INTRODUCTION

A very high level of resistance to the green mites was observed in four inter-specific hybrid families, CW05, CW06, CW07 and CW09, from the wild Manihot accession MTLA-457-007 with an almost equal number of susceptible and resistant genotypes. Bulk segregant analysis (BSA) was used to identify four SSR markers (RS874, RS217, RS260 and RS587) polymorphic for poor 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 BC1 families were developed. Selected individuals of these BC1 families were crossed extensively with CMD resistant parents used in CSRAT to obtain CMD and COM resistant genotypes from progenies meant for Africa. The resulting BC2 families were screened for resistance to CMD and COM using molecular markers to select parents for breeding.

Cassava can serve as a cheap reservoir 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 backcross QTL (ABCQTL) has been initiated to introgress high protein content from wild relatives into cassava. Inter-specific hybrids 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 RS874, RS217, RS580 and RS587-30 polymorphic in the bulks segregant analysis were evaluated in the 4 F1 inter-specific families of CW05, CW06, CW07 and CW06B (Fig. 1) and a simple regression analysis conducted using Microsoft Excel. The markers found to explain a significant part of phenotypic variance of COM resistance in the analysis of the 4 F1 families were then analyzed in the parents of the 45 BC1 families. The BC1 families had earlier been evaluated for resistance to green mites. Based upon the COM resistance levels, a large number of putatively resistant BC1 progenies were crossed to CMD resistant parent for the generation of BC2 families from which CMD and COM resistant lines can be selected by marker-assisted selection for the generation of parents for breeding in African gene pools.

For protein content, total protein was measured in root flour obtained from a bulked sample of 3 root per plant from F1 inter-specific hybrids in a seedling trial, using the Kjeldahl method. In collaboration with the starch company AVEBE, an amino acid profile was obtained from root flour from 1 genotype each of Manihot subsp. 'Bellofalko' and M. trifida high in protein, as well as from 2 inter-specific hybrids high in protein. Pooling 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]. About 100mg of root flour was used in each case, suspended in a 500ul volume of sample buffer. The samples were centrifuged in an eppendorf tube at 5 min at 10,000 rpm and the supernatant was transferred to a new eppendorf tube and mixed in a 1:1.1 bd ratio with SDS-PAGE disruption buffer [Lowemill, 1970]. Proteins were completely dissolved by incubating the samples in a boiling water-bath for 5 min, then briefly centrifuging at 14,000 rpm to pellet cellular debris. The resulting supernatants [total protein extracts] were stored at -20°C. Protein were analyzed on 10% SDS-PAGE, according to Laemmli (1970), and stained with coomassie brilliant blue R250.

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

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

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Components</th>
<th>SDS-PAGE</th>
<th>Protein Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer A</td>
<td>0.1 M Tris-HCl, 0.1 M DTT, 2% SDS</td>
<td>Good</td>
<td>Low</td>
</tr>
<tr>
<td>Buffer B</td>
<td>0.1 M Tris-HCl, 1% SDS, 5% Glycerol</td>
<td>Fair</td>
<td>High</td>
</tr>
<tr>
<td>Buffer C</td>
<td>0.1 M Tris-HCl, 2% SDS, 5% Glycerol</td>
<td>Poor</td>
<td>Very Low</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Simple regression analysis of SSR markers RS 74, RS 217, RS260 and SSR 330 in the 4 inter-specific families had coefficients (R2) of 40%, 30%, 30% and 56% in the 4 families respectively. The resistance in the inter-specific families transferred successfully to CMD resistance. The results in this study revealed that similar low regression coefficients falling into question the probability to the genes of the marker, all the markers belong to the same region of the genome. An effort has therefore been initiated to identify more markers linked to CMD resistance using additional SSR and RAPD markers and bulk segregant analysis. Several promising RAPD and SSR markers were identified (Fig 2). Individuals of the BC1 families that possess resistance to CMD were crossed to CMD resistant parent to obtain recombinants that carry CMD and COM resistance. These crosses have been established in vitro from embryonic axes to enable sharing with collaborators in Africa and also evaluated with markers for CMD and COM resistance to select for resistant phenotypes.

Fig. 2. Molecular marker associated with resistance to COM

A total of 4,271 sexual organ lines were obtained from 58 families were obtained from inter-specific crosses between high protein accessions of M. esculenta subsp. 'Bellofalko', M. trifida and elite parents of the cassava gene pool. An evaluation of root protein content was made of 570 genotypes, based on a direct assay that revealed that some high-gene genotypes such as CW 231-1, 230-2, CW 132-2, and CW 264-1 have good combing ability for root protein content. It was also observed that crosses between 2 wild parents, both high in protein (Wf), had more uniform high root protein profiles, compared to wild by cultivated crosses, suggesting that a number of genes for protein content might be recessive or additive. The inter-specific hybrids were being evaluated for a second year in a single cross trial (SFT) experiment, simultaneously BC1 families are being produced from putative high protein lines. Extraction buffer number 3 and 5 gave the best quality protein extract. The success of SDS-PAGE protocols with selection for high protein amount that is a compromise between 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.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Protein Content</th>
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<tbody>
<tr>
<td>CW 231-1</td>
<td>High</td>
</tr>
<tr>
<td>CW 230-2</td>
<td>High</td>
</tr>
<tr>
<td>CW 132-2</td>
<td>High</td>
</tr>
<tr>
<td>CW 264-1</td>
<td>High</td>
</tr>
</tbody>
</table>

CONCLUSIONS AND ONGOING WORK

Markers associated with COM 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 families upon selection of individuals which in turns depend on 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.

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REFERENCES


