

# **Summary**

## **Annual Report Project SB-2**

### **Conservation and Use of Tropical Genetic Resources**

*Formerly know as: Assessing and Utilizing  
Agrobiodiversity through Biotechnology*

**November, 2003**

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## SUMMARY ANNUAL REPORT

### SB-2: ASSESSING AND UTILIZING AGROBIODIVERSITY THROUGH BIOTECHNOLOGY

#### a. SB2 Personnel: Discipline and time allocation

Name	Discipline	Time dedication %
Alves Alfredo	CBN Regional Coordinator	100
Beebe Steve	Bean Breeding	30
Bellotti Anthony	Cassava Entomology	20
Blair Mathew	Bean Genetics and breeding	70
Ceballos Hernan	Cassava Breeding	40
Chavarriga, Paul	Transgenesis, Cassava	100
Debouck Daniel	Botany	20
Fregene Martin	Cassava Genetics and breeding	60
Ishitani Manabu	Molecular Biologist	100
Lentini Zaida	Biology/Genetics	80
Lorieux Mathias	Rice Genetics and Biotechnology	50
Martínez César	Breeding	49
Mejía Alvaro	Cell Biology	100
Sperling Louise	Seed Systems	20
Tohme Joe	Genomics, Project manager	100

#### b. Collaborators and their affiliations

**Within CIAT:** Genetic resource unit, Bean, Forage, Cassava, Rice, IPM, and Participatory Research projects. CBN, CLAYUCA and FLAR

**Outside CIAT:** **Australia:** Center for Applied Molecular Biology in International Agriculture (CAMBIA) **Brazil:** Embrapa-Cenargen, Embrapa-CTA, Embrapa-CNPAF, Embrapa-CNPMF **CGIAR, and International organizations:** CIP, Perú; FAO, Italy; IAEA, Australia; ICARDA, Syria, ICRISAT, India, IFPRI, US; IITA, Nigeria; IPGRI; IRRI, Philippines; **Colombia,** Cenicaña, Cenicafe, Universidad Javeriana CIB, COLCIENCIAS, Colombian Ministry Agriculture and Rural Development, Corpoica, Corporacion Biotech, Instituto Humboldt, UniAndes, UniValle, Universidad Nacional at Palmira and Bogotá, Universidad del Tolima; **Japan:** JIRCA-Tsukuba, **France:** CIRAD, IRD, Montpellier. Universite de Perpignan.; ETH, Switzerland; United UK: University of Bath, ; University of Aarhus, Denmark, **Germany:** University of Freiburg, University of Hanover, University of Hoesheimm. **Sweden:** USLU, Uppsala. **NARS- Latin America:** Centro Tecnológico Polar, Venezuela; National Bean Programs of the Dominican Republic (INIAF) INVIT, Cuba, Ministerio de Agricultura, Nicaragua; **NARS –Africa:** National Root Crops Research Institute, Nigeria, University of Nairobi, Kenya ; Namunlonge Agricultural and Animal Research Institute, Kampala, Uganda. Medical Biotech Laboratories, Kampala, Uganda. CEGA, Colombia; FIDAR, Colombia, PBA, Colombia REDBIO, Latin America, Parque del Software, Cali, Colombia;. **Private sector Colombia:** Corn product, Barranquilla, LIMSYS, Cali, DATABIO, Cali; **United States:** Clemson University, Cornell University, Danforth Center, Kansas Sate University, Michigan State University, National Center for Genome

Research, (NCGR), Ohio State University, Penn State University, Rutgers University, Smithsonian molecular systematic lab, University of Nebraska, University of Puerto Rico; USDA-Plant Soils and Nutrition Lab at Cornell University, USDA at Children Hospital Baylor University, USDA-Soybean genomics, at Beltsville, Yale University,

**c. Budget:**

<b>BUDGET 2003</b>		
<b>PROJECT SB2: Conservation and Use of Tropical Genetic Resources</b>		
<b>SOURCE</b>	<b>AMOUNT US\$</b>	<b>PROPORTION (%)</b>
Unrestricted Core	849,107	24%
Restricted Core	471,802	13%
Carry over from 2002	193,947	5%
<b>Sub-total</b>	<b>1,514,856</b>	<b>43%</b>
Special Projects	2,015,627	57%
<b>Total Project</b>	<b>3,530,483</b>	<b>100%</b>

**d. Highlights of outputs**

**Staff changes:**

- Dr. Manabu Ishitani joined CIAT in April 2003 to lead the area of molecular biology for abiotic stress and nutritional traits. Manabu worked for several years with the two leading research drought groups at Arizona State University. In the past three years, he worked at BASF, in North Carolina as the principal investigator on gene discovery for drought. In that capacity, Dr. Ishitani brings to CIAT several years of knowledge on gene discovery for environmental stress, and a working experience with the private sector.
- Dr. Chikelu Mba, acting coordinator of the Cassava Biotechnology Network (CBN) left CIAT to join the Atomic Energy molecular lab in Vienna as a permanent staff. Dr. Mba did an outstanding job as acting CBN coordinator under the difficult situation faced last year due to the tragic loss of Dr. Chusa Gines, Dr. Mba's new position will allow CIAT to established a working relationship with the IAEA.
- Dr. Alfredo Alves joined CIAT from EMBRAPA-CNPMPF as part of a sabbatical to coordinate the Cassava Biotechnology Network in replacement of Dr. Chikelu Mba. Dr. Alves is a physiologist with a long experience in cassava under stress environment. His presence at CIAT besides leading the CBN, will strengthen CIAT relationship with EMBARAP – CNPMPF.

- The merge of the SB-1 and SB-2 project initiated last year proceeded smoothly ensuring a full visibility of the Genetic Resources Unit.

**Team member received the following awards in 2002:**

- Election as Chair of the Board: Sustainable Agriculture and Natural Resource Management CRSP (Collaborative Research Support Project) – L. Sperling.
- Best Presentation: Annual Review CIAT (2003): “ Keeping Research Off the Shelves: Making Emergency Relief More Effective” L. Sperling
- Leonardo M. Galindo received the Laureada Award for his Bsc. thesis on “Aislamiento y caracterización de las secuencias LTR de retrotransposones del grupo TY1-copia en *Phaseolus vulgaris* . Universidad Nacional de Colombia. Sede Bogotá.
- Catalina Romero received the Laureada Award for her Bsc. thesis on “Aproximación genómica al fenómeno de resistencia de *Brachiaria* al salivazo (*aeneolania varia*): Correlación con homólogos de genes de resistencia (HGR) y aislamiento de genes expresados diferencialmente en la respuesta de defensa”. Universidad Nacional de Colombia. Sede Bogotá.
- Claudia Flores received the Meritorio Award for her PhD thesis “Desarrollo y uso de la transferencia genética de *Brachiaria decumbens* Stapf en fitomejoramiento”. Universidad Nacional de Colombia, Sede Palmira
- Mauricio Soto received the Meritorio Award for his BSc. thesis “Herramientas de genómica funcional: Análisis de ESTs, Librerías sustractivas y microarreglos de ADN para avanzar en el conocimiento de las respuestas de defensa de la yuca a la infección por *Xanthomonas axonopodis* pv *manihotis*”. Universidad Nacional de Colombia, Sede Bogotá.
- Manuel Quintero received the Meritorio Award for his BSc. thesis Ajuste del sistema RITA para la inducción de callos embriogénicos y regeneración de plantas a partir del cultivo de anteras de arroz.

## Overall view of the SB2 project

The project lead within CIAT and with IFPRI the fund raising, structuring and organization of the Biofortification Challenge Program, called as October 2003, Harvest Plus. The team members have interacted successfully with researchers from Advanced Research Institute, National Programs, NGO and the private sector. They also pursued successfully all three outputs related to:

- Output 1: Genomes of wild and cultivated species of mandated and non mandated crops, associated organisms characterized.
- Output 2: Genes and genes combination made available for broadening the base of mandated and non mandated crops.
- Output 3: Collaboration with public and private partners enhanced

Only the following achievements are summarized.

### **1) Marker development is leading to improved genetic mapping, diversity assessment and marker assisted selection in common beans**

This year, the bean genomics group has concentrated on developing new molecular markers, especially microsatellites and sequence characterized amplified regions (SCAR). A new genome-wide anchored microsatellite map was made for common bean that serves as a bridge for integrating the central CIAT genetic map with the integrated and RFLP maps developed for the species by Freyre et al. (1998) and Vallejos et al. (1992). This genetic map has been efficiently applied for the QTL mapping of drought tolerance, of nutritional quality (for iron, zinc and tannin content) and of insect resistance (to Thrips palmi and Apion goodmani). The microsatellites have been used in diversity assessment on a collection of accessions mostly red mottled and other putatively Andean genotypes from the Caribbean collected by the University of Puerto Rico and the National Bean Programs of the Dominican Republic (INIAF) and Haiti (Ministry of Agriculture). Many genotypes showed possible introgression of Mesoamerican alleles. These results confirm previous phaseolin characterization of Caribbean common bean germplasm showing hybrid Mesoamerican-Andean origin to red and pink mottled "Andean" landraces. Discrimination power and allelic diversity values were established for the range of microsatellite markers developed at CIAT. In addition, to the microsatellites, we are developing SCAR markers for Apion resistance genes and applying previously developed SCAR markers for bean common mosaic virus resistance for the bc-3 BCMV resistance gene. Microsatellite markers have been identified which are tightly linked to the bruchid resistance gene encoding the arcelin protein and which are being used effectively in marker assisted selection. These new molecular markers are being used in practical, real-life plant breeding situations and a total of almost 3000 advanced breeding lines were evaluated with these new DNA based markers this year. The bc-3 marker was found to work well in Andean breeding lines especially for screening of climbing beans, where it resulted in substantial savings in planting area and agronomic effort. Meanwhile the arcelin markers were found to work as well or better than previous serological and protein based tests in distinguishing the resistance alleles derived from wild beans. All of this work builds on last year's successes in using SCAR markers for bgm-1 and for QTLs for resistance to bean golden yellow mosaic virus (BGYMV) and common bacterial blight (CBB). Next year we plan to

implement additional SCAR markers for the I gene and for resistance to angular leaf spot and anthracnose.

## **2) Realizing the potential of nutritional genomics: Candidate gene analysis of micronutrient content in common beans:**

Nutritional genomics is being used as part of the Biofortification Challenge Program to discover the basic mechanisms for mineral uptake and accumulation. As part of the overall genomics approach, information from other well studied species such *Medicago truncatula*, peas and soybeans, as well as other model species such as *Arabidopsis thaliana* which have extensive genetic and molecular resources are being used for gene discovery and functional analysis. The underlying concepts of this work are to take advantage of metabolic unity among plants to characterize gene function and to apply bioinformatics and molecular cloning approaches to identify potential orthologous genes. Advances were made on identifying candidate genes for mineral uptake and storage in root cDNA libraries from common beans. Our immediate targets were the genes for ferritin, the major iron storage protein in plants, for zinc transporters from the ZIP family and for enzymes involved in the synthesis of nicotianamine, an iron chelator. Common bean homologues were identified by hybridization with soybean clones. The number of positive hits per hybridization gave a good idea of the relative levels of gene expression of each gene. A collaboration with the Grusak lab at USDA-Houston is producing very interesting results on the analysis of iron reductase as a mechanism for enhanced iron uptake in common beans. So far, there is evidence that there are differences between parents of several mapping populations for their ability to reduce iron and that these differences are evident more at low Fe concentration than at high iron concentration. More information on this trait will be reported next year when a set of recombinant inbred lines have been fully tested and the QTLs for this trait localized. In addition to increasing iron content per se, we are studying the inheritance of soluble and insoluble tannins, which are implicated as anti-nutrients in mineral absorption, through a QTL analysis of parents and segregating populations of common bean. This builds on our research reported last year on the QTL analysis of mineral content (especially of iron and zinc accumulation). All of this research forms part of the Biofortification challenge program; Harvest Plus.

## **3) *Empoasca* resistant lines found in the progeny of *P. vulgaris* x *P. acutifolius* interspecific hybrids**

After a field screening performed by the IP-1 project, at least seven common bean lines derived from Double Congruity Backcrosses (DCBC) were identified as *Empoasca* resistant. All these hybrids include in its pedigree the genotype of *P. acutifolius* G40036 and one of them the genotype G40019, which are resistant to *Empoasca*. These accessions of tepary bean may be the source of the resistance detected in the hybrids. In the past 10 years several common x tepary hybrid lines produced using other backcross strategies like recurrent or congruity backcrosses, were evaluated in the field for *Empoasca* resistance with no success in identifying resistant genotypes. This is an additional proof of the usefulness of the DCBC-strategy to allow introgression of traits from alien germplasm (last year the IP-1 project reported the identification of drought tolerant lines derived also from DCBC hybrids).

#### **4) Dissection of a cluster of Resistance Gene Candidates (RGCs) associated with resistance to angular leaf spot (ALS) in common bean (III)**

Using candidate resistance genes as molecular markers, we have previously detected a locus (RGC7) that explains 47% and 64% of the resistance to two isolates of angular leaf spot (ALS) in common bean (López et al., 2003 *Phytopathology* 93:88-95). This result prompted us to sequence a large genomic region that contains a cluster of RGCs highly similar to the original molecular marker associated with the ALS resistance locus. The final phase of the sequencing of the BAC clone 57-M14 derived from the susceptible ALS cultivar Sprite; and the annotation of the 90 Kb of genomic sequence were completed. Homology searches and annotation tools revealed the presence of five highly similar Resistance Gene Candidates (RGC) that constitute a part of the gene family. All of the members contain a TIR domain with an intron splicing site highly conserved that separates the TIR domain from other domains, a modular feature that is usual in the TIR-NBS-LRR class of R-genes. The NBS and LRR domains appear complete only in two members while two more are truncated in the NBS domain. The fifth member contains the TIR and NBS but sequence information beyond this point is not available yet. Moreover, two out of the five RGCs showed stop codons, suggesting that they are pseudogenes in the Sprite cultivar. Sequence data provide a valuable platform for the cloning of the corresponding R-genes in the source of ALS resistance, the variety G19833, a process that is currently in progress. The sequence information allowed the identification of additional molecular markers in order to verify that the physical region encompassed by the BAC mapped at the same genetic locus represented by the original RGC (RGC7) molecular marker. Single Marker Analysis (Qgene software) of the microsatellite SSR07, positioned at the beginning of one member of this cluster, shows a correlation of 75% and 69% of the resistant phenotype to the two different isolates of the pathogen. This even higher correlation with the resistant phenotype to ALS confirm that this physical region must contain (a) major component(s) of the resistance to ALS.

#### **5) A Molecular marker-assisted, farmer-participatory breeding project to improve local cassava varieties in Tanzania with resistance to pest and diseases**

Cassava is the main stay of many rural communities in many African countries. Overlapping attacks of the cassava mosaic disease (CMD) and the cassava green mites (CGM), two of the principal disease and pest problem of the region, often leads to a complete loss of the harvest and famine. With funding from the Rockefeller foundation, a molecular marker-assisted, farmer-participatory, breeding project has been initiated this year to improve local farmer preferred germplasm in Tanzania for resistance to CMD and CGM within a period of 5 years. This project builds upon the marker-assisted selection program for CMD at CIAT as well as a study of genetic diversity of local land races in Tanzania, carried out by CIAT in collaboration with the Tanzanian National Root and Tuber program. Improved parents that combine adaptation to the semi-arid tropics and the acid savannahs with resistance to CMD, CBB and CGM, has been developed at CIAT for shipment to Tanzania. The project is a first and experience gained is expected to guide the application of molecular markers in breeding cassava for small holders.



## **6) Development of Waxy Cassava Starch via the anti-sense and sense down regulation of the granule bound starch synthase (GBSSI) gene**

Higher incomes from cassava in the developing world where the crop is generally found will require the industrialization of the crop and the development of novel industrial products from cassava. With funds from the Colombian Ministry of Agriculture and Rural Development, a project was initiated to genetically engineer industrial cassava varieties for the production of waxy starch using an anti-sense and sense construct of a full length GBSSI gene. The constructs were successfully transformed into friable embryogenic Callus (FEC) of the model transformation genotype MNG11 via *Agrobacterium tumefaciens* as demonstrated by a GUS transitory assay revealed a successful incorporation of the gene. Regeneration of the transformed calli is nearing completion and will be tested for waxy phenotype.

## **7) Utilization of new alleles from wild rice to improve cultivated rice**

In previous years we have reported that some regions in *O. rufipogon*, *O. glaberrima* and *O. barthii* carry alleles with positive effects on traits of agronomic importance including grain yield and its components, as well as tolerance to biotic stress. This year further analysis on the performance of advanced lines from the cross BG90-2/*O. rufipogon* over eleven locations in Latin America showed that these lines are stable over diverse environments. The top yielder lines gave 14--24 percent yield advantage over the recurrent improved parent Bg90-2(6ton/ha). Molecular analysis showed that these lines carry introgressions from the wild parent. Top yielder lines have a yield potential (7.7ton/ha) similar to Fedearroz50, the top yielder commercial variety grown in Colombia. Results suggest that the advanced populations developed could be cornerstone for a further yield increase through a marker assisted selection program.

## **8) Identification of genes induced during the defense response of *Brachiaria* to the spittlebug**

The molecular basis of plant defense responses to insects is a challenging area whose understanding should make feasible the use of natural immunity in economically important plants. To clarify the molecular mechanisms of resistant *Brachiaria* cultivars to spittlebug, we have used a subtractive hybridization technique that allowed us to isolate gene fragments expressed during the defense response to *Aeneolamia varia* infestation. Sequencing analysis of ~240 clones from the subtractive library revealed that they corresponded to 74 unique expressed genes. Putative functions were assigned to 41 transcripts through sequence similarity searches. Additional biological information such as precise biochemical roles, metabolic pathways and protein motifs and domains of the predicted amino acid sequences were established from annotated secondary databases. Through this bioinformatic analysis we were able to classify the predicted proteins in several functional groups. Some of them are involved in the biosynthetic pathways of three important plant signaling hormones related with defense responses: Jasmonate, a central regulator of the systemic response to wounding, ethylene and brassinosteroids. Other putative sequences take part in cell signaling, transcriptional regulation, cell wall modification and the homeostasis of the plant during the water stress caused by the insect. Finally, we found two putative effector proteins that may be the ultimate cause of the antibiotic action of the resistant plants on the insect. These results outline part of the

physiological, biochemical and cellular processes that seem to be involved in the defense responses of *Brachiaria* to the spittlebug. To our knowledge, this is the first report on the molecular mechanisms of resistance in the interaction of a monocotyledonous plant with a xylem-sucking insect, an interaction that is poorly understood at the molecular level given the peculiarity of this feeding habitat.

### 9) Rice T-DNA insertional mutagenesis platform

In the framework of its work plan for functional analysis of cereal genomes, the Génoplante consortium decided to construct a rice T-DNA insertional mutagenesis collection. A T-DNA mutant collection is a library of lines obtained from transformation by *Agrobacterium tumefaciens*, using a specific construct called the *T-DNA insert*. Typically, dozens of thousands are produced (35,000 in this case). A T-DNA mutant collection is a powerful tool for discovering *gene functions* through *functional genomics* studies. As a collaboration between CIAT and Génoplante, a program of seed multiplication and phenotypic analysis of a rice T-DNA mutant collection was initiated. The planned activities were: 1) to carry out greenhouse and field phenotyping of a first set of 5,000 lines; 2) to produce a mutant phenotypic database; 3) to produce T2 entire panicles for further detailed analyzes (Génoplante project); 4) to multiply T2 seeds to constitute a rice T-DNA mutant stock center for future distribution and collaboration with partners.

A list of possible phenotypic traits was established from data mining of several rice phenotypic databases, and was used as a guide for observations. An English-Spanish-French lexical of botanical and agronomic terms was established to facilitate phenotype identification. Seed multiplication of a collection of 5,000 T-DNA mutants of rice was successful, entire panicles will be sent to Génoplante, France and a backup storage will be made at CIAT headquarters. Phenotypic analysis of the collection was conducted, first in the greenhouse and then on transplanted lines in the field. Numerous interesting phenotypes were observed, including modification of size, tillering, lesion mimics, panicle development, general architecture, leaf color, chlorotic or albino. A phenotypic database was set up and will serve for functional genomics studies.

### 10) Rice Genetic Transformation and Biosafety

The use of genetic transformation of rice at CIAT is evolving as a tool for complementing the breeding program as well as for evaluating functional expression of novel genes that are key for other CIAT commodities. Backcross conversion breeding of transgenic rice resistant to RHBV progressed in the field in 2003. Advanced backcrossed or hybrids lines containing the RHBV nucleoprotein gene were selected based on their resistance to RHBV, high fertility, vigor, and yield potential. Some of these lines also showed tolerance to sheath blight in the greenhouse. Genetic transformation for sheath blight resistance, an increasing important disease for which there is not source of genetic resistance in rice, was advanced. Transgenic lines, derived from three generations of self cross from varieties Cica 8 and Palmar containing the PAP gene, showed a stable inherited of sheath blight resistance respect to non-transgenic control and the inter-specific cross *Oryzica 3/O. rufipogon*, when inoculated with the hyper virulent strains of the pathogen. Transgenic rice was also used as a model for functional analysis of a caffeic acid *O*-methyltransferase (COMT) gene cloned from *Brachiara decumbens* and sequenced at CIAT. This gene encodes for a key enzyme in the lignin biosynthesis pathway. This gene could be a candidate to manipulate the lignin

composition of pastures for increasing their quality. Environmental biosafety research is conducted at side of the development of transgenic rice at CIAT. Specific microsatellites alleles were identified and are being used to study red rice genetics, dispersion of red rice genotypes, degree of hybridization between red rice and cultivated rice, and genetic introgression and persistence of domesticated genes in red rice and wild species populations.

### **11) Scaling up cassava genetic modification: Incorporating new genes, cultivars, and regeneration methods, and testing plants in the field**

Genetic modification of cassava will render new varieties with traits valued by small and industrial farmers, as well as consumers. For this to be true, cassava genetic transformation has to be scaled up. Eight cultivars from Brazil, and five  $\beta$ -carotene rich cultivars, are being transformed with marker genes to select the ones that perform the best. New genes for starch modification, to increase the amount of amylopectin in the roots, are now introduced in cassava cultivars. A new variety with herbicide tolerance is being regenerated. Regeneration of transgenic plants via organogenesis is being tested with Brazilian cultivars to reduce time and somaclonal variation. Finally, beginning 2004, plants carrying marker genes, and a Bt gene, will be evaluated in the field for agronomic performance.

### **12) Farmer- produced, certified cassava seed augurs yield boost**

Farmers in Santa Ana, a small village in the Department of Cauca, Colombia, have finally started to evaluate the performance of the cassava plants that they produced *in vitro*. They set up small field trials for six varieties, all adapted to different edapho-climatic conditions of the Department, and all free from frog skin disease. Initial results indicate that each plant can produce between 3.4 to 4.5 kilos of roots, which anticipates an average 20 t/h yield, well above current yields. This advance has been possible thanks to the establishment of an appropriate scheme to produce mother plants *in vitro* at CIAT, transfer and propagate them in Santa Ana to finally test them in farmer's field.

### **13) Tropical Fruits**

A large number of fruits of Andean origin have great potential to become premium products for local and export markets with a high economic return for the farmers. *Solanum quitoense*, locally known as lulo in Colombia and as naranjilla in other countries, is among these fruits. A major constraint for the rapid adoption of naranjilla by the local farmers is the limited availability of elite germplasm free of pathogens from clonal propagation. Rapid multiplication of high quality planting materials is of paramount importance. An efficient protocol was developed for the maintenance and propagation *in vitro* of lulo (*Solanum quitoense*) elite clones selected from farmers' fields. Currently, this protocol is being evaluated jointly with small farmers that are commercial producers of lulo, to test with farmers the suitability of using *in vitro* propagated plants as planting materials.

#### **14) HarvestPlus (Biofortification Challenge Program)**

Project team members continued to play a major role in the formulation, organization and fund raising of the Biofortification Challenge program convened by CIAT and IFPRI. The Biofortification Challenge Program (now called HarvestPlus) was approved in October, 2002. World Bank funding of \$3 million for 2003 was made available in December, 2002.

A first Project Advisory Committee (PAC) meeting was held in March to initiate/approve the selection of the Program Director and to set up/approve initial operating procedures and initial project activities for 2003. A second PAC meeting will be held in November 2003 to discuss/approve the workplans developed for 2004, coming out of the various planning meetings.

Renewed contacts with the Gates Foundation were initiated by CIAT and IFPRI in January, 2003. A formal proposal for 25 millions over four years was submitted on behalf of the CGIAR lead consortium to the Gates Foundations including the submission of the external reviews commissioned by the interim Science Council (iSC) and iSC. While the proposal was approved in Aug 1, 2003 a formal announcement and the launch of Harvest Plus took place in Washington on October 14, 2003. A press release was issued announcing the grant, and a press conference was held in Washington, DC.

Discussions continue with a number of additional donors in the hope of meeting the proposal target budget of \$12.5 million per year over the first four years. Full start-up of the project is envisioned in January, 2004.

Several planning meetings were organized during the year. The most relevant ones were: 1) Planning meeting of core collaborators and selected stakeholders, June 2-6, CIAT headquarters: 75 persons attended the five-day meeting. The planning committee of 15 persons met for the first time as a group for one full day on May 30; 2) Private sector meeting, IFPRI headquarters, July 20: Representatives from DuPont/Pioneer and Monsanto corporations, ILSI, and USAID met with Howarth Bouis and Joe Tohme of the BCP to discuss collaboration. Further meetings are planned for early 2004; 3) Nutritional breeding objectives and Crops meetings: Team members attended the Nutritional Breeding Objectives, and the maize, sweet potato and rice crop meeting and organized the bean and cassava meetings. The project team members are leading the bean and cassava teams and acting as breeding and biotech coordinator.

#### **15) CBN's Activities for 2003**

The Cassava Biotechnology Network for Latin America and the Caribbean (CBN-LAC) is a network of cassava researchers and end-users aiming to enable small-scale cassava farmers, processors and consumers to benefit from advances in cassava biotechnology, enhancing the value of cassava for food security and economic development in the poorest rural areas of the LAC. In this third year (2003) of operation the CBN-LAC devoted to the following main activities: a) Monitoring and guidance for 3 on-going projects and

implementing 11 new projects under the CBN-LAC small grants scheme in 4 countries (Brazil, Colombia, Cuba, and Ecuador); b) Scholarships for postgraduate studies in biodiversity under the Ginés-Mera Memorial Fellowship Fund, which granted 7 awards in the first round; and c) Organizing the Sixth International CBN meeting (CBN-VI), which will take place at CIAT on 8-14 March 2004. CBN also undertook activities in the areas of communication, capacity building of NARS partners and staffing.

## **16) Workshop on Models of Food Safety Assessment of Transgenic Crops**

A workshop was organized by Joe Tohme and Hector Quemada from Crop Technology to assist developing country and the CGIAR researchers in gaining an understanding of the types of food safety assessments needed for the deployment of transgenic crops, and to determine which requirements are appropriate for developing countries. The workshop was held in May, 2003, with funding from the USAID and the Rockefeller Foundation.

The goals of the workshop were to provide researchers and regulators with the means to examine 1) the protocols used in the safety assessments of current commercialized transgenic crops, and 2) the rationale for the requirements imposed (or are likely to be imposed) on crops representative of different transgenic technologies. The workshop focused only on food safety assessment. The participants were from NARS representatives from Brazil, Colombia, Mexico, Egypt, India, Kenya, South Africa, Philippines, Thailand and Uganda; from CG centers: CIAT, CIP, CIMMYT, ICRISAT, ICARDA and IITA and from international Institutions and donors: AATF, AgBios, USAID, Rockefeller Foundation and USDA. Three representative case studies were commissioned to leading experts and were presented at the workshop: 1) Bt potato, 2) virus resistant papaya, 3) mustard oil with enhanced levels of vitamin A.

The outputs of the workshop were the followings: 1) Examination of the protocols used in the food safety assessments of current commercialized transgenic crops; 2) Understanding of the rationale for the requirements imposed (or likely to be imposed) on crops representative of different transgenic technologies; 3) Awareness of the regulatory steps to enable researchers to integrate regulatory issues and experiments related to regulatory approval in their overall research and development strategy; 4) Definition of a roadmap for food safety assessment.

### **e. Problems encountered and their solutions**

Some of the major problems facing the project are similar to the encountered last year (space, *Cassava Frog Skin disease*) while others surfaced during the year (additional charges for infrastructure). Various solutions to the three constraints raised last year (bioinformatics and molecular biology expertise, and access to genes -freedom to operate) were successfully implemented.

***Space availability:*** Lab and offices spaces continue to be a major constraint facing the different sections of the project. The availability of lab and desk space for assistants and students is becoming hard to manage. The purchase of new equipment, funding of new special projects, and the incorporation of a new staff are the major causes of such constraint. The restructuring made last year has

proven to be insufficient. In addition the office space allocated to IRD was lost to the Science Park after Dr. Verdier departure. Her possible reincorporation to the project next year will further put an additional strain on the office and lab space. The project will continue to seek efficient use of the lab space, however contrary to last year, no obvious solution seems in sight.

***Cassava Frog Skin disease:*** The problem endemic to the Palmira station continues to limit the progress of several cassava projects by delaying 1) the planting of the cassava populations from mapping and 2) by putting additional burden on SB-2 tissue culture facility to produce disease free plants for diagnostic efforts and the increase of planting material for new released varieties. It is expected that progress in diagnostic developed by the Cassava and IPM projects will alleviate this constraint.

***Additional charges for infrastructure:*** The project faced this year new requests to cover the use of the greenhouses. Currently the project is paying for the internal maintenance of the greenhouses. While the additional charges are not that high, such unplanned charging for facilities without prior authorization by the project managers is eroding a proper budget management as well as reducing the operational budgets of the project. The project will engage next year CIAT management to understand the nature of the services being charged and to suggest that proper mechanism are in place to avoid unauthorized charging.

#### ***Solutions to constraints raised in 2003:***

***Expertise in molecular biology:*** A new staff, Dr. Manabu Ishitani, was hired to lead gene discovery activities for abiotic stress and nutritional traits. A start up fund allocated by the director of research provided the new staff with needed equipment such as real time PCR.

***Access to genes and freedom to operate:*** Discussions with several private sector companies such as Syngenta, universities in the US and Europe and other CG centers such as CIP were initiated to obtain the freedom to operate for key technologies. More time and efforts will be dedicated next year for this area as part of the Harvest Plus challenge program..

***Bioinformatics:*** Responding the concerns raised last year the director of research re-assigned one of the IT staff to work almost full time for the SB-1 and SB-2 projects. The new staff addition allowed the project to proceed with various activities, setting up of local databases and interacting with other bioinformatics units in the CG. The Lab information management system outsourced last year is proceeding on schedule and a full implementation is expected by early January 2004.

#### **f. Plans for next year**

- Teams members will lead the implementation of the bean and cassava Harvest Plus activities and coordinate the breeding and biotechnology within the Challenge programs. Team members will also pursue a fund raising on behalf of

NARS partners to complement the funds allocated by the World Bank, Gates and USAID. Projects on bean (iron and Zinc) biofortification already funded by USAID will be expanded as part of the Harvest Plus and a beta carotene Cassava will be initiated with new funding.

- Teams members will also interact with the CGIAR genetic resources challenge program and participate in the formulation of the different clusters established in the CP. Members of the project will continue their active participation in the coordination of the Global Partnership for Cassava Improvement and in the organization of the VI International Cassava Biotechnology Network meeting at CIAT.
- As part of the overall strategy of the project, special efforts will be taken to reinforce the integration with breeding of marker assisted selection in bean, cassava and rice. High throughput markers will be tested and implemented. The full implementation of CIAT LIMS and bioinformatics tools will be completed to provide efficient support to the genomic platform.
- The newly established molecular biology section for abiotic stress and nutritional traits will lead within CIAT the effort for the formulation of innovative strategies for genes discovery.
- In the area of transgenic research, the team members will continue to develop the tools for risk assessment and gene flow studies for rice and bean as part of the biosafety risk assessment project. The issue of deregulation of transgenic will be analyzed and a strategy will be formulated to move CIAT product towards field and food safety testing. Public awareness in the area of biotechnology and biosafety for policy makers and journalists will continue.
- In the area of new tools, microarray technologies already implemented and Single Nucleotide Polymorphism (SNP), will be incorporated into the screening of germplasm and marker assisted selection. Microarray and real time PCR analysis will be expanded to gene expression studies to better understand abiotic and biotic stresses. A cassava set of 6000 unigenes will be produced for stress and starch studies. Virus Induced Gene Silencing (VIGs) protocols will be implemented to assist in the gene cloning of disease resistance genes.
- A strategy to integrate CIAT work on biotechnology in Africa with crop improvement will be devised. Biotechnology projects with colleagues in Africa will be developed in the area of MAS in beans and Cassava and training with African NARS will be strengthened. Collaborations with NARS in Latin America will be pursued in the area of genetics, genomics, tissue culture and genetic transformation.

## PROJECT PERFORMANCE INDICATORS

### 1. TECHNOLOGIES, METHODS & TOOLS

**1.1. Genes tagged: more than 14 genes and QTLs identified in bean, rice, cassava and *Brachiaria***

**1.2: Methods implemented:** Microarray, Substraction cDNA libraries construction, Resistance genes analog, Single Nucleotide polymorphism, EST, BAC sequencing, Biosafety field assesement

**1.3 Support Tools: Libraries developed: Gene Libraries/Construct**

#### cDNA

- 13824 clones from cassava roots, variety MPER 297, for isolating carotene pathway genes.
- 1536 clones from cassava stems, varieties MBRA 685 and SG 107-35, subtractive hybridization library was constructed using Differential Subtractive Chain method for isolating of sequences related with cassava defense response to *Xanthomonas axonopodis* pv. *Manihotis*.
- 2688 clones from cassava stems, variety MCOL 1522, subtractive hybridization library was constructed using suppression subtractive hybridization method for isolating of sequences related with cassava defense response to *Xanthomonas axonopodis* pv. *Manihotis*.
- 1440 clones from cassava stems, variety SG 107-35, Subtractive hybridization library was constructed using suppression subtractive hybridization method for isolating of sequences related with cassava defense response to *Xanthomonas axonopodis* pv. *Manihotis*.
- 4992 clones from cassava roots, variety MPER 297, for isolating associated genes with starch content process and 5376 clones from cassava roots, variety CM 523-7, for isolating associated genes with starch content process.
- Early, 7680 clones from cassava (variety CM523-7) roots at 0,6 and 12 hours after harvesting, and late, 3052 clones cDNA library from cassava roots at 24, 48 and 96 hours after harvesting, For isolating the full set of major genes involved in the postharvest physiological deterioration using cDNA microarrays.
- 960 clones from *Brachiaria* roots, hybrid 36062, subtractive hybridization library was constructed using Differential Subtractive Chain method for isolating of sequences related with *Brachiaria* resistance to spittlebug (*Aeneolamia varia*).



- 768 clones from *Brachiaria* roots, cDNA pool from aluminium Tolerant population subtracted from sensitive population, subtractive hybridization library was constructed using Differential Subtractive Chain method for isolating of sequences related with aluminum tolerance.
- 2304 clones from *Brachiaria* roots, cDNA pool from aluminium tolerant population subtracted from tolerant population, subtractive hybridization library was constructed using Differential Subtractive Chain method for isolating of sequences related with aluminum tolerance.
- 1536 clones from *Brachiaria* roots, cDNA from *Brachiaria ruziziensis* subtracted from *Brachiaria decumbens*, subtractive hybridization library was constructed using Differential Subtractive Chain method for isolating of sequences related with aluminum tolerance.
- 768 clones from *Brachiaria* roots, cDNA from *Brachiaria decumbens* subtracted from *Brachiaria decumbens*, subtractive hybridization library was constructed using Differential Subtractive Chain method for isolating of sequences related with aluminum tolerance.
- Forward and reverse subtractive libraries from 3 to 20 days old pistils of seven-bulked apomictic and six-bulked sexual advanced-generation interspecific *Brachiaria* hybrids (*B. ruziziensis* X *B. decumbens* X *B. brizantha*), using suppression subtractive hybridization, mirror orientation selection and differential subtraction chain. Each library contains 384 clones. For isolating differentially expressed genes between apomictic and sexual plants, using cDNA microarrays.

## DNA

- 3840 clones from cassava leaves, varieties TAI-8, Bra383, Fla444-002, Ven 25, TMS 60-444 PER 413-003, MCOL 2246, CM 2177-2 and Ecu 72. DNA digested with *MseI* for evaluating diversity with DarT.
- 3840 clones from cassava leaves, varieties TAI-8, Bra383, Fla444-002, Ven 25, TMS 60-444 PER 413-003, MCOL 2246, CM 2177-2 and Ecu 72. DNA digested with *PstI* for evaluating diversity with DarT.
- 768 clones from potato leaves, varieties 703508, CHS 625, PS 3, 704746, 705428, 700347, DNA digested with *MseI* for evaluating diversity with DarT.
- 768 clones from potato leaves, varieties 703508, CHS 625, PS 3, 704746, 705428, 700347, DNA digested with *PstI* for evaluating diversity with DarT.
- 1152 clones from bean leaves, variety G19833, for isolating Ty1-copia retrotransposon RNase-LTR sequences.
- 3840 clones from bean leaves, variety G19833, for isolating Ty1-copia retrotransposon PPT downstream sequences.

- 70 isolated reads obtained from Genome Walker PCR and direct primer walking on BAC 57-M14 (*Phaseolus vulgaris* Sprite cv.), reaching 20.3 Kb of new sequence. The total 90.3 Kb were distributed in 12 contigs with sizes between 30 Kb – 3Kb and an average 7,3 Kb.

## 1.2. Data Bases united/ improved

- Updating of BeanGene and ACEDB YUCA DB
- Database about wild relatives of crops

## 2. PUBLICATIONS

- 2.1. Refereed Journals: published: 27  
 2.2. Refereed Journals: submitted: 6  
 2.3. Book Chapters: published: 5

## 3. STRENGTHENING NARS

3.1. **Training Courses:** A total of more than 177 persons from 2 national and international institutions received training with SB-2 Project Staff

3.2. **Individualized Training:** 71 SB-2 individual training

3.3. **Visitors:** Approx 400 persons visited the facilities of the project, which amount to 40 percent of the total of CIAT visitors

### 3.4. Current graduate Students

PhD: 7  
 MSc: 5  
 BSc: 7  
 Undergraduate: 10  
 Thesis: 20

## 4.0 RESOURCE MOBILIZATION

*Projects submitted, in preparation and concept notes : 25*

- Combating hidden hunger in Latin America: Biofortified crops with improved Vitamin A, essential minerals and quality protein A collaborative project submitted to CIDA on behalf of a partnership of International Agricultural Research Centers, and National Agricultural Research Systems in Latin America
- Using biotechnology tools and GIS to conserve biodiversity in Colombia

- Development of micro-satellite markers to facilitate use of the cassava
- Molecular genetic maps by African collaborators working on gene mapping resistance to CMD
- Towards the development of industrial cassava varieties: genetic and molecular analysis of early bulking in cassava germplasm collection
- Marcadores moleculares asociados a resistencia a pudrición radical por *Phytophthora drechsleri*, *Phytophthora nicotianae* y *Phytophthora cryptogea* en una población segregante de yuca.
- Sustainable oil palm production as a source of employment and income for rural communities and small-scale farmers in tropical Latin America.
- Molecular Characterization of Genetic Diversity and the Definition of Heterotic Groups in Cassava.
- Applications of Spatial Statistics and GIS to Cassava Bacterial Blight Management.
- Developing and exploiting expressed sequence Tags for cassava Starch and Bacterial Blight Resistance.
- Expanding the range of uses of cassava starch: A source of income generation.
- Seed aid and germplasm restoration in disaster situations: síntesis of lessons learned and promotion of more effective practices.
- Identificación de marcadores moleculares para la resistencia a la enfermedad de la hoja blanca del arroz en programas de mejoramiento.
- Development of an *in vitro* protocol for the production of cassava doubled-haploids and its use in breeding.
- Combating hidden hunger in Latin America: Biofortified crops with improved Vitamin A, essential minerals and quality protein.
- “Comparative genomics and genetics in legumes” a collaborative research Project between CIAT and University of Aarhus, concept note prepared for DANIDA.
- “Phaseomics” wRUIG-GIAN. (Submitted by Univ. Of Geneva with CIAT collaboration)
- “Utilización de hierro y zinc en modelo animal y respuesta clínica al consumo habitual de frijol de alta densidad mineral en mujeres y niños” Submitted by Universidad del Valle (with CIAT) to Colciencias.
- The molecular diversity network of Cassava (MOLCAS).

- Mutagenesis of Cassava (*Manihot esculenta* Crantz) for the generation, identification and Molecular Análisis of Novel traits. Research Contract submitted to the International Atomic Energy Agency (IAEA), Viena, Austria.
- Workplan in sub-programs of the Genetic Resources Project for 2004. Fregene
- Challenge Program “Unlocking Genetic Resources in Crops for the Resource-Poor”.
- Genoplante. Project for phenotypic and characterization of a new series of T-DNA mutants.
- Development and use of inbred lines in cassava breeding. Submitted to the Rockefeller Foundation. New York .
- Development of an In vitro Protocol for the Production of Cassava Doubled-Haploids and its Use in Breeding. Submitted to ZIL, Switzerland.

***Projects approved or on going : 14***

- High through-put genetic diversity characterization of germplasm with a DNA chip. Donor: IPGRI
- Expanding the range of uses of cassava starch: A source of income generation. Donor: USAID
- Development of strategies for better targeting of seed relief and linking relief and rehabilitation. Donor: FAO
- Models of food safety assessment of transgenic crops – Workshop, Donor: USAID and Rockefeller Foundation
- Situations: Síntesis of Lessons Learned and Promotion of More Effective Practices. Donor: IDRC
- Nutritional Genomics. Donor: WB/USAID
- Maize – Vit A Biofortification. Donor: WB/USAID
- Biofortified Crops for Improved Human Nutrition. Donor: WB/IFPRI.
- Bean Genomics for Improved drought Tolerance in Africa and Latin America. Donor: GTZ

- A molecular marker-assisted, farmer-participatory breeding project to improve local cassava varieties in Tanzania with resistance to pest and disease (Rockefeller Foundation)
- Genetic Mapping of the Linamarin biosynthetic genes CYPD1 and D2 and the development of markers for CNP in Cassava, in collaboration with Prof. Birger Moller, Royal Agriculture and Veterinary University, Copenhagen (DANIDA)
- Genoplante. Project for phenotypic and characterization of a series of T-DNA mutants
- Development of protocols for genetic transformation (USAID)
- Expanding the range of uses of cassava starch: a source of income generation (USAID)