BIOTECHNOLOGY RESEARCH UNIT
ANNUAL REPORT 1991

EXECUTIVE SUMMARY

In 1991, the Biotechnology Research Unit (BRU) has successfully continued to integrate efforts with CIAT's programs in the constant pursuit of developing germplasm well adapted to selected agroecosystems, the specific role of the BRU being the application of modern biological and biochemical technology to address critical research constraints in productivity and stress tolerance. The activities of the BRU can be subdivided into the following interrelated areas: (1) characterization and utilization of genetic variability, (2) research on crop productivity, (3) mechanisms of plant adaptation to the environment, (4) institution building.

1. Characterization and Utilization of Genetic Variability

1.1 Molecular Mapping of Crops

A solid basis has been worked out for the upcoming project of the molecular mapping of the **cassava** genome. A basic set of polymorphic markers from cassava genomic libraries has been selected within the frame of an IBPGR sponsored project. Now, with support from the Rockefeller Foundation, we are going into the generation of the linkage map using these markers. The map will be useful in the characterization of genetic variability in cassava, and will assist in future breeding projects.

A hundred clones from a common **bean** cDNA library have been selected to complement the existing bean maps generated by C.E. Vallejos (Gainesville, FL) using genomic clones (this map contains about 200 markers by now), and P.Gepts (Davis, CA). The saturation of the map is an important goal, as it is a prerequisite to get tight linkages in future gene tagging projects. The level of polymorphism of the selected clones is currently being analyzed using selected parental lines. The genome of tepary bean will be mapped as part of a project, in collaboration with the University of Ghent, Belgium, which includes the characterization of resistance factors against bruchids in beans. RFLPs (Restriction Fragment Length Polymorphism) as well as RAPDs (Randomly Amplified Polymorphic DNA) will be used for this purpose.

The genome size is an important parameter in mapping studies. In a collaboration with the Rice Program (C. Martínez) and the University of Cornell, flow cytometry was used to assess nuclear DNA contents of *P. vulgaris*, *P. acutifolius* and *P. lunatus*. The genome size seems to be smaller than expected. Differences were observed between wild and cultivated accessions, while no relationship was found between genome and seed size, as had been speculated.

The mapping of Brachiaria has been recognized as a high priority project by the **Pastures Program**, apomixis being one of the targets for gene tagging. Contacts have been established with the Salamini group at the Max-Planck-Institute in Cologne for this mapping project. We will start by selecting parentals and providing a set of polymorphic markers. The RAPD technique will be used to generate a primary map.

1.2 Molecular Fingerprinting of Pathogenic Microorganisms

The fingerprinting of **rice blast** (*Pyricularia oryzae*), which is a cooperation between **Rice Pathology** and Purdue University, has been a very successful project, opening the doors to detailed studies on genetic variability and stability of the rice blast fungus.

Another very successful ongoing project is the fingerprinting of *Xanthomonas campestris pv phaseoli* utilizing molecular probes, developed through a cooperation between **Bean Pathology** and Wisconsin.

Both projects are being continued at CIAT in close collaboration with the BRU. The results achieved so far and the experience gained by the programs have led to new initiatives concerning three fungal pathogens, which are the causative agents of Angular Leaf Spot Disease and Anthracnose in **beans**, and Anthracnose in **Stylosanthes**, respectively. The high degree of observed variability of these pathogens justifies the efforts being undertaken. A better understanding of the epidemiology and phylogeny will assist in the design of new resistant varieties and integrated pest management.

1.3 Cryopreservation

Cryopreservation has reached a very advanced stage now. We are able to regenerate up to 70% of material stemming from frozen shoot tips. The experimental development of this IBPGR sponsored project was successfully finished this year. We have gone now into testing the applicability of the methodology to multiple genotypes of the collection. The importance of this project resides in the stable long-term storage of such a unique germplasm collection as CIAT's, thus solving a logistic problem and conserving potentially useful germplasm for future breeding programs.

2. **Crop Productivity Research**

2.1 Gene Tagging

The final application of any genetic map is its use in breeding. We have been very successful in using molecular markers from the **rice** map (acquired from S. Tanksley, Cornell University) to map resistance loci for the Rice Hoja Blanca Virus (RHBV) and Rice Blast. For tagging resistance genes to blast, bulk analysis of susceptible and resistant doubled haploids was performed. The RAPD markers linked to blast resistance are currently being checked on larger F2 populations and additional doubled haploid lines from the same cross. We have gone very early into the utilization of newly developed techniques, e.g. RAPD, which are very promising as to their direct applicability in breeding programs because of their simplicity and their potential to handle large numbers of crosses, a major constraint to other technologies.

In **beans** we are aiming at tagging the resistance loci against BGMV and the Common Bean Weevil. Another important issue will be the merging of the **bean** maps generated by Vallejos (Gainesville, FL) and Gepts (Davis, CA), which will lead to a more saturated map. The RAPD technique, combined with the bulk screening method, is currently being used to tag the resistance locus to the Kappa race of anthracnose.

2.2 Interspecific Hybridization

The introgression of tepary bean (*Phaseolus acutifolius*) characteristics into common bean using a congruity backcross approach, has advanced further this year. The fourth congruity backcross generation (CBC4) has now been crossed with *P. vulgaris* and the first resulting embryos from that cross are presently being grown in vitro. The goal of this recurrent crossing scheme, is to force recombination between the two genomes as to attain fertile and commercially useful plants with important tepary bean traits, like drought and heat tolerance, as well as *Empoasca* and bacterial blight resistance. We have already morphological and biochemical evidence for stable introgression of tepary bean into common bean.

2.3 Genetic Transformation

Genetic transformation is a prerequisite for the non-classical introduction of useful traits into plants. Such traits include at the moment genes for insect resistance, protection against viral infection, and herbicide resistances. Sources of resistance against fungal pathogens, genetic manipulation of protein contents, genes leading to cold-tolerance, these are some of the upcoming new possibilities for the future.

The forage legume ***Stylosanthes guianensis*** is CIAT's first transgenic plant. It was transformed using a construct which includes two selectable marker genes that confer resistance to an antibiotic and a herbicide, and a scorable marker gene whose expression can be detected by a histochemical assay. Regenerated plants have conserved their herbicide resistance trait. The Mendelian segregation of the trait is presently being studied in the F1 progeny in the greenhouse.

Another pasture, ***Brachiaria***, is passing through the first step that is needed for successful transformation: regeneration. *Brachiaria* has responded very well to preliminary experiments dealing with somatic embryogenesis, thus we can now start phase number two, which is the introduction of foreign DNA and selection of the transgenics. Another crop that is responding well to preliminary regeneration experiments is **rice**. In a collaboration between the **Rice Program** and the BRU, viable protoplasts have been successfully isolated from indica type rice embryogenic suspension cultures stemming from mature seeds. Regeneration experiments are underway. Protoplasts are amenable to direct DNA transfer, and have been used to produce transgenic rice of the japonica type.

In **beans** we have adapted a powerful regeneration protocol starting from cotyledonary nodes. The organogenic meristematic ring in the cotyledonary area will be a target for Agrobacterium-mediated or direct DNA transfer. Virulent Agrobacterium strains have been selected after a meticulous screening procedure, as well as susceptible bean cultivars. Adventitious shoot formation has been histologically demonstrated. Transient gene expression of a scorable marker gene has been detected after infection with *Agrobacterium* and after particle bombardment with DNA coated tungsten microprojectiles.

Cassava can be regenerated from secondary embryos forming on embryogenic calli stemming from apical meristems. This is presently our target tissue for transformation experiments using *Agrobacterium* and the particle gun.

2.4 Doubled Haploids

The incorporation of pollen derived doubled haploids into breeding programs reduces the time to obtain fixed lines to two generations of selection without affecting genetic variability and stability. The **Rice Program** is using anther culture to introgress early grain maturity and good grain quality into cold tolerant germplasm, to increase the recovery of useful recombinants from wide crosses, to facilitate the transfer between savanna and irrigated materials, and to produce fixed lines for the mapping of resistance gene loci. We have been able to achieve a 35 fold enhancement in the production of green plants from indica types, which had proved unresponsive to anther culture up to now. This has been a constraint, particularly in the breeding for irrigated rice.

2.5 Somaclonal Variation

14 ***Stylosanthes guianensis*** somaclonal lines generated through in vitro regeneration have been tested for their agronomic performance. A main goal is the search for plants tolerant to acid soils. Some of the lines have shown a superior agronomic performance than the check, others have shown some special morphological characteristics, like dwarfism, chlorotic foliage, 1-2 leaflet leaves, and one tetraploid line. These traits have shown to be inheritable through four generations.

2.6 Utilization

Another topic that is related to productivity, is the fermentation of **cassava** starch to produce bitter starch. This artisanal product is very important for Colombia, and needs input with respect to well defined parameters and possibly inocula, to enhance starch quality and reproducibility of the process. Together with the Cassava Utilization Unit in a collaborative project with CIRAD/CEEMAT (France), the BRU has been working on the characterization of microbial amylolytic activities involved in the process. This results, added to the studies being done at the microbiological and physicochemical levels, will provide a more accurate picture of the fermentation process, which is not a simple one, as we are dealing with a solid state fermentation and mixed microbial populations.

3. Mechanisms of Plant Adaptation to the Environment

3.1 Resistance to Pests

A major constraint in **bean** storage is the bruchid *Acanthoscelides obtectus* or Common Bean Weevil. Very few resistant accessions have been found in wild beans collected in only a small number of locations in Mexico. Resistance has also been found in wild lima and tepary beans. A few years ago resistance to the Mexican Bean Weevil (*Zabrotes subfasciatus*) was found in a similar way. After identification of the factor involved, a protein from the lectin family named arcelin, an immunoassay was developed that accelerated breeding enormously. We have set for the identification of the factor involved in the resistance to the Common Bean Weevil. Some promising results have been obtained with a protein fraction stemming from the resistant accessions; artificial seeds enriched with this fraction lead to high mortality rates in insects feeding on them. Other approaches include the analysis of inhibitors of digestive enzymes, as well as other types of biomolecules, using separation techniques like HPLC and other analytical tools, combined with enzymology and feeding experiments.

In a similar way we are looking for an antibiotic factor involved in the resistance against the spittlebug in ***Brachiaria***, together with the **Pastures Entomology** group. A phytoecdysteroid has been postulated as the putative resistance factor, due to the observed effects on the insect, which resemble ecdysone action on the development of the insect. We are trying to develop an immunoassay for positive identification of the substance as well as for the development of a screening procedure to assist in breeding programs.

3.2 Photosynthesis

Cassava is well known for its drought and heat tolerance. Together with the **Cassava Physiology** group, we are interested in the adaptation mechanisms of cassava for dealing with this climatic constraints without loosing productivity. Cassava has shown some photosynthetic characteristics that range between typical C3 and C4 plants. Additionally to the physiological experiments being performed, we want to analyze possible compartmentalization of photosynthetic enzymes in the leaf by in situ hybridization, using labelled gene probes. We have shown that a high degree of homology exists between maize and cassava photosynthetic genes by hybridization techniques (maize clones from T. Nelson, Yale). We are in the process of generating a genomic cassava library from where we want to fish out the correspondent cassava clones, as heterologous probes are not useful for in situ hybridization. A mechanistic explanation of the phenomenon will aid us in searching for better-performing genotypes in the cassava collection, as there is variation of the physiological parameters among them.

4. Institution Building

4.1 Networking

Financiation of the Advanced Research Network for Cassava by the Dutch Government has been confirmed. The budget includes the position of a coordinator, who will be placed at CIAT, the publishing of a newsletter, and bridging funds for the initiation of projects. A meeting of the members of the network is scheduled for August 1992 in Cartagena.

Some members of the Steering Committee of the Advanced Phaseolus Beans Research Network (founded in September 1990) met at the BIC Meeting in

November in Nebraska, to discuss the needs of the network. A recommendation was made for CIAT to actively seek for potential donors for the network.

4.2 Training

Four PhD students have been doing practical research at the BRU. **Alvaro Mejía** (Bonn University, FRG, funded by the BMZ), who is working on the tepary bean introgression into common bean using congruity crosses and embryo rescue techniques, and on regeneration from embryogenic suspension cultures. **Martine Korban** (McGill University, Montreal, funded by IDRC), who has been working on bean regeneration and transformation. **Bill Welsh** (University of Manitoba, Winnipeg, funded by CIDA) is working on the characterization of recombinant inbred lines from crosses between Mesoamerican and Andean gene pools. **Rodrigo Hoyos** (Michigan State University) spent some time working on regeneration of bean plants from embryogenic suspension cultures.

It is already a tradition for biology and agronomy students from the Universidad del Valle and the Universidad Nacional de Colombia, Seccional Palmira, Universidad de San Buenaventura and Universidad Santiago de Cali to do their thesis research at the BRU. Their one year research work usually reaches levels of MSc thesis elsewhere.

The BRU has organized introductory courses to Molecular Genetics for CIAT's programs as to acquire a common language that will facilitate exchange of ideas, and to make clear what kind of contributions the BRU could make to their specific problems. On the other hand, we have been offered introductory courses to different areas of the programs, like breeding and pathology, for the same purpose. Courses have also been held at the Universidad Nacional in Bogotá and Medellín.

4.3 Biosafety

The BRU assumed a major responsibility in the editing of the Institutional Biosafety Guidelines. These have been approved by external highly qualified reviewers, thus representing the state-of-the-art situation of biosafety regulations in the world. We are already in contact with representants of the Colombian government, in order to develop national regulations in the spirit of reaching uniform regulations all across Latin America, observing the highest standards of developed countries.