Mining the Primary Gene Pool of Cassava: Introgression of Resistance to the Cassava Green Mite and High Root Protein from Accessions of *Manihot esculenta* sub spp *fabellifolia* and *Manihot tristis* into Cassava

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INTRODUCTION

A very high level of resistance to the green mites was observed in four inter-specific hybrid families, CW65, CW66, CW67 and CW68, from the wild *Manihot* accession MFLA 437-007 with an almost equal number of susceptible and resistant genotypes. Bulk segregant analysis (BSA) was used to identify 4 SSR markers NS74, NS217, NS260 and SSRY330 polymorphic in the bulks and individuals of the inter-specific families resistant to green mites (CIAT 2002). An attempt was made to transfer the resistance observed in the inter-specific families into elite cassava parents. A total of 45 BC_1 families were developed. Selected individuals of these BC_1 families were crossed extensively with CMD resistant parents used for MAS at CIAT, to combine CMD and CGM resistance in progenitors meant for África. The resulting BC_2 families were screened for resistance to CMD and CGM using molecular markers to select parents for breeding.

Cassava can serve as a cheap means of deploying adequate protein requirement amongst the poor and for feeding animals. A major effort has therefore been embarked upon to increase protein content of cassava roots. The advanced back cross QTL (ABC-QTL) has been initiated to introgress high protein content from wild relatives into cassava. Inter-specific hybridization were made between selected high protein lines and some improved elite parents, including some yellow varieties. Wild by wild crosses were also carried out to investigate if combining favorable alleles from different populations or species of the wild accessions can further increase protein content. In addition many cassava varieties from Central America were found to be high in protein from an evaluation conducted in 2001, they were re-established in the field from tissue culture plants for another round of evaluations. If the previous results are confirmed, genetic crosses will be made with elite parents of the cassava gene pools for breeding high protein content and QTL mapping studies.

MATERIALS AND METHODS

The SSR markers NS74, NS217, NS260 and SSRY330 polymorphic in the bulks segregant analysis were evaluated in the 4 F_1 inter-specific families CW65, CW66, CW67 and CW68 (Fig. 1) and a simple regression analysis conducted using Microsoft Excel. The markers found to explain a significant part of phenotypic variance of CGM resistance in the analysis of the 4 F_1 families were then analyzed in the parents of the 45 BC_1 families. The BC_1 families had earlier been evaluated for resistance to green mites. Based upon the CGM resistance evaluation of the 45 BC_1 families a large number of putatively resistant BC_1 progenies were crossed to CMD resistant parent for the generation of BC_2 families from which CMD and CGM resistant lines can be selected by marker-assisted selection for the generation of parents for breeding in African gene pools.

Fig. 1 Crosses of susceptible and highly resistance plants to the cassava green mites.

For protein content, total protein was measured in root flour obtained from a bulked sample of 3 root per plant from F_1 inter-specific hybrids in a seedling trial, using the Kjedahl method. In collaboration with the starch company AVEBE, amino acid profile was obtained from root flour from 1 genotype each of *M.esculenta* sub spp *flabellifolia* and *M. tristis* high in protein, as well as from 2 inter-specific hybrids high in protein. Pooled leaf flour sample from 10 cassava varieties with high protein was also analyzed.

Several protein extraction protocols were evaluated for SDS-PAGE analysis of root protein in cassava in order to select the most suitable one (Table 1). About100mg of root flour was used in each case, suspended in a 500µl volume of sample buffer. The samples were centrifuged in an eppendorf tube for 5 min at 14000 rpm and the supernatant was transferred to a new eppendorf tube and mixed in a 1:1 (v:v) ratio with SDS-PAGE disruption buffer (Laemmli, 1970). Proteins were completely dissociated by immersing the samples in a boiling water bath for 5 min, then briefly centrifuging at 14000 rpm to pellet cellular debris. The resulting supernatants (total protein extracts) were stored at –20°C. Protein were analysed on 10% SDS-PAGE, acording to Laemmli (1970). and stained with coomassie brillant blue R250.

Table 1. Diferent extraction buffers for isolation of proteins in cassava root 3

Buffer	Reference
1. 1M Tris HCl pH: 7.5, 0.5M EDTA, 1% Ascorbic acid, 0.1M PMS	Gutierrez 2003,Personal communication
1ª. 1M Tris-HCl pH: 7.5, 0.5M EDTA, 1% Ascorbic add, 0.1M PMS and boiling for 5 min.	
2. 0.005M Sodium phosphate, sucrose 5% 0.005M Sodium phosphate, sucrose 5%	Suiter, 1988
3. 0.1M KCl, 20 mM cystein, pH 7.3	Bourdon, 198
4. 0.0625 M Tris-HCl, 2% SDS, 10% Glycerol, 5% (ß-mercapthoethanol, 0.001 bromophenol	Laemmli, 1970
5. 50mM Sodium phosphate 5ª. 50mM Sodium Phosphate, 10mM PMSF, 10% PVP	Shewry, 1992 Shewry, 1992
6. 0.5M NaCl, pH 3.2, 2mM EDTA, 2% SDS, 40% Sucrose, 1% (ß-mercapthoethanol, 0.01% Bromophenol blue in 0.0625M Tris HCl pH 6.8	CIAT, Gutierrez 2003, Personal communication

RESULTS AND DISCUSSION

Simple regression analysis of SSR markers NS 74, NS 217, NS260 and SSR 330 in the 4 inter-specific families had coefficients (R2) of 46%, 30%, 30% and 5% in the 4 families respectively. The regression coefficients are suprisingly low for resistance that is apparently controlled by a major gene (CIAT 2002). BSA analysis of the BC_1 also revealed similar low regression coefficinents calling into question the proximity to the gene of the markers, all the markers belong to the same region of the genome. An effort has therefore been initiated to identify more markers linked to CGM resistance using additional SSR and RAPD markers and the bulk segregant analysis. Several promising RAPD and SSR markers were identified(Fig 2). Individuals of the BC_1 families that possess resistance to CGM were crossed to CMD resistant parent to obtain recombinats that carry CMD and CGM resistance. These crosses have been established *in vitro* from embryo axes to enable sharing with collaborators in Africa and also evaluated with markers for CMD and CGM resistance to select for resistant phenotypes

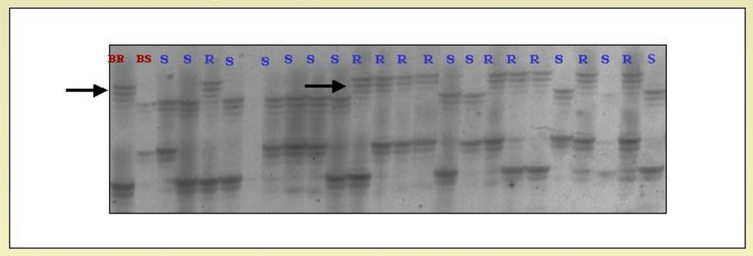

Fig. 2. Molecular marker associated with resistance to CGM

A total of 4,271 sexual seeds organized into 58 families were obtained from inter-specific crosses between high protein accessions of *M. esculenta* sub spp *flabellifolia*, *M. tristis* and elite parents of the cassava gene pool. An evaluation of root protein content was made of 579 genotyopes , based on root size. Results reveal that some wild genotypes such as OW 231-3, 280-2, OW132-2, and OW284-1 have good general combing ability for root protein content. It was also observed that crosses between 2 wild parents, both high in protein (WW), had more uniform high root protein progenies, compared to wild by cultivated crosses, suggesting that a number of genes for protein content might be recessive or additive. The inter-specific hybrids are being evaluated for a second year in a single row trial (SRT) experiment, simultaneously BC_1 families are being produced from putative high protein lines. Extraction buffers number 3, and 5 gave the best quality protein extract. The success of SDS-PAGE relies upon selection of the buffer which in turns depends of the characteristics of the tissue. The ratio of buffer to tissue needs to be optimized in materials with low content of proteins like cassava root flour. Efforts continue to standardize the SDS-PAGE protocol for characterization of root proteins in cassava.

Table 1. List of cassava varieties with high protein content in an evaluation conducted in 2001 that are to be re-evaluated again this year.

Clone and protein (%)		Clone and protein (%)		Clone and protein (%)	
CM 5620-3	8.31	MCOL 2436	6.25	MBRA 101	5.94
SM 1406-1	8.13	MBRA 26	6.25	MCOL 219	5.94
MCOL 689B	7.75	MCR 136	6.13	MGUA 33	5.94
MCOL 1563	7.38	MGUA 9	6.13	CM 7310-1	5.88
MGUA 76	6.94	MGUA 91	6.06	MCOL 678	5.88
MCR 142	6.63	MMEX108	6.06	MMEX 95	5.81
CM 696-1	6.44	SM 629-6	6.00	MGUA 79	5.81
CM 3199-1	6.44	SM 673-1	6.00	MBRA 300	5.75
SM 734-5	6.44	MCOL 2532	6.00	MCOL 2459	5.75
MCR 38	6.31	MGUA 19	6.00	MBRA 1384	5.75
MGUA 86	6.31	CM 3236-3	5.94	MCOL 2694	5.75

CONCLUSIONS AND ONGOING WORK

Markers associated with CGM resistance in bulks of 4 inter-specific families were evaluated in the entire families and rather low regression coefficients were found. This is most likely due to the distance of the markers from the gene. BC2 families have been generated from BC1 individuals towards an introgrssion of this resistance into elite cassava gene pools. On the protein work, inter-specific hybrids from wild relatives with high root protein and cassava were evaluated and results reveal the consistency of the trait, amino acid profile and preliminary SDS-PAGE analysis was also conducted with the root proteins. Future perspectives include genetic back crosses, to cassava, of inter-specific hybrids with high protein and evaluation of putative high root protein cassava varieties

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