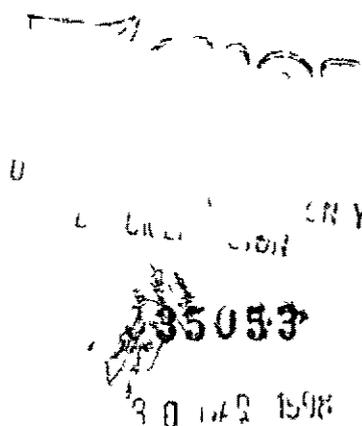




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GENOME RESEARCH IN AGROBIODIVERSITY AT CIAT

Prepared by Agrobiodiversity Team of CIAT



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GENOME RESEARCH IN AGROBIODIVERSITY AT CIAT

SUMMARY

Research on agrobiodiversity assessment, conservation, and enhancement is a central element of CIAT's response to the challenges posed by current concerns about agricultural productivity and competitiveness and natural resource conservation. Given CIAT's historical and current strengths, the Center is well positioned to undertake a genetic-based approach to agrobiodiversity research. Utilization of modern genetic tools at the molecular and cellular level is considered central to CIAT's agrobiodiversity research. The focus of the research will be the characterization and modification of genomes and the conservation of genetic resources. Thematic research on agrobiodiversity with CIAT's mandate crops will form the basis for extending genomic methodologies to other promising tropical crops. General themes of this research include assessment of genetic diversity and identification of useful genes, modification of plant genomes for broadening crop genetic bases and improving germplasm conservation strategies. Special attention will be given to wild relatives and land races as sources of genetic variability for improving economic traits. Agrobiodiversity research at CIAT will comprise two projects: Conservation (SB-1) and Genome Research (SB-2) with their corresponding research infrastructures: the Genetic Resources Unit to support project SB-1 and the Genome Research Laboratory to support project SB-2. To house genome research on crops outside CIAT's mandate, the development of a Biodiversity Regional Lab is proposed.

Introduction

The need to expand agricultural production at a rate that exceeds population growth, probably with declining water and land resources, is a major concern of agricultural research and development. A related concern is the urgency of adopting agricultural practices that favor biodiversity conservation, particularly in tropical countries (Srivastava, et al 1996). Additionally, the globalization of economies requires increased agricultural competitiveness through enhanced productivity, product diversification, and value adding.

In responding to these challenges, CIAT has integrated research in genetic resources with research on the management of natural resources (CIAT, 1997). The characterization, conservation, and utilization of agrobiodiversity are key to the Center's strategies for increasing crop productivity while enhancing diversity.

Biodiversity can be addressed from different perspectives, requiring the integration of many areas and disciplines. CIAT's approach to the conservation and utilization of the biological resources that support agricultural production (agrobiodiversity) should be based on its historical record and current strengths. Hence, we believe CIAT is well positioned to develop a successful genetic-based approach to agrobiodiversity research, integrating diversity at the genome level with that at the agroecosystem level.

Using the modern tools of molecular and cellular genetics, we propose to concentrate our efforts on the assessment and modification of plant genomes, as well as the massive propagation of material to develop improved strategies for germplasm conservation and to broaden crop genetic bases. We propose that CIAT undertake thematic research on agrobiodiversity, using its mandate crop germplasm and extending genome methodologies and tools to other promising tropical crops. This effort will involve projects SB-1 and SB-2. Enhanced cooperation with other CIAT projects and partners in developing and developed countries will be necessary to effectively link genomic research with field applications.

Justification

Although most biological diversity is potentially relevant to agriculture, genetic diversity at the intra- and inter-specific levels offers immediate value for improving agriculture. A large proportion of economically useful genetic variation has yet to be tapped among the wild relatives, land races, and folk varieties of most crop plants (Tanksley and McCouch, 1997). Therefore, through its agrobiodiversity research, CIAT proposes to assess these resources (from the genome to agroecosystem level) and to conserve and modify them for enhancing crop germplasm. Recent developments in genetics and biology have added new dimensions to our ability to carry out these tasks. This also affects our priorities in terms of investments and timing.

Molecular technologies permit the direct characterization and modification of genotypes, making it possible to solve intractable problems and modify specific traits with greater precision and speed. A gene-based approach is the most effective way to deploy and distribute germplasm, for example, inherent traits can improve the utilization of seeds with minimum inputs. The preservation of natural resources, including the conservation of agrobiodiversity *in situ* and *ex-situ*, is necessary for a genetic approach, but not sufficient, unless it is strongly linked to the utilization of these resources.

Another important benefit of a genetic approach to agrobiodiversity research is that it addresses the narrowing of the genetic base of many crops resulting from events during domestication and from modern agricultural practices. As a result, modern cultivars have become more susceptible to pests and diseases. The possibilities for incorporating new gene combinations from wild relatives or related cultivated species have now been greatly enhanced with the development of DNA-based technologies. This is particularly relevant for agronomic traits such as yield potential and quality.

Building on strengths

Background

For the last 20 years, CIAT has engaged in a global effort for genetic improvement of cassava, common bean, tropical forages, and rice. In support of this research, the Center maintains the world's most complete collections of the genetic resources of cassava, common beans, and tropical forages. The contrasting reproduction and breeding systems, growth cycles, life spans, and other characteristics of these species have contributed to a broad base of knowledge on their conservation and utilization. These include the use of contrasting domesticated gene pools, wild species, and interspecific crosses, all of which require creative strategies for the utilization of diversity. We propose to undertake agrobiodiversity research at the genome level that builds on this experience and takes advantage of CIAT's unique capacities and infrastructure for research using DNA and cellular technologies.

By bringing this experience to bear on neotropical agrobiodiversity, our work should bridge the gap between biodiversity inventorying, which is currently going on in most countries, and the eventual utilization of that diversity. CIAT has much to offer toward a better understanding of genetic diversity and better strategies for making this resource more productive for the developing world. CIAT's particular niche is in ecoregions of the

neotropics where its mandate crops originated and where it has long experience with plant genetic resources in collection, characterization with agronomic and molecular techniques, conservation, geographic information systems (GIS), and genetic improvement

Main research achievements

At an early stage in its work, CIAT appreciated the value of biotechnology for conservation, characterization, and utilization of plant genetic resources. The Center's past contributions in this area include

- Studies on patterns of diversity and domestication in *Phaseolus*, *Manihot*, and tropical forages (Debouck, et al 1993), using classical taxonomy and phylogeny and more recently molecular techniques (Kamari, et al 1995, Tohme, et al 1996)
- Studies on the genetic structure of crop gene pools with molecular markers, leading to the establishment of core collections of beans and cassava (Tohme, et al 1996, Roa, et al 1997)
- Spatial distribution of diversity, based on the association between passport information on the collections and climatological data, using GIS for *Phaseolus*, *Manihot*, and forages (Jones, et al 1997)
- Methods of germplasm conservation, using seed banks and *in vitro* banks, including cryopreservation, developed mainly for beans and cassava (Escobar, et al 1997)
- Interspecific hybridization as a means of broadening the crop genetic base, carried out with *Phaseolus* (Mejia-Jimenez, A et al 1994, Singh, et al 1997)
- Use of anther culture to accelerate the production of improved plant types from inter-subspecies and inter-species crosses in rice (Lentini, et al 1995, Perez-Almeida et al, 1995)
- Methods for gene transfer, using genetic transformation, developed for cassava, rice, and forages such as *Stylosanthes* and *Brachiaria*, and being developed for beans (Sarría, et al 1994, Lentini, et al 1996, Sarría, et al 1996, CIAT 1996)
- Exploitation of wild relatives of *Oryza* and *Phaseolus* as sources of useful traits through back crosses, aided by QTL analysis, using molecular markers (Martinez, et al 1997)
- Gene tagging of economic traits in cassava, using the molecular map constructed at CIAT (Fregene, et al 1996)
- Genetic fingerprinting of blast pathogen populations and tagging resistance genes to pathogen lineages (Levy, et al 1993)
- Some of these activities have also been carried out on a few non-CIAT mandate species, such as passifloras, chili peppers, and plantain

CIAT has been eager to monitor and participate in the international agenda on biodiversity and genetic resources. Rio 1992 and Agenda 21, the Convention on Biological Diversity, inputs to the CBD SSBSTA on technology transfer, the Latin American preparatory meeting for the FAO Leipzig Conference, and the Global Plan of Action on Genetic Resources. CIAT has also participated actively in biotechnology, biosafety, and related activities at the Latin American (REDBIO) and international (The RF Rice Biotechnology Network, the Cassava Biotechnology Network) levels and more recently in the CGIAR initiative on biotechnology

The experience described above provides a solid background and justification for our proposal to strengthen agrobiodiversity research at CIAT

Research focus

Themes

A thematic focus in agrobiodiversity research will allow us to deal with strategic issues that are relevant across plant species and countries. Some areas of strategic genomic research in agrobiodiversity include

- Genomic research on diversity at the intra- and inter-species levels and its relationship to populations in agroecosystems and to their geographic distribution. This information in turn can be used to improve the conservation of genetic resources *ex-situ*, linked with *in-situ* conservation
- Genotyping of germplasm accessions and genetic analysis of populations, with the use of molecular markers, leading to the characterization of gene pools and the identification of qualitative and quantitative genes responsible for economic traits. Marker-assisted selection (MAS) schemes can be developed for integration into crop pre-breeding strategies
- Single-gene cloning has already extended the realm of useful variability for broadening the crop genetic base beyond sexual compatibilities. Genome mapping, modifications of genes through genetic engineering, and more complex genetic transformation will include quantitative traits. This will open the way to more effective utilization of wild germplasm
- The contribution of agrobiodiversity to productivity and conservation will very often require the massive multiplication of existing or genetically improved germplasm. Modern tissue culture and bioreactor technology, associated with the use of synthetic seed production, can be applied

Germplasm

CIAT has accumulated a wealth of experience in the biology, genetics, and agronomy of its mandate crop germplasm

- The wild species of *Manihot* as well as other *Phaseolus* cultivated spp and their wild relatives and wild *Oryza*, will receive more attention at CIAT as sources of genes for valuable qualitative and quantitative traits
- The diversity of breeding systems, including apomixis, makes it possible to draw implications for the conservation of alien species. Through comparative genome mapping in cassava, common bean, or rice, one can infer genetic structure and localization of homologous traits in other *Euphorbiaceae*, other *Phaseolus* species, or other grass species, such as *Brachiaria* (CIAT, 1996)
- Over the last few years, CIAT has engaged increasingly in research activities on crops outside the Center's mandates. In most cases, this work has resulted from requests by partners in national organizations, and it has taken the form of counseling, training, and sharing of facilities. Latin American and Caribbean countries are developing research

agendas that respond to economic globalization and the deregulation of markets, including those for tropical fruits and horticultural crops. As a consequence, there is a steady increase in requests for our involvement with nonmandate crops. CIAT's experience would be quite valuable in collaborative research on the further domestication of less known plants and their improvement. This is also the case with the domestication of wild plants, whose basic biology and genetics are not well understood and for which comparative genome mapping could save time.

- We believe our work should consist in extending CIAT's strategic research and tools to similar themes in other species. Our involvement should focus on specific research topics, and it should be supportive. Our strength lies in establishing the knowledge base for the breeding efforts of our partners.
- Finally, CIAT must maintain an international focus, i.e., the themes and outputs of our genetic approach to agrobiodiversity should be relevant at least on the regional level.

Research organization

Mission

To contribute to the increased productivity of food crops and the preservation of the natural resource base through research on the assessment, conservation, enhancement, and diffusion of tropical agrobiodiversity. Working in partnership with a range of national institutions, we are committed to integrating molecular and cellular genetic approaches in agrobiodiversity research.

Objectives

To identify, document, and collect genetic diversity of CIAT mandated crops, while maintaining the existing collections at CIAT in trust for FAO under international standards.

To improve or develop conservation techniques, integrating conventional and modern biological technologies and focusing on *ex-situ* collections of mandate germplasm, with linkages to *in situ* conservation on-farm or in protected areas.

To assess and characterize the structure and diversity of genetic resources of wild and cultivated mandate species, selected nonmandate species, and associated organisms (e.g., pathogens, arthropods, and symbionts) through the use of analytical genomic technologies and agroecological information.

To enhance the diversity and broaden the genetic base mainly of mandate and selected nonmandate crops through the use of cellular and molecular gene transfer technologies.

To generate indicators of gene flow between cultivated and major outcross relatives in ecological settings, with the aid of genome analysis and GIS.

To make genetic resources, databases, genetic stocks, molecular maps, probes, strains, and pertinent information available to users at CIAT and in partner institutions.

To collaborate with CIAT partners in organizing and conducting conferences, workshops, and training activities on agrobiodiversity assessment, conservation, and enhancement through genomic approaches

Outputs

- CIAT mandate germplasm collections maintained with state-of-the art conservation techniques and globally available Germplasm samples tested for freedom from major pests and pathogens
- Distributed neotropical genetic resources better known at the species and genetic levels
- Improved germplasm conservation methods, using seeds and field techniques
- Improved germplasm conservation methods, using *in vitro* and cryopreservation techniques
- Genetic structure characterized within and between gene pools of *Phaseolus* and *Manihot*, using molecular markers
- Genetic diversity of cassava bacterial blight pathogen characterized and resistance genes identified, using molecular markers
- Genetic diversity of rice blast pathogen characterized and resistance genes identified, using molecular markers
- Molecular mapping of apomixis gene(s) and genes for resistance to spittle bug in *Brachiaria*
- Agroecological information integrated with genetic diversity, using GIS and molecular markers (beans, cassava, and tropical forages)
- Transgenic rice with resistance to RHBV field tested in Latin America and incorporated into IPM schemes
- Novel genes (Bt, proteinase inhibitors, etc) accessed and utilized in IPM schemes for insect resistance by genetic transformation in cassava, beans, rice, and *Brachiaria*
- Novel genes (starch quality, carbohydrate metabolism, postharvest conservation, seedling vigor, etc) accessed and utilized for quality modifications, using genetic transformation of cassava, beans, and rice
- Apomixis gene(s) isolated and cloned for homologous (*Brachiaria*) and heterologous (other crop spp) transformations
- QTLs for yield and quality traits identified and utilized in rice, beans, and cassava
- Useful traits (biotic/abiotic stress resistance) transferred through interspecific hybridization in *Phaseolus*
- Field studies of risk assessment (biosafety) organized with the cooperation of the biotech private sector and NARS
- Cellular and molecular genetic techniques developed for assessing and enhancing agrobiodiversity (novel DNA markers, biomolecular statistics, genetic engineering)
- Genetic material (plant stocks, microorganism strains, etc) distributed to partners
- Databases, maps, probes, strains assembled and made available to partners
- Capacity building activities in conservation technologies and processes organized with national partners
- Awareness programs and capacity building of NARS organized for assessing and enhancing agrobiodiversity through molecular and cellular techniques

Partnerships

As described above, CIAT's agrobiodiversity research will focus on genomic research for the assessment and modification of germplasm. Linkages with other CIAT projects, and with partners outside the Center, will be necessary to relate genomic information and technologies with research in agroecosystems. In developing partnerships, issues such as technology transfer, cooperation with the public and private sector, and intellectual property rights (IPR) are often involved.

Research linkages The immediate partners of CIAT's agrobiodiversity research are Center scientists involved in projects focusing on productivity, integrated pest management, land use, participatory research, and regional cooperation. Cooperation with NARS and other developing country institutions will preferably take place through projects. Shared organization of conferences, workshops, and training courses will be a strong component of this cooperation.

Through networks, such as the Cassava Biotechnology Network, the *Manihot* Genetic Resources Network, and the Rice Biotechnology Program of the Rockefeller Foundation, end user perspectives and priorities will be obtained and analyzed. Such information will be incorporated into CIAT's priorities for agrobiodiversity research through a dynamic, on-going process that is closely monitored.

Public Advanced Research Institutions (ARIs) in developed and developing countries will be a first source of basic information and technologies for CIAT's agrobiodiversity research. A number of such partnerships have been established already with biotechnology ARIs. These will be expanded to include biodiversity organizations in and outside the region.

Private sector CIAT has cooperated with the private sector in biotechnology research through the promotion of "public good" activities with some companies, involving the transfer of material, genes, etc. Future cooperation with the private sector can take a variety of forms. One would consist of developing "business plans," in which the end products(s) and end user(s) are identified first. From there all the necessary inputs (e.g., technologies, materials, operational budget, personnel, additional partnerships, etc.) would be worked out back to the starting point. Many research topics can be adjusted to this mode of cooperation (e.g., cassava starch modification). Under this mode of cooperation, a long-term project can be divided into interdependent but self-contained phases. Thus, funding for a given phase would depend on successful completion of the prior phase. Funding could be variable in source and amounts. Another mode of cooperation with the private sector would involve "outsourcing" jointly with companies for specific services, such as large-scale sequencing, gene construction, etc. In this case funding would come from developed country public agencies. With a third mode of cooperation, CIAT would offer services related to specific technologies or processes to the private sector (mostly national or regional). Contractual arrangements would be necessary.

Intellectual property rights (IPR) This issue is unavoidable in developing partnerships in the current global environment. Increasingly, private as well as public institutions in developed and developing countries request assurance that their IPR be honored and that mutual research will somehow be protected. Regarding genetic resources, CIAT follows the international agreement with FAO to maintain the designated germplasm collections in trust for the world.

community, and we make these collections freely available upon request. Distribution is subject to material transfer agreements with the recipient. The recipient agrees not to appropriate the genetic material or its derivatives.

CIAT is updating its IPR policy. It will allow the establishment of strategic alliances for biotechnology research, involving intellectual property arrangements, if necessary, to ensure that the results of its research reach the intended beneficiaries in developing countries.

Biosafety Because familiarity with genetically engineered organisms is still incipient, there is a need to closely monitor the performance of transgenic crops upon their release into the environment. This is particularly important in the tropics where cropping is continuous, with more diseases and pests, and where biodiversity is highest. CIAT's biosafety guidelines and committee have been in place since 1991. No field testing of transgenic plants will be conducted at CIAT until the Colombian regulatory framework is in place. Current activities by Colombian institutions indicate that the biosafety regulations are forthcoming.

In the area of biosafety there is an increasing need to generate basic field information on risk assessment with particular attention paid to gene flow analysis. This is a strategic issue with implications across crops and countries. There is a need to validate the information generated in temperate areas under tropical conditions. The outputs of this work will be essential for each country's decision regarding the level of risk acceptance and the management of risk that follows for a given location. CIAT can contribute to this task through collaborative research involving NARS in the region and private sector institutions. Strong capacity building and awareness of agrobiodiversity and biotechnology will be included.

Structure

The agrobiodiversity research area at CIAT will comprise two interdependent projects:

1. Project SB-1: Conservation of neotropical genetic resources. This project will have basic responsibilities, such as the maintenance, documentation, and distribution of the world germplasm collections at CIAT and the development and use of techniques to test germplasm accessions for pests and diseases. This project will conduct research aimed at developing information strategies and techniques for genetic resource conservation, and it will contribute to capacity building in genetic resources conservation in tropical countries.

The project will concentrate on linking *ex situ* collections with *in situ* conservation by providing scientific input into on-farm management of landraces and into studies on the significance of domestication transitions, such as weed-crop complexes, and on the distribution of wild relatives for inclusion into protected areas and gene reserves. It will test protocols for reliable and affordable conservation of seeds of tropical species, including, where appropriate, *in vitro* and cryogenic techniques.

The Genetic Resources Unit, including its facilities and operations, will be the operational structure supporting Project SB-1.

2. Project SB-2. Given that the potential of biotechnology has not yet been realized for cassava, common beans, and tropical forages and that significant advances have been

made in rice, we propose to rename the current SB-2 project Genome Research for Promising Tropical Crops

The domain of this project includes basic responsibilities at CIAT, such as monitoring advanced research in plant molecular and cellular biology worldwide, bringing to CIAT and developing countries selected outputs with potential pay-off in terms of applications, "outsourcing" of special services in public and private ARIs (e.g., large-scale sequencing, genetic constructs for transformation, etc), biosafety in DNA research and testing of transgenic plants, and updating and training CIAT personnel in biotechnology

Research The genomic work carried out in this project will be linked through projects with the research of national institutions in developing countries. In dealing with nonmandate crops, the role of project SB-2 will consist of providing the necessary biotechnology inputs for integration into crop improvement at the national and regional levels

This project will use molecular/cellular genetic tools for assessment, modification, and mass propagation of agrobiodiversity in research areas such as analysis of diversity and relationship with the spatial distribution of genetic resources, genotyping genetic resources with value in prebreeding strategies, identification and localization of genes, gene combinations, and chromosome parts responsible for agronomic traits, development of marker-assisted-selection strategies, genetic transformation using single genes first, later on, more complex transformation using map-based cloned genes, clonal multiplication of plants by bioreactor and artificial seed technologies

Lab Infrastructure to support project SB-2 We propose to change the name of the Biotechnology Research Unit to the Genome Research Laboratory. This lab will support Project SB-2, and related research under project SB-1 and other CIAT projects by housing operational activities in basically three research areas

- (i) genome characterization of plants and associated organisms (pathogens, arthropods, and symbionts), using molecular genetic markers and maps,
- (ii) genome modification of plants, using cellular and molecular biology and genetic engineering techniques,
- (iii) clonal propagation of plants, using cell and tissue culture, and related technologies

Infrastructure to support regional cooperation This requires a lab for genomic research on crops outside CIAT mandates. The lab would provide a center for cooperation with partners in Latin America and the Caribbean. Institutions such as the A von Humboldt Institute in Colombia, Inbio in Costa Rica, the Smithsonian Institute in Panama/USA, and numerous NARS in the region would be the users of this facility. The Biodiversity Assessment Regional Lab would be implemented and equipped through joint partners' contributions. The lab will benefit from links to the CIAT Genome Research Lab, and likewise, project SB-2 will benefit from facilities/equipment of the Regional Lab

Operations under the new structure would start in January 1998

The proposed changes reflect a more sharply defined research focus. The changes will allow CIAT to extend its thematic research to include other promising crops. Hence, phasing out some current activities and upgrading current facilities and resources are envisaged. Research with crops outside CIAT's mandate will be possible only with additional resources.

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