysis

Seven short sequence repeat (SSR) primer pairs (Chavarriga-Aguire et al., 1998) were used in multiplex polymerase chain reaction (PCR) amplifications. GA126, GA134, and GA136 were multiplexed using FAM labelled forward primers (set 1). GA57, GA127 and GA131 were multiplexed using HEX labelled forward primers (set 2), and GA161 using forward primers labelled with TET (set 3). Each reaction mix consisted of 2 μ l of 10 ng/ μ l target DNA, 1 μ l 10 \times buffer, 0.4 μ l 10 mM deoxynucleotide triphosphates (dNTPs), 2.5 pmol of each primer, 0.6 μ l Dynazyme (Finnzyme) and H₂O to 10 μ l. Temperature cycling included denaturation at 95 °C 3 min., followed by 35 cycles of denaturing at 93 °C 1 min., annealing at

Table 2. Allele frequencies in genotypes found in plants identified as belonging to three 'cool' and seven 'bitter' cultivars

SSR locus ^a	Allele (bp ^b)	Cultivar type		P ^c
		cool	bitter	
GA161a	105	0.89	0.80	0.524
GA161b	131	0.39	0.72	0.006
	129	0.35	0.08	0.008
GA131	118	0.27	0.46	0.140
	116	0.35	0.01	0.013
	114	0.27	0.14	0.216
	106	0.12	0.18	0.530
GA57	183	0.23	0.20	0.773
	179	0.46	0.52	0.639
	160	0.23	0.28	0.786
GA127	232	0.62	0.86	0.021
	230	0.12	0.04	0.331
	228	0.08	0.00	0.120
	216	0.12	0.08	0.685
GA136	151	0.69	0.66	0.804
GA126	219	0.15	0.08	0.434
	213	0.19	0.12	0.496
	183	0.12	0.10	0.999
	181	0.46	0.66	0.140
GA134	319	0.85	0.76	0.555
	309	0.12	0.24	0.238

^a From each locus the least frequent allele (i.e., GA161a – 99; GA161b – 123; GA131 – 96; GA57 – 181; GA127 – 240; GA136 – 187; GA134 – 333).

^b bp is number of base pairs in each allele.

^c The probability (P) of allele frequency homogeneity by Fisher's Exact Test.

52 °C 1 min. and extension at 72 °C 1 min., and a final extension at 72 °C 10 min.

After PCR amplification, pooling was done for each set of three PCR products in the following proportions: 1 μ l set 1, 4 μ l set 2, 0.6 μ l set 3 and H₂O to, 20 μ l. From the pool, 0.5 μ l was mixed with 1.5 μ l formamide, 0.5 μ l GeneScan 500 (Perkin Elmer/Applied Biosystems) and 0.2 μ l Blue dextran loading buffer. After denaturation, 1.5–2 μ l was loaded on 6% denaturing gel (7 M urea) acrylamide:bisacrylamide (19:1) gels. The samples were separated by electrophoresis in 1 \times TBE at 29 W for a minimum of 3 hours on an automatic DNA sequencer (Perkin Elmer/Applied Biosystems model 377XL). Allele sizes were determined with GeneScan version 2.1, and genotyping was performed manually from gel images. Each primer pair amplified one locus except GA161 that amplified two linked loci designated as

GA161a and GA161b and the study thus included a total of 8 loci (Table 2). Null alleles were omitted.

Statistical analysis

Fisher exact tests and chi-square tests were used to compare proportions. Principal component analysis was done on SSR allelic data. Each allele at each locus was treated as a separate variable (attaining values 0, 1 or 2). The least frequent allele (among all plants) at each locus was omitted in order to reduce dependencies between alleles at the same locus. A multivariate fit (JMP, 1994, Multivariate fitting) was done on 7 of the 11 morphological variables of the 181 plants having any of the ten genotypes most frequent in the cultivars (one genotype dominated in each cultivar). Morphological variables that did not vary were excluded.

Results

Morphological description of the 18 to 26 plants identified as belonging to each of the 10 most grown cultivars are given in Table 1. The data on leaf shape and colour, inner root skin and colour of root pulp are not shown, since no differences were observed among the 232 plants studied.

Most of the eight SSR loci included in the laboratory analysis were highly polymorphic (Table 2). More than one allele was common at each locus, as shown by gene diversity (Nei, 1973) ranging from 0.30 to 0.74. The pattern of alleles found in the eight loci is regarded as the genotype for each plant studied, and thus null alleles were not considered.

The allele patterns of all genotypes found in the 232 plants are presented in Table 3. One single genotype was found in the majority (54–100%) of the 18 to 26 plants identified as belonging to each of the 10 most commonly grown cultivars. These genotypes are henceforth referred to as the ‘typical’ genotype of that cultivar. In Table 3 these genotypes are given the genotype code ‘typical-’ followed by a two-letter abbreviation for each cultivar name. Of the 181 plants with ‘typical’ genotypes, the farmers identified 14 as belonging to the wrong cultivar (Table 4). Table 3 also shows the allele composition of the other 29 genotypes found, henceforth referred to as ‘non-typical’ followed by a two-digit number. Most of the plants with ‘non-typical’ genotypes that were identified by farmers as belonging to the same cultivar had different

‘non-typical’ genotypes. Twelve ‘non-typical’ genotypes were found in more than one plant, one in six, two in four, three in three and six in two. Four of these were found in plants identified as belonging to different cultivars of which two, 32 and 29, were found in plants referred to as belonging to both ‘cool’ and ‘bitter’ cultivars. A parent offspring analysis of the 51 plants with a ‘non-typical’ genotype showed that for those classified as belonging to the cultivars ‘chimpuno’, ‘nyachikundi’ and ‘nyankhata’ more than half, 4/5, 3/5 and 7/8 respectively, could not be offspring of the typical genotype. For the ‘non-typical’ genotypes belonging to other cultivars, the majority could be offspring, but since we only have recorded the genotypes for eight SSR markers the risk is high for type II errors, that is, an individual is accepted as progeny although it is not. On average the ‘non-typical’ plants differed in five alleles from the ‘typical’, but three of the plants of the cultivar ‘ngwenyani’ had ‘non-typical’ genotypes that only differed by one allele from the ‘typical’, suggesting that they may possibly have been cloned from a mutant plant.

The mean cyanogenic glucoside levels expressed as mg HCN equivalents per kg fresh weight were three to ten fold higher in plants with ‘bitter’ ‘typical’ genotypes compared to those of ‘cool’ ‘typical’ genotypes (Table 4). The cyanogenic glucoside levels in plants with ‘non-typical’ genotypes, except a few, were several fold higher when identified as belonging to a bitter cultivar than when identified as belonging to a ‘cool’ cultivar (Table 3).

Table 4 shows the percent agreement between the farmer’s identification of plants belonging to each name-given cultivar and the molecular marker identification of these plants having the ‘typical’ genotype for that cultivar. The first column gives the farmers’ name of the cultivars and the second column the number of plants identified by farmers as belonging to that cultivar. Columns 3–12 show the number of plants with each ‘typical’ genotype, named as a two-letter abbreviation of the corresponding cultivar. Columns 13 and 14 show the number of plants identified as belonging to each cultivar and having a ‘non-typical’ genotype. The two bottom rows show the mean and standard error of cyanogenic glucoside levels of the roots of the plants for each genotype. The last column shows the percent agreement between the farmers’ identification of the plant as belonging to the particular cultivar and the molecular marker identification of the plants as having the ‘typical’ genotype of that cultivar. The highest agreement between what

Table 3. Allele composition of genotypes in 232 plants of ten cultivars collected in 1996

Cultivars	Genotypes	No. of plants	Alleles at the eight SSR GA-loci ^c								HCN ^a mean
			161a	161b	131	57	127	136	126	134	
Mbundumali	typical-Mb	22	11	23	23	13	13	12	14	22	26
	non-typical-01	1	11	12	24	23	13	12	14	22	22
	non-typical-02	1	11	23	22	23	12	11	24	22	15
Chimphuno	typical-Ch	13	11	23	12	23	12	12	24	22	25
	non-typical-03	1	11	12	34	23	11	12	14	22	39
	non-typical-04	1	11	22	12	14	11	12	44	23	26
	non-typical-05	1	11	23	12	14	11	12	24	22	32
	non-typical-06	1	11	23	13	23	23	12	24	22	93
	non-typical-07	1	12	11	14	12	11	11	13	12	66
	non-typical-08	1	12	11	13	12	15	11	35	12	39
NyaChikundi	typical-Nc	15	11	13	23	22	14	11	24	22	32
	non-typical-09	2	11	13	23	22	14	11	44	22	17
	non-typical-10	1	12	11	13	12	15	12	35	12	79
	non-typical-11	1	12	11	45	23	11	11	34	12	38
Gomani	typical-Go	24 ^b	12	11	15	12	11	11	34	11	163
Depweti	typical-De	28 ^b	12	11	14	12	11	11	44	12	111
	non-typical-12	4	11	11	14	13	11	12	34	12	132
	non-typical-13	1	11	12	14	23	13	12	14	22	83
	non-typical-14	1	11	23	12	23	11	12	14	22	113
Koloweki	typical-Ko	14	11	12	24	23	11	11	14	22	123
	non-typical-15	1	11	13	15	13	11	11	23	12	193
	non-typical-16	1	11	13	23	23	11	11	34	22	95
	non-typical-17	2	11	13	34	23	12	12	24	22	66
	non-typical-18	1	12	33	15	23	11	12	45	12	107
NyaHarawa	typical-Nh	13	11	11	13	12	11	22	44	22	112
	non-typical-19	4	11	11	11	22	15	12	44	22	79
	non-typical-20	3	11	11	13	12	11	12	44	22	151
	non-typical-21	1	12	33	13	12	11	22	44	22	102
NyaNkhata	typical-Nk	16 ^b	12	33	15	13	11	12	45	12	203
	non-typical-07	2	12	11	14	12	11	11	13	12	43
	non-typical-15	4	11	13	15	13	11	11	23	12	112
	non-typical-22	1	11	23	12	23	11	12	14	22	88
	non-typical-23	1	12	11	12	23	11	12	14	22	79
Ng'wenyani	typical-Ng	14 ^b	11	11	11	22	13	12	24	22	177
	non-typical-14	1	11	23	12	23	11	12	14	22	165
	non-typical-15	1	11	13	15	13	11	11	23	12	167
	non-typical-24	2	11	11	11	22	13	12	24	12	148
	non-typical-25	1	11	11	11	22	13	12	44	22	220
	non-typical-26	1	11	12	33	23	12	12	22	22	101
	non-typical-27	2	12	11	12	12	11	11	44	12	166
	non-typical-28	1	12	11	14	12	11	11	44	22	74
Nyamakozi	typical-Nm	22 ^b	11	11	14	23	11	11	44	12	251
	non-typical-11	2	12	11	45	23	11	11	34	12	151
	non-typical-29	1	12	11	45	23	11	11	44	12	127

^a Mean levels of cyanogenic glucosides are given as mg HCN equiv. per kg dry weight.

^b Include plants classified to other cultivars (see Table 4).

^c The alleles are given as numbers, number 1 to 5 refer to the allele in order of how commonly they occurred as shown in Table 2.

Table 4. Agreement between farmer's identification of cultivar and laboratory identification of genotypes of 232 plants

Cultivars ^{a,b}	No. of plants	Number of plants with genotypes that are:											Agreement ^c		
		typical										non-typical			
		Mb	Ch	Nc	Go	De	Ko	Nh	Nk	Ng	Nm	cool		bitter	
Cool															
Mbundumali(Mb)	24	22											2	92	
Chimpuno (Ch)	18		13										5	72	
NyaChikundi(Nc)	20			15									5	75	
Bitter															
Gomani (Go)	22				22									0	100
Depweti (De)	26					19					1			6	73
Koloweki (Ko)	24					5	14							5	58
NyaHarawa (Nh)	24				1	1		13	1					8	54
NyaNkhata (Nk)	26				1	1				15		1		8	58
Ng'wenyani (Ng)	24					2					13			9	54
Nyamakozo (Nm)	24											21		3	88
Total	232	22	13	15	24	28	14	13	16	14	22	12	39		
HCN mean ^d		26	25	32	163	111	123	112	203	177	251	40	119		
HCN s.e. ^d		4	7	7	17	7	11	16	19	28	26	26	4		

^a Within 'cool' and 'bitter' groups, cultivars are listed in descending order according to the proportion of farmers in the area growing the cultivar.

^b The abbreviation in parenthesis designates the 'typical' genotype of each cultivar.

^c Percentage of plants with 'typical' genotype among plants identified as belonging to each cultivar.

^d Mean and s.e. of the level of cyanogenic glucosides in root parenchyma of all plants belonging to each 'typical' genotype and plants with 'non-typical' genotypes among 'cool' and 'bitter' cultivars, respectively, expressed as HCN equivalents per kg fresh weight.

the farmers said and the molecular marker findings were for 'gomani' (100%), 'mbundumali' (92%), and 'nyamakozo' (88%).

Farmers classified 170 plants as belonging to 'bitter' and 62 as belonging to 'cool' cultivars. The relative frequency of plants with a 'non-typical' genotype was not significantly different between plants from the 'bitter' or 'cool' cultivars. The cyanogenic glucoside levels were much higher in plants classified as belonging to 'bitter' cultivars compared to those classified as belonging to 'cool' cultivars, irrespective of whether the genotype was 'typical' or 'non-typical'.

A comparison of farmers in Thowolo-B and Matyenda-1 shows that they differ in their cultivar maintenance. In an earlier interview study the farmers in Thowolo-B reported growing an average of 5.8 and those in Matyenda-1 an average of 3.6 cultivars (Chiwona-Karlton et al., 2000). In this study we found that the frequency of plants with 'typical' genotypes was significantly lower in Thowolo-B (62%) than in Matyenda-1 (82%). However, this difference only applies to the cultivars 'depwete', 'nyankhata' and 'nyamakozo', for which 0/6, 7/18 and 5/8, respectively, were typical in Thowolo-B and, 19/20, 8/8 and 16/16, respectively, were 'typical' in Matyenda-1. Out

of the six plants classified as 'depwete' in Thowolo-B, only two had the same 'non-typical' genotype. One of the other cultivars, 'ng'wenyani', differed in the other direction, 13/20 being typical in Thowolo-B and 0/4 in Matyenda-1. The other six cultivars, which include all 'cool' ones, did not differ between the two blocks. For three of the cultivars all the plants had a 'typical' genotype and for four cultivars all but one plant have a 'typical' genotype in 'Matyenda-1'.

The allele pattern of the genotypes found in 45 plants collected in a complementary survey in 1997 is presented in Table 5. During this survey plants of the less common cultivars were collected. Farmers identified, 20 plants as belonging to 'cool' and 25 as belonging to 'bitter' cultivars. Among these 45 plants 28 new genotypes were identified using DNA analysis and they are presented in Table 5 as consecutive numbers. Only three plants had a 'typical' genotype of the ten most grown cultivars. Five plants had a 'non-typical' genotype that was previously identified in the 1996 study among the ten most grown cultivars studied in 1996. Although some of the plants, collected from different farmers, had identical names, the genotypes were different except for two plants named 'kanonono'.

Table 5. Alleles of genotypes of 45 plants collected in complementary survey in 1997

Cultivar		Genotype ^a	Alleles at the eight SSR GA-loci							
Name	Taste		161a	161b	131	57	127	136	126	134
Chibisa	Cool	1	12	23	13	23	12	22	25	22
Fyoka	Cool	2	11	33	34	23	25	11	25	22
Fyoka	Cool	3	12	33	11	12	12	12	23	12
Kabinyu	Cool	4	11	33	22	22	13	12	44	22
Kachamba Mtuba	Cool	5	11	13	34	12	11	12	11	22
Kalomu	Cool	6	11	22	14	22	13	12	45	22
Kamwala	Cool	4	11	33	22	22	13	12	44	22
Kanjuchi	Cool	7	11	22	23	12	11	12	45	22
Kanonono	Cool	8	12	33	11	12	12	12	35	22
Kanonono (New)	Cool	9	11	12	13	23	13	11	25	22
Kweti Chimulole	Cool	10	12	22	13	12	11	12	35	12
Mnyakayuni	Cool	11	11	23	13	23	12	22	25	22
Mnyakayuni	Cool	4	11	33	22	22	13	12	44	22
Mwaya	Cool	typ-Ch	11	23	12	23	12	12	24	22
Ng'ung'uta	Cool	4	11	33	22	22	13	12	44	22
Ntheghama	Cool	12	11	12	33	23	11	11	14	22
Nyasungwi	Cool	13	12	11	14	12	11	11	44	11
Palamu	Cool	14	12	11	14	12	11	11	13	12
Palamu	Cool	typ-De	12	11	14	12	11	11	44	12
Virginia	Cool	12	11	12	33	23	11	11	14	22
20:20	Bitter	15	11	11	34	22	11	12	14	22
Cakubaba	Bitter	non-typical-03	11	12	34	23	11	12	14	22
Chigwalantha	Bitter	16	11	22	36	33	12	11	25	12
Ching'anya	Bitter	17	11	23	22	23	25	12	15	22
Gomani Admarc	Bitter	non-typical-15	11	13	15	13	11	11	23	12
Kachamba Muyera	Bitter	18	12	22	13	12	15	12	35	12
Kachamba Muyera	Bitter	19	11	13	15	13	12	12	23	12
Kachamba Muyera	Bitter	20	11	12	35	12	11	12	45	12
Kalomu	Bitter	typ-De	12	11	14	12	11	11	44	12
Kanonono (old)	Bitter	9	11	12	13	23	13	11	25	22
Kanonono (old)	Bitter	11	11	23	13	23	12	22	25	22
Kawalika	Bitter	15	11	11	34	22	11	12	14	22
Kolobeki Chibala	Bitter	21	11	12	12	13	13	12	14	22
Kweti Chimulole	Bitter	22	11	11	11	23	13	12	45	22
Mbayani	Bitter	non-typical-15	11	13	15	13	11	11	23	12
Mnyakayuni	Bitter	23	11	11	11	13	11	22	45	22
Mpuma	Bitter	22	11	11	11	23	13	12	45	22
Mpuma	Bitter	24	11	13	15	11	12	12	23	12
Mwatatu	Bitter	25	11	34	33	13	13	12	13	22
Nyankhonjerwa	Bitter	26	11	11	13	12	12	11	23	12
Nyasalima	Bitter	27	11	23	33	13	12	22	24	22
Palamu	Bitter	non-typical-15	11	13	15	13	11	11	23	12
Thipula	Bitter	7	11	22	23	12	11	12	45	22
Thipula	Bitter	28	11	12	13	23	11	12	15	22

^a Genotype in bold occurs in more than one plant. Numbers from 1 to 27 indicates genotypes found in 1997. The others are as labelled in Table 3.

^b The alleles are given as numbers, number 1 to 5 refer to the allele in order of how commonly they occurred as shown in Table 2.

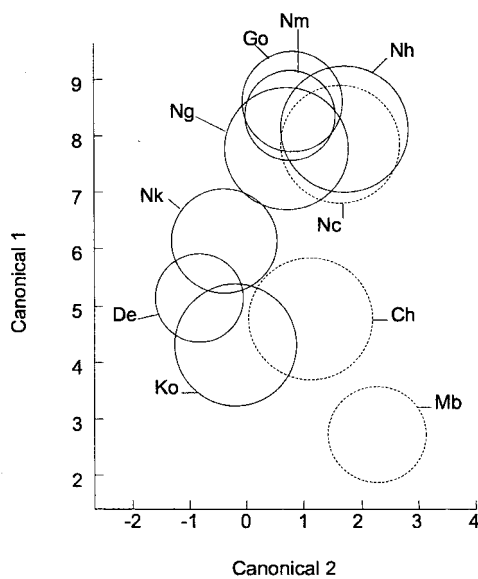


Figure 1. Centroid plots of the first two canonical variables from discriminant analysis. The analysis was done on seven morphological variables of the 13 to 28 plants with typical genotypes of the three 'cool' (dashed perimeter) and seven 'bitter' (continuous perimeter) cultivars. The abbreviations (as in Tables 3&4) indicate each genotype and the perimeter marks the 95% confidence region.

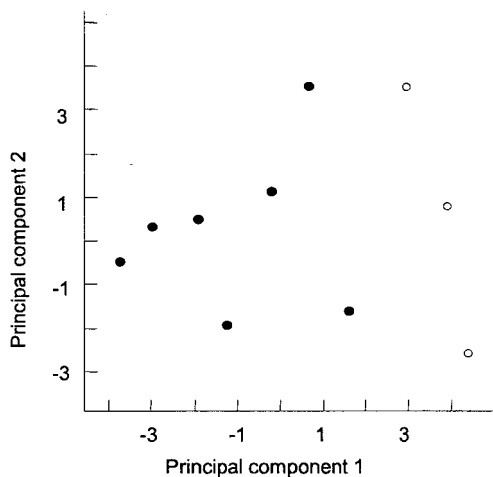


Figure 2. Scattergram based on principal component analysis of the typical genotypes of the three 'cool' (○) and seven 'bitter' (●) cultivars. The analysis was done on the allelic composition of eight SSR loci. The first component explained 36% and the second 18% of the total variation.

Figure 1 shows the results of the discriminant analysis of the seven varying morphological characters observed in the 181 plants with 'typical' genotypes. The circles illustrate the morphological variation of each cultivar. Each cultivar shows considerable morphological overlap with at least one other cultivar, except for 'mbundumali'. This shows that the investigators were unable to separate the plants of the ten 'typical' genotypes into different groups using the standard botanical descriptors. The morphology of plants with the 'typical' genotype of the 'cool' cultivars 'chimpuno' and 'nyachikundi' overlaps with that of plants with 'typical' genotypes of several 'bitter' cultivars. The morphological overlap between the plants with 'typical' genotypes of the seven 'bitter' cultivars is particularly pronounced for the cultivar 'gomani' and 'nyamakozo' although Table 4 shows that farmers were able to separate all plants with these two genotypes.

Figure 2 shows a plot of the 'typical' genotypes using the first two principal components of the composition of the SSR alleles of these ten genotypes. The genotypes of the three 'cool' cultivars were distinct from those of the seven 'bitter' cultivars. Already the first principal component was significantly ($p < 0.001$) different for the two groups. All 39 'typical' and 'non-typical' genotypes found in plants identified as belonging to 'cool' and 'bitter' cultivars were also clustered into two significantly ($p < 0.001$) different groups in the principal component analysis.

Discussion

Cassava (*Manihot esculenta* Crantz) is an important staple crop in Africa (Cock, 1982; Romanoff & Laynam, 1992). Almost everywhere where cassava is of significant importance as a staple crop the 'bitter' cultivars with high cyanogenic glucoside content dominate (Jones, 1959; Purseglove, 1968; Fresco, 1986). These compounds may cause adverse effects if not reduced to negligible levels during processing (Tylleskär et al., 1992). Advances in molecular genetics now offer the possibility to develop acyanogenic cassava (IITA, 1993; Dixon et al., 1994; Kereztesy et al., 1994a; Kereztesy et al., 1994b; McMahon et al., 1995; Andersen et al., 2000) but its relevance to small-scale farmers remains controversial (Wambugu, 1999; Machuka, 2001). To partly clarify this issue we used molecular genetics to elucidate how cassava farmers understand and manage cassava genetic di-

versity in relation to cassava cyanogenesis. Malawian women farmers were studied since they, like many other small-scale farmers in Africa, grow a repertoire of both 'cool' and 'bitter' cassava cultivars in the same field (Chiwona-Karlton et al., 1998) that morphologically appear to be similar. Roots from 'cool' cultivars are regarded as non-toxic, whereas those of 'bitter' cultivars are regarded as toxic and requiring extensive processing that, as a norm, is done solely by women (Chiwona-Karlton et al., 1998; Chiwona-Karlton et al., 2000). Despite this additional labour and time requirement for processing these farmers still prefer 'bitter' cultivars since toxicity is perceived as conferring protection from theft. Furthermore, these farmers claim that they are able to distinguish plants from the 'bitter' and the 'cool' cultivars by looking at their morphological appearance. The present study combined participatory farmer guided plant collection (Sperling & Sheidegger, 1995; Fujisaka, 1999) with laboratory DNA assay using PCR amplified SSR markers (Chavarriaga-Aguire et al., 1998). The quality of comparisons between laboratory DNA assays and the farmers' ability to identify the genetic diversity of their crop depend equally on the methodological stringency in the field survey and on the quality of the laboratory analysis. Mutual rapport was a fundamental issue when requesting information from the farmers. This study entailed tedious activities for the farmer and the investigators therefore remunerated them for the pre-emptory harvest of their cassava.

The SSR, or microsatellite, DNA sequences mutate at rates several orders of magnitude higher than that of the bulk of DNA. SSR-markers are therefore very informative for assessment of intra-species genetic diversity (Chavarriaga-Aguire et al., 1999). In the main study of 232 plants of the ten most grown cultivars we found it sufficient to use eight SSR loci since each one had high polymorphism. A complementary survey of the less common cultivars in 1997 was conducted to confirm that the eight SSR loci were sufficient to separate the genotypes grown in the area. Scoring with the same eight SSR loci in DNA from the 45 plants collected identified 27 new genotypes. Only three plants had the 'typical' genotype of the ten most grown cultivars, which is what one could expect if farmers occasionally misidentify varieties.

Cultivar and genotype

The finding of one single genotype in 92 to 100% of the plants that farmers identified as belonging

to the two most grown cultivars, 'mbundumali' and 'gomani', strongly indicate that the studied farming communities endeavour to maintain the most common cultivars as single clones. It would appear that farmers strive to maintain each of the ten most grown cultivars as one specific genotype as 72% of all plants, 167 of 232, had the typical genotype of the cultivar it was identified to belong to. Among plants not having the 'typical' genotype of each cultivar only 6%, 14 of 232, had a 'typical' genotype of another cultivar. More common was that the lack of agreement of the results between farmers and molecular marker identification was due to a 'non-typical' genotype. This was found in 22% of the plants, 51 of 232. Although the farmers in these two blocks appear to strive for cultivars consisting of single clones their fields contain a considerable proportion of plants of more than 30 'non-typical' genotypes that farmers fail to distinguish from the 'typical' genotypes of the ten most common cultivars. Since farmers were free to identify plants that they considered as belonging to any of the ten named cultivars the results do not tell the proportion of plants with 'non-typical' genotypes in their fields. However, among the plants considered to belong to one of the ten most common cultivars the 'typical' genotype was always at least three times more frequent than were any of the 'non-typical' genotypes.

All 'non-typical' genotypes were found in low frequencies and only four of the non-typical genotypes (07, 11, 14 & 15) were found in plants identified as belonging to different cultivars (Table 3). Mutations in the SSR loci may only explain a few of the 'non-typical' genotypes in the main survey since merely 8 of the 51 plants with 'non-typical' genotypes differed by only a single allele when compared with the 'typical' genotype. We cannot exclude that some 'non-typical' genotypes are offsprings of the 'typical' genotypes. We deduce that farmers manage to maintain the most preferred cultivars as single genotypes but that high proportions of plants of the less grown cultivars have 'non-typical' genotypes. Our findings have similarities with recent findings from a study of American Indian cassava farmers in Guyana (Elias et al., 2000; Elias et al., 2001). Our study design and sample size, however, did not enable us to conclude if the 'non-typical' genotypes were permanently accepted as part of the cultivars or if they were offsprings that would soon be discontinued once their characteristics had been observed by the farmers.

The difference in the number of plants with 'typical' genotypes between the two studied communit-

ies, situated only 3 km apart, suggests that a high agreement between named common cultivars and one specific genotype may be a local phenomenon. The cultivar 'depwete' was reported as being recently adopted in 'Thowolo B' (Chiwona-Karlton et al., 2000), and none of the reported six plants identified as 'depwete' had the 'typical' genotype, whereas in Matyenda-1 (where 'depwete' was frequently grown since many years back) all but one of, 20 identified plants had the 'typical' genotype. It is noteworthy that the 'Matyenda-1' farmers, who reported growing fewer cultivars, managed to identify a higher proportion of plants (82%) with 'typical' genotypes. In addition to 'mbundumali' and 'gomani' that had high agreement in both communities the cultivars 'depwete', 'nyankhata' and 'nyamakozo' had similarly high agreement in Matyenda-1. This further supports that small-scale cassava farming communities strive to maintain distinct cultivars that are single genotypes. An equally high agreement between the farmers' cultivar concept and one single genotype may well be found in most of the small-scale cassava farming systems in Africa. However, the probability that specific agreement between cultivar name and genotype may be restricted to quite small areas cannot be excluded. The names of the most popular cultivars, 'gomani' and 'mbundumali', are referred to over large parts of Malawi. It remains to be shown if the use of these names refers to the same genotypes in other parts of Malawi.

Molecular markers can help breeding and extension programmes to understand the contemporary pattern of genotypes used in the small-scale farming communities. This would require collection of plant material in a participatory interaction with local farmers and analysis of DNA from several plants of each common cultivar from each community before drawing generalisable conclusions about the relationships between name and genotype.

Farmers' accuracy in identifying cultivars and genotypes

Our morphological classification of plants was based on a modified and extensively used botanical key for cassava (Nweke et al., 1994). However, the discriminant analysis showed that the plants with the 'typical' 'mbundumali' genotype were the only group that could be distinctly differentiated by our morphological classification of the plants (Figure 1). On the other hand, when the farmers examined the plants they could

with relative ease differentiate 167 (92%) of the 181 plants with 'typical' genotypes. If the 'depwete' genotype, the farmers' only major problem, is excluded, farmers correctly differentiated 97% of the remaining 153 plants with nine different 'typical' genotypes. The farmer's skilled accuracy was further supported by the findings in the complementary study in 1997. Only 3 out of 45 plants identified as belonging to less common cultivars turned out to have a 'typical' genotype of one of the ten most grown cultivars. Once more, if 'depwete' is excluded, the farmers' mistakes concerned less than 3% of the plants. It seems most peculiar that farmers could correctly identify 43 (93%) plants of the 46 with 'typical' genotypes of 'gomani' and 'nyamakozo', since when using the botanical key we found considerable morphological overlap between the plants of these two genotypes (Table 1 & Figure 1).

The reason for the farmers' high accuracy in identifying 'gomani' and 'nyamakozo' plants cannot be attributed to the abundance of plants of these two genotypes in the fields, since 'nyamakozo' was the least grown cultivar of the ten (Chiwona-Karlton et al., 2000). Neither can the high accuracy be attributed to the farmers having planted cultivars in some easily recognisable pattern in the fields. We clearly observed that the farmers' identification of the plants of each cultivar always involved thorough searching throughout each field and a careful examination of a considerable number of plants. The farmers' high accuracy in identifying plants with the 'typical' genotypes of the ten most common cultivars is most probably explained by their use of more detailed morphological characters than the ones used by the investigators in this study. The local classification of cassava plants into cultivars with local names thus reflects a skilful morphological recognition of several locally preferred genotypes (Chiwona-Karlton et al., 2000). Farmers in this part of Africa have cultivated cassava for only one to two centuries (Jones, 1959; Carter et al., 1992). In spite of this they appear to be as good at identifying their local cassava genotypes as the Amerindians who have been growing cassava for several thousands of years (Boster, 1985; Bellon, 1996; Elias et al., 2000; Salick et al., 1997; Moore, 1998). Careful use of botanical keys developed for the whole species cannot match the accuracy of local farmers in morphological differentiation of their most common cassava cultivars. We did not study which morphological markers farmers used to be able to identify the genotypes with such high accuracy, but our results strongly indicate

that botanical keys for cassava should be developed in close collaboration with the local farmers.

These findings would imply that a cultivar would not be adopted within a farming system if it cannot be morphologically distinguished from the cultivars already grown in the area (Boster, 1985). A cassava cultivar thus needs to be recognised as a distinct morphological type before its yield, taste and other qualities can be assessed in the local environment. This has also been reported from cassava farming systems in South America (Boster, 1985; Sambatti et al., 2001). Studies in the Amazonian basin of Peru have shown that farmers select cultivars using perceptual taxonomic characters that show the greatest variation, i. e. leaf shape, petiole colour and stem colour (Boster, 1985; Salick et al. 1997; Elias et al., 2000, 2001; Sambatti et al., 2001).

In addition to the handful of cultivars occupying most of the land planted with cassava the small-scale cassava farming systems also preserve a high genetic bio-diversity of cassava. This is done by maintaining some plants of many old name-given cultivars and by testing new name-given cultivars from outside their community. It is also done by collecting new *volunteer* seedlings from sexually propagated cassava in their fallow fields (Chiwona-Karlton et al., 1998; Chiwona-Karlton et al., 2000). The DNA analysis suggests that the least grown cultivars kept for bio-diversity are more genetically heterogeneous than the common cultivars. This is in accordance with what has been found in small-scale Indian potato farming systems (Johns & Keen, 1986) and in cassava diversity studies (Bellon, 1996; Salick et al., 1997). Studies in small-scale Indian cassava farming systems in South America using morphological classification of plants suggest that farmers in the same community may differ in their accuracy in recognising cultivars (Boster, 1984; Boster 1985; Salick et al., 1997; Sambatti et al. 2001). Besides the differences observed between the two communities, we could not find any such pattern among the 28 farmers included in this study.

Differences between 'bitter' and 'cool' cultivars

Farmers classified 8% of the plants with 'typical' genotype as belonging to the wrong cultivar, but all 14 mistakes were made among the seven 'bitter' cultivars. We saw no cases whereby the farmers misclassified plants with 'typical' genotypes as 'cool' when they were supposed to be 'bitter' or vice-versa. The root cyanogenic glucoside levels in the 'typical' plants

of 'bitter' cultivars were four to seven fold higher (Table 3 and 4) compared to the 'cool' cultivars with 'typical' genotypes. Only two of 11 'non-typical' genotypes found among plants identified as belonging to 'cool' cultivars were also found among plants identified as belonging to 'bitter' cultivars (Table 3). The mean glucoside levels in roots of the plants with 'non-typical' genotype were three fold higher for those classified as belonging to 'bitter' cultivars than those classified as 'cool' cultivars as shown in Tables 3 and 4. These findings indicate that farmer's ethno-classification of cassava cultivars into 'cool' and 'bitter' reflects their knowledge of the potential risk of poisonous effects from cyanogenic glucosides in the roots.

These differences between 'cool' and 'bitter' cultivars support the results from interview studies in which the farmers in this part of Malawi were found to regard 'bitter' and 'cool' cassava cultivars as two different crops in their farming and food system (Chiwona-Karlton et al., 1998; Chiwona-Karlton et al., 2000). They maintained that roots from the 'cool' cultivars were safe for consumption in the raw form whereas roots from the 'bitter' group of cultivars could only be consumed safely as the staple food *kondowole* after processing by soaking and fermentation, drying and pounding into flour (Chiwona-Karlton et al., 2000). This method of processing (Lancaster et al. 1982; Dufour, 1989) reduces cyanogenic compounds to negligible levels (Banea et al., 1992).

The principle component analysis displayed in Figure 2, shows that the three genotypes of the 'cool' cultivars differed from all the seven genotypes of the 'bitter' cultivars. This suggests that the farmers' necessity to differentiate between 'cool' and 'bitter' cultivars might have influenced the genetic structure of cassava by the way farmers over time decide which cultivars to plant and which ones to discard (Wilson & Dufour, 2002). The division observed in contemporary Malawi might have occurred locally, elsewhere in Africa, or already in South America, many centuries to millennia ago. A more speculative hypothesis is that the two groups of cultivars constitute separate domestication events (Allem, 1994; Dufour, 1995; Allem, 1999; Rogers & Fleming, 1973; Ugent et al., 1986). It should be noted that Amerindians also appear to regard 'bitter' and 'sweet' cultivars as two different crops (Box & Box-Lasocki, 1982; Sauer, 1963; Dufour, 1993; Dufour, 1995). However, studies using botanical taxonomy have not recognised any morphological signs of genetic division (Nordenskiöld, 1924;

Rogers 1965; Renvoize, 1972; McKey & Beckerman 1993). This supports Boster's hypothesis (Boster, 1985) that morphologic characters do not relate to agronomic characters since differences in morphology are an effect of farmers' need to perceptually distinguish cultivars.

Conclusion

This study has shown that each of the most common cultivars in the study area was composed of a majority of plants with identical genotypes. On the other hand, plants of the less common cultivars had in addition to a typical genotype in most plants, mixture of several rare atypical genotypes. Though farmers made some mistakes in identifying plants as belonging to a common cultivar, they did not make any mistake between the groups of bitter and cool, respectively. The molecular genetic and biochemical results are compatible with a genetic separation between the cool and bitter genotypes in this study area.

We conclude that the biological knowledge of cassava cyanogenesis among small-scale African farmers enables them to benefit from its protection against theft with very limited risk, as the toxicity can be removed by processing that takes almost one week. The potential thieves, predominantly hungry young males, are deterred by the duration of processing, which is traditionally done only by women. Dietary cyanide exposure from cassava is rare, and causes severe health effects when short cuts in processing brought about by food shortages induced by environmental degradation, drought, severe poverty or war. Ironically, toxic effects are most common where protection against theft is most needed. As the farmers at risk will not plant non-toxic cultivars, transgenic acyanogenic cassava will not help much in prevention of cassava toxicity. Notwithstanding, acyanogenic cassava may be useful where theft is rare and 'cool' roots are consumed without processing, as well as to further the understanding of the biology of cyanogenesis. Further analyses of the genetic differences between 'cool' or 'sweet' and 'bitter' cultivars will tell how and when they became genetically separated. The differentiation may have affected many agronomic characters and is therefore important to consider in the maintenance of breeding populations. It seems to be taxonomically important for cassava farmers that the new cultivars fit into either of these two categories. Scientists need to understand the mechanisms that de-

termine the adoption of new cultivars by small-scale farmers.

Biotechnology indisputably has the potential to improve food security among small-scale farmers in Africa by the use of tissue culture, marker assisted breeding and development of transgenics. We advocate that the advances in molecular genetics also should be used to improve the understanding of farming and food systems of poor small-scale farmers by combined use of in-depth interviews, molecular techniques and farmer participation.

Acknowledgements

The authors thank the farmers, chiefs, communities, James Ngoma and Sidney Simukoko for their unwavering support, field assistants, the agricultural extension staff, the agricultural research division staff, in particular the then director Dr C Matabwa, for their patience and collaboration. We are much obliged for the institutional support from Mkondezi and Lunyangwa Agricultural Research Stations, Chancellor College, University of Malawi and the International Institute for Tropical Agriculture. The Swedish International Development Agency (Sida/SAREC) and The International Science Programme (ISP) at Uppsala University financially funded this work.

References

- Akoroda, M.O., 1995. Alleviating hunger in Africa with roots and tuber crops. *Afr J Root Tuber Crops* 1: 41–43.
- Allem, A.C., 1994. The origin of *Manihot esculenta* Crantz (Euphorbiaceae). *Gen Resour Crop Ev* 41: 133–150.
- Allem, A.C., 1999. The closest wild relatives of cassava (*Manihot esculenta* Crantz). *Euphytica* 107: 123–133.
- Andersen, M.D., P.K. Busk, Ib Svendsen & B.L. Moller 2000. Cytochromes P-50 from cassava (*Manihot esculenta* Crantz). Catalyzing the first steps in the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin – cloning, functional expression in *Pichia pastoris*, and substrate specificity of the isolated recombinant enzymes. *J Biol Chem* 275: 1966–1975.
- Banea, M., N. Poulter & H. Rosling, 1992. Shortcuts in cassava processing and risk of dietary cyanide exposure in Zaire. *Food Nutr Bull* 14: 137–143.
- Bellon, M.R., 1996. The dynamics of crop infraspecific diversity: A conceptual framework at farmer level. *Econ bot* 50: 26–39.
- Berry, V. & C. Petty, 1992. The Nyasaland Survey Papers 1938–1943 Agriculture, Food and Health. Academy Books Limited: London.
- Boster, J.S., 1984. Classification, cultivation, and selection of Aguaruna cultivars of *Manihot esculenta* (Euphorbiaceae). *Adv Econ Bot* 1: 34–47.

- Boster, J.S., 1985. Selection for perceptual distinctiveness: Evidence from Aguaruna cultivars of *Manihot esculenta*. *Econ Bot* 39: 310–325.
- Box, L. & B. Box-Lasocki, 1982. Bread of the earth: Cassava cultivation, processing and consumption among Amerindians. In Man and manihot. L. Box & F. Doorman (Eds.), Case Studies on Cassava Cultivars, pp. 1–6. Wageningen, Agricultural University: Wageningen.
- Brimer, L., J.D.K. Saka & T. Pedersen, 1997. Extraction of plant materials. A new blender design and the extraction of fresh cassava roots in dilute orthophosphoric acid. *J Food Comp Anal* 10: 358–367.
- Carter, S.E., L.O. Fresco & P.G. Jones, with J.N. Fairbairn, 1992. An atlas of cassava in Africa. Historical, agroecological and demographic aspects of crop distribution. Cali, Colombia, CIAT. 86 p.
- Chavarriaga-Aguire, P., M.M. Maya, M.W. Bonierbale, S. Kresovich, M.A. Fregene, J. Tohme & G. Kochert, 1998. Microsatellites in cassava (*Manihot esculenta* Crantz): discovery, inheritance and variability. *Theor Appl Genet* 97: 493–501.
- Chavarriaga-Aguire, P., M.M. Maya, J. Tohme, M.C. Duque, C. Iglesias, M.W. Bonierbale, S. Kresovich & G. Kochert, 1999. Using microsatellites, isozymes and AFLPs to evaluate genetic diversity and redundancy in the cassava core collection and to assess the usefulness of DNA-based markers to maintain germplasm collections. *Mol Breed* 5: 263–273.
- Chiwona-Karlun, L., J. Mkumbira, J. Saka, M. Bovin, N.M. Mahungu & H. Rosling, 1998. The importance of being bitter – A qualitative study on cassava cultivar preference in Malawi. *Ecol Food Nutr* 37: 219–245.
- Chiwona-Karlun, L., T. Tylleskar, J. Mkumbira, M. Gebre-Medhin & H. Rosling, 2000. Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. *Int J Food Sci Nutr* 51: 33–43.
- Cock, J.H., 1982. Cassava: A basic energy source in the tropics. *Science* 218: 755–762.
- Cousins, H.H., 1903. Cassava from Colombia. *Bull Dept Agr Jamaica* 1: 35–38.
- Dixon, A.G.O., R. Aseidu & M. Bokanga, 1994. Breeding of cassava for low cyanogenic potential: Problems, progress and prospects. *Acta Hort* 375: 153–161.
- Dufour, D.L., 1988. Cyanide content of cassava (*Manihot esculenta*, Euphorbiaceae) cultivars used by Tukanoan Indians in Northwest Amazonia. *Econ Bot* 42: 255–266.
- Dufour, D.L., 1989. Effectiveness of cassava detoxification techniques used by indigenous peoples in Northwest Amazonia. *Interciencia* 14: 86–91.
- Dufour, D.L., 1993. The bitter is sweet: A case study of bitter cassava (*Manihot esculenta*) use in Amazonia. In: C.M. Hladik, A. Hladik, O.F. Linares & H. Pagezy (Eds.), Tropical Forests, People and Food: Biocultural Interactions and Applications to Development, pp. 575–588. UNESCO/Parthenon, Paris.
- Dufour, D.L., 1995. A closer look at the nutritional implications of bitter cassava use. In: L.E. Sponsel (Ed.), Indigenous Peoples and the Future of Amazonia. An Ecological Anthropology of an Endangered World, pp. 149–65. The Tucson, University of Arizona.
- Edwards, K., C. Johnstone & C. Thompson, 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucl Acids Res* 19: 1349.
- Elias, M., O. Panaud & T. Robert, 2000. Assessment of genetic variability in a traditional cassava (*Manihot esculenta* crantz) farming system, using AFLP markers. *Heredity* 85: 219–230.
- Elias, M., D. McKey, O. Panaud, M.C. Anstett & T. Robert, 2001. 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.
- FAO, 2000. FAO Statistical Database.
- Fresco, L.O., 1986. Cassava in Shifting Cultivation: A Systems Approach to Agricultural Technology Development in Africa. Amsterdam, Royal Tropical Institute.
- Fujisaka, S., 1999. Systems and Farmer Participatory Research. Developments in Research on Natural Resource Management. CIAT Publication; No 311. Cali, Colombia, International Centre for Tropical Agriculture, CIAT.
- IITA, 1993. Breeding Cassava for 'Safer' Consumption, Higher Protein, pp. 25–27. Ibadan, International Institute for Tropical Agriculture.
- JMP, 1994. Statistics and Graphics Guide, SAS Institute Inc. Cary NC, USA.
- Johns, T. & S. Keen, 1986. Ongoing evolution of the potato on the Altiplano of Western Bolivia. *Econ Bot* 40: 409–424.
- Jones, W., 1959. Manioc in Africa. Stanford: Stanford University Press.
- Keresztessy, Z., L. Kiss & M. Hughes, 1994a. Investigation of the active site of the cyanogenic beta-D-glucosidase (linamarase) from *Manihot esculenta* Crantz (cassava). I. Evidence for an essential carboxylate and reactive histidine residue in a single catalytic centre. *Arch Bioch Biophys* 314: 142–152.
- Keresztessy, Z., L. Kiss & M. Hughes, 1994b. Investigation of the active site of the cyanogenic beta-D-glucosidase (linamarase) from *Manihot esculenta* Crantz (cassava). II. Identification of Glu-98 as an active site carboxylate group with acid catalytic function. *Arch Bioch Biophys* 315: 323–330.
- Lancaster, P.A., J.S. Ingram, M.Y. Lim & D.G. Coursey, 1982. Traditional cassava-based foods: survey of processing techniques. *Econ Bot* 36: 12–45.
- Lathrap, D.W., 1973. The antiquity and importance of long-distance trade relationships in the moist tropics of pre-Colombian South America. *World Archaeology* 5: 170–186.
- Machuka, J., 2001. Agricultural biotechnology for Africa. African scientists and farmers must feed their own people. *Plant Phys* 126: 16–19.
- Malawi, Government, 2000. Malawi Population and Housing Census 1998: Summary of Final Results. Zomba, Malawi, National Statistics office.
- McKey, D. & S. Beckerman, 1993. Chemical ecology, plant evolution and traditional manioc cultivation systems. In: C. Hladik, A. Hladik, O.F. Linares, H. Pagezy, A. Semple & M. Hadley (Eds.), Tropical Forests, People and Food. Biocultural Interactions and Applications to Development, pp. 83–112. Paris, UNESCO.
- McMahon, J., W. White & R. Sayre, 1995. Cyanogenesis in cassava (*Manihot esculenta* Crantz). *J Exp Bot* 46: 731–741.
- Moore, P.D., 1998. Getting to the roots of tubers. *Nature* 395: 330–331.
- Nei, M., 1973. Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci US* 70: 3321–3323.
- Nordenskiöld, E., 1924. The Ethnography of South America seen from Mojos in Bolivia. *Comparative Ethnographic Studies*. Vol. 3. Göteborg, Erlanders Boktryckeri Aktiebolag.
- Nweke, F., 1995. Processing cassava for wider market opportunities in Africa. In: Postharvest Technology and Commodity Marketing. Accra, Ghana, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.
- Nweke, F.I., A.G.O. Dixon, R. Asiedu & S.A. Folayan 1994. Cassava varietal needs of farmers and the potential for production

- growth in Africa. COSCA working paper No. 10. Collaborative study of cassava in Africa. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.
- Nweke, F., Spencer, D. & J. Lynam, 2001. The Cassava Transformation: Africa's Best Kept Secret. East Lansing: Michigan State University.
- Pelletier, D.L. & L.A.H. Msukwa, 1990. The role of information system in decision-making following disasters: Lessons from the mealy bug disaster in northern Malawi. *Hum Org* 49: 245–254.
- Purseglove, J., 1968. *Tropical Crops: Dicotyledons*. Vol 1: 171–180. London: Longmans, Green.
- Renvoize, B.S., 1972. The area of origin of *Manihot esculenta* as a crop plant: a review of the evidence. *Econ bot* 26: 352–360.
- Rogers, D.J., 1965. Some botanical and ethnological considerations of *Manihot esculenta*. *Econ bot* 19: 369–377.
- Rogers, D.J. & S.G. Appan, 1973. *Manihot, Manihotoides* (Euphorbiaceae). New York, Hafner Press.
- Rogers, D.J. & H.S. Fleming, 1973. A monograph of *Manihot esculenta* with an explanation of the taximetric methods used. *Econ Bot* 27: 1–113.
- Romanoff, S. & J. Lynam, 1992. Cassava and African food security: Some ethnographic examples. *Ecol Food Nutr* 27: 29–41.
- Saka, J.D.K., A.R.K. Mhone & L. Brimer, 1998. An improved microdiffusion method with solid-phase detection for the determination of cyanogens in fresh cassava. *J Sci Food Agric* 76: 334–340.
- Salick, J., N. Cellinese & S. Knapp, 1997. Indigenous diversity of cassava: Generation maintenance, use and loss among the Amuesha, Peruvian Upper Amazon. *Econ Bot* 51: 6–17.
- 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.
- Sauer, C.O., 1963. Cultivated plants of South and Central America. In: J. Steward (Ed.), *Handbook of South American Indians*, pp. 507–532. New York, Cooper Square.
- Schery, R.W., 1947. Manioc—a tropical staff of life. *Econ Bot* 1: 20–25.
- Scott, G.J., M.W. Rosegrant & C. Ringler, 2000. Global projections for roots and tuber crops to the year 2020. *Food Policy* 25: 561–597.
- Spencer, D.S.C., 1994. Infrastructure and technology constraints to agricultural development in the humid and sub-humid tropics of Africa. IFPRI EPTD discussion paper no. 3.
- Sperling, L. & U. Scheidegger, 1995. Participatory Selection of Beans in Rwanda: Results, Methods and Institutional Issues. IIED, Gatekeeper series 51.
- Tylleskär, T., M. Banea, N. Bikangi, L. Fresco, L.A. Persson & H. Rosling, 1991. Epidemiological evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. *Bull WHO* 69: 581–590.
- Ugent, D., S. Pozorski & T. Pozorski, 1986. Archaeological manioc (*Manihot*) from coastal Peru. *Econ Bot* 40: 78–102.
- Wambugu, F., 1999. Why Africa needs agricultural biotech. *Nature* 400: 15–16.
- Wilson, W.M. & D.L. Dufour, 2002. Why 'bitter' cassava? Productivity of 'bitter' and 'sweet' cassava in Tukanoan Indian settlement in the Northwest Amazon. *Econ Bot* 56: 49–57.