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

The use of wild relatives in regular breeding programmes is complicated by the long reproductive breeding cycle of cassava, high genetic load that is released on backcrossing, and linkage drag associated with the use of wild relatives in crop improvement. A project was initiated at CIAT to accelerate the process of introgression of useful genes from wild relatives into cassava via a modified Advance Back Cross QTL (ABC-QTL) breeding scheme. We describe here advances in the last 6 months in the introgression of resistance to delayed post harvest physiological deterioration (PPD), whiteflies, cassava green mites, and horn worm from wild *Manihot* species into cassava. We also describe new collections of wild *Manihot* species carried out by project partners in Brazil as well as progress in molecular breeding of resistance to CMD and cassava green mites resistance by partners in Africa.

1. PPD:

A source of delayed PPD had earlier been identified in an inter-specific hybrid CW429-1 obtained by crossing *Manihot walkerae* with cassava. This line was crossed extensively to the elite cassava genotypes MTAI8, CM523-7, and SM909-25 to create 3 BC_1 families (BC_1 only in the sense of crosses to cassava) or selfed to generate an S_1 family. The F_1 hybrid and 8 elite cassava genotypes namely: MCOL 1505, MPER 183, MTAI 8, CM523-7, HMC-1, MBRA 337, MCOL 2279 and CM 2772-3, having the widest variation for delayed PHD in the cultivated gene pool, were re-evaluated to obtain 3rd year phenotypic data for the novel source of delayed PPD and a wide variation of the trait in the cultivated gene pool. Results of mean PPD values at 5 days after harvest (DAH) ranged from 0% in CW 429-1 and MBRA 337 to 44.85% in CM523-7 (Table 1). At 10 DAH, mean values ranged from 0% in CW429-1 to 58% in CM523-7, respectively. The same trend was observed 15 DAH with CW 429-1 still displaying no visible sign of deterioration (Annex 1). Previous evaluation of delayed PPD shows nothing higher than 7 DAH has been found. Evaluation of inter-specific hybrid CW429-1 reveals that genes for delayed PPD have been transferred into cassava from a wild relative. BC_1 and S_1 mapping populations, a total of 3,400 plants representing 550 genotypes organised into 4 families, for delayed PPD were hardened in the screen house and transferred to the field March this year. They will be evaluated in December for delayed PPD.

2. Hornworm and Whiteflies:

The only known source of resistance to the cassava hornworm was identified in 4th backcross derivatives of *M. glaziovii* – MNGA11 (60444). High levels of resistance to white flies were also identified in inter-specific hybrids of *M. esculenta* sub spp flabellifolia (CIAT, unpublished data). The variety MNG11 was to several cassava varieties and selfed to produce BC_1 and S_1 families respectively. Several genotypes of the inter-specific hybrid CW67, a progeny of M.esculenta sub

spp flabellifolia, showing a high level of resistance to white flies was crossed to MTAI 8 or selfed to produce BC_1 and S_1 families respectively. After embryo rescue of seeds from the above crosses and screen house hardening, the number of seeds obtained was below that required for mapping of resistance genes. The low number of recovered plants was due to an unusual level of precipitation and flooding at CIAT recently. Additional crosses were made last year with MNGA11 and several inter-specific hybrids with *M. esculenta* sub spp *flabellifolia*, the family CW67, that flowers more profusely and many more seeds obtained (Annex 2). The seeds are currently being put *in vitro* from embryo axes and will be micro propagated and established in the field by August this year for 2 cycles of evaluation for the appropriate pests.

3. Cassava Green mites (CGM)

Good resistance to cassava green mites (CGM) was identified in 4 inter-specific hybrid families, CW68, CW65, CW67, and CW66, derived from a cross between cassava and *Manihot esculenta* sub spp *flabellifolia* accession. In order to identify markers associated with resistance to CGM the BC_1 derivates of the inter-specific crosses were evaluated over three growing cycles to identify resistant and susceptible individuals. Genotypes with symptom damage of less than 3, resistant, and those with symptom score of 4-6, susceptible, were bulked respectively. Molecular markers for bulked segregant analysis (BSA) were 530 SSR markers with broad coverage of the cassava genome. Till date 131 SSR markers have been evaluated in the resistant and susceptible bulks and 44 markers were polymorphic between the bulks and parental lines (Annex 3). After completion of the bulk and parental survey with all markers, individuals of the bulks and eventually the entire BC1 family would be evaluated with the polymorphic markers.

4. Molecular breeding of resistance to CMD and CGM

A total of 2,400 plantlets representing 11 F_1 families were shipped from CIAT, Colombia, in March 2006 (Annex 4) to each NARs of Ghana, Uganda, and Nigeria. This is in addition to BC_2 for CMD and CGM resistance shipped last year to Brazil and the African NARs. The materials are being hardened for field establishment during this season. The materials would be evaluated in the field for CMD and CGM resistance and put in a crossing block together with local farmer preferred and improved varieties for making crosses later in the year. The F_1 seedlings will be selected for resistance to CMD and CGM using molecular markers and phenotypic evaluations next year as well as for other traits of agronomic importance for example high starch

5. Collection of new accessions of *Manihot* species

Collection of sexual seeds of additional wild species accessions were carried out in three places:

i) From the CNPMF field collection

916 sexual seeds were collected from different accessions of 4 species: *M. anomala* (527); *M. dichotoma* (228); *M. flabellifolia* (78); *M. peruviana* (83). Most of these seeds were sowed under greenhouse condition and the seedlings will be planted at the target sites for field evaluation later in the season.

ii) Germplasm collections in the semi-arid region of Bahia State where the following cassava wild species were found and collected:

1) *M. caerulescens*
2) *M. diamantinensis*
3) *M. jacobinensis*
4) *M. glaziovii*
5) *M. dichotoma*
6) *M. maracasensis*

iii) Another collection was carried out in the "cerrado" region of Brasília and surrounds, in which 28 populations of cassava wild species were found. A total of

16 species were found and collected. The probable species found in this expedition were:

1) *M. pentaphylla*
2) *M. irwinii*
3) *M. violacea*
4) *M. falcata*
5) *M. salicifolia*
6) *M. fruticulosa*
7) *M. cecropiaefolia*
8) *M. tripartita*
9) *M. stipularis*
10) *M. triphylla*
11) *M. tristis*
12) *M. anomala*
13) *M. mossamedensis*
14) *M. mana*
15) *M. gracilis*
16) *M. tomentosa*

A part of the seeds of these collections have been established in seedling nurseries for eventual transfer to the field at CNMPF and to the evaluation sites at Petrolina, São Miguel das Matas and Tancredo Neves for field evaluation

6. Evaluation of *Manihot* species for additional genes of interest

i) Field establishment of the cassava wild species collection at Embrapa/CNPMF.

The first species/accessions established in this collection came from: a) Sexual seeds of 7 wild species sent from Embrapa/CENARGEN; b) Stakes of 3 wild species previously collected at Bahia's semi-arid region; c) Stakes of "Maniçobas" and "Pornúncias" accessions collected at Embrapa/CPATSA and at Bahia Federal University. Up to now, accessions of the following genotypes were field established:

1) *M. anomala*
2) *M. caerulescens*
3) *M. dichotoma*
4) *M. flabellifolia*
5) *M. glaziovii*
6) *M. peruviana*
7) M. tomentosa
8) Maniçoba - probably is *M. glaziovii* or *M. pseudoglaziovii*
9) Pornúncia – probably is a natural hybrid between *M. esculenta* and *M. glaziovii*

ii) Inter specific hybrids introduced from CIAT at Embrapa/CNPMF.

1098 F_1 seeds of 34 inter specific hybrids from crosses between *M. esculenta* and wild species, produced at CIAT, were sowed at CNPMF under greenhouse condition. A total of 450 seedlings from 32 hybrids were obtained. The number of seedlings per hybrid varied from 1 to 74. Some seedlings of 21 hybrids were planted in Petrolina, São Miguel das Matas and Tancredo Neves for field evaluation

iii) Inter specific hybrids introduced from CIAT to NARs of Nigeria, Ghana, and Uganda.

These were nursed in the screen house and evaluated as part of the effort to introgress Latin America material for use in the development of improved varieties of cassava. A selection would be made upon evaluation for the crossing block. The seeds were planted in September 2005 and some transplanted in November. These were crosses between *Manihot esculenta*, between

Manihot esculenta and wild relatives; and between the wild relatives. The germination rates were moderate to low. The seedlings were evaluated for disease pressure reaction

7. Training

Following the initiation of the GCP project, CIAT appointed a visiting scientist, Dr. Emmanuel Okogbenin, a breeder with experience in molecular genetics to technically backstop project activities in the NARs. The visiting scientist is placed at the NRCRI, NARs of Nigeria but oversees project activities at the African NARs as well as serving as liaison officer between CIAT headquarters (PIs) and NARs partners (collaborating scientists). The visiting scientist has successfully assisted the NRCRI, Nigeria to establish a MAS laboratory in addition to backstopping the institute in its cassava breeding activities. The NRCRI Molecular Biology Laboratory which was established last year started operations early this year. Test running of the laboratory started in February and it started functioning since March. PCR reactions and polyacrylamide gels are being run at the moment. This laboratory is now been used for diversity studies as part of preliminary activities towards applying the laboratory in molecular breeding activities. CIAT has recently provided the laboratory with primer kit (of 36 primers) for the diversity studies. The laboratory will be used for marker assisted selection activities later in the GCP project. Similar molecular marker activities have also been initiated at CRI Ghana and NAARI, Uganda.

Tangible outputs delivered:

1. Confirmation of delayed post-harvest physiological deterioration (PPD) in inter-specific hybrid CW429-1 transferred into cassava from a *M.walkerae*
2. Initiation of bulked segregant analysis (BSA) of resistance to cassava green mites in BC_1 derivatives and the identification of 44 polymorphic markers for further evaluation
3. Introduction of CMD and CGM resistant genotypes from CIAT to African NARs and establishment of a crossing block for genetic crosses to local and improved farmer preferred varieties
4. Collections of accessions of 20 species of *Manihot* germplasm in the semi-arid region of Bahia State and in the "cerrado" region of Brasília.
5. Establishment of sexual seeds of wild and inter-specific hybrids for evaluation in an attempt to identify new sources of genes for resistance to pests and diseases and other traits of agronomic interest
6. Introduction of F_1 and BC_2 molecular breeding parents with CMD resistance and tolerance to Mites to NARs partners in Nigeria, Ghana, Uganda, and Brazil for molecular breeding; setting up of crossing blocks in Nigeria, Ghana, and Uganda

Deviations from the work plan:

1. A delay in the field establishment of resistance to white flies and hornworm mapping populations: the need to increase the mapping population sizes for resistance to white flies and hornworm has meant that these mapping populations were not moved to the field at the same time with the delayed PPD populations, they will be transferred in August to the field.
2. A delay in the shipment of F_1 populations with resistance to CMD and cassava green mites to NARs partners in Nigeria, Ghana, and Uganda for molecular breeding, the plants were eventually shipped in April this year as against the original date of October last year, 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.
3. On project activities in Brazil, the main delays were: 1) bureaucratic bottle necks (plant quarantine); on the release of the plant materials (imported from CIAT) by the Ministry of Agriculture to CENARGEN 2) Delay in the transfer of the materials from CENARGEN to CNPMF; and 3) damage to the *in vitro* plants; the in vitro BC_2 plants of 28 genotypes, sent from CIAT on Dec 20, 2005, were delivered to CENARGEN on Jan 24, 2006. All the plants inside the bottles were lost due to the damages that occurred during transportation 4) delay in

the release of more than 3000 sexual seeds of five wild species shipped from CIAT on Sep 01, 2005 to CENARGEN (for quarantine), they were released to CNPMF after 5 months. These seeds were sowed at CNPMF and none sprouted. The second batch of F_1 seeds of inter-specific hybrids shipped from CIAT on Oct 18, 2005 remained 3 months in São Paulo before being releasing to CENARGEN. These constraints have caused significant delay of the original work plan and can be minimised by no cost extension of the project's activities.

4. New introductions were received from CIAT in April in Nigeria. The materials were inspected by PQS and about 1100 in vitro plants were transplanted for the hardening stage. However, due to fungal infections experienced during the hardening stages substantial losses were experienced, and efforts are underway to re-introduce more materials by June.

10. Exploring Natural Genetic Variation: Developing genomic resources and introgression lines for four AA genome rice relatives

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Development of Chromosome Segment Substitution Lines populations
Completion of two *O. sativa* x *O. glaberrima* populations
A1. IR64 x TOG 5681 cross

New BC1F1, BC2F1 and BC1F2 populations of this cross have been developed at CIAT in order to try to introgress some missing fragment of the *O. glaberrima* genome that were lost in the former population (see last year report).

A set of 357 SSRs was optimised in the frame of the core map project. From this set of SSRs, 120 SSRs that show polymorphism were chosen for the genotyping of the new BC1F1s. To this date, 35 SSRs have been tested on chromosomes 3 and 6. Those chromosomes were targeted because they bear two important interspecific sterility genes.

For the BC3F3s/BC2F4s (first population), a set of 40 new SSRs has been selected to complete the genotyping in order to have evenly distributed SSRs.

As a result of the genotyping of the BC1F1s, we found that the plants that were showing a high fertility don't bear the *O. glaberrima* allele at the location of the sterility genes. Crosses between those lines and the two *O. sativa* and *O. glaberima* parent are on going to verify the hypothesis that they could serve as interspecific bridges. Also, we found lines with introgressions for most of the genome segments from the *O. glaberrima* genome that were lost in the former BC3F3/BC2F4 population, especially on chromosomes 4 and 10.

In the next months, we are going to continue with the evaluation of the SSRs that we select for each one of the populations.

A2. Caiapo x IRGC103544 cross

A cross between Caiapo (an elite tropical *japonica* from Brazil) and *Oryza glaberrima* (IRGC103544) was made at CIAT HQs (C.P. Martinez), using IRGC103544 as the male parent. The F1 was backcrossed with Caiapo in the subsequent 2 generations until taking the population