

- genotyping a random sample of 45 individuals from both cycles. At present, the null hypothesis of drift could be rejected for 47 of the SSR loci after correcting for multiple tests. Many of these loci correspond to previously published QTL for NCLB.
21. As mentioned above, saturation of selected chromosomal segments has been conducted. Specifically, a region on chromosome 8 has been examined with some 20 SSR loci, nine of which exhibited significant departures from drift. In this region the following resistance loci have been reported: (a) four QTL for NCLB, (b) two major genes for NCLB, (c) three QTL for GLS, (d) two QTL for common rust, (e) one QTL for common smut, and (f) one QTL for maize streak virus. We are now using the maize disease QTL consensus map to select additional SSR loci in the vicinity of previously reported QTL (typically where NCLB QTL co-localize with QTL for several other diseases) for study.
 22. Efforts are underway to determine if allelic differences can be associated with phenotypic differences in disease resistance. A random sample of individuals from intermediate cycles (n=40 for cycle 1; n=20 for cycle 3) from four (including Pool 30) of the eight populations were previously crossed with a common maize inbred line, B73. In the summer 2005 season in upstate NY, F₂ populations were derived from 10 random progeny of each F₁ line (n=2,400 F₂ families). Selected F₂ families will be used to conduct an association analysis of putatively selected alleles versus B73 alleles (similar to a bulk-segregant analysis), and to develop further derivatives of the material (e.g., NILs).

Tangible outputs delivered:

1. Panels of disease resistant maize lines and genetic stocks derived from them.
2. Synthesis of disease QTLs in maize submitted for publication.

We would like to include Southern Corn Leaf Blight as one of the target diseases for the project. An analysis of the literature indicates that this can be justified based on importance in the developing world.

9. Development of Low-Cost Technologies for Pyramiding Useful Genes from Wild Relatives of Cassava into Elite Progenitors

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MID-YEAR REPORT

Wild *Manihot* germplasm are a wealth of useful genes for the cultivated species *M. esculenta* but their use in regular breeding programs is restricted due the long reproductive breeding cycle of cassava and linkage drag associated with the use of wild relatives in crop

improvement. This project seeks to identify useful genes for pest and disease resistance, and post-harvest deterioration in cassava and to develop low cost marker tools for their rapid introgression into cassava. During the first six months of the project the following outputs were obtained:

Previous work revealed that a RAPD marker RME1 and an SSR marker NS158 are the closest markers to the gene CMD2 that confers resistance to the cassava mosaic disease (CMD), they are located at distances of 9 and 4 cM respectively, and are being routinely used for marker-assisted selection (MAS) of CMD resistance at CIAT. To reduce the cost and time as well as accuracy of assaying the most important marker, RME1, the polymorphic RAPD fragment in the CMD resistant parent was eluted from an agarose gel, cloned into pGEMT-easy (Promega inc, Madison) and sequenced. Primers were designed from the sequences (Appendix 1) and the RAPD marker successfully converted into a SCAR marker, this marker is now routinely being used for MAS at CIAT and primer sequences has been sent to NARs partners in preparation for its use in MAS in their breeding programs.

Several previous reports have revealed moderate to high levels of resistance to many pests and diseases that attack cassava. Some of these species are being used in this project to introgress the resistance genes into cassava. Additional evaluations of 5 Wild *Manihot* species accessions, F1 Inter-specific hybrids, and BC1, derivatives growing in the field at CIAT were conducted to identify high levels of resistance to green mites, mealybugs, whiteflies, and cassava bacterial blight (CBB). Results reveal excellent sources of resistance to white flies, and moderate sources of resistance to mites and mealybugs (Appendix 2). Preliminary results of the evaluation of CBB resistance in BC2 derivatives of *M. esculenta* sub spp *flabellifolia* revealed moderate to high levels of resistance in some genotypes.

Sexual seeds of natural wild populations of many wild *Manihot* species and their inter-specific hybrids with cassava were distributed to NAS participants for field establishment and evaluation for pest and diseases endemic in their own environment. Seed lots of a total of 1740 sexual seeds from 175 families representing 5 wild *Manihot* species namely: *M. esculenta* sub spp *flabellifolia*, *M. esculenta* sub spp *peruviana*, *M. tristis*, *M. carthaginensis*, and *M. Fomentosa* were each shipped to Brazil, Uganda, Ghana, and Nigeria. Also sent to participating NARs were 1072 sexual seeds of F1 hybrids representing 171 inter-specific families obtained from crossing selections from accessions of the 5 species and elite cassava varieties

Wild relatives of cassava are important sources of genes for resistance to pests and diseases and longer shelf life. The only source of dramatically delayed PPD has been identified in an inter-specific hybrid between cassava and *Manihot walkerae*, a unique source of resistance to the cassava hornworm was also identified in 4th backcross derivatives of *M. glaziovii*. Moderate to high levels of resistance to white flies have been found in inter-specific hybrids of *M. esculenta* sub spp *flabellifolia*. BC1 and S1 mapping populations for the identification of molecular markers for the introgression of delayed PPD, resistance to the cassava hornworm and white were developed last year. They include a cross between CW429-1 (F1 hybrid of *M. walkerae*) and MTAI 8 (BC1), a total of 205 progenies a cross between MNG11 (BC4 derivative of *M. glaziovii*) and MTAI8, 157 individuals, and a cross between CW67-7 (F1 hybrid of *M. esculenta* sub spp *flabellifolia*) and MTAI 8, 230 genotypes. The above-mentioned crosses were established *in vitro* from embryo axes and are currently being multiplied, at least 8 plants per genotype, for transfer to the screen house for hardening and eventually to the field during next year's planting season.

An advanced field-based and molecular marker-assisted selection (MAS) breeding course in cassava was held at CIAT from April 11 to May for NARs partners in the GCP competitive grant project from Uganda, Ghana, Nigeria, and Brazil. The purpose of the course was to expose the NARs cassava breeders to methodologies being used at CIAT for MAS and to update them on current methods in scientific field-based breeding of cassava. Specific objectives of the course were to teach participants the theory and practice of every aspect of cassava breeding and to expose them to new approaches, for example, molecular markers in cassava breeding, doubled haploid technology, tissue culture, and genetic transformation. Molecular marker labs have also been established at CRI, Kumasi, Ghana and NRCRI, Umudike, Nigeria, the lab in NAARI, Namulonge, Uganda is still under construction.

Tangible outputs delivered:

- Development of a low-cost SCAR marker for MAS for breeding resistance to the cassava mosaic disease (CMD)
- Evaluations of several natural populations of 5 *Manihot* species, their F1s and BC1s for resistance to whiteflies and green mites
- Shipment of sexual seeds of several natural populations of 5 *Manihot* species to NARs collaborators in Brazil, Nigeria, Ghana, and Uganda for establishment in the field and eventual evaluations
- *In vitro* establishment of BC1, and S1 gene mapping populations for delayed post-harvest deterioration, resistance to Horn Worm, and whiteflies
- Training of NARs partners from Brazil, Nigeria, Uganda, and Ghana in the theory and practice of field-based and molecular breeding during a one month intensive course at CIAT

A delay in the shipment of BC2 populations with CMD resistance and tolerance to Mites to NARs partners for Molecular breeding, the plants will now be shipped in early October, the delay has been due to the large volume of in vitro culture work involved in establishing the mapping populations for PPD, whiteflies, and hornworm.