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Molecular markers as a tool for participatory cassava breeding

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Abstract. It is of fundamental importance to know the genetic identity of the plants used in the breeding process and in the case of asexually reproduced crops to know to which clone a plant belongs. Since cassava small-scale farmers do not maintain a one-to-one relationship between variety and clone participatory cassava breeding has a problem using farmer's plants. This study uses molecular markers to identify clones popular by small-scale cassava farmers and to describe the impact of genotype by environment interaction (GxE) on farms. Popular clones are particularly important when choosing local parents for crossings. Better knowledge of GxE at the farm level would improve the selection schemes. Eight SSR markers were found to be sufficient to discriminate between clones. A wide diffusion of a clone was used as an estimate of its popularity. Plants were collected from ten districts of Malawi. Most clones were limited in their geographic distribution. A majority was represented by a single plant but a few were found in several districts. Plants of the clones 'gomani' and 'mbundumali', that for decades have been distributed by the national breeding programme, were collected from farmers in three and nine districts, respectively. There was genetic identity between all 'gomani' collected from the farmers and the 'gomani' in the breeding programme. Plants that belonged to the program clone 'mbundumali' had seven variety names among farmers and these varieties also included a fraction of other clones. Consequently farmers can maintain varieties as single clones but mostly molecular markers are needed for identification of a plant's genotype. The study also suggests that the major part of the small-scale farmers' clones is used for breeding the rest for production. The analysis of GxE in farmers' fields showed that interaction occurred within farms for variables related to cultivation activities as well as to natural environmental variation.

Introduction

The starchy roots of cassava, *Manihot esculenta* Crantz, have become the most important source of dietary energy in Sub-Saharan Africa (Hillocks, 2002). Cassava is mainly grown by small-scale farmers and about a third of the villages in the cassava growing areas rate it as the most important crop (IITA, 1997). The farmers predominantly grow cassava in marginal environments, that is, under conditions that vary over space and time. Chemical fertilisers are rarely used (Nweke, 1994) but the yields are sustainable over many years, possibly because the foliage is recycled back into the soil. A threat to cassava's survival as a dominant crop in subsistence communities is cassava's vulnerability to diseases. The African cassava mosaic virus, the causal agent to the historically most serious disease to African cassava cultivation (Hahn *et al*., 1980), has made farmers give up cultivation of cassava

at least for periods (Jameson, 1964; Otim-Nape, 2001). Still it seems realistic to assume that the cassava farming system will remain important to many Sub-Saharan communities and for their development because of its capacity to provide food and market opportunities (FAO, 2000).

The breeding of cassava for Sub-Saharan Africa is conducted by the international community of formal cassava breeders as well as by small-scale farmers. Cassava is an out-crossing crop, but since it is vegetatively propagated it is composed of groups of genetically identical plants, that is, clones. Formal breeders primarily use recurrent selection and multilocation clone trials to develop new varieties (IITA, 1990) but have also started to use marker-assisted selection (Fregene pers com). Farmers base their selection on phenotypic characters (Chiwona-Karltun *et al*., 1998) although evidence of parent-offspring relationships between their varieties (Fregene *et al.*, 2003) indicates that recombination could be an important component in their breeding. However, interviews with small-scale farmers has shown that farmers adopt few new improved varieties, partly as an effect of dismal performance and deficiency in traits that the farmers like (Nweke *et al*., 1994). This would indicate that formal breeding has a minor impact on the variety composition in Sub-Saharan Africa despite clear evidence of its capacity to reduce disease problems in subsistence farming (Jennings, 1994; Otim-Nape *et al*., 2001) and to adapt cassava to market needs (CIAT, 2001). As a consequence cassava-breeding programs in this area have opted for participatory plant breeding (PPB) schemes, e.g. Bua (1998) or Fregene (pers. com), in which new varieties are produced from crosses between local and improved varieties, a scheme that has shown promising results under conditions similar to those of cassava in Sub-Saharan Africa (Ceccarrelli *et al*., 2001).

As discussed by Sperling *et al.* (2001) PPB involves formal breeders and farmers but also consumers, extensionists, vendors, industry and rural co-operatives in plant breeding research. However, in this paper it is only considered how formal breeders could interact with farmers to make the PPB more efficient. The first aim is to identify clones that have proven to be widely appreciated by local farmers. As Witcombe and Virk (2001) have pointed out such clones would improve the chances of producing valuable recombinants from crosses between local and improved varieties and therefore reduce the number of necessary crosses in a breeding scheme. The second aim is to analyse if there are GxE interactions within subsistence communities. To our knowledge, such GxE are not considered in the formal breeding of cassava and therefore become a part of the non-repeatable GxE, a condition that makes breeding for small-scale farmers non-optimal. By considering the factors on the farm level that affect GxE in the planning of selection experiments it would be possible to reduce the very high non-repeatable GxE observed for cassava (Mkumbira *et al*., 2003b).

An obvious approach to identify clones that have proven to be widely appreciated by local farmers would be to look for varieties that occur over large areas, since one could assume that farmers' cassava -varieties, like the varieties produced by formal breeders, are maintained as single clones (Mkumbira *et al*., 2003a). One way to identify such clones would be to analyse if there exists local names that are used over wide areas. This method was used by Jameson (1964) when he described the diffusion of local varieties in Uganda in the 1950's, and implied that some few clones were dominating parts of Uganda during that period. Otim-Nape (2001), describing the variety composition just before the severe outbreak of mosaic virus in Uganda, also assumed that local names represent single clones and therefore concluded that some few clones dominated parts of Uganda before the outbreak. Otim-Nape (unpublished) also checked their statements by conducting morphologic analysis of the plants.

Identifying clones by local names and morphology may very well be sufficient, but

caution should be applied about jumping to wrong conclusions for different reasons. Plants of a local variety might not all belong to a single clone but the variety could consist of a dominating clone and plants belonging to other clones. Thus molecular marker analysis indicated that varieties kept by subsistence farmers in French Guyana either consisted of mixtures of plants belonging to unrelated genotypes, mixtures of plants that are genetically related or plants of single clones (Elias *et al*., 2001). Molecular marker analysis in Malawi by Mkumbira *et al.* (2003a), on the other hand, did not suggest that small-scale farmers keep varieties as mixtures of plants belonging to related genotypes. Here a few commonly grown varieties were kept as single clones while most varieties consisted of a dominating clone and a fraction of plants belonging to other genotypes as well. Other reasons for no one-to-one relationship between a local variety and a single clone are the observations of farmers' naming habits (Chiwona-Karltun *et al.*, 1998; Jones, 1959). These indicate that the same name could purposefully be given to different clones and that a local variety, as observed by Fregene *et al.* (2000), could be adopted under different names. There is also evidence that farmers miss-classify varieties that are recently introduced to a village (Mkumbira *et al.*, 2003a), also causing different names for the same dominating clone. Mkumbira *et al.* (2003a) also showed that some botanical keys for cassava are insufficient in making a clear distinction between plants belonging to different clones, since plants of the same clone varied considerably in morphology. This suggests that the use of morphology could be a poor tool to establish one-to-one relationships between local varieties and clones. Molecular marker analysis therefore could be used as a complement to local names and morphological descriptions to analyse the diffusion of single clones.

As regards GxE on small-holder farms, observations under a previous study (Mkumbira *et al*., 2003a) indicated that it probably exists since farmers did not grow varieties randomly on the farm but some varieties were preferred on certain sites. However, considering the risk that plants could be classified to the wrong clone by local names or botanical keys the analysis of GxE will be improved if the plants are genotyped by molecular markers.

In this study farmer interviews and molecular markers were combined to analyse the diffusion of cassava clones in Malawi. Plants genotyped in a previous study (Mkumbira *et al*., 2003a) and belonging to the ten most frequent clones in two adjacent villages in northern Malawi were also used to study GxE in farming communities.

Material and Methods

Study area. The study of GxE in microenvironments and a major part of the analysis of the diffusion of clones was conducted in two blocks, 'Thowolo-B' and 'Matyenda-1', in Nkhata-Bay district (Figure 1), a district with a predominantly rural population of approximately 165,000 (Malawi, 2000). In this district, about 70% of the farmed land is allocated to cassava (Musukwa and Pelletier, 1990). The two blocks are located at the lakeshore zone along Lake Malawi, 475 – 600 meters above sea level. The farming community in Nkatha Bay district has a long tradition of growing cassava and many of the varieties have been grown for more than fifty years (Berry and Petty, 1992; Chiwona-Karltun *et al*., 1998). The crop is mainly grown in small fields with no inter-cropping. Since essentially everyone in the area is a cassava farmer mainly using cassava for household consumption, the harvesting 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 at different ages that belong to up to 15 different varieties. The analysis of the diffusion of clones were also made in other parts of Nkhata Bay, besides the two blocks mentioned above, and in several other districts but mainly in Mulanje, Nkhotakota, Mangochi and Zomba (Figure 1).

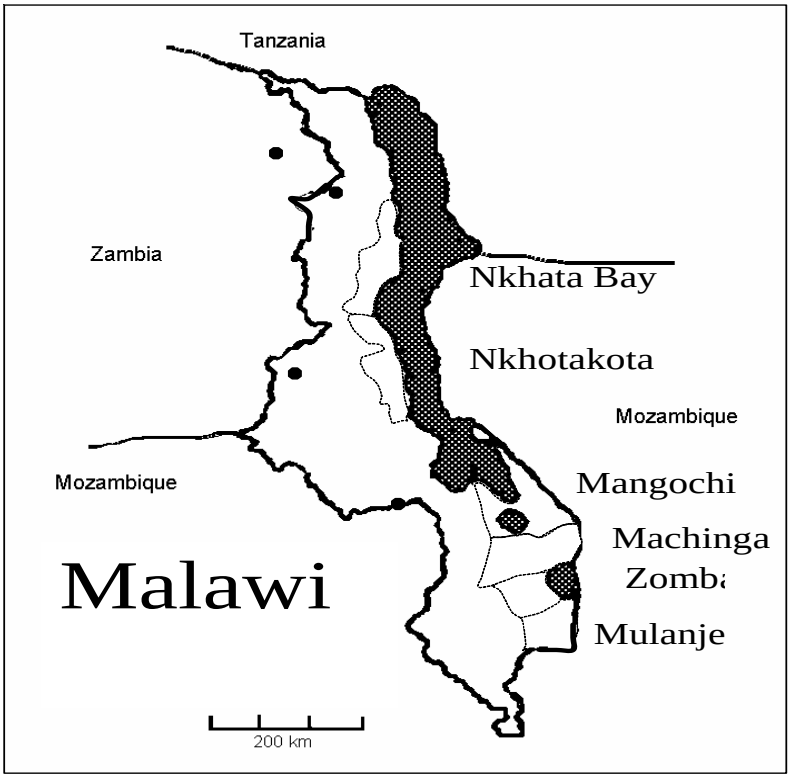

Figure 1: Map of Malawi showing districts where the major part of the collections were conducted.

Nkhotakota, like Nkhata Bay is situated along the coast of Lake Malawi and this district is also similar to Nkhata Bay with respect to agro-ecology and socio-economy but maize is more commonly grown than in Nkhata Bay. The villages studied in Mulanje have frequent rains due to their location in relation to Mount Mulanje and many of the farmers are employed in the tea estates.

They also grow sorghum, millet and maize in addition to cassava. In Machinga and Zomba farmers grow rice, sorghum, millet and maize in addition to cassava.

Interviews with farmers and collections of plants. One of the members of the research team, Linley Chíwona-Karltun, conducted qualitative interviews in Nkhata Bay district during two months in 1994 and 1995, respectively, to elucidate why farmers grow bitter cassava (Chiwona-Karltun *et al*., 1998). In July and August 1996 she interviewed all the 176 households in the blocks 'Thowolo-B' and 'Matyenda-1' (Chiwona-Karltun *et al*., 2000). Later that year a large team collected around 25 plants from each of the ten most grown varieties in the two blocks to study how farmers predict taste and score bitterness of cassava (Chiwona-Karltun *et al*., 2004) and how farmers classify these varieties (Mkumbira *et al*., 2003a). These plants were included in this study. The details of the collection procedure, where the farmers were asked to bring two plants from each variety, are described in Mkumbira *et al*. (2003a). In 1997 interviews were conducted in the same blocks with the purpose to understand the farmers breeding practices. Plants from the less common varieties were also collected and genotyped that year and are included in this study. Nearly all the farmers' varieties in the two blocks 'Thowolo-B' and 'Matyenda-1'

were recorded and genotyped since the farmers had been interviewed for the past four years about their varieties and very few new were recorded the last year.

Areas outside the two blocks were visited in 1997, 1999 and 2000 (Table 1 and Figure 1). The research team conducted in-depth interviews with knowledgeable cassava farmers, primarily women, in prior to collecting the plants. In most cases the discussions were in the local language, with simultaneous translation to English, but quite often English was used intermittently. The interviews were conducted to get information on how the farmers acquired, selected and maintained cassava varieties. After the interviews the farmers were asked to bring one plant from each of their varieties. Each farmer only contributed with one plant per variety. In Nkhata Bay and Mulanje the aim was to get as many varieties as possible while the focus was on the commonly grown varieties in the other districts. In addition to the 555 plants collected and genotyped in 1996 – 2000, 68 plants from a national collection, made earlier in the 1990's, were genotyped and included in the study.

Plants of the bitter variety 'gomani' and the cool/sweet variety 'mbundumali' in the Uniform Yield Trial kept at the Research station Makoka in Zomba district were also genotyped. Since these two varieties had been multiplied and distributed through the national breeding program since the 1970's, the purpose was to check their identity with the plants collected from small-scale farmers.

Molecular marker analysis. Eight highly polymorphic SSR loci, used in an earlier study (Mkumbira *et al.*, 2003a) and possible to combine in more than 10 million genotypes when all alleles are considered and in more than half a million when alleles with frequencies below 0.1 are disregarded, were recorded on all genotyped plants. The DNA techniques were as described in the earlier study. Analysis of molecular variance, AMOVA (Excoffier *et al*., 1992), was used to estimate variance components within and between districts. It showed that only 4.2% of the molecular variation was between districts indicating that they had minor differences in allele frequencies. The statement 'the probability for finding identical genotypes that are not clones is low' made by Mkumbira *et al*. (2003a) is therefore valid even in this study. Plants genotyped in this study and belonging to the same eight-locus SSR genotype are therefore considered to belong to the same clone.

Identification of appreciated clones. To be able to analyse the occurrence of cassava clones that are liked by the farmers, here called 'appreciated' clones, a definition of such clones is needed. Recently introduced clones,

Table 1: Genotyped plants and number of variety names, clones and 'appreciated' clones.

Area	Genotyped plants	Variety names	Clones	Appreciatedclones
Thowolo + Matyenda[1]	290	45	69	29
Chisinga+ Milonde+Nande[2]	76	31	29	20
Nkhata Bay (0utside T+M)	64	37	44	25
Nkhotakota	19	9	9	7
Mangochi	25	6	8	4
Zomba	47	15	20	12
Outside the above areas	102	68	52	26
All Malawi	623	165	178	66

[1]The two blocks in Nkhata Bay that were intensively studied. [2]The three blocks in Mulanje that were most carefully studied.

via cuttings or seedlings, that are represented by some few plants should most probably not be classified as 'appreciated' since they could be poorly evaluated by the farmers. The 18 plants that the farmers classified as belonging to the ten common varieties in Nkhata Bay but that all had unique genotypes (Mkumbira *et al*., 2003a) could be examples of such clones. A clone that is represented on two different farms, on the other hand, is probably evaluated and accepted by the farmers and such a clone is therefore accepted as an 'appreciated' clone in this study. Since, as mentioned above, the AMOVA indicated that the probability is small to find two identical genotypes that are not clones in the populations from Malawi with the markers used in this study. The minimum requirement for an 'appreciated' clone in this study would therefore be fulfilled if plants with the same genotype were found on two different farms.

Plant characters in the GxE study. The GxE interaction in farmers' fields was analysed using the plants genotyped in the earlier study in the two blocks 'Thowolo-B' and 'Matyenda-1'. In that study a total of 246 plants belonging to the ten most grown varieties in the area were collected from 38 fields, and genotyped using SSR markers (Mkumbira *et al.*, 2003a). For each variety there was one dominating clone and the 181 plants belonging to the ten clones were used for this study. After uprooting, two roots per plant were cleaned by hand to remove the soil, and then weighed. The roots were placed in marked paper bags and transported to Mkondezi Agricultural Research Station laboratory. Root dry matter and cyanogenic glucoside content were determined every afternoon for every sample on the day they were collected. In the laboratory, each root was peeled, washed with tap water and split longitudinally with a sharp knife. One half of the root was used for determining the taste (data not used in this study) and the other half used to determine the dry matter and cyanogenic glucoside content (Chiwona-Karltun *et al*., 2004). The gravimetric method was used for dry matter determination, while cyanogenic glucoside levels were determined using the methods of Brimer *et al*. (1997) and Saka *et al*. (1998).

Soil analysis in the GxE study. Soil samples were collected from the top 30-cm at each position where a cassava plant was uprooted and placed in a marked plastic bag. These were transported together with the root samples to Mkondezi Agricultural Research Station laboratory. Soil moisture was determined by the gravimetric method every afternoon on the day the samples were collected. For each sample, 100gm of soil was weighed out and placed in a paper bag and dried in an oven at 110°C until constant weight. The remaining soil samples were later sent to Chitedze Agricultural Research Station for determination of soil pH, organic matter, nitrogen, phosphorus, potassium, calcium and magnesium.

Classification of microenvironments in the GxE study. For each field in which plants were collected the microenvironment was classified for the variables that were considered important for the performance of cassava. The following landforms or physical features of each field and surrounding area as well as operations that farmers had done in their fields at the time of the study were considered:

Anthills. Presence/absence of large termite mounds of soil. The soil is carried and deposited there by the termites while constructing their nest holes underground and could affect an area of up to 100 m^2.

Topography. Topography was narrowed down to upland or lowland. The uplands were areas on relatively high ground while the lowlands or *dambos* were the relatively small depressions or sunken places and valleys in the area with fairly high water table for most part of the year.

River. Presence/absence of fairly shallow streams of fresh water flowing into Lake

Malawi. The course of most of these streams slightly changes over time due to sedimentation of soil particles eroded from the high grounds. The deposited soil could either predominantly be composed of sand particles or be rich alluvial soils. Farmers often use these patches of land to grow some crops.

Road. Presence/absence of open ways providing passage from one place to another. The soils within and around the 'road' often become more compacted. Plants growing by the 'road' may be disturbed or their leaves/ branches may get broken and also more dust particles may settle on their leaves than the plants off the 'road'.

Vegetation. The vegetation around the fields was categorised into two distinct groups: savannah and woodland. The savannah vegetation was characterised by open grassland, usually with scattered trees or shrubs, while the woodland was land mostly covered with woods or dense growth of trees and shrubs.

Land preparation. Traditionally, farmers in this area prefer to grow cassava on mounds, which are small raised mass of soil made at random in the field. The ministry of agriculture, in Malawi, however, has been promoting use of ridges (long narrow raised strips or elevations of soil in ploughed land) as a soil and water conservation measure.

Plant age. A particular feature of cassava is its flexibility in time of harvesting. Farmers, therefore, have the liberty to decide when they can harvest their cassava. A classification into three groups, 6-9, 10-15 and 16-20 months, was used.

Field age. Some fields had been cultivated for more than two years without a fallow break and often grown with the same crop, cassava, while a few were opened and used for the first or second time.

Cropping system. Most often, small-scale farmers plant their crops as mixtures of a number of varieties of different crops (inter-cropping) so as to avoid the risk of crop failure and/or due to limited land for cultivation. Others, however, may grow one or more varieties of a single crop (mono-crop) in each field.

Farm management. Due to one reason or another, farmers fail to weed their fields and this affects the performance of their crops.

Statistical analysis in the GxE analysis. The states of the microenvironments were numerically coded. Regression analysis with stepwise selection was used to analyse the effect of the microenvironments (SAS version 8.2). A moderate significance level of 15% was used at the SLENTRY/SLSTAY= level. Thus the *F* statistic for a variable, that is a microenvironment, to be added to the model must be significant at the SLENTRY = level. After a variable is added, however, the stepwise method looks at all the variables already included in the model and deletes any variable that does not produce an *F* statistic significant at the SLSTAY = level. Plant age was not included in this analysis since it masked the effect of the other microenvironments.

Results

Farmers' breeding activities. The interviews resulted into some insights into the farmers' breeding practices. The discussions showed that the farmers knew a lot about their varieties. In-depth interviews with some farmers showed that they had developed special techniques to store cutting material and evaluate new introductions. They looked for new varieties from other villages but also from distantly located woods where they found a considerable variation among the cassava plants that were growing wild. In the two areas most intensively studied 'Thowolo-

than one plant and only five of the 34 varieties represented by more than two plants belonged to a single clone. The frequency of plants belonging to a varieties dominant clone, excluding the ten varieties studied by Mkumbira *et al*. (2003a) was 53%. Among the ten studied by Mkumbira *et al.* (unpublished) this frequency was 78%.

Farmers also use several variety names for some of the 'appreciated' clones (Table 2) and particular variety names were given to more than one 'appreciated' clone (Table 2). Even within the two blocks 'Thowolo-B' and 'Matyenda-1' in Nkhata Bay, where ten varieties were observed for 18 to 26 plants, four of them were observed to consist of two 'appreciated' clones each, one grown by many farmers, the other by few.

The plants classified as 'gomani' and 'mbundumali' in the Uniform Yield Trial kept at the Research station Makoka belonged to the genotypes 50 and 41, respectively. All 33 plants called 'gomani' or 'gomani mfipa' by the farmers also belonged to the clone with genotype 50 (see Table 2). The 'mbundumali' plants at the research station belonged to the same clone as 25 of the 28 plants called 'mbundumali' by the farmers. This clone was also found among all the plants that were called 'balaka' (4 plants), 'kabuthu' (2) and 'white' (1) and among the majority of the plants called 'mangochi' (3), 'manyokola' (19) and 'mwaya' (9). Thus the 'mbundumali' plant in the trial belonged to the same clone as 63 of the 79 plants that the farmers claimed belonged to any of the varieties in which this clone dominated and it occurred in nine of the ten districts studied (Table 2).

The effect of microenvironment on GxE. The analyses of farmers' fields from which cassava plants were collected showed the following mean and range over microenvironments (in parenthesis) for soil variables: soil moisture content 8.3 (7.2 – 11.4) %, pH 5.4 (5.2 – 5.8), organic matter 2.0 (1.7 – 3.0) ppm, nitrogen 0.10 (0.08 - 0.20) %, phosphorus 32 (9 – 80) ppm, potassium 0.4 (0.3 - 0.6) Cmol(+)/kg, magnesium 0.5 (0.1 – 0.8) Cmol(+)/kg and calcium 1.7 (0.9 – 2.5) Cmol(+)/kg. Substantial differences due to topography were shown for all soil factors except pH.

Table 3 shows the number of plants from the ten clones in each microenvironment state. The number and composition of the microenvironments differ considerably between clones. Microenvironments that were included by the stepwise regression model as having a significant contribution to the observed variation for a root yield component are shown in Table 4. More microenvironments were included for the bitter clones compared to the cool/sweet ones. Results of the analysis that included microenvironments interactions have not been included due to small numbers of observations.

Discussion

Diffusion of clones. This study supports earlier findings of a lack of a one-to-one relationship between the farmers' variety names and a single clone. The percentage of plants belonging to a dominating clone was 78% among the ten varieties studied by Mkumbira *et al*. (2003a) in the two blocks in Nkhata Bay but only 53% in the other parts of Malawi. The higher percentage of plants in the two blocks belonging to dominant clones could be due to a more careful sampling and/ or an effect of a higher dependency of cassava. None of the 14 clones that occurred in more than one district had only one variety name but eight of them had more than three names. Seven names of the clones in Table 2 were used for more than one clone. One explanation for several names for the same clone could be differences in local languages between farming communities both within and between districts. One probable reason for the occurrence of several clones with the same name could be classification mistakes when new varieties are introduced as indicated in Nkhata Bay (Mkumbira *et al.*, 2003a). Marker analysis shows that the conclusions about

Table 2: Variety names[1] of clones that occurred in more than one district.

District	Genotype of the clone													
	17	19	22	36	37	41	43	46	50	81	114	116	156	160
Nkhata Bay			102	16	19 20	23	32		29 38 39 40	10 40				
Kasungu		8				22								
Nkhotakota		?[3]	11 12		20		28		38	11		11		
Salima												51		
Mangochi	7			13 18	21	22 24		36 37				13 46 47	52	
Balaka				13		22								
Machinga				13		22	31					13 45 49 50	53	
Zomba				13	20	23 28 29 30	22 29 31	13 34	38			13	54	55
Blantyre		9		17			29	35						
Mulanje				14 15		25 26 27		33			22 41 42	48		
Thyolo				7		22					43			

[1] A number in the table stands for a variety name

[2] Numbers in bold indicate that the variety name occurs in more than one clone

[3] Unknown.

B' and 'Matyenda-1' in Nkatha Bay, and the blocks Chisinga, Milonde and Nande in Mulanje, there was a difference in the use of 'bitter' varieties. In the latter only 10% of the clones were classified as 'bitter' whereas in Nkhata Bay they were as many as 60%.

Distribution of clones. The 623 collected and genotyped plants belonged to 165 locally named varieties and 178 clones. Sixty-six clones were classified as 'appreciated ' clones but 112 only occurred on a single farm (Table 1). Of the 'appreciated' clones 48 of 66 (73%) occurred in more than one village. Between the adjacent blocks in Nkatha Bay ('Thowolo-B' and 'M atyenda-1'), and in Mulanje (Chisinga, Milonde and Nande), the percentage of clones cultivated in more than one block was 43 and 45, respectively. The distribution of the 14 'appreciated' clones that were grown in more than one district is presented in Table 2. Since there is a risk that the markers used do not discriminate among all clones the number of variety names used for a clone could be overestimated and the number of names used for more than one clone could be underestimated.

Relation between clones and local variety names. There was no one-to-one relationship between clones and local variety names. Thus only 22 of the 56 varieties represented by more

Table 3: Number of plants belonging to any of the ten clones in each micro-environment state.

Micro-environment	State	Clone									
		Ch[1]	Mb	Nc	De	Go	Ko	Ng	Nh	Nm	Nk
Anthill	Present	4	12	8	19	19	10	10	6	18	11
	Absent	9	8	7	8	3	2	4	5	2	5
Topography	Upland	10	21	13	20	18	11	7	11	22	9
	Dambo	3	1	2	8	6	3	7	2	.	7
River	Present	7	6	4	12	8	10	2	4	8	8
	Absent	6	16	11	16	16	4	12	9	14	8
Road	Present	5	12	7	20	10	9	4	5	14	9
	Absent	8	10	8	8	14	5	10	8	8	7
Vegetation	Savannah	13	19	12	26	20	14	11	11	21	16
	Woodland	.	3	3	2	4	.	3	2	1	.
Land preparation	Ridges	11	22	13	24	20	12	14	12	16	14
	Mounds	2	.	2	4	4	2	.	1	6	2
Plant age (months)	6 to 9	7	17	10	17	8	7	6	10	13	5
	10 to 15	3	4	3	5	6	2	4	.	5	6
	16 to 20	3	1	2	6	10	5	4	3	4	5
Field age (years)	1 to 2	.	5	3	5	4	2	2	4	6	.
	3 to 4	13	17	12	23	19	12	11	9	16	15
Cropping system	Mono-crop	12	12	12	19	17	9	10	9	14	11
	Inter-crop	1	8	3	8	4	3	3	2	6	4
Farm management	Clean	10	13	13	22	12	6	7	10	12	3
	Weedy	3	7	2	5	9	6	6	1	8	12

[1]The two letters refers to the varieties in which these clones dominated in the study site Ch=Chimphuno; Mb=Mbundumali= genotype 41; Nc=Nyachikundi; De=Depwete; Go=Gomani= genotype 50; Ko=Koloweki; Ng=Ng'wenyani; Nh=Nyanhalawa; Nm=Nyamakozo; Nk=Nyankhata.

the diffusion of cassava clones based on occurrence of local names (e.g. Fregene *et al.*, 2000; Otim-Nape *et al.*, 2001; Nweke *et al.*, 1994) could be misleading.

Marker analysis also suggests that two clones that have been chosen for multiplication and distribution by the national program in Malawi, around two decades before this study, have been widely diffused among the farmers. These are the clones with the genotypes 41 and 50, that is the clones of the varieties 'mbundumali' and 'gomani' in the Uniform Yield Trial kept at the Research station in Makoka. They were also common under these names in Nkatha Bay district and dominated in the two blocks 'Thowolo-B' and 'Matyenda-1' at the time of two earlier studies (Chiwona-Karltun *et al.*, 2000; Mkumbira *et al.*, 2003a). The sweet 'mbundumali', which means 'one cannot finish eating it' in the languages spoken in the area (Chiwona-Karltun *et al.*, 1998), could have been grown

Table 4: Micro-environments with significant effect on root yield components.

Clone	Cyanogenic glucoside	Root weight	Dry matter
All	Land preparation Farm management Anthill	Land preparation Topography Field age	River Land preparation River
All bitter	Land preparation Farm management Field age River	Topography	Land preparation
All cool	Land preparation River	Topography Road	Topography
Chimphuno	Anthill Land preparation Farm management	Farm management	
Mbundumali	River	Cropping system	Road
Nyachikundi	Land preparation	Road	Vegetation Road
Depwete	Land preparation Field age	Land preparation Cropping system Topography Farm management	River
Gomani	Farm management Cropping system Field age	River	Road Farm management
Koloweki	River	Road Field age	Land preparation
N'gwenyani	Farm management		
Nyahalawa	Road		Anthill
Nyamakozo	Land preparation Field age Cropping system	River Field age	
Nyankhata			Topography Cropping system Farm management

there for more than five decades (Chiwona-Karltun pers. com.). 'Gomani', named after the male introducer (Chiwona-Karltun *et al.*, 1998), could also have been grown at least that long in the country. The clone with genotype 41 was represented by most plants in this study (66). It occurred in nine districts (Table 2) and was the dominant clone in seven varieties. On average 80% of the plants belonging to these varieties belonged to the clone with genotype 41. The study therefore gives evidence that the clone with genotype 41 was widely distributed in a large part of the country, although under different names, and

rarely was mixed up with other clones. However, it is unrealistic to assume that a large fraction of the plants belonging to this clone would be an effect of recent introductions from the national program since the interviews indicate that the variety has been cultivated for decades in many of the villages covered by the study.

It is more probable that the clone, in a majority of the areas, has been multiplied and distributed by the farmers independent of the national program for several decades, an indication that clones can be widely accepted by farmers. Possible reasons for the wide distribution could be its fairly high yields and its relatively wide adaptation, although it does not perform exceptionally well in these respects compared with other local varieties in a nation-wide field test (Mkumbira *et al*., 2003b). Another reason could be its distinct morphology from other clones. Plants belonging to genotype 50, the bitter clone distributed as 'gomani' in the national program, was, with one exception, called 'gomani' or 'gomani-mfipa' by the farmers. Contrary to the clone with genotype 41, this clone only occurred in three districts (Table 2) but, even for this clone, its wide and dominant occurrence is difficult to explain with a recent introduction by the national program. However, in this case it would also be difficult to explain it as an effect of its distinct morphology considering how it has been shown to overlap with other clones (Mkumbira *et al.,* 2003a) or due to its high and stable productivity (Mkumbira *et al.*, 2003b). The farming communities' capacity to maintain 'gomani' as a single clone is therefore difficult to explain but it indicates that when a variety is important to them the farmers find ways to keep it genetically pure. The take-home lesson seems to be that clones already accepted by farmers have a good chance to be widely distributed.

This study also indicates that farmers' plants should be classified into those that belong to clones evaluated and grown for their production value (a production population) and those under test or kept for future needs (a breeding population). Thus the interviews corroborate the findings from earlier studies in the two blocks in Nkhata Bay that farmers keep a high genetic diversity, a breeding population, by maintaining old varieties, testing new introductions and use seedlings (Chiwona-Karltun *et al.*, 1998; Mkumbira *et al*., 2003a). Fregene *et al.* (2003) have also shown that the population structure of locally grown cassava in Africa suggest that the use of seedlings is quite frequent in the development of new material. The observations in the two blocks in Nkhata Bay also imply that the plants belong to many more clones than those found in this study. Thus, as indicated above, 18 plants among those belonging to the ten most commonly grown varieties in the two blocks in Nkhata Bay had unique genotypes, that is belonged to 18 different clones (Mkumbira *et al*., 2003a) Since the total number of plants of these varieties, among the 176 farmers, could be estimated to have been much over 100 000, the total number of clones could be ten fold of that observed, that is several hundred (Table 1). The number of clones that are grown for their production valueon the other hand, is most probably much smaller. Thus, as mentioned above, most of the varieties in these two blocks were recorded and plants from a majority of these were grown by more than one farmer (data not shown). Therefore the number of varieties recorded in these blocks, 45, could be considered the maximum number of clones grown for their production and the number of 'appreciated' clones, 29, a lower limit. Considering that the frequency of plants with dominating clones is higher among the ten common clones in the two blocks (78%) than among the other varieties studied (53%), the fraction of clones used for their production value of the total number of clones would be small even in these areas. Consequently our data suggest that the size of the breeding population, measured in number of clones, is more than five times bigger than the production population.

Untangling non-repeatable GxE. The generally acidic soils, low in almost all soil nutrients and in water holding capacity indicate that the study area, the two blocks 'Thowolo-B' and 'Matyenda-1', was indeed a marginal environment. The results of this study show that microenvironments affect root yield components of cassava clones. However, the effect of each microenvironment varied with clone. Furthermore, the number and composition of microenvironments with a significant contribution varied from one clone to the other (Table 4). This strongly suggests the presence of GxE interaction at a farm level. However, the unbalanced design and the large amount of possible variables analysed did not allow an estimate of the size of effects but only show their significance level.

There were more microenvironments with significant effect on the bitter clones compared to the sweet when analysed as a group. This may be attributed to the difference in total number of observations (sweet, n=50; bitter, n=131). However, genetic difference observed between the 'sweet' and 'bitter' clones may also contribute to this difference (Mkumbira *et al*., 2003b). Mkumbira *et al*. (1996) also observed a similar trend with cyanogenic glucoside levels measured on the same cassava clones grown at seven different environments in Malawi. They reported that cassava clones with low cyanogenic glucoside levels varied less between agro-ecologies compared to those with high levels.

Both agronomic practices and physical features of the land in farmers' fields were important microenvironments causing GxE interaction (Table 4). For the agronomic practices, like land preparation and field management, it may be possible to minimise GxE interaction by uniformity in agronomic practices done in farmers' fields. However, this is practically impossible for small-scale farmers to achieve considering their physical and socio-economic environment. A possible approach to minimise the GxE would therefore be to select for varieties that can buffer variation in management.

All the physical features of the land were included as being important at least in one clone. However, topography is shown to have significant contribution in seven clones for cyanogenic glucoside content. Thus, the contrast between *dambo* and upland may affect a wide range of clones as opposed to other microenvironments.

Conclusions

Using molecular markers to identify clones shows promise. The possibility to find widely appreciated clones in Malawi is certainly much higher when using markers than when only using local names only. By using the marker to identify the clone the plants growing in the farmers fields can be used for finding microenvironments causing GxE on farming communities.

A necessary requirement to identify clones is a discriminative set of molecular markers. In this study eight SSR markers were used, but to be on the safe side a higher amount is recommended. The evaluation of multiplication and distribution of improved varieties would benefit from molecular marker analysis. Sampling schemes for genetic resources of cassava ought to consider that farmers conduct and maintain production and breeding populations.

The analysis of GxE on the farm level is favoured by using communities where the farmers grow many varieties and where the growing conditions vary but also by considering similar representation of each clone in all environments.

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