OUTPUT 8
Development and use of biotechnology tools for cassava improvement

Cassava is a remarkable crop with many advantages over other crops, particularly in relation to stability of performance, capacity to achieve acceptable productions in low fertility soils, and general tolerance to biotic and abiotic stresses. However, the crop also has some clear disadvantages. Among some of the limitations, the low reproductive rate and the length of each cycle of selection slows down the genetic progress achieved and limits the amount of genetic information available for the species. New biotechnology tools can help in overcoming or reducing some of these problems specific to cassava.

Activity 8.1. Marker-Assisted Selection (MAS) for Breeding Resistance to the Cassava Mosaic Diseae (CMD) at CIAT

Collaborators: Dr Alfred Dixon (IITA), Emmanuel Okogbenin, Edgar Barrera, Jaime Marin, Martin Fregene (CIAT)
Funding: The Rockefeller Foundation

Important Outputs

1) *Implementation of MAS for CMD resistance at CIAT*
2) *Proof of concept that the SSR marker NS158 can be used to predict CMD resistance in different crosses for resistance breeding.*
3) *More than 7000 seeds have been obtained for the second phase of the MAS project.*

Rationale

The absence of cassava mosaic disease (CMD), the most important production constraint in Africa and India, limits the usefulness of CIAT cassava germplasm in those areas. With the discovery of a dominant CMD resistance gene, *CMD2*, and 3 molecular markers tightly associated with it, it is now possible to breed for CMD resistance at CIAT. A pilot experiment was set up together with IITA in 2000, as a proof of concept of the utility of molecular markers in CMD resistance breeding. Six crosses, and reciprocals, were made between TME3 and TME9, two cassava land races from Nigeria that carry *CMD2*, and: a susceptible Nigerian land race and 2 elite cassava varieties from IITA, one tolerant and the other susceptible to CMD. We describe here findings of that experiment.

A second phase of molecular breeding for CMD resistance breeding at CIAT has also been initiated. CMD resistant progenies derived from TME3 that were obtained from IITA in 2000 were crossed to elite parents of CIAT's cassava gene pools, and to high carotene or high protein content genotypes. Seeds harvested from the crosses were germinated *in vitro* from embryo axes, to permit sharing of the CMD resistant genotypes with collaborators in Africa and India, in preparation for MAS. These plants will also be the basis of breeding for CMD resistance in CIAT cassava gene pools..

Methodology

The MAS crosses were made in 2000 and a seedling nursery was established at the IITA Mokwa sub-station, a low CMD pressure site in Nigeria, in June 2001. The crosses were harvested December last year and re-established as a clonal observation trial at the IITA headquarters in Ibadan, a high CMD pressure area. Table 8.1 summarizes the families that are in the clonal observation trial. The plants were evaluated at 3, 4 and 6 months after planting for resistance to CMD.

Table 8.1 Crosses from TME3 and TME 9 planted in IITA for to test markers associated with *CMD2* for molecular marker-assisted selection

Family name	Female	Male	Seeds harvested	Plants in field	Total plants in field
M1	TME 3	TME 117	36	18	
M2	TME 117	TME 3	220	95	113
M5	TME 3	91934	103	49	
M6	91934	TME 3	60	12	61
M7	TME 3	30572	70	49	
M8	30572	TME 3	846	791	840
M17	TME 9	TME 117	368	309	
M18	TME 117	TME 9	174	107	416
M21	TME 9	91934	370	282	
M22	91934	TME 9	27	12	294
M23	TME 9	30572	264	214	
M24	30572	TME 9	700	552	766
				Grand Total	2490

DNA was isolated from 1-2g of young leaves and dried for 24h in an oven at 48°C. The dried leaves were ground using a power drill and washed sand. DNA isolation was from 200mg leaf tissue using a miniprep version of the Dellaporta (1983) protocol. Molecular marker analysis was at CIAT. A single dilution, 10X, was employed for all samples. The samples were analyzed with the SSR marker NS158, the closest marker found to date to *CMD2*. PCR analysis and acrylamide gel analyses were carried out as described by Akano et al. 2002. Gel image was captured by scanning and transferred to a Microsoft Excel file for the inclusion of resistance data and interpretation.

A large number of crosses were made between CMD resistant progenies, introduced from IITA to CIAT, and elite parents of CIAT cassava genepools, high beta-carotene varieties and wild *Manihot* accesions and inter-specific hybrids with high protein and dry matter content (Table

8.2). To permit for sharing of this invaluable germplasm with collaborators in India and Sub Saharan Africa as well as keeping a copy here for breeding at CIAT, the seeds are being germinated from embryo axes. Once germinated, the plantlets will be multiplied, molecular-assisted selection (MAS) will be performed using the marker NS158, and CMD resistant genotypes will be shipped to collaborators.

Results

DNA isolation using dried leaves, a power drill and the mini Dellaporta protocol allowed for the processing of 130-150 samples daily. Yield of DNA was between 10-20ug/ 200mg of leaves, which provides enough DNA for more than 200 PCR reactions. This DNA also stores very well and can be used at again at a later time. For more routine MAS work, DNA extraction using the protocol currently used for bean MAS at CIAT will be tested (CIAT 2001).

Molecular marker analysis, using the NS158 SSR marker that is tightly associated to *CMD2*, revealed the marker to be an excellent prediction tool for CMD resistance in some crosses but to a lesser extent in others (Figure 8.1 and Table 8.2). Scrutiny of the data reveals a problem of shared allele sizes in the molecular marker NS158. Examination of allele sizes for marker NS158 in the parental genotypes TME3, TME9, CMD resistant genotypes, and TME117 and TM91924, the susceptible genotypes, reveals they have the same allele size for the allele that is associated with resistance in TME3 and TME9. Although NS158 is tightly linked to *CMD2*, less than 1 cM, it has the same allele size for certain resistant and susceptible genotypes. This could be due to a close relationship of these genotypes and also highlights the need to develop a marker that is truly unique to CMD2. Efforts are underway to clone, by positional cloning, *CMD2* and to develop an allele specific PCR fragment for use as a sequence characterized amplified region (SCAR) marker.

The predominance of genotypes designated as resistant from field evaluation but without the resistant allele, termed as resistant "recombinants", underscores a fundamental problem in field evaluation of disease resistance, i.e uneven pathogen pressure. The crosses employed to test the MAS concept have been in the field for only 6 months and uneven disease pressure can lead to susceptible genotypes being erroneously scored as resistant.

Table 8.2. Summary of the molecular marker analysis of the crosses and recombinants.

Cross	No. genotypes	Recomb. (R)	Recomb. (S)	% total R.
TME3xTME117 (+reciprocals)	110	31	0	28.1
TME3xTMS91934 (+reciprocals)	61	15	0	24.6
TME3x TMS30572 (+reciprocals)	815	40	4	5.3
TME9xTMS91934 (+reciprocals)	223	44	4	22.5
TME9xTMS30572 (+reciprocals)	733	32	3	4.7
TME9xTME117(+reciprocals)	395	93	4	23.5

In an earlier study where a similar set of crosses had been in a field in Uganda for 2 years under heavy disease pressure there was no recombinants (CIAT 2001). This strengthens the case for MAS in CMD resistance breeding.

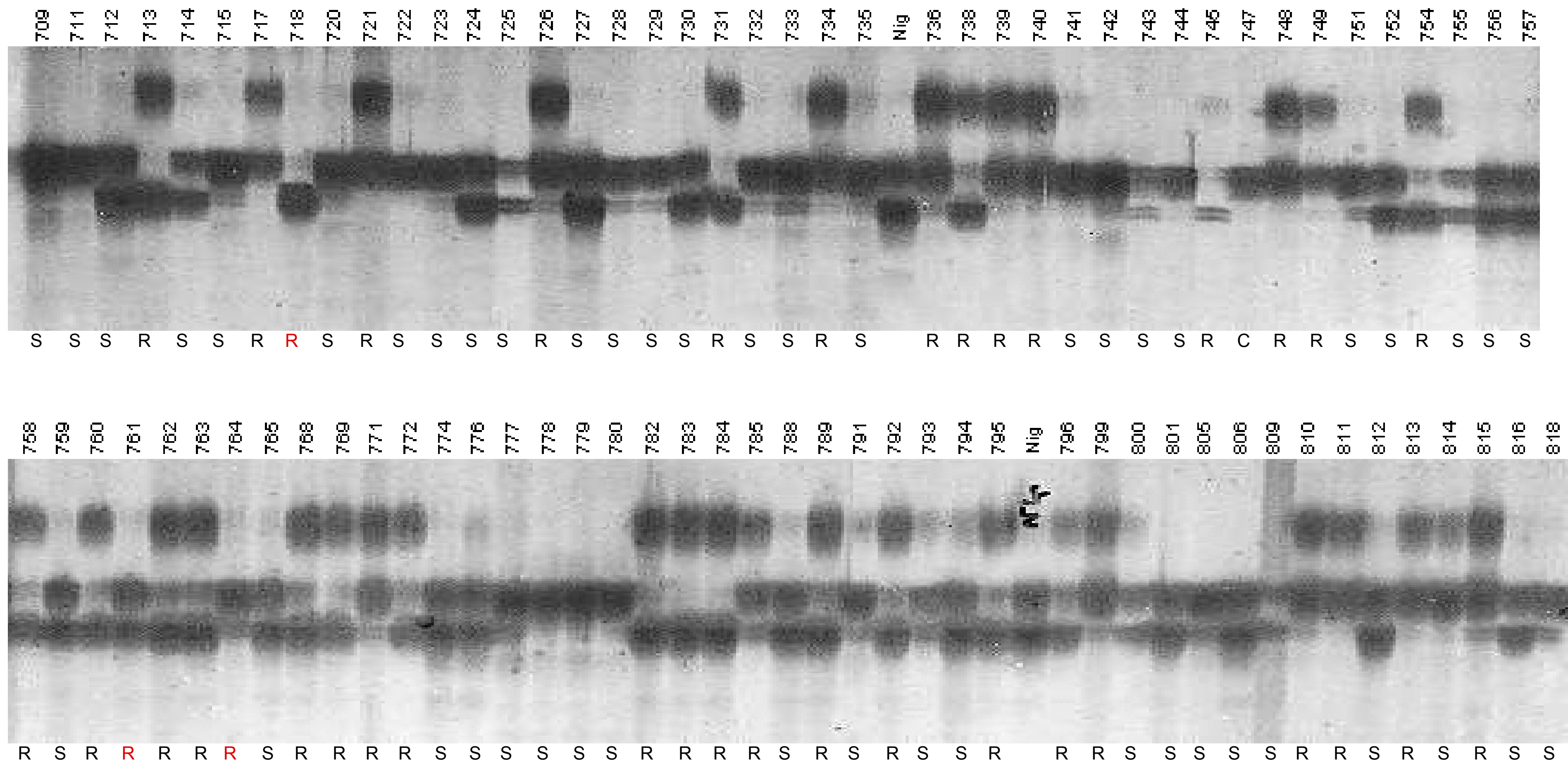

Figure 8.1. Silver stained gel polyacrylamide gel showing PCR analysis of SSR marker NS158 in the cross TME3 x TMS30572 and field resistance data of resistant (R and susceptible (S) genotypes. The topmost alleles are associated with resistance. Three recombinant genotypes can be observed (in red).

More than 7000 seeds were obtained from crosses between the CMD donor parents and elite parents of CIAT's cassava gene pools (Table 8.3). These seeds are being germinated *in vitro* and MAS will be employed to select resistant genotypes for shipment to collaborators in India and Africa.

Table 8.3. List of seeds obtained this year from genetic crosses between CMD donor parents and parents at CIAT for multiple purposes

Mother	Father	Fuente	Purpose 1	Purpose2	No. of Seeds
CMD donor parents x elite parents of agro-ecology zone one (tropical lowlands)					
CG 1141- 1	C 413	GY200122	Z01	ACMD	10
CM 3306- 4	C 4	GY200122	Z01	ACMD	56
CM 3306- 4	C 18	GY200122	Z01	ACMD	11
CM 3306- 4	C 33	GY200122	Z01	ACMD	30
CM 3306- 4	C 39	GY200122	Z01	ACMD	8
CM 3306- 4	C 243	GY200122	Z01	ACMD	5
CM 3306- 4	C 413	GY200122	Z01	ACMD	24
CM 6754- 8	C 33	GY200122	Z01	ACMD	1
SM 1411- 5	C 33	GY200122	Z01	ACMD	1
C 4	MTAI 8	GY200122	ACMD	Z01	470
C 33	CM 3306- 4	GY200122	ACMD	Z01	34
C 33	CM 6754- 8	GY200122	ACMD	Z01	18
C 33	MTAI 8	GY200122	ACMD	Z01	9
C 39	CM 3306- 4	GY200122	ACMD	Z01	19
C 127	MTAI 8	GY200122	ACMD	Z01	54
C 243	MTAI 8	GY200122	ACMD	Z01	8
C 413	MTAI 8	GY200122	ACMD	Z01	6
MTAI 8	C 4	GY200122	Z01	ACMD	28
MTAI 8	C 18	GY200122	Z01	ACMD	6
MTAI 8	C 33	GY200122	Z01	ACMD	32
MTAI 8	C 39	GY200122	Z01	ACMD	35
MTAI 8	C 243	GY200122	Z01	ACMD	44
MTAI 8	C 413	GY200122	Z01	ACMD	33
				SUBTOTAL	**943**

Table 8.3 (cont.)

CMD donor parents x elite parents of agro-ecology zone 2 (acid savannas)						
CM 523- 7	C 4	GY200122	Z02	ACMD		11
CM 523- 7	C 33	GY200122	Z02	ACMD		138
CM 523- 7	C 39	GY200122	Z02	ACMD		79
CM 523- 7	C 243	GY200122	Z02	ACMD		26
CM 4574- 7	C 18	GY200122	Z02	ACMD		1
SM 909- 25	C 4	GY200122	Z02	ACMD		95
SM 909- 25	C 18	GY200122	Z02	ACMD		4
SM 909- 25	C 33	GY200122	Z02	ACMD		58
SM 909- 25	C 39	GY200122	Z02	ACMD		37
SM 909- 25	C 413	GY200122	Z02	ACMD		16
SM 1219- 9	C 243	GY200122	Z02	ACMD		3
C 4	CM 523- 7	GY200122	ACMD	Z02		16
C 4	CM 4574- 7	GY200122	ACMD	Z02		8
C 4	SM 909- 25	GY200122	ACMD	Z02		18
C 4	SM 1219- 9	GY200122	ACMD	Z02		1
C 18	CM 4574- 7	GY200122	ACMD	Z02		2
C 33	CM 523- 7	GY200122	ACMD	Z02		3
C 33	CM 4574- 7	GY200122	ACMD	Z02		31
C 33	SM 909- 25	GY200122	ACMD	Z02		7
C 39	CM 4574- 7	GY200122	ACMD	Z02		9
C 39	SM 1219- 9	GY200122	ACMD	Z02		2
C 243	CM 4574- 7	GY200122	ACMD	Z02		2
C 243	SM 1219- 9	GY200122	ACMD	Z02		26
						596

CMD donor parents x elite parents of agro-ecology zone 4 (mid-altitude Andean)						
CM 7951- 5	C 4	GY200122	Z04	ACMD		44
CM 7951- 5	C 18	GY200122	Z04	ACMD		13
CM 7951- 5	C 33	GY200122	Z04	ACMD		82
CM 7951- 5	C 39	GY200122	Z04	ACMD		27
CM 7951- 5	C 243	GY200122	Z04	ACMD		22
CM 7951- 5	C 413	GY200122	Z04	ACMD		8
SM 1741- 1	C 4	GY200122	Z04	ACMD		61
SM 1741- 1	C 18	GY200122	Z04	ACMD		8
SM 1741- 1	C 33	GY200122	Z04	ACMD		133
SM 1741- 1	C 39	GY200122	Z04	ACMD		28
SM 1741- 1	C 413	GY200122	Z04	ACMD		26
C 4	AM 244- 31	GY200122	ACMD	Z04		6
C 4	SM 1741- 1	GY200122	ACMD	Z04		5
C 4	MCOL 1734	GY200122	ACMD	YRT		135
C 4	MCOL 2206	GY200122	ACMD	YRT		195
C 18	SM 1741- 1	GY200122	ACMD	Z04		2
C 33	SM 1741- 1	GY200122	ACMD	Z04		31
C 39	SM 1741- 1	GY200122	ACMD	Z04		4
C 127	SM 1741- 1	GY200122	ACMD	Z04		6
C 127	MCOL 1734	GY200122	ACMD	YRT		28
C 243	SM 1741- 1	GY200122	ACMD	Z04		5
						869

Table 8.3 (cont.)

CMD donor parents x wild species (high protein content and CGM resistance)					
OW 183- 4	C 127	GY200122	ALW	ACMD	28
C 4	CW 66- 60	GY200122	ACMD	CGM	2
C 4	CW 66- 73	GY200122	ACMD	CGM	12
C 4	CW 67- 42	GY200122	ACMD	CGM	3
C 4	OW 280- 1	GY200122	ACMD	PTN	13
C4	OW230-3	GY200123	ACMD	PTN	159
C4	OW 231- 4	GY200122	ACMD	PTN	183
C 243	OW 280- 1	GY200122	ACMD	PTN	12
					412

CMD donor parents x high beta carotene content varieties					
C 243	MCOL 1734	GY200122	ACMD	YRT	2
C 243	MCOL 2206	GY200122	ACMD	YRT	13
C 18	MCOL 2056	GY200122	ACMD	YRT	4
C 33	MCOL 2056	GY200122	ACMD	YRT	19
C 33	MCOL 2206	GY200122	ACMD	YRT	16
C 127	MCOL 2206	GY200122	ACMD	YRT	47
MBRA 1A	C 18	GY200122	YRT	ACMD	4
MBRA 1A	C 39	GY200122	YRT	ACMD	10
MCOL 1734	C 4	GY200122	YRT	ACMD	54
MCOL 1734	C 18	GY200122	YRT	ACMD	5
MCOL 1734	C 33	GY200122	YRT	ACMD	48
MCOL 1734	C 127	GY200122	YRT	ACMD	37
MCOL 2206	C 4	GY200122	YRT	ACMD	7
MCOL 2206	C 18	GY200122	YRT	ACMD	22
MCOL 2206	C 127	GY200122	YRT	ACMD	8
MMAL 66	C 18	GY200122	YRT	ACMD	30
MTAI 2	C 18	GY200122	YRT	ACMD	13
Total					**338**

Open pollinated seeds of CMD donor parents					
C 4		GY200122	ACMD		4200
C 18		GY200122	ACMD		20
C 127		GY200122	ACMD		100
C 243		GY200122	ACMD		50
			Total:		**4370**
			Grand Total:		**7527**

Future perspectives

1. The need to develop a marker that is truly unique to CMD2 to eliminate confounding effects of alleles from susceptible genotypes having the same size with the allele associated with CMD resistance
2. MAS of the new crosses

References

Akano A., Barrera E., Mba C., Dixon A.G.O., Fregene M. (2002). Genetic Mapping of a Dominant Gene Conferring Resistance to the Cassava Mosaic Disease (CMD). Theoretical and Applied Genetics (published online May 8, 2002).

CIAT, (2001). Annual Report Project SB2, Assessing and Utilizing Agrobiodiversity through Biotechnology, CIAT, Cali, Colombia, pp 239-241.

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Activity 8.2. Development of Expressed Sequence Tags (ESTs) from TME3, the source of CMD2, the Dominant Cassava Mosaic Disease (CMD) Resistance Gene

Collaborators: Dr Ryohei Terauchi, Dr Hideo Masamura (IBRC, Kitakami, Japan), Martin Fregene (CIAT)

Funding: The Rockefeller Foundation.

Important Outputs

1) *Four thousand expressed sequence tags (ESTs) developed from the CMD resistant genotype, TME3.*

b) *Annotation of several hundreds of the ESTs and their use in annotation of SAGE tags differentially expressed in TME3 under heavy disease* pressure.

Rationale

Attempts to clone genes expressed down stream of the single dominant gene, designated *CMD2*, that confers high levels of resistance to the cassava mosaic disease (CMD) by the serial analysis of gene expression (SAGE) has led to identification of many differentially expressed tags – 11bp cDNA sequences. Two methods were employed to annotate the tags obtained, PCR amplification of a cDNA library, using the tag sequence as sense primer and a primer designed from the 3' end of the multiple cloning site of the vector (pYES, Invitrogen Inc.), and ESTs from CMD resistant genotypes. We describe here the generation of 4000 ESTs expressed in a CMD resistant genotype challenged with the virus.

Methodology

A cDNA library was constructed in pYES (Invitrogen Inc.) using mRNA from the CMD resistant bulk. Two microlitre of the cDNA library was electroporated into 40ul of *E.Coli* HB101 cells (Gibco BRL) and plated on LB agar plates + ampicillin (100ug/ml). A total of 5,000 colonies were picked into 70ul of LB media + ampicillin (100ug/ml) in 384 well plates. Plasmid isolation was by the MONTAGE 96-well plate system (Millipore Inc), 4 96-well plates or 384 clones were processed at a time. The 3' end sequencing of the cDNA clones was with

a primer designed from the 3' end of the multiple cloning site of pYES (Invitrogen Inc.) and 5ul of plasmid miniprep. Sequencing PCR reaction was with the big dye terminator kit (Applied Biosystems) on a 9600 Perkin Elmer Machine or an MJ Research DNA engine (Tetrad). The sequence reaction was cleaned using the multi screen 96-well plate format (Millipore Inc.) and analyzed on a Shimadzu RISA 384-capillary sequencing machine. Sequences obtained were cleaned from vector sequences by eye and combined into one single text file using a program written in perl, running on a SunSparc Station (Sun Microsystems Inc.). A program was written in perl to perform batch BLAST (Altschul et. al at 1990) similarity searches for sequence identification using the CIAT local BLAST site (http://gene2/BLAST/inicio.htm).

Results

The 3' end sequencing of about 5000 cDNA clones generated a total of 4000 ESTs. Homology with known genes and proteins deposited in public data bases were sought using the local BLAST (Altschul et. al at 1990) at CIAT, the identity of about 2500 sequences could be ascertained with a good confidence level which corresponds to about 800 unique sequences. Redundancy found in sequences of known functions was about 30%. The ESTs were used for tag annotation and results are summarized Table 8.4. The most abundant tags were easily annotated, for example, identity of the ten genes that make up 5% of all expressed transcripts were found by ESTs, but annotation of less abundant tags is not as efficient. This suggests that the PCR method of tag annotation is a more powerful route to annotating SAGE tag compared to ESTs from regular cDNA libraries. On the other hand ESTs from a normalized cDNA library may be a more efficient means of tag annotation compared to non-normalized libraries. EST data will be be submitted to the Gene Bank.

Future Perspectives

1. Submission of the ESTs to GeneBank.

Table 8.4. Putative identity of SAGE tags annotated by cassava ESTs.

Tag Seq.	Suscep	Resist	Total	Putative identity
CCAGGTTGT	88	72	160	Chlorophyll A-B binding protein type II 1B, chloroplast precursor
CTGCAATGG	58	59	117	NONSPECIFIC LIPID-TRANSFER PROTEIN PRECURSOR (LTP) (ALLERGEN PYR
TTTGGATTC	58	37	95	Chlorophyll A-B binding protein type II 1B, chloroplast precursor
TTTGGGTGC	34	31	65	RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN PRECURSOR
GATTTCATT	29	26	55	Photosystem I reaction center subunit X, chloroplast precursor
ATGATATCA	18	23	41	THIAZOLE BIOSYNTHETIC ENZYME, CHLOROPLAST PRECURSOR.
GATTTGTGT	25	18	43	RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN PRECURSOR
AACTCCTTT	13	18	31	HISTONE H2B
TTCTTGTAT	33	16	49	CHLOROPHYLL A-B BINDING PROTEIN 7 PREC
TTCTGTTGA	24	16	40	Chlorophyll A-B binding protein 151, chloroplast precursor
TAGTCTTAT	18	14	32	PROBABLE NONSPECIFIC LIPID-TRANSFER PROTEIN AKCS9 PRECURSOR (LTP).
GCGTTGGTG	15	12	27	CYTOCHROME C OXIDASE POLYPEPTIDE III
AATGACCTT	1	12	13	TUBULIN BETA CHAIN
CGCCAGACA	3	11	14	ELONGATION FACTOR 1-ALPHA (EF-1-ALPHA).
CATTGTACA	8	11	19	Chlorophyll A-B binding protein 4, chloroplast precursor (LHCII
ATGTGGTCT	6	11	17	GERMIN-LIKE PROTEIN 1 PRECURSOR.
AAGAAGCTC	6	11	17	40S RIBOSOMAL PROTEIN S15A (PPCB8).
CGTAATCAG	30	10	40	PROBABLE NONSPECIFIC LIPID-TRANSFER PROTEIN AKCS9 PRECURSOR (LTP).
CCTGACCTC	23	9	32	Chlorophyll A-B binding protein 151, chloroplast precursor (LHCII
TTAATATGG	1	6	7	CYCLIN A/CDK2-ASSOCIATED PROTEIN P19
TACTTTGTA	13	5	18	Carbonic Anhydrase, Chloroplast Precursor (Carbonate Dehydratase).
GGTGTCTCT	13	5	18	40S RIBOSOMAL PROTEIN S4.
CGATTAAAA	1	5	6	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE (PPIASE) (ROTAMASE)
TTGGATCTT	0	4	4	HYPOTHETICAL 59.9 KD PROTEIN IN SGA1-KTR7 INTERGENIC REGION.
TAGAATCTT	1	4	5	superfamily: myrosinase-associated protein MyAP;
GCACAACAC	8	4	12	CHLOROPHYLL A-B BINDING PROTEIN 4 PREC
AGAACCACT	1	4	5	ELONGATION FACTOR TU, CHLOROPLAST PRECURSOR (EF-TU).
AATTTGATG	1	4	5	SUCCINATE DEHYDROGENASE [UBIQUINONE] I
AAGTGGTGC	0	4	4	60S RIBOSOMAL PROTEIN L17/protein tyrosine phosphatase e
GTGGTGGTA	0	3	3	60S RIBOSOMAL PROTEIN L2 (L8) (RIBOSOMA
GCTTCATTA	0	3	3	UBIQUITIN-CONJUGATING ENZYME VARIANT M...
CCTCAATCC	0	3	3	cholecystokinin B receptor - rat
ATTCCTGAT	0	3	3	putative protein [Arabidopsis thaliana]
AGGGAGGCA	0	3	3	PHOTOSYSTEM II CORE COMPLEX PROTEINS PSBY PRECURSOR (L-ARGININE
AAATTGAAA	0	3	3	unknown [Euphorbia esula]./recA protein A.thaliana

Activity 8.3. Simple Sequence Repeat (SSR) Marker Diversity in Cassava (Manihot esculenta Crantz) Landraces from Nigeria

Collaborators: Dr Alfred Dixon (IITA), Ms Adebola Raji (IITA, and Ph.D. student University of Ibadan, Ibadan, Nigeria), Mr Jaime Marin, Martin Fregene (CIAT)

Funding: IPICs, University of Uppsala, Sweden

Important Outputs

1) *Completion of the Nigerian country study, 270 land races with 31 SSR markers and and observation of high geneic diversity.*
2) *Confirmation of moderate to high genetic differentiation between land races from Nigeria and Guatemala and also observation of secondary structure of genetic diversity in Nigerian collection that may represent heteroic* groups.

Rationale

Nigeria is the world's largest producer of cassava with an annual production of 32 million tons a year (Nweke et al. 2001). This is a more than 200% increase from production twenty years ago. Reasons for the leap in production have been attributed to government policies that favor cassava, population increase and the adoption of improved cassava varieties (Nweke et al. 2001). Nigeria has the highest area in Africa planted to improved varieties, 60%, and cassava is more of an urban staple and cash crop than a food security crop. The impact of the increased commercialization of cassava in Nigeria is expected to lead to an even greater adoption of improved varieties and an erosion of land races and the inevitable loss of diversity. A high level of genetic diversity of cassava has been demonstrated in land races found in traditional Ameri-Indian and African farming communities (Doyle 1997; Fregene et al. 2002), principally a product of the allogamous nature of cassava, agricultural practices, natural and farmer selection. This genetic diversity is an important resource and needs to be collected and preserved for future use. Besides a study of genetic diversity might reveal genetic differentiation amongst accession that might represent heterotic pools. A study to assess the genetic diversity of cassava land races in Nigeria was initiated in July 2000, we describe here completion of the SSR characterization component and present insights gained from the study.

Specific Objectives

a) To study the genetic diversity of cassava land races on a country-wide basis.
b) Assess genetic differentiation between the Nigerian and Latin American accessions, for example accessions from Guatemala.
c) Genetic crosses between Nigerian and Neo tropical land races that are highly differentiated to test for heterotic groups.

Methodology

The original study plan was to characterize by SSR markers the 148 accessions held at the cassava germplasm banks of the National Root Crop Research Institute (NRCRI) and the International Institute of Tropical Agriculture (IITA). The IITA collection was made during the collaborative study of cassava in Africa (COSCA) from 65 villages in the entire country. The lack of passport data for more than half of the NRCRI collection and the incomplete IITA

COSCA collection lead to a decision to conduct a fresh collection in all 65 Nigerian villages surveyed by the COSCA study. The collection was carried out by 3 teams from IITA during the period of May through June 2001. All 65 COSCA villages were visited and an average of 4-5 of the most commonly grown varieties were collected. Farmers were also asked questions on where they got their varieties, disease and pest incidence and end uses. A total of 285 accessions were collected. The names and passport data of the land races collected can be seen at the following URL: http://www.ciat.cgiar.org/Molcas, under Nigeria country study. The collection was planted at IITA, Ibadan and will be maintained there.

DNA was isolated from young leaf tissue harvested from field plants according to a modified miniprep method of Dellaporta et al. (1983) at the biotechnology research unit, IITA, Ibadan. DNA was quantiated by flourimetry and shipped to CIAT for molecular analysis. A student from IITA participated in the molecular analysis as a means of transferring the technology to Nigeria. A set of 36 SSR markers, two from each of the 18 linkage groups of the cassava genome, selected based on their clear banding patterns and robustness across several SSR diversity studies, had been selected earlier, this set of markers was employed in characterizing the land races. PCR amplification, gel electrophoresis, and silver staining were as described earlier for these SSR markers (Fregene et al 2002). A previous study had shown high differentiation between African and Guatemalan land races, a set of 13 land races from Guatemala was therefore included to confirm the earlier observation. SSR allele data captured off the gels using the computer software “Quantity One” (Bio-Rad Inc.) Genetic distance, based upon the proportion of shared alleles (PSA), was obtained using the computer program “microsat” (Minch 1993). The distance matrix obtained was displayed graphically using a principal component analysis (PCA) using the computer program JMP (SAS Institute 1997). Parameters of genetic diversity and differentiation were calculated from allele data using the computer packages GENSURVEY (Vekeman et al 1997) and FSTAT (Goudet 1990).

Results

Data from a total of 31 unlinked SSR loci was available for statistical analysis the other 5 markers had poor overall data quality requiring their elimination. Genetic diversity parameters, including total heterozygosity (Ht) and genetic differentiation (Gst) ranged widely from locus to locus (Table 8.5). The average number of alleles for each locus was roughly four and is similar to that found for a study of land races from Tanzania and 7 Neo-tropical countries (Table 8.6). The probability that 2 randomly selected alleles in a given accession are different, average gene diversity, was 0.5832±0.0482 and it is quite high as also found for the previous study (Fregene et al. 2002). However, average gene diversity was higher for land races from Guatemala (0.62 - 0.650) compared to those from Nigeria 0.49 - 0.57). Cassava land races from the humid and sub humid regions of Nigeria had higher average gene diversity compared to those from the semi-arid region of the country. The results found here buttress earlier findings that agricultural practices of cassava farmers and the allogamous nature of cassava produces a large pool of volunteer seedlings that natural and human selection acts upon to maintain a high level of land race diversity of a clonally propagated crop (Doyle et al. 2001; Fregene et al. 2002).

Unique alleles were found in the Guatemalan accessions and pair-wise comparison of the genetic differentiation estimator F_{ST} revealed moderate to high genetic differentiation between the Nigerian and Guatemalan land races (Table 8.7). Of particular interest are Guatemalan land races from the town El Progresso.

Genetic distances between all pairs of individual accessions was calculated by the 1-proportion of shared alleles (1-PSA) and presented graphically by a principal coordinate analysis (PCA) (Figure 8.2). The PC1 and PC2 accounted for 26% and 16% of the total variance respectively. The PCA clearly separates the accessions from Guatemala from those from Nigeria, but it also reveals a sub-structure in the accessions from the Semi-arid region of Nigeria. The presence of a defined sub-structure in the genetic relationship of cassava land races from Africa has been demonstrated before in Tanzania (Fregene et al. 2002). It is yet to be understood the underlying basis for the sub-structure. These results also agree with a previous AFLP marker study of 29 African and 11 Neo-tropics land races that placed African and Neo-tropics land races in two distinct cluster with a sub structure for the African accessions (Fregene *et al.* 2000).

The differentiation amongst land races from Guatemala and Nigeria observed in a previous study (Fregene et al. 2002) and confirmed here may well represent heterotic pools as have been found for maize (Shull *et al.* 1952). One of the principal reasons for this study was to assess genetic diversity in cassava land races as a first step to delineating heterotic pools for a more systematic improvement of combining ability via recurrent reciprocal selection. Activities ongoing include diallel crosses of representative land races from Nigeria and Guatemala.

Future perspectives

1. Genotype a larger land race collection from Guatemala with the 36 SSR markers
2. Analyze the SSR marker results
3. Genetic crosses between Nigerian and Guatemalan land races.

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Table 8.5. Parameters of Genetic diversity, Ho, Hs, Ht, Dst, Gst and Gst' (correction for differences in sample size) by SSR locus

LocName	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'
SSRY4	0.874	0.729	0.751	0.022	0.027	0.756	0.029	0.036
SSRY12	0.751	0.678	0.691	0.014	0.017	0.695	0.02	0.024
SSRY19	0.538	0.606	0.708	0.102	0.127	0.734	0.144	0.174
SSRY20	0.878	0.812	0.821	0.009	0.011	0.823	0.01	0.013
SSRY21	0.692	0.534	0.555	0.021	0.026	0.56	0.038	0.047
SSRY34	0.328	0.412	0.45	0.039	0.049	0.46	0.086	0.106
SSRY38	0.125	0.152	0.171	0.019	0.024	0.176	0.112	0.136
SSRY51	0.34	0.652	0.655	0.002	0.003	0.655	0.004	0.005
SSRY52	0.61	0.612	0.653	0.041	0.051	0.663	0.062	0.077
SSRY59	0.526	0.646	0.716	0.07	0.088	0.733	0.098	0.119
SSRY61	0.482	0.527	0.548	0.021	0.026	0.553	0.038	0.048
SSRY63	0.264	0.523	0.503	-0.02	-0.025	0.498	-0.04	-0.05
SSRY64	0.621	0.643	0.663	0.02	0.025	0.668	0.03	0.037
SSRY69	0.707	0.645	0.68	0.034	0.043	0.688	0.051	0.062
SSRY82	0.775	0.725	0.779	0.055	0.068	0.793	0.07	0.086
SSRY10	0.823	0.703	0.752	0.049	0.061	0.765	0.065	0.08
SSRY10	0.507	0.472	0.501	0.029	0.036	0.508	0.057	0.071
SSRY10	0.303	0.36	0.373	0.013	0.016	0.376	0.034	0.043
SSRY11	0.284	0.314	0.315	0.001	0.001	0.315	0.003	0.004
SSRY13	0.798	0.585	0.634	0.05	0.062	0.646	0.078	0.096
SSRY14	0.626	0.571	0.634	0.063	0.079	0.65	0.099	0.121
SSRY15	0.837	0.744	0.789	0.045	0.056	0.8	0.057	0.07
SSRY15	0.605	0.551	0.624	0.073	0.091	0.642	0.117	0.142
SSRY16	0.873	0.589	0.711	0.122	0.152	0.741	0.172	0.206
SSRY16	0.5	0.655	0.718	0.063	0.078	0.734	0.087	0.107
SSRY16	0.621	0.496	0.502	0.006	0.008	0.504	0.012	0.015
SSRY17	0.12	0.251	0.325	0.074	0.093	0.343	0.228	0.269
SSRY17	0.793	0.637	0.702	0.065	0.081	0.718	0.092	0.113
SSRY17	0.817	0.767	0.814	0.047	0.059	0.826	0.058	0.072
SSRY18	0.726	0.678	0.75	0.072	0.09	0.768	0.096	0.117
SSRY18	0.807	0.725	0.79	0.065	0.082	0.807	0.083	0.101
Overall	0.598	0.58	0.622	0.041	0.052	0.632	0.067	0.082

Table 8.6. Intra-population and inter-population estimates of genetic diversity parameters of cassava land races from different agro-eclogies of Nigeria and Guatemala

Population	n	#loc.	#l oc_P	PLP	K	K_P	HO_p	HE_p	HEc_p
Nig-Humid	50	31	30	96.8	4.3	4.4	0.5823	0.5683	0.5742
Nig-Semi Arid	111	31	29	93.5	4.2	4.4	0.5517	0.4972	0.4995
Nig-Sub humid	81	31	30	96.8	4.5	4.5	0.576	0.5677	0.5713
GUA-pro	5	31	30	96.8	2.9	3.0	0.678	0.558	0.6212
GUA-otro	6	31	31	100.0	3.5	3.5	0.6044	0.5977	0.6501
Mean :				96.77	3.9	3.97	0.5985	0.5578	0.5832
std				2.28	0.64	0.66	0.0482	0.037	0.0573

n: number of genotypes per sample
#loc: number of SSR loci;
#loc_P: number of polymorphic loci
PLP: percentage pf polymorphic loci
K: average number of allele per locus
K_P: average number of allele per polymorphic loci
Ho: observed heterozygosity
He: Average gene diversity
Hec_p: Average gene heterozygosity corrected for small samples size

Table 8.7. Pair-wise estimates of genetic differentiation estimated by F_{ST} (theta) between cassava land races from the humid, sub humid and semi-arid regions of Nigeria and 2 regions of Guatemala

	Nig.Sub-humid	Nig. Semi Arid	Nig. Humid	Gua-pro	Gua-otro
Nigeria Sub humid	0	0.0715	0.0026	0.1287	0.0843
Nigeria Semi Arid	0.0715	0	0.0511	0.1741	0.1133
Nigeria Humid	0.0026	0.0511	0	0.1288	0.0844
Guatemala-pro	0.1287	0.1741	0.1288	0	0.0047
Guatemala-otro	0.0843	0.1133	0.0844	0.0047	0

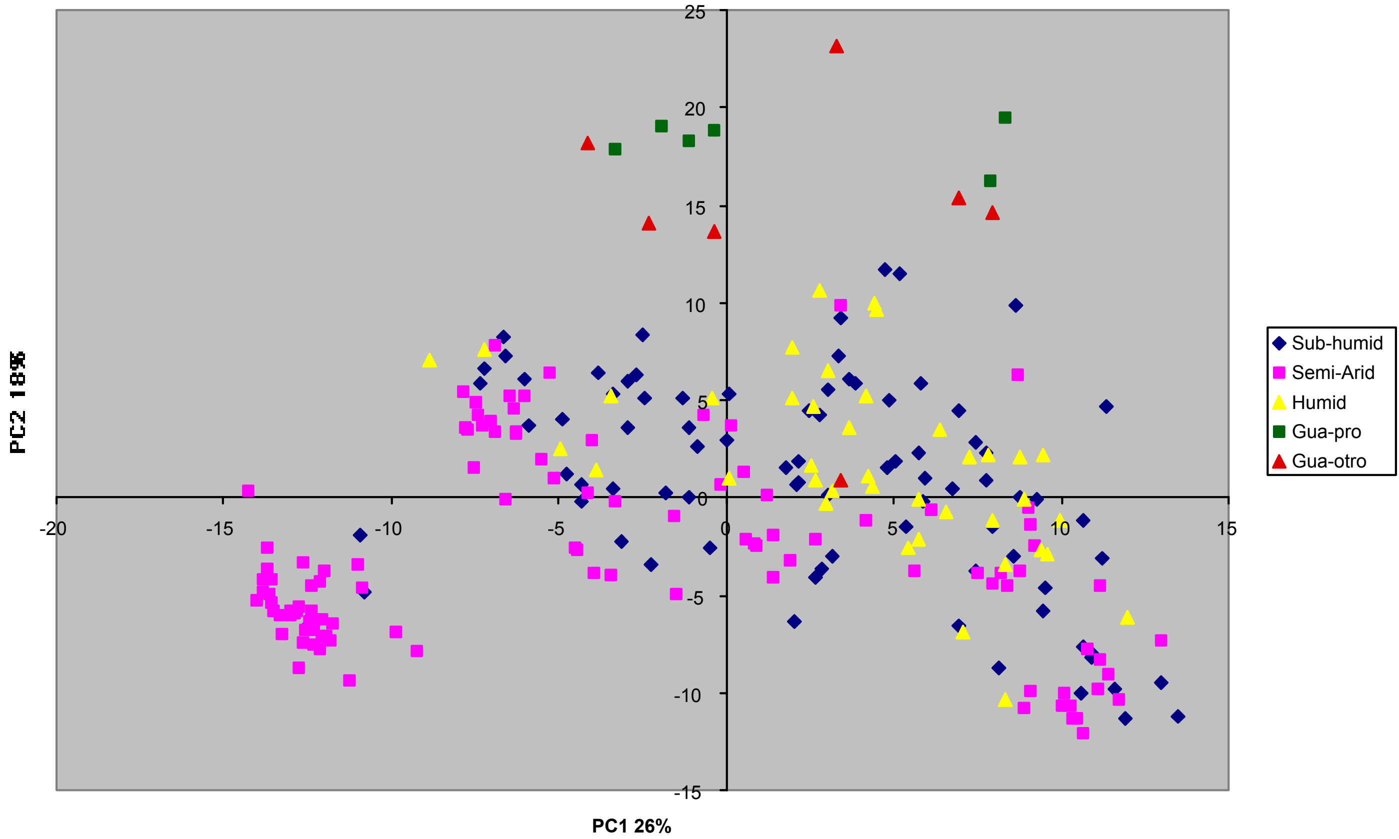

Figure 8.2 PCA of cassava land races from Nigeria and Guatemala based on genetic distances (1-proportion of shared alleles) from 31 SSR markers

Activity 8.4. Simple Sequence Repeat (SSR) Marker Assessment of Genetic Diversity of Cassava Land Races from Guatemala

Collaborators: Dr Cesar Azudia Luis Monte (Facultad de Agronomia, Universidad de San Carlos de Guatemala), Dr Daniel Debouck, Martin Fregene (CIAT)

Funding: IPICs, University of Uppsala, Sweden

Important Outputs

1) *Collection and establishment of 128 cassava accessions from all over Guatemala*
2) *Characterization of the collection with 30 SSR markers to date*

Rationale

Two primary centers of diversity, one in South America and the other in Meso-America have been postulated for the genus *Manihot* (Roger and Appan 1973). Although several studies have demonstrated a likely South American origin for the crop (Allem, 1994; Fregene et.al 1994; Roa et al. 1997; Olsen and Schaal 1999), the diversity of cassava and its wild relatives in Meso- America is great enough to suggest it as a second center. Besides, the potential of Meso-American diversity in cassava improvement has not been properly assessed. Three recent studies of genetic diversity in land races from South America and Meso-America (Chavariagga et. al. 1999; Fregene et. al. 2002; Raji et. al. unpublished data) have revealed unique alleles in land races from Guatemala at a frequency high enough to suggest a Meso American center of cassava diversity. The results of the three studies were based upon 6, 4, and 13 Guatemalan land races. The small sample size of the previous study could distort the allele frequencies and lead to wrong conclusions. A larger collection and SSR characterization of land races from Guatemala was therefore planned to confirm preliminary data of a Meso-American center of diversity and to secure the largely untapped diversity in Guatemala before it becomes extinct. In addition, a selection from the Guatemalan collection will be crossed to CIAT elite parents to evaluate the utility of the Meso-American diversity in cassava breeding.

The present study was to confirm the high genetic differentiation between cassava land races from Guatemala and Nigeria, Brazil, and Colombia. If the uniqueness of the Guatemalan germplasm is confirmed, genetic crosses to CIAT's elite breeding lines will be made to test hybrid vigor and delineate heterotic pools. Plant materials are a collection of cassava from all over Guatemala and a representative group used in previous studies from Nigeria, Colombia and Brazil to confirm earlier results. It is hoped that results of the uniqueness and the utility of the Guatemalan germplasm will give collection and conservation of this germplasm in regions of Meso-America high priority (Azurdia and Gomez 2002)

Methodology

A collection of cassava land races was carried out all over Guatemala in May this year (Azurdia and Gomez 2002). A total of 128 accessions were collected in the departments of Baja Verapaz, Quiche, Huehuetenango, Alta Verapaz, San Marcos, Escuintla y Santa in Guatemala (Figure 8.3). For comparison with results of previous studies, DNA from 6, 11 and 12 cassava land races from Nigeria, Colombia y Brazil respectively were included. DNA from the Guatemalan accessions was isolated at the Facultad de Agronomia, Universidad de

San Carlos de Guatemala using a micro-prep protocol of the Dellarporta (1983) methodology and transferred to CIAT. DNA from the other accessions was obtained from previous studies at CIAT.

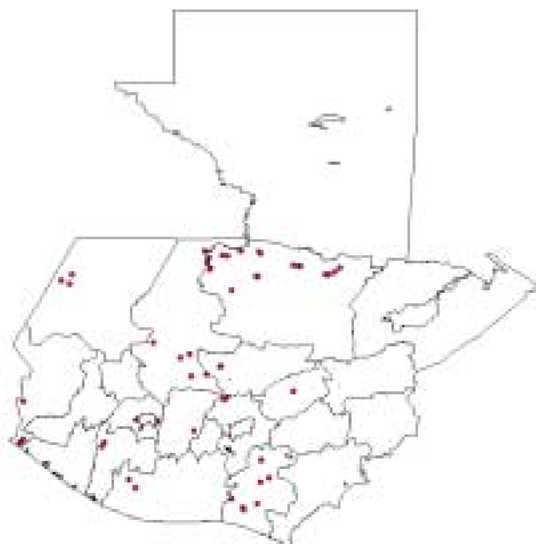

Figure 8.3. Collection sites of cassava germplasm in Guatemala May 2002.

The concentration and quality of DNA samples was accessed by flourimetry and agarose gel electrophoresis respectively. The DNA samples were diluted to10 ng/ml for subsequent PCR analysis. A set of 36 SSR markers, carefully chosen to represent a broad coverage of the cassava genome with moderate to high polymorphism information content (PIC) and robust amplification, were used in this study. SSR diversity studies PCR amplification, polyacrylamide gel electrophoresis, and silver staining were as described earlier for these SSR markers (Fregene et al 2002).

Results

A total of 30 SSR markers have been analyzed to date in the Guatemalan germplasm. Results so far reveal a number of unique alleles in the Guatemalan accessions not found in those from other regions (Figure 8.4). The allele data was captured using the program "Quantity One" (Bio-Rad Inc) and entered directly into EXCEL (Microsoft Inc) in preparation for statistical analysis (Figure 8.5). Statistical analysis to be carried out include: principal component analysis (PCA) of a distance matrix based upon 1-proportion of shared alleles, and parameters of genetic diversity and differentiation as described in Fregene et al (2002).

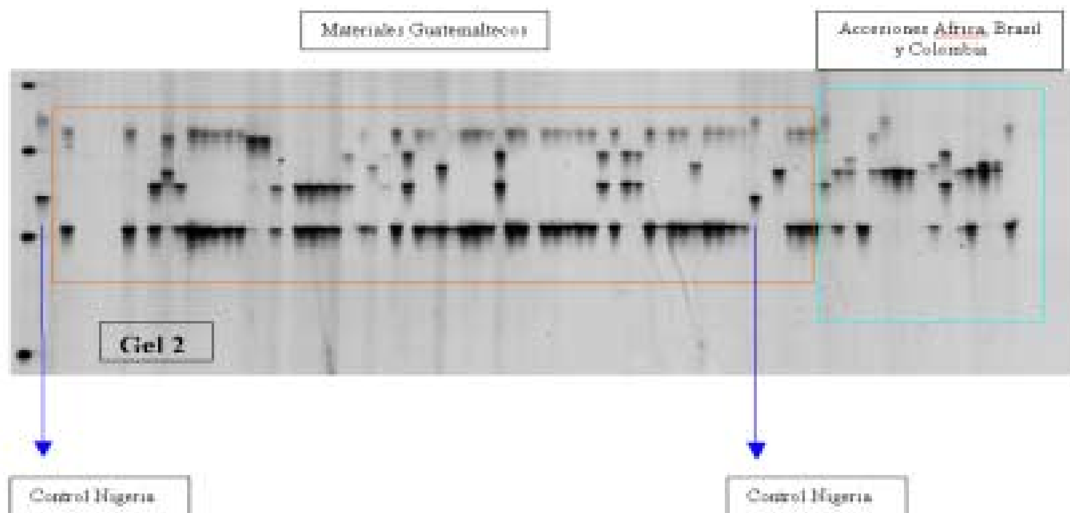

Figure 8.4. Silver-stained polyacrylamide gel of PCR amplification of cassava accessions from Guatemala, Nigeria, Colombia and Brazil with primers of SSR marker SSRY20. A unique allele can be observed in the Guatemalan accessions with a high frequency.

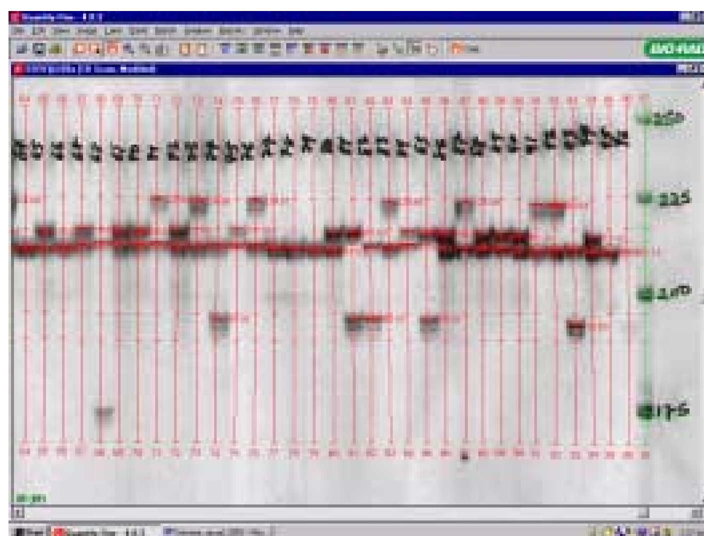

Figure 8.5 . Determination of SSR allele sizes on silver-stained polyacrylamide gels using the software "Quantity One" (Biorad)

Future Perspectives

1. Statistical analysis of the SSR data to estimate genetic diversity and differentiation
2. Genetic crosses between representative accessions from Guatemala and elite cassava parents at CIAT.

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Activity 8.5. Report on the Molecular Characterization of Ghanaian cassava (Manihot esculenta Crantz) Land races and Predictability of Heterosis.

Collaborators: Elizabeth Okai, Dr John Otoo (Crop Research Institute, CRI, Kumasi, Ghana) Martin Fregene (CIAT) Dr Alfred Dixon (IITA)

Funding: IPICs, University of Uppsala, Sweden

Important Outputs

1. Collection of more than 300 cassava land races from 62 villages in the major cassava growing regions of Ghana.
2. Establishment of the collection at the University of Legon and IITA Head quarters, Ibadan.

Rationale

Cassava is an important food crop in developing countries where it is the fourth source of calories, after rice, sugarcane and maize, for more than 400 million people (El-Sharkawy, 1993). Africa is now the largest producer of cassava with a production of 90million metric tones in 1999(FAO 2000). It is cultivated mainly for its storage roots, which provide 390-400 calories/10g dry matter. The leaves when consumed as vegetable provide 7g protein per 100

g edible portion (IITA 1991). The collaborative study of cassava in Africa (COSCA) revealed that cassava serves as a family food staple, a famine reserve crop, and a cash crop.

Cassava was introduced from Brazil, its country of origin, to the tropical areas of Africa, the Far East and the Caribbean Islands by the Portuguese during the 16th and 17th centuries (Jones, 1959). In Ghana, the then Gold Coast, the Portuguese grew the crop around their trading ports, forts and castles. It was a principal food eaten by both the Portuguese and the slaves. By the second half of the 18th century, cassava had become the most widely grown and used crop of the people of the coastal plains (Adams 1957)

The spread of cassava from the coast into the hinterlands was very slow. It reached Ashanti region, Brong Ahafo and the northern Ghana, mainly around Tamale in the 1930.Until the early 1980s,the Akans of the forest belt preferred plantain and cocoyams and sorghum and millet in the north. Cassava became firmly established in most areas after the serious drought of 1982/83 when all other crops failed completely (Korang-Amoakoh,Cudjoe and Adams 1987).

Cassava ranks first in the area under cultivation and utilization. Cassava contributes 22% of the agricultural gross domestic product AGDP compared to 5% for maize, 2% for rice, and 14% for cocoa (Al-Hassan, 1989;Dapaah, 1996). According to the Ghana Living Standards Survey (GLSS), for 1.73 million sampled households 83% were found engaged in cassava production. The spread of cassava into the upper west and upper east of Ghana is an indication of growing trend in cassava production through area expansion(MOA, 1990).

In the traditional bush-fallow system, some cassava plants are allowed to grow during the fallow period, which is long enough to allow cassava to flower and set seeds. The usual out-crossing habit of cassava leads to the production of numerous heterozygous gene pools, which create phenotypic diversity and new hybrid combinations from self-sown seed from which farmers select and propagate desirable types. This process creates pools of new land races, which are adapted to the different agro-ecological zones of Ghana. Coupled with this are the several names that farmers give to cassava as they distribute among themselves. Several land races have been found with the same name and morphological characteristics yet genetically different and the same land races could have different names in several places (Fregene et al., 2000). Doku (1969) recorded 30 such named local varieties in 1930 and by 1960 the number had increased to over 90. Selection for desirable traits has been done by farmers over 1000s of years. Hence the landraces possess higher frequencies of genes required for adaptation to biotic and abiotic stresses, food quality characteristics than unadapted materials. Vegetative propagation also leads to the accumulation of pest and diseases and good varieties susceptible to these biotic stresses disappear. These factors lead to a fairly high turnover of varieties and has implications for gene pool structure of cassava in any center of diversity. Selection is one of the principal factors at work in cassava's gene differentiation in Africa. Evidence for genetic drift has not been demonstrated given that cassava is vegetatively propagated crop, however the use of spontaneous sexual seeds by farmers has been documented (Fregene et al. 2002). High heterosis for yield components, starch, and number of roots have been observed in cassava, and hence considered a promising method of genetic improvement (Easwari Amma and Sheela, 1996). Heterotis groups identified in maize in the early 20th century (Shull et al 1952) have been the basis of a very successful hybrid seed industry.

Specific Objectives

a) The objective for this study is to assess the genetic diversity in Ghanaian landraces

b) To detect heterotic patterns in the collection and between the Ghanaian collection and land races from other countries and regions.

c) To generate hybrids between the Ghanaian land races and genotypes from putative heterotic groups and select together with farmers superior hybrids from the crosses.

Methodology

In January 2002 a collection of cassava land races from all the agro ecological zones in Ghana was done. A total of 45 villages visited during the collaborative study on cassava in Africa (COSCA) were visited. Another 28 villages, important for cassava production, were also visited. Farmers were assembled and asked to share information on cassava varieties grown by them, characteristics of their varieties, and reasons for keeping them. Farmers volunteered to give mature cassava stems, which were labeled.

Fresh young leaf samples of the accessions were collected on ice and used for DNA extraction. An amount of 0.1g of the fresh young leaf was ground in liquid nitrogen and the DNA extracted using the Qiagen kit. The extraction was carried out in IITA, Ibadan, Nigeria. The DNA was carried in absolute ethanol to CIAT. DNA quantification was done using the fluorometer. The DNAs were diluted to 10ng/ul and used for SSR reactions. A sub-set of 36 SSR markers, two from each of the 18 linkage groups of the cassava genome, was employed to obtain an estimate of genetic diversity and differentiation in the land races. PCR amplification, gel electrophoresis, and silver staining was as described earlier (Fregene et al 2002). An internal control of 10 genotypes was included to permit comparison between this study and other ones. The PAGE gels containing SSR data will be scanned and allele sizes determined using the computer software "Quantity One" (Bio-Rad Inc.) based upon an internal gel molecular marker size standard. Genetic distance, based upon the proportion of shared alleles (PSA), will be obtained from the raw allele size data using the computer program "microsat"of Eric Minch (http://www.lotka.stanford.edu/microsat.html). Distances between the accessions will be subjected to principal component analysis (PCA) using JMP (SAS Institute 1995) to obtain a structure of relationship between the land races. Parameters of genetic diversity and differentiation will be calculated from allele data using the computer packages GENSURVEY (Vekeman et al 1997) and FSTAT (Goudet 1990).

Results

A total of 320 landraces were collected including 18 genotypes with yellow roots. Farmers who responded were predominantly women. Among the land races were very early bulking ones 3-9 months after planting. The various local names given suggest a lot of useful traits farmers had associated with the cultivars. Cassava hard wood stems were cut to 20-30cm sizes and planted in plastic pots in a nursery. These were sent to the field after 4weeks and planted in an irrigated field at the Ashiaman office of the Ghana Irrigation Authority. A copy of the collection was packaged and sent to IITA. Accessions were planted in single rows at 1m x 1m spacing with improved varieties as checks.

To date, seventeen out of a set of thirty-six primers used routinely for SSR characterization of cassava genetic diversity have been analyzed. The rest of the analysis is on going. Once the SSR marker analysis is completed, genetic distance and estimates of genetic diversity and differentiation will be calculated. A principal component analysis (PCA) will also be carried

out to graphically display the genetic distance matrix. Based upon clusterings obtained above, genotyopes representative of the clusters will be selected as parents for a diallel experiment to search for heterotic patterns.

Future Perspectives

1. Complete SSR marker analysis of the entire collection
2. Obtain estimates of genetic diversity and differentiation from the SSR data
3. Test heterotic patterns present within the collection or between the collection and others.

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Activity 8.6. A Web-Based Data Base of Simple Sequence Repeat Characterization of Genetic Diversity of Cassava Land Races.

Collaborators: Charles Buitrago, Fernando Rojas, Danny Mauricio Montero, Martin Fregene (CIAT)
Funding: IPICs, University of Uppsala, Sweden

Important Output

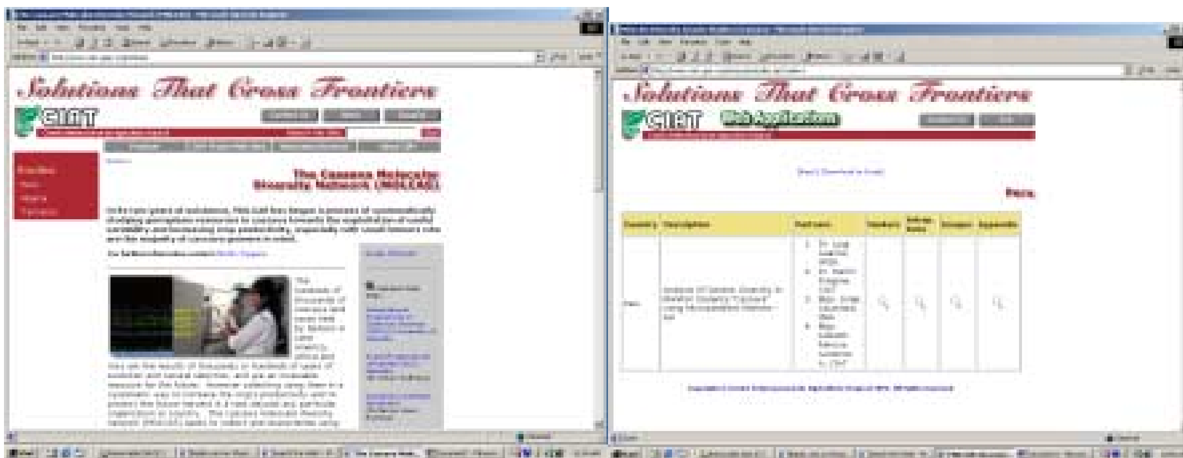
1. A web-based data base to share results of SSR diversity studies of cassava with the cassava research community.

Rationale

One of the objectives of the cassava molecular diversity network (MOLCAS) is to make available to cassava researchers everywhere results of the molecular characterization of cassava genetic diversity. With the completion of the Peruvian, Nigerian and Tanzanian study, a web-based database was constructed in Oracle to accommodate the results of the above and other studies. Data available for viewing include passport data of the accession, raw SSR gel data, allele sizes, SSR locus information, parameters of genetic diversity and differentiation and principal component analysis (PCA) of genetic distances. The database will be updated as other country studies become available.

Results

The database is organized according to country studies, the Peruvian, Tanzanian and Nigerian country studies are the available ones at the moment. The first page of each country study has links to raw marker data namely, allele sizes per accession and genome location of the marker, where available (Fig 1). Other links are intra-population estimates of genetic diversity, gel images of individual markers and appendixes of additional information from the country study (Figure 8.6). The URL for the database is: http://www.ciat.cgiar.org/Molcas. Since the inception of the database in June, average monthly hits for the first three months averaged 2500 hits per month (Table 8.8). The high number of visits to the site confirms the importance of the MOLCAS database for the cassava community.



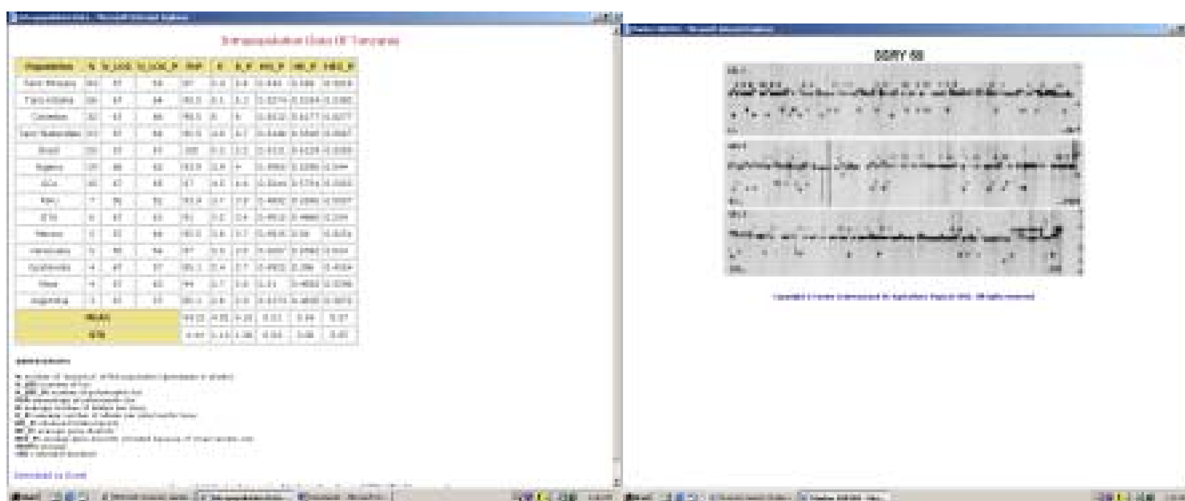


Figure 8.6. An illustration of the different pages of the SSR diversity data-base on MOLACS web site **http://www.ciat.cgiar.org/molcas**.

Table 8.8. Monthly hits at the MOLCAS web site **http://www.ciat.cgiar.org/molcas** for June, July and August and the total for the 3 months.

Request	Aug 2002	Jul 2002	Jun 2002	Total Hits
/molcas/imagen.jsp	1,033	723	816	2,572
/molcas/locus.jsp	982	743	713	2,438
/molcas/alelosp.jsp	556	84	486	1,131
/molcas/	123	114	94	350
/molcas/markers-det.jsp	110	45	63	220
/molcas/estudios.jsp	96	93	0	196
/molcas/studies.jsp	35	16	67	119
/molcas/intrap_data2.jsp	55	37	22	114
/molcas/pcr_cond.jsp	50	22	20	93
/molcas/imagenbioquim.jsp	50	25	0	75
/molcas/appendix1.jsp	44	19	0	65
/molcas/appendix2.jsp	45	10	0	55
/webapps/molcas/	4	2	46	52
Total	**3,183**	**1933**	**2327**	**7,480**

Future Perspectives

1. Include results of other country studies as they become available
2. Seek for a way to unite data sets from diverse studies.

Activity 8.7. Mining the Primary and Secondary Gene Pool: Protein and Dry Matter Yield Genes from Wild Manihot Species

Collaborators: Nelson Morantes, Teresa Sanchez, Martin Fregene (CIAT)
Funding: CIAT core funds

Important Outputs

1. Second year evaluation of wild and inter-specific accessions for the AB-QTL scheme
2. Identification of an inter-specific hybrid with a putative yield of 114t/ha

Rationale

The advanced back cross QTL (AB-QTL) identification and introgression of favorable alleles of gene for high protein and dry matter content, pest resistance and starch quality in cassava is in its second year. During the first year more than 1,200 accessions of wild species and inter-specific hybrids representing 7 wild *Manihot* species were evaluated for the above traits and genotypes with high protein and dry matter content, excellent resistance to white flies and very high amylopectin content were identified (CIAT 2001). The best genotypes were selected for second year evaluation of six plants (clonal observation or single row trial, SRT) and also planted in the hybridization block for genetic crosses to elite CIAT parents. Genetic crosses between the selected wild accessions and CIAT elite parents will provide F_1 families to initiate the AB-QTL scheme. This year we report on the SRT trial, 6 plants as against 1 last year, of the wild accessions and also on genetic crosses made.

A major problem this year was the high incidence of frog skin disease (FSD) in the clonal observation trial, more than 70%, which has lowered considerably dry matter content and affected protein content in an unknown way. Some genotypes turned out with an exceptionally high amount of protein, while others showed a significant reduction compared to last year's result. FSD infected materials that showed high values for the above traits have been re-planted in Santander the Quilichao pending a virus clean-up of these genotypes by tissue culture and thermotherapy. Nevertheless, seeds obtained from crosses to the selected wild accessions are supposedly virus free and have been planted in the seedling nursery for transfer to the field and trait evaluation at harvest.

Methodology

Accessions of inter-specific hybrids and wild *Manihot* species with high protein and dry matter content, good resistance to white flies, and high amylopectin content starch identified from last year's evaluation were established at CIAT Palmira this year as a clonal observation trial. A selection index program developed by the cassava breeding unit (CIAT annual report 2000) was used to select the best 12 genotypes for protein content, dry matter content, and white fly resistance and the best 4 genotypes low amylose content, a total of 145 wild accessions and 343 inter-specific hybrids for genetic crosses. Due to very poor germination of a majority of the wild species planted directly in the fields from woody stakes, a principal problem with wild species, it was necessary to plant these accessions again in bags in the green house. The stakes were treated with growth hormones to aid germination and transferred to the field after 2 months in the green house. For some materials, the problems with poor germination continued and open pollinated seeds obtained last year from these

genotypes had to be planted to obtain information on the trait of interest.

At 10 months after planting, all six plants were harvested and measured for fresh root yield, dry matter content, foliage weight, harvest index, culinary quality, starch content/quality, and frog skin disease according to standard CIAT procedures. Dried roots from genotypes that had high protein content last year were sent for total protein determination at the CIAT analytical service lab. Seeds from the genetic crosses were also collected and germinated in a seedling nursery in preparation for transfer to the field. Due to the lateness in field establishment some of the wild accessions, particularly the high protein content genotypes, mature seeds could not be harvested before the end of the hybridization season, the immature seeds were therefore harvested and germinated from embryo axes (see activity 6 for more details).

Results

The second year evaluation of inter-specific hybrids and wild accessions selected for high protein and dry matter content, and other traits of interest confirmed the stability of the trait value across years (Table 8.9). However, the experiment was greatly inhibited by the difficulty of establishing the wild accessions from woody stakes.

Table 8.9. Crude protein percentage of dry root scored over 2 years in wild *Manihot* accessions with high protein content. Data from 2002 is based on 6 plants.

Genotype	Mother	%Protein 2001	%Protein 2002	FSD
OW 284- 1	TST XXX- 77	7.00		Infected
OW 131- 2	TST XXX- 2	7.13	9.48	Infected
OW 134- 1	TST XXX- 8	7.26		Infected
OW 136- 3	TST XXX- 13	8.22		Infected
OW 230- 2	FLA 441- 5	9.20		Infected
OW230-3	FLA 441- 5	10.50	9.63	
OW230-4	FLA 441- 5	10.34	6.99	
OW 230- 5	FLA 441- 5	9.14		Infected
OW 231- 3	FLA 444- 7	7.16	5.24	Infected
OW 231- 4	FLA 444- 7	11.00	7.69	Infected
OW 231- 6	FLA 444- 7	8.27		
OW 280- 1	TST XXX- 51	7.24	7.28	Infected
OW181-2	FLA 423- 6	5.89		Infected
OW132-2	TST XXX- 3	11.71	9.48	

Note: Missing values are due to no storage roots, a effect of severe FSD infection

The inter-specific hybrids fared much better, an observation worthy of mention is a genotype, CW67-30, from an inter-specific hybrid family between cassava and its progenitor, *M. esculenta* sub species *flabellifolia* that had a fresh root yield yield of 114 t/ha or 39t/ha dry matter yield (Table 8.10). The genotype CW67-30 showed very vigorous growth and profuse production of foliage, it has been planted again with more replications to examine if the extraordinary yield will be repeated. Also planted again are the above best 25 inter-specific hybrids.

A large number of crosses were made between wild accessions and inter-specific hybrids having high protein and dry matter content, waxy starch, and pest resistance and more than 2000 seeds in total were obtained (Table 8.11). Good size populations exist for the different traits and for the identification of F_1 genotypes for the AB-QTL scheme. The seeds have been planted at CIAT Palmira and they will be evaluated for the relevant traits at 9-10 months after planting.

Table 8.10. Dry matter yield and yield components of the best 25 inter-specific hybrids from 4 families having the same *M. esculenta* sub spp *fabellifolia* accession as male parent.

Clone	Mother	Father	Harvest index	% Dry matter	Yield t/ha	Dry matter yield (t/ha)	Starch	Taste
CW 67-30	MFLA 437- 007(3)	MCOL 2215	0.46	34.08	114.42	39.00	1.00	4.00
CW 66-10	MFLA 437- 007(3)	CM 2766- 5	0.33	38.40	49.00	18.82	2.00	2.00
CW 67-33	MFLA 437- 007(3)	MCOL 2215	0.36	36.73	44.92	16.50	5.00	5.00
CW 65- 75	MFLA 437- 007(6)	CG 501-16	0.50	23.66	61.60	14.58	1.00	4.00
CW 67-24	MFLA 437- 007(3)	MCOL 2215	0.50	33.76	40.92	13.81	2.00	5.00
CW 67-116	MFLA 437- 007(6)	MCOL 2215	0.49	32.57	40.58	13.22	3.00	5.00
CW 66-28	MFLA 437- 007(3)	CM 2766- 5	0.45	34.62	36.00	12.46	3.00	3.00
CW 67- 40	MFLA 437- 007(6)	MCOL 2215	0.43	31.71	36.83	11.68	3.00	3.00
CW 66-76	MFLA 437- 007(3)	CM 2766- 5	0.36	35.40	29.83	10.56	1.00	4.00
CW 67-124	MFLA 437- 007(6)	MCOL 2215	0.62	33.48	28.00	9.38	2.00	5.00
CW 67-55	MFLA 437- 007(6)	MCOL 2215	0.27	36.31	25.75	9.35	1.00	2.00
CW 67-152	MFLA 437- 007(6)	MCOL 2215	0.51	36.00	25.83	9.30	2.00	3.00
CW 66-61	MFLA 437- 007(3)	CM 2766- 5	0.28	35.37	26.17	9.25	3.00	5.00
CW 67-136	MFLA 437- 007(6)	MCOL 2215	0.25	34.71	26.40	9.16	3.00	4.00
CW 67-18	MFLA 437- 007(3)	MCOL 2215	0.24	34.90	26.20	9.14	1.00	3.00
CW 67-77	MFLA 437- 007(6)	MCOL 2215	0.21	32.14	26.33	8.46	4.00	5.00
CW 67-98	MFLA 437- 007(6)	MCOL 2215	0.35	29.70	27.17	8.07	3.00	5.00
CW 64-7	MFLA 437- 007(6)	CG 487-2	0.22	29.70	27.17	8.07	3.00	5.00
CW 65-79	MFLA 437- 007(6)	CG 501-16	0.20	33.72	23.92	8.06	1.00	4.00
CW 66- 35	MFLA 437- 007(3)	CM 2766- 5	0.31	35.87	21.50	7.71	1.00	3.00
CW 67-129	MFLA 437- 007(6)	MCOL 2215	0.71	35.87	21.33	7.65	3.00	5.00
CW 67-121	MFLA 437- 007(6)	MCOL 2215	0.30	28.22	25.25	7.12	3.00	3.00
CW 66-21	MFLA 437- 007(3)	CM 2766- 5	0.24	33.64	21.00	7.06	3.00	3.00
CW 67-44	MFLA 437- 007(6)	MCOL 2215	0.40	31.13	22.60	7.04	1.00	4.00
CW 67-126	MFLA 437- 007(6)	MCOL 2215	0.62	31.13	22.00	6.85	3.00	5.00
CW 66-49	MFLA 437- 007(3)	CM 2766– 5	0.43	20.03	27.00	5.41	3.00	4.00

Table 8.10 (cont.)

	Statistics of best 25 inter-specific hybrids evaluated								
Maximum				0.71	38.40	114.42	39.00	1.00	3.00
Minimum				0.20	20.03	21.00	5.41	5.00	5.00
Average				0.39	32.80	33.76	11.07	2.35	3.96
Standard Dev.				0.14	4.06	19.16	6.54	1.09	1.00
	Statistics of 343 inter-specific hybrids evaluated								
Maximum				0.71	55.88	114.20	39.00	1.00	5.00
Minimum				0.01	16.30	5.80	1.19	5.00	2.00
Average				0.16	27.59	9.66	2.70	2.80	4.10
Standard Dev.				0.12	7.17	9.90	3.46	1.37	1.00

Table 8.11. Summary of sexual seeds obtained from crosses between inter-specific hybrids and wild *Manihot* accessions high in protein, dry matter content, and waxy starch.

	Family	Mother	Father	Purpose of cross		No. of seeds
	Protein					
175	CW 179	OW 132- 2	MTAI 8	PTN	Z01	21
178	CW 185	OW 180- 1	MTAI 8	PTN	Z01	9
186	CW 204	OW 231- 3	AM 244- 31	PTN		14
187	CW 205	OW 231- 3	MTAI 8	PTN	Z01	11
188	CW 206	OW 280- 1	AM 244- 31	PTN		64
189	CW 207	OW 280- 1	MTAI 8	PTN	Z01	291
190	CW 207	MTAI 8	OW 280- 1	Z01	PTN	16
						426
		Protein and yellow roots				
221	CW 73	CM 1585- 13	OW 284- 1	YRT	PT-MS	15
222	CW 177	OW 132- 2	CM 1585- 13	PTN	YRT	128
223	CW 184	OW 180- 1	MCOL 1734	PTN	YRT	13
224	CW 186	OW 181- 2	CM 1585- 13	PTN	YRT	13
225	CW 188	OW 181- 2	MCOL 1734	PTN	YRT	23
226	CW 207	OW 280- 1	CM 1585- 13	PTN	YRT	215
227	CW 212	OW 284- 1	MCOL 1734	PT-MS	YRT	13
228	CW 251	MCOL 1734	OW 189- 1	YRT	PT-MS	18
229	CW 256	MCOL 1734	OW 280- 1	YRT	PTN	13
						451
	Dry matter and yellow roots					
21	CW 69	CM 1585- 13	CW 30- 29	YRT	DMC	6
22	CW 69	CW 30- 29	CM 1585- 13	DMC	YRT	6
23	CW 70	CM 1585- 13	OW 234- 2	YRT	DMC	58
24	CW 71	CM 1585- 13	OW 240- 8	YRT	DMC	19
25	CW 72	CM 1585- 13	OW 280- 2	YRT	DMC	1
26	CW 100	CW 30- 29	MCOL 1734	DMC	YRT	3
27	CW 100	MCOL 1734	CW 30- 29	YRT	DMC	4
28	CW 101	CW 30- 31	CM 1585- 13	DMC	YRT	9
29	CW 115	CW 30- 73	CM 1585- 13	DMC	YRT	1
30	CW 127	CW 30- 73	MCOL 1734	DMC	YRT	1
31	CW 127	MCOL 1734	CW 30- 73	YRT	DMC	46
32	CW 147	CW 47- 3	MCOL 1734	DMC	YRT	3
33	CW 147	MCOL 1734	CW 47- 3	YRT	DMC	11

Table 8.11 (cont.)

34	CW 153	CW 48- 1	MCOL 1734	DMC	YRT	54
35	CW 154	MCOL 1734	CW 48- 1	YRT	DMC	66
36	CW 155	CW 56- 5	CM 1585- 13	DMC	YRT	23
37	CW 166	CW 56- 5	MCOL 1734	DMC	YRT	38
38	CW 181	OW 146- 1	MCOL 1734	DMC	YRT	11
39	CW 211	OW 280- 2	MCOL 1734	DMC	YRT	11
40	CW 249	MCOL 1734	CW 28- 38	YRT	DMC	34
41	CW 250	MCOL 1734	CW 30- 65	YRT	DMC	44
42	CW 252	MCOL 1734	OW 234- 2	YRT	DMC	16
43	CW 253	MCOL 1734	OW 240- 6	YRT	DMC	113
44	CW 254	MCOL 1734	OW 240- 8	YRT	DMC	16
45	CW 255	MCOL 1734	OW 269- 4	YRT	DMC	71
						665
	Dry matter					
46	CW 82	CM 7951- 5	OW 240- 8	Z02	DMC	22
66	CW 111	CW 30- 31	MTAI 8	DMC	Z01	16
82	CW 128	CW 30- 73	MTAI 8	DMC	Z01	4
90	CW 137	CW 30- 87	MTAI 8	DMC	Z01	4
107	CW 167	CW 56- 5	MTAI 8	DMC	Z01	168
108	CW 169	CW 60- 7	SM 1036- 8	DMC		5
109	CW 170	CW 60- 7	MTAI 8	DMC	Z01	27
112	CW 180	OW 146- 1	AM 244- 31	DMC		70
118	CW 197	OW 213- 4	MTAI 8	DMC	Z01	71
121	CW 216	SM 1219- 9	CW 48- 1	Z02	DMC	11
122	CW 221	SM 1460- 1	CW 30- 65	Z02	DMC	3
123	CW 222	SM 1460- 1	CW 48- 1	Z02	DMC	19
124	CW 228	SM 1460- 1	OW 240- 8	Z01	DMC	6
125	CW 262	MTAI 8	OW 234- 2	Z01	DMC	83
126	CW 263	MTAI 8	OW 240- 6	Z01	DMC	4
127	CW 264	MTAI 8	OW 240- 8	Z01	DMC	33
						546
	Waxy starch					
2	CW 143	CW 39- 2	MTAI 8	ALW	Z01	5
5	CW 191	OW 183- 4	MCOL 1734	ALW	YRT	1
						6
Grand total						**2094**

Notes: PTN-protein; ZO-agro-ecological zone; YRT-yellow root; DMC/MS- dry matter content

Future Perspectives

1. Evaluate the inter-specific hybrids generated for the target traits
2. Continue making crosses of

References

CIAT, (2000). Annual Report Project SB2, Assessing and Utilizing Agrobiodiversity through Biotechnology, CIAT, Cali, Colombia, pp 239-241.

8.8 Mining the Primary and Secondary Gene Pool: Resistance Genes for Green Mites from Manihot esculenta sub species flabellifolia

Collaborators: Nelson Morantes, Jose Maria Guerrero, Anthony Bellotti, Martin Fregene (CIAT)
Funding: CIAT core funds

Important Outputs

1. Discovery of a very high level of resistance to CGM in inter-specific hybrids of *M. esculenta* sup spp *flabellifolia*
2 A putative marker associated with a high level of resistance to the cassava green mite

Rationale

The cassava green mites (*Mononychellus tenajoa*) is a biotic stress of cassava that becomes prominent during periods of prolonged dry periods. In East Africa, overlapping outbreaks of CMD and CGM during the dry season tend to result in very heavy losses and a severe reduction in farm profits (Legg et al. 1998). January this year at CIAT Palmira was particularly dry and, not surprisingly, a very heavy incidence of mites was recorded on the station. The CIAT cassava entomology group conducted a thorough evaluation of cassava plants in the field and while most plants had damage ratings of 4 on the CIAT scale of 1-5, where 1 is no symptoms, and 5 is severe leaf damage and stunted growth, 4 inter-specific hybrid families from the wild *Manihot* accession MFLA 437- 007 showed an almost equal number of susceptible(score of 3-4) and resistant (score of 1-2) genotypes.

The very high level of resistance found in the inter-specific hybrids and the almost equal number of susceptible and resistant genotypes suggests a simple mode of inheritance of the resistance gene(s), which makes deployment of this source of mite resistance very attractive. Bulk segregant anlysis (BSA) using 500 SSR markers was quickly used to identify molecular markers associated with resistance. At the same time, highly resistant hybrids were crossed to CMD resistant parents, to combine CMD and CGM resistance for Africa, and also to elite cassava parents at CIAT.

Methodology

A clonal observation trial of 6 plants per genotype of inter-specific hybrids between the cassava varieties CG487-2, CG501-16, MCol2215, and CM2766-5 and the *M. esculenta* sup spp *flabellifolia* accession MFLA 437- 007, designated CW68, CW65, CW67, and CW66 respectively were planted at CIAT Palmira August last year. They were evaluated for resistance to mites during a very heavy mite infestation January this year. A high level of resistance was observed in about half of the inter-scpefic hybrids. It was thought desirable to transfer this high level of resistance to elite parents of the cassava gene pools. Genetic crosses where therefore made to elite parents of the cassava gene pool.

Following the interesting distribution of resistant genotypes observed in the families, the family CW67 was chosen for bulk segregant analysis of CGM resistance. Ten resistant and ten susceptible genotypes were used for molecular analysis. DNA was isolated from 1-2g of young leaves and dried for 24h in an oven at 48°C. The dried leaves were ground using a

power drill and washed sand and DNA was isolated from 200mg leaf tissue using a miniprep version of the Dellaporta (1983) protocol. DNA from the cassava parent Mcol2215 was also isolated for inclusion in the analysis. The wild parent no longer exists it was eliminated from the field in 2000 during an eradication of the wild *Manihot bank*, many of which were contaminated with frog skin disease, the crosses were made in 1995. DNA from the bulks and parent was genotyped with the 500 available cassava SSR markers. Markers that were polymorphic in the bulks were analyzed in individual genotypes that make up the bulks.

Results

Resistance response to CGM in the families CW65, 66, 67 and 68 was qualitative, i.e., all 6 plants of resistant genotypes showed no visible symptom, while all plants of susceptible genotypes were always heavily infected. The percentage of resistant to susceptible plants was about the same (Figure 8.12). A chi square of the ratio of resistant to susceptible plants was not significantly different from a 1:1 ratio at a probability level of 0.05 for CW65, 66, and 68. This fits the expected segregation ratio for a single dominant gene heterozygous in the wild accession. BSA revealed an allele of the SSR marker, SSRY330, is present in the resistant parent and in the resistant bulk but absent in the susceptible bulk and the susceptible parent (Figure 8.13).

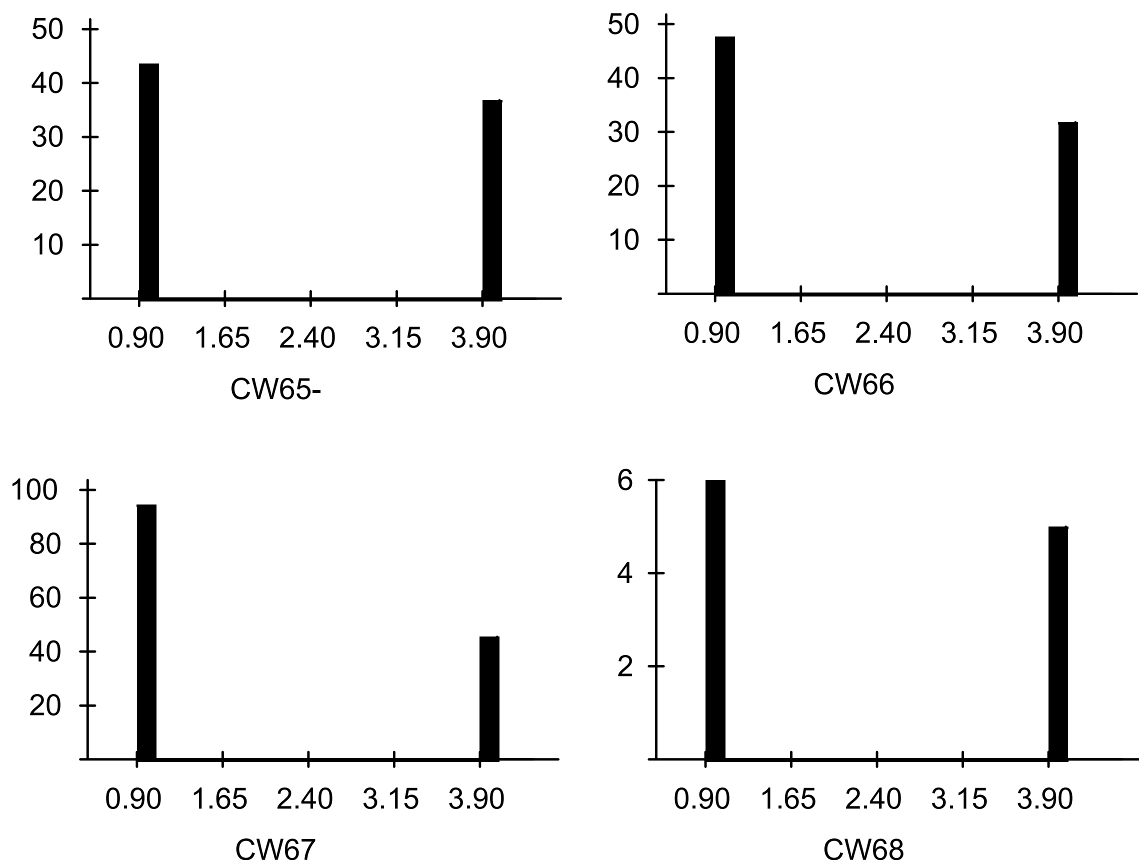


Figure 8.12. Distribution of response to CGM in 4 inter-specific hybrids derived from a single accession of *M. esculenta* sup spp *flabellifolia* MFLA 437- 007 as father.

The polymorphism was confirmed when the individuals of the bulks were screened with the SSR marker although 3 and 1 recombinant could be observed in the resistant and susceptible bulk respectively (Figure 8.13). The SSR marker is currently being analyzed in all individuals of CW67, as well as those of the other three families.

Genetic crosses have been made between inter-specific hybrids having high CGM resistance and elite parents of CIAT gene pools, a total of 832 seeds were obtained (Table 8.7). This cross can be loosely described as a back cross and it is the second step of the AB-QTL scheme. Seeds obtained have been planted at CIAT Palmira and they will be evaluated for the relevant traits at 9-10 months after planting.

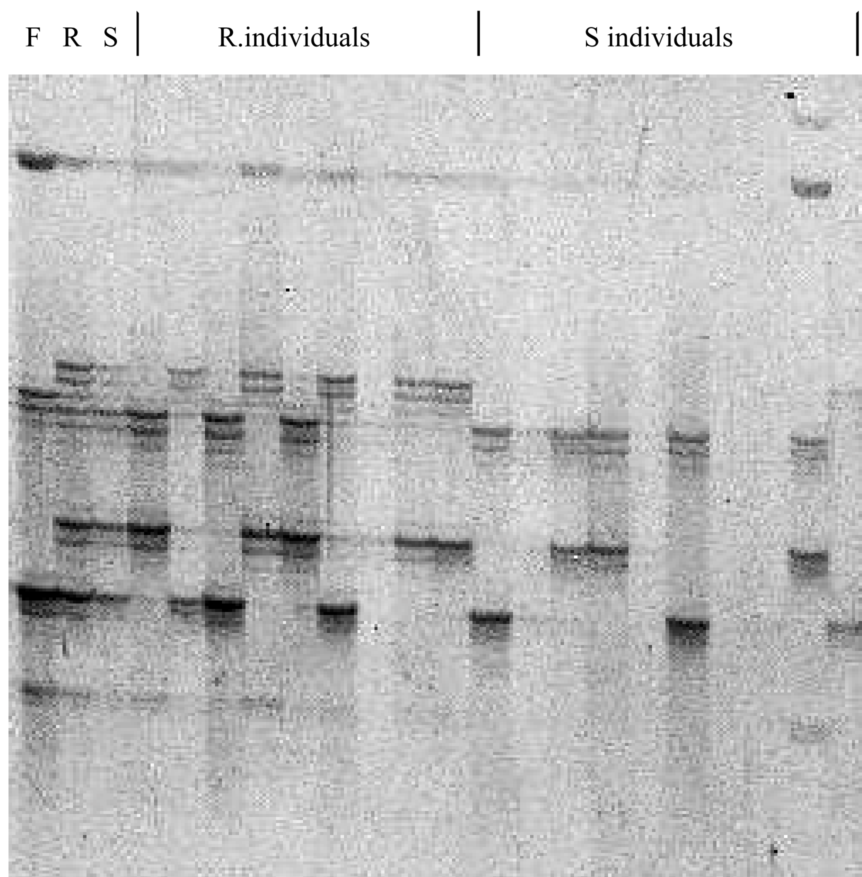


Figure 8.13. Silver stained polycrylamide gel of bulk segregant analysis (BSA) of CGM resistance in the CW67 family, the topmost fragment segregates with resistance. Three and one recombinants respectively can be observed in the CGM resistant and susceptible varieties. F stands for father, the male parent (MFLA 437- 007), while R and S stands for the resistant and susceptible bulk respectively.

Future Perspectives

1. Analyze the marker SSRY330 in the entire individuals of all 4 families namely, CW65, CW66, CW67, and CW68.
2. Second year evaluation of the inter-specific hybrids in Santander the Quilichao.

Table 8.7. Seeds obtained from crosses between the inter-specific hybrids with high levels of resistance to CGM and elite parents of cassava gene pools at CIAT.

Family	Mother	Father	No. of seeds
CW 75	CM 3306- 4	CW 66- 60	8
CW 76	CM 3306- 4	CW 68- 3	12
CW 77	CM 7951- 5	CW 65- 77	7
CW 78	CM 7951- 5	CW 66- 19	4
CW 79	CM 7951- 5	CW 66- 62	2
CW 80	CM 7951- 5	CW 67- 42	5
CW 81	CM 7951- 5	CW 67- 98	4
CW 213	SM 805- 15	CW 67- 39	1
CW 214	SM 805- 15	CW 67- 87	11
CW 215	SM 909- 25	CW 66- 60	10
CW 217	SM 1219- 9	CW 65- 77	23
CW 218	SM 1219- 9	CW 66- 73	24
CW 219	SM 1219- 9	CW 66- 74	4
CW 220	SM 1219- 9	CW 67- 123	15
CW 223	SM 1460- 1	CW 66- 19	18
CW 224	SM 1460- 1	CW 66- 60	22
CW 225	SM 1460- 1	CW 66- 62	42
CW 226	SM 1460- 1	CW 66- 73	16
CW 227	SM 1460- 1	CW 68- 3	3
CW 229	SM 1511- 6	CW 67- 87	35
CW 230	SM 1565- 15	CW 66- 19	5
CW 231	SM 1565- 15	CW 66- 60	27
CW 232	SM 1665- 2	CW 66- 19	31
CW 233	SM 1665- 2	CW 66- 60	4
CW 234	SM 1665- 2	CW 66- 74	49
CW 235	SM 1665- 2	CW 67- 87	129
CW 236	SM 1669- 5	CW 66- 19	58
CW 237	SM 1669- 5	CW 66- 60	12
CW 238	SM 1669- 5	CW 66- 62	2
CW 239	SM 1669- 5	CW 66- 73	7
CW 240	SM 1669- 5	CW 66- 74	36
CW 242	SM 1669- 7	CW 67- 87	13
CW 241	SM 1669- 5	CW 67- 123	8
CW 243	SM 1741- 1	CW 66- 19	9
CW 244	SM 1741- 1	CW 66- 60	18
CW 245	SM 1741- 1	CW 66- 62	3
CW 246	SM 1741- 1	CW 67- 91	12
CW 247	SM 1778- 45	CW 66- 19	4
CW 248	SM 1778- 45	CW 67- 45	4
CW 257	MTAI 8	CW 65- 77	33
CW 258	MTAI 8	CW 66- 60	31
CW 259	MTAI 8	CW 66- 73	59
CW 260	MTAI 8	CW 66- 74	6
CW 261	MTAI 8	CW 67- 123	5
			832

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