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BIOTECHNOLOGY RESEARCH AND
COOPERATION AT CIAT

AN INVITATION



A photograph of a lush tropical landscape. In the foreground and middle ground, there are numerous banana trees with large green leaves and some yellowing, ripe fruit. A small, simple hut with a thatched roof is nestled among the trees in the lower right. The background is a dense forest of tall trees. The overall scene is vibrant and green, with some brownish-yellow patches of soil or fallen fruit visible.

THIS IS AN INVITATION TO
AGRICULTURAL RESEARCHERS IN
DEVELOPING AND DEVELOPED
COUNTRIES.

We want to collaborate with more of you in applying the powerful tools of biotechnology to preserve, study, and use plant genetic diversity. Through effective management of this diminishing natural resource, we can confront together the most pressing challenges of agriculture today.

THE BALANCING ACT

Over the last 30 years or so, the single most urgent task of agricultural researchers in the tropics has been to help farmers put food on the developing world's table. Now, we've got to work with them toward a balance between three competing objectives:

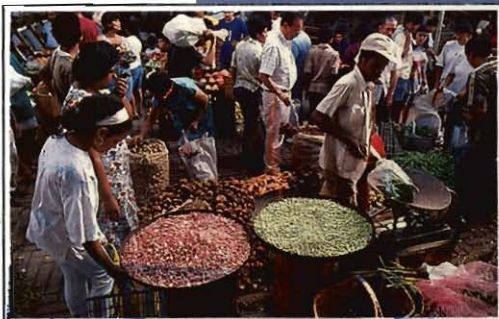
1. Achieve and maintain food security.

In many parts of the developing world, food security is still tenuous at best. To help abolish hunger, agricultural researchers must make an all-out assault on major constraints of food production and storage.

2. Make agriculture more competitive.

Openness is now the by-word of economic policy in much of the developing world. The nations that benefit most are the ones that better enable agriculture to compete by linking production of staple crops to new markets and by tapping a wide range of plant diversity to develop new products. One way researchers can help is to generate crop varieties whose quality traits satisfy market demands.

JUAN CARLOS QUINTANA



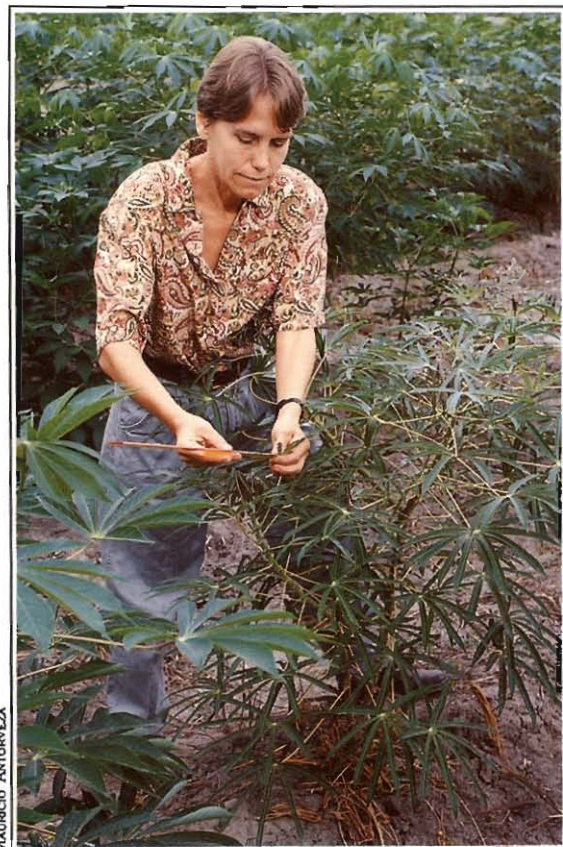
Biotechnology can help improve the efficiency, not only of large-scale agroindustries, but also of small-scale operations, such as the manual extraction of starch from cassava.

Dominique Dupour



THE LAB AND FIELD WORK RUN PARALLEL

For the CIAT scientists shown here, constructing a molecular genetic map of cassava—the first such map to be developed in its entirety at an international agricultural research center—is an exacting step on the road to crop improvement. Here's why two of them think it's worth the investment of their time and energy and the Rockefeller Foundation's money:



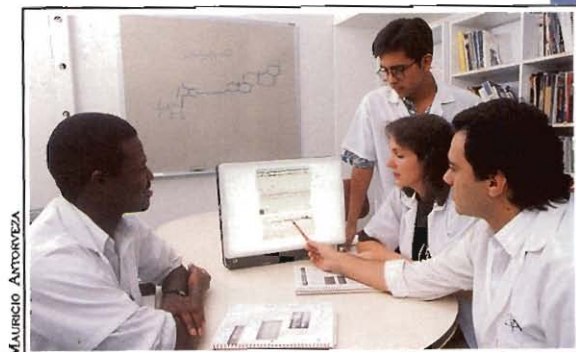
MAURICIO ANTORVEZA

"To find molecular markers related to valuable traits, we're studying the differences between diverse cassava genotypes under a range of field conditions and in the lab."

"Actually, I've done this before. At Cornell University I developed a molecular map of potato, which is now being integrated into applied breeding. I switched jobs because I wanted to be involved more directly with food issues in the developing world."

"The map should explain some of the complications we face in cassava improvement and help us find ways around them. In fact, I've already started orienting my breeding program to make better use of the map. The lab and field work have to run parallel."

The mapping team: Martín Fregene (also opposite page), Fernando Rodríguez, Rocio Gómez, Fernando Angel, Merideth Bonierbale (above), and Joe Tohme (not shown).



MAURICIO ANTORVEZA

Merideth Bonierbale,
Cassava Geneticist,
CIAT

BIOTECHNOLOGY WITH MUD ON ITS LAB COAT

For some people biotechnology conveys an image of upstream science remote from the everyday demands of agriculture. And perhaps in some cases the image fits.

But what we're doing at CIAT is *biotechnology with mud on its lab coat*. That means we integrate molecular and cellular methods with field research to gain two important advantages:

1. New techniques can enhance the efficiency of conventional methods and improve our chances of solving problems that would otherwise be difficult or impossible to overcome.
2. Close links to well-targeted, participatory research on crops and their wild relatives ensure that what we do in the lab is relevant to the needs of farmers and consumers.

In the accompanying *Project Updates* (see pocket), we describe research on selected topics. These examples illustrate how biotechnology, integrated with field research, can enhance the problem-solving power of agricultural science.

"One of the most important applications of biotechnology to agriculture to date resulted from collaboration between CIAT and Purdue. Scientists worldwide are using their findings to produce rice varieties with more durable blast resistance."

Gary Toenniessen,
Deputy Director,
Agricultural Sciences,
Rockefeller
Foundation, USA

"At CIAT biotechnology research seems well connected with other key research activities, like plant breeding, evaluation of genetic resources, and plant pathology."

H.-J. Jacobsen,
Professor, University of
Hannover, Germany



LUIS FERRANDO PINO

John Miles, CIAT
plant breeder, checks
Brachiaria grass for
spittlebug infestation.



3. *Reduce environmental and economic risks in agriculture.*

In market-oriented agriculture, there is a powerful tendency toward excessive pesticide use, which adds to production costs, causes ecological damage, and may give rise to trade barriers. One way biotechnology can combat this problem is by speeding the development of disease- and pest-resistant varieties that eliminate or reduce the need for chemical controls. Another is to enhance natural associations between plants and beneficial microorganisms that reduce populations of harmful pathogens and pests.

To help farmers maintain the balance, we must do more than simply master new techniques. We must apply them creatively to practical ends, such as conserving plant genetic diversity (in gene banks, farmers' fields, and nature) and using valuable genes for plant improvement.

Fernando
Correa, CIAT
plant
pathologist,
examines the
reaction of rice
genotypes to the
blast pathogen.



"The map is like a jigsaw puzzle with thousands of pieces. We take one DNA segment or probe at a time and determine where it fits on the crop genome."

"As a Nigerian I'm concerned about Africa's food supply.

When I was in primary school, we ate cassava maybe twice a week. In secondary school, it was once a day. By the time I got to university, families I knew ate cassava twice a day. If production is threatened, a lot of people will be in trouble.

"So, we have to start looking for new sources of resistance to diseases and other stresses. Then, we have to find efficient ways to deploy genes for the resistance in well-adapted germplasm. This map will help us streamline the breeding."

*Martin Fregene,
Cassava Geneticist,
CIAT*



Participants in a course on biotechnology for the conservation of agrobiodiversity learn how to transfer DNA from an electrophoresis gel to a nylon membrane for analysis of genetic diversity at the molecular level.

"As part of its cooperation with national and international programs in the region, CIAT has provided training to members of our Network for Technical Cooperation in Agricultural Biotechnology."

Juan Izquierdo, Latin America Regional Officer for Crop Production, Food and Agriculture Organization (FAO), Chile

"From the outset CIAT understood the importance of an adequate policy environment for developing biotechnology capabilities and stimulated the few initiatives (for example, in biosafety) that have been undertaken in Latin America."

Walter Jaffé, Specialist in Technology Generation and Transfer, Instituto Interamericano de Cooperación para la Agricultura (IICA), Costa Rica

"CIAT can play an important role in strategic research, adapting new technologies to deal with problems that current research methods cannot solve or to improve the efficiency of conventional methods."

Javier Narváez Vásquez, Coordinator of the National Program for Agricultural Biotechnology, Corporación Colombiana de Investigación Agropecuaria (CORPOICA)

A LAB WITHOUT WALLS

When CIAT established the Biotechnology Research Unit (BRU) in 1985, we sought a way to integrate new techniques into the Center's strategic research, which today includes a wide range of activities in germplasm development and natural resource management. To accomplish this purpose, says BRU head William Roca, "we built a lab without walls—a lab open to people who can help identify problems and develop innovative solutions."

Key functions of the BRU are to:

- Research the potential of new techniques, using basic information and materials from advanced laboratories (see *Inside Biotechnology's Toolbox*, in pocket).
- Design biotechnology applications and make them available to plant breeders and other agricultural researchers.

Since 1989, CIAT scientists and colleagues in other institutions have taken up rice anther culture, in vitro storage of cassava, isozyme fingerprinting, and the use of molecular genetic maps and markers. More applications are in the pipeline.



An easy, cost-effective method for rice anther culture breeding.

LUIS FERNANDO PINO



"Through your shipment of 16 cassava clones in vitro, we developed a high-yielding cassava cultivar, Nan-Zhi 188, which was quickly distributed to farmers by tissue culture in rural areas at low cost."

Kuo Chun-Yen,
Professor, South
China Institute of
Botany

"My relationship with biotechnology specialists is a marriage of convenience. I need them and they need me."

César Cardona,
Bean Entomologist,
CIAT

AN OPEN DOOR

An open door is one of the hallmarks of biotechnology research and cooperation at CIAT. We keep the door open to flexible arrangements for research and training on a wide variety of topics. And we open doors of opportunity for colleagues through:

- Research networks, which provide a framework for many of our collaborative activities.
- Training and other activities aimed at strengthening national research capacity.
- Support for scientists conducting undergraduate or postgraduate thesis research.
- Active participation in the debate on biosafety, intellectual property rights, and exchange of genetic resources.

(For details on these activities, see *Building Biotechnology Capacity*, in pocket.)

Many developing country institutions already have excellent staff and infrastructure for biotechnology research. Even so, the gene technology gap between these countries and the industrialized world remains wide.

A good way to bridge the gap is through cooperative research projects that put biotechnology specialists in contact with scientists from other disciplines. In recent years CIAT has carried out 10 such projects with support from various donor agencies. The opportunities for further cooperation are abundant.

If we didn't think so, we wouldn't have sent you this invitation.

"CIAT has realized for some time the need for client- and problem-oriented application of biotechnology and has been a logical partner for us in operating the Cassava Biotechnology Network."

Th. J. Wessels,
Head,
Biotechnology and
Development
Cooperation,
Directorate
General for
International
Cooperation
(DGIS), The
Netherlands

"Graduate student and postdoctoral exchanges between CIAT and Purdue University have been one of the continuing rewards of our enjoyable and fruitful collaboration."

Morris Levy,
Professor, Purdue
University, USA

IMPROVING THE CONSERVATION OF CASSAVA GENETIC RESOURCES

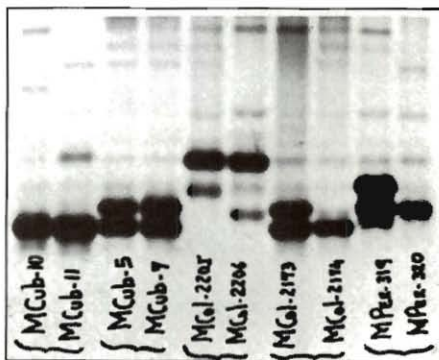
The cassava collection at CIAT comprises nearly 6,000 accessions from the species' primary centers of diversity in the Americas and secondary centers in Africa, Asia, and Oceania. The Center is pursuing several approaches to improve conservation of these genetic resources.

One is to identify duplicates in the collection, which contains several local clones (each with a different name) of the same genotype. This should increase the cost-effectiveness of germplasm conservation and management.

Analysis of isozyme profiles (see photo), along with morphological and agronomic descriptors, has shown that about 20% of the collection consists of duplicates. Among varieties already screened on the basis of morphology and isozymes, we're applying DNA fingerprinting to detect genetic differences more precisely. For example, in a sample of 100 apparently similar accessions, we determined that 20 are genetically unique (and the remainder duplicates), using the gene for protein III of the bacteriophage M13 as a probe. RAPD markers confirm these results.

Effective conservation of genetic resources requires a combination of in situ and ex situ approaches. In situ conservation is preferred for populations of crop wild relatives, primitive cultivars, and landraces. Ex situ conservation complements that approach by safeguarding germplasm threatened with genetic erosion or other dangers. We can achieve this end by various means, ranging from preservation of landraces and primitive cultivars in the field to in vitro conservation, DNA storage, and cryopreservation.

In cooperation with the International Institute for Plant Genetic Resources (IPGRI), CIAT has developed an in vitro active gene bank that contains more than 5,900 clones, representing over 95% of the world collection of cassava germplasm (see photo). The Center's Genetic Resources Unit maintains these clones under slow-growth conditions (i.e., at reduced temperature in a special medium). Even so, the accessions must be renewed every 12-18 months. The entire in vitro bank occupies 35 m² of laboratory space, about a thousandth of the area needed to maintain the same materials in the field. From this active collection, we've distributed nearly 2,000



α , β -esterase isozyme profiles of cassava germplasm accessions. Note the similarities and differences among profiles within the five morphologically similar groups.



The in vitro active cassava gene bank at CIAT.



Cryopreservation: The ideal approach to long-term conservation of cassava genetic resources.

pathogen-tested cassava clones to the national research institutions of 35 countries in Africa, Asia, and Latin America.

An even more ideal approach is cryopreservation or ultrafreezing (see photo). By stopping cell functions and senescence, this technique makes it possible to preserve the plant genome indefinitely. CIAT has been working on the cryopreservation of cassava shoot tips since 1989. By 1991 we were able to recover complete cassava plants from shoot tips frozen in liquid nitrogen (-196°C). Further improvements in the technique (involving changes in tissue dehydrating treatments, the rate of cooling, and culture media) enable us to consistently recover plants from frozen shoot tips with a success rate of more than 60%.

Currently, we're developing a simple protocol for more efficient and less costly freezing, which will open the way to long-term conservation of a base gene bank of cassava clones in liquid nitrogen.

Windows of cooperation: In vitro conservation of cassava figures importantly in CIAT training on genetic resources. For example, it was among the main topics of a course entitled Biotechnology for the Conservation of Agrobiodiversity, which was held at Center headquarters in November 1994.

CIAT and IPGRI jointly established the in vitro active cassava gene bank in 1991, after a 3-year study of its technical and logistical requirements. In collaboration with national programs, the two centers are now planning a comparable pilot project to implement a base gene bank using cryogenics.

CONTACT: W. Roca, M. Bonierbale, and C. Guevara

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GETTING A PREVIEW OF *PHASEOLUS* GENETIC DIVERSITY

The 26,500 *Phaseolus* accessions stored in CIAT's Genetic Resources Unit contain enough genetic diversity to keep bean researchers busy for decades. But because the complete collection is so large and has not been thoroughly characterized, it's an unwieldy tool for studying the structure and distribution of *Phaseolus* genetic diversity and identifying valuable genes.

To provide a more convenient way of performing these tasks, CIAT researchers (including specialists in bean genetics, biotechnology, and agricultural geography) recently formed two *core* collections. The first, containing 1,420 accessions, represents cultivated common beans (*Phaseolus vulgaris*), while the second, with 100 accessions, covers wild *P. vulgaris*. The core collections are intended, not to replace the complete holdings, but to give researchers a preview of them as a guide to further investigation.

But core collections are useful only if they accurately represent the genetic diversity of the species sampled. To meet this requirement, we based the composition of our core collections on a combination of factors related to the evolution of common bean and to the agroecologies in which it is found. For example, in forming the collection of cultivated *P. vulgaris*, we included more accessions from primary than secondary centers of diversity and gave more weight to primitive seed types and growth habits than to modern, commercial ones.

To guarantee that the core collection covers the crop's entire range of adaptation, we developed a simple agroecological classification. It's based on four factors (such as soils and rainfall) and includes a total of 54 distinct agroecologies. Using map coordinates for the sites where seed of bean landraces was collected, we identified the agroecology to which each accession belongs.

In cooperation with the University of Wisconsin, USA, we used RAPD markers to verify that the genetic variability of the cultivated *P. vulgaris* core collection truly represents that of the base collection.

Now we need to evaluate and use the core collections. One example is our screening of the cultivated bean collection for phosphorus use efficiency and selection of desirable genotypes.

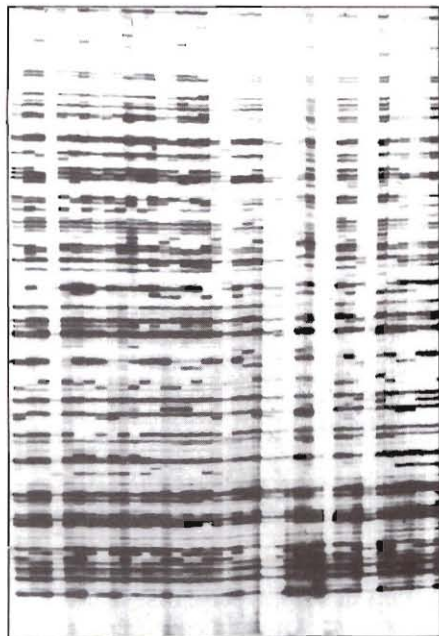
In addition, we're characterizing the collection of wild accessions, using phaseolin and other polymorphic seed proteins as biochemical indicators of genetic diversity as well as molecular markers, such as AFLPs and RAPDs (see photo). These techniques help us study the genetic structure of the wild germplasm, determine the extent to which cultivated beans evolved from only a limited fraction of wild populations (referred to as the *founder*

effect), and trace the gene flow between wild and cultivated germplasm and between the Andean and Mesoamerican gene pools of common bean.

Windows of cooperation: We see the core collections and procedures by which they are being formed as new opportunities for cooperation with gene banks and breeding programs. The collections could provide a convenient, common focus for further study and evaluation of *Phaseolus* diversity.

But we're also seeking partners interested in applying the new procedures to form core collections of other species, which will help researchers study a wide range of agrobiodiversity. Tools like molecular markers can magnify the precision of such studies and the insights we gain from them. Geographic information systems (GIS) can help us describe and analyze genetic diversity in relation to key agroecological variables.

Such research will improve our understanding of the relationship between genetic diversity at the molecular level and the biological diversity of agroecosystems. It will also help us develop strategies for more effective in situ and ex situ conservation and use of germplasm. Through this work CIAT can help developing countries implement agreements under the Convention on Biological Diversity.



AFLP fingerprints of different accessions in the core collection of wild *P. vulgaris*.

CONTACT: J. Tohme, S. Beebe, and
D. Debouck

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TAMING THE WILD RELATIVES OF COMMON BEAN

Many useful genes have been identified in wild forms and distant relatives of important staples but often lie beyond the reach of conventional plant breeding. Through several projects at CIAT, we're exploring new ways to ease the transfer of such genes to domesticated common bean (*Phaseolus vulgaris*).

Some important traits occur in wild or primitive forms of *P. vulgaris*. But getting them into domesticated common bean can be far from straightforward, even though the two are sexually compatible.

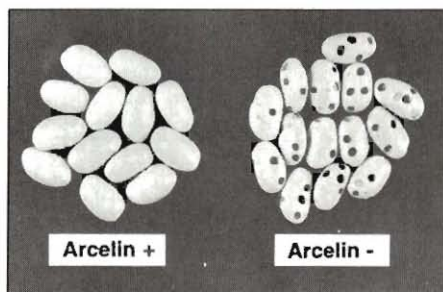
Take the case of resistance to the Mexican bean weevil (*Zabrotes subfasciatus*) and bean weevil (*Acanthoscelides obtectus*), the two major pests of stored dried beans in Africa and Latin America. By screening thousands of accessions of wild *P. vulgaris*, CIAT entomologists identified some resistant to Mexican bean weevil, others resistant to bean weevil, and a couple resistant to both insects.

Among Mexican bean weevils that died in resistant seed, studies conducted in cooperation with the University of Wisconsin pointed to a single cause of death—the protein arcelin, which the insect apparently cannot digest well (see photos). The protein's name is based on that of a town in Mexico, Arcelia, where wild bean accessions containing the gene were collected.

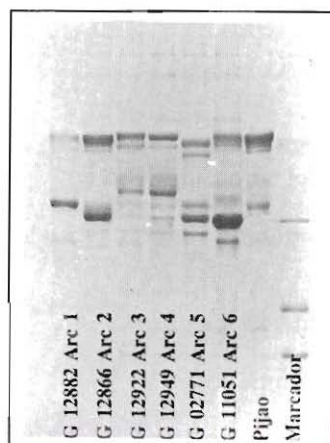
Since the biosynthesis of arcelin in resistant genotypes is a monogenic or simply inherited trait, CIAT bean researchers were able to breed resistance to *Z. subfasciatus* into experimental lines of domesticated beans. These lines are now being tested in Africa and Latin America.

Our only disappointment with arcelin is that it has no effect on *A. obtectus*. With support from Belgium's General Administration for Development Cooperation (AGCD), we're trying to determine what factors do account for resistance to this species in wild *P. vulgaris* germplasm. So far, we've identified a protein fraction that inhibits larval development in resistant accessions of wild beans (see figure). We also know that another fraction contains general resistance factors, which limit insect growth.

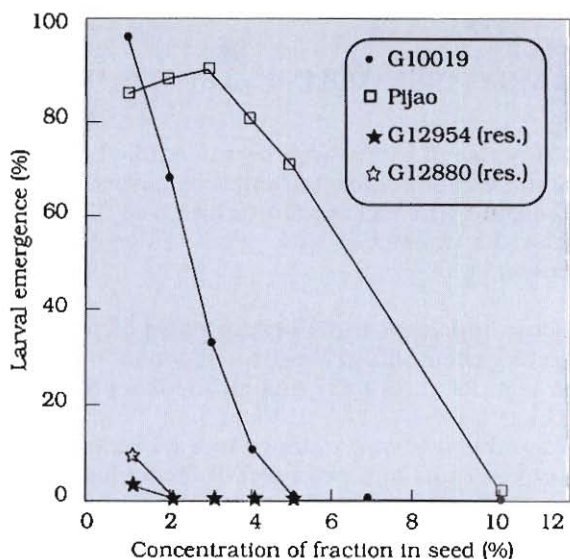
These results account in part for the quantitative or complex genetic character of resistance to *A. obtectus*. Once we know exactly what the factors are, we expect to develop a biochemical assay or molecular probes that permit efficient selection for multigenic, durable resistance to this important pest.



Effect of *Z. subfasciatus* on accessions with and without the arcelin gene.



A biochemical marker is used to identify the arcilin gene in bean accessions. Each of the six resistant materials designated "G" carries a different variant of the arcilin gene.



Effect of the 0-20% acetone protein fraction on emergence of *A. obtectus* larvae in bean artificial seed.

Windows of cooperation: CIAT is a logical candidate to characterize proteins possibly responsible for resistance to *A. obtectus* in wild common bean. If we succeed, the window will be wide open for cooperation in cloning the associated genes and transferring them to common bean.

Such cooperation is already taking place in research on a particular variant of the gene corresponding to arcilin. With funding from AGCD, scientists at the University of Ghent in Belgium are cloning the arcilin-5 gene with a view to transforming common bean and other crops that need resistance to the Mexican bean weevil. Brazil's Centro Nacional de Recursos Genéticos (CENARGEN) is also investigating this possibility.

CIAT is searching the genetic resources of other *Phaseolus* species, such as *P. lunatus* and *P. acutifolius*, for additional sources of resistance. Alternative approaches to developing resistance, including antinutritive factors such as avidin and cystatin, are also being explored.

CONTACT: J. Mayer and C. Cardona

December 1994



KNOW YOUR ENEMY: TOWARD DURABLE RESISTANCE TO RICE BLAST

Rice blast is the most widespread and damaging disease of the staple food of 2.5 billion people. In seeking a genetic solution to this constraint, CIAT scientists and their research partners have gained new insights into the genetic basis of interactions between the host plant and its number one enemy.

The fungal pathogen of blast (*Pyricularia grisea*) produces a large number of races or pathotypes, whose extreme diversity complicates the development of resistant cultivars. Most varieties released so far have contained single resistance genes, which are effective only against certain pathotypes. Invariably, this resistance loses its effectiveness after only two or three years, as a result of shifts in the frequency of pathotypes, immigration of existing compatible races, or rapid emergence of new ones through mutation or other mechanisms. Lacking varieties with durable blast resistance, many farmers rely heavily on fungicides.

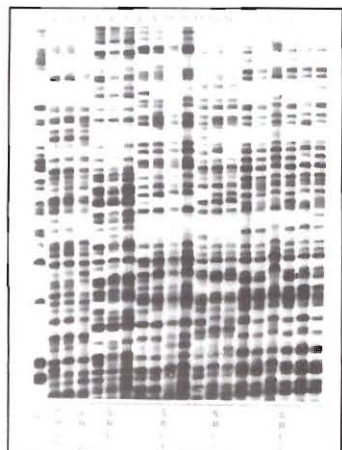
In 1989 an experimental line developed by CIAT researchers at Santa Rosa, a blast "hot spot" in Colombia, was released as Oryzica Llanos 5 by the Instituto Colombiano Agropecuario (ICA). During six years of commercial production, the blast resistance (immunity) of this variety has shown no signs of breaking down. Scientists at the International Rice Research Institute (IRRI) in the Philippines report that it has also shown resistance at several blast hot spots in Asia.

To explain the durability of this resistance and facilitate the development of similar varieties, an interdisciplinary group at CIAT, in cooperation with colleagues from Purdue University in the USA, has carried out virulence diversity studies and characterized the genetic structure of the pathogen, using MGR-DNA "fingerprinting" (MGR, *Magnaporthe grisea* repeat, refers to a molecular probe developed at Dupont and designated MGR586).

Studies of the genetic structure and virulence frequency of the fungus indicate that certain combinations of virulence genes are absent or occur at low frequency where resistance genes in the host plant are specific to a given lineage or family of pathotypes.

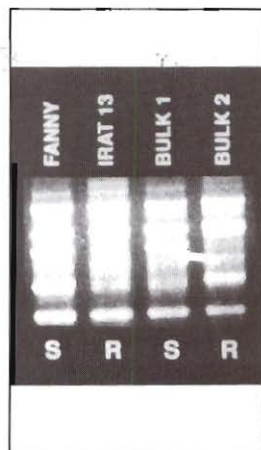
Based on these results, CIAT scientists are increasing the precision of breeding for durable blast resistance. Their strategy is to match combinations of resistance genes in the host plant with the combination of virulence genes that are absent or occur at low frequency in the pathogen population. This will render the various genetic lineages of the pathogen incompatible with the host plant.

With funding from the Rockefeller Foundation, we're making rapid progress in using the lineage data derived from MGR-DNA fingerprinting (see photo), together with molecular markers (RFLPs and RAPDs), to identify



MGR-DNA fingerprints of blast isolates from Santa Rosa, Colombia. Note the similarity of patterns for isolates within lineages and the clear differences between lineages 1 to 6.

Through bulk segregant analysis, a RAPD marker is used to distinguish susceptible from resistant genotypes infected with an isolate of a Colombian blast lineage. Note that the RAPD pattern for the susceptible parent is the same as that for the susceptible bulk 1.



chromosome segments carrying resistance to the lineages found in Colombia (see photo). Within a few years, we expect to have markers that make it possible to identify combinations of resistance genes more efficiently.

Windows of cooperation: IIRI and CIAT are strongly promoting international cooperation in the development of germplasm with durable blast resistance, so that developing countries can more easily reap its benefits (estimated at US\$210 million annually, on average, in Latin America alone).

IIRI is coordinating research globally, while CIAT focuses on needs in Latin America. Together with scientists from Cornell and Purdue universities, staff of the two international centers have agreed on a joint breeding strategy. Our work in Colombia is critical, because it takes place at a site (Santa Rosa) where the causal fungus is extremely variable.

To make new findings and technology more widely available to national institutes, CIAT held a rice blast workshop during October 1994 in cooperation with the Programa Cooperativo para el Desarrollo Tecnológico Agropecuario (PROCISUR). It focused on molecular marker-aided analysis of pathogen diversity and virulence diversity studies. The participants were multidisciplinary teams of scientists (each consisting of a breeder, pathologist, and biotechnology specialist) from the five countries of South America's Southern Cone.

CONTACT: F. Correa and J. Tohme

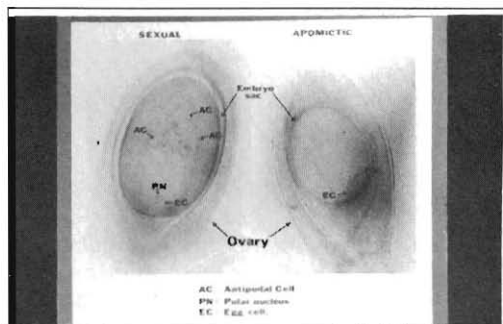
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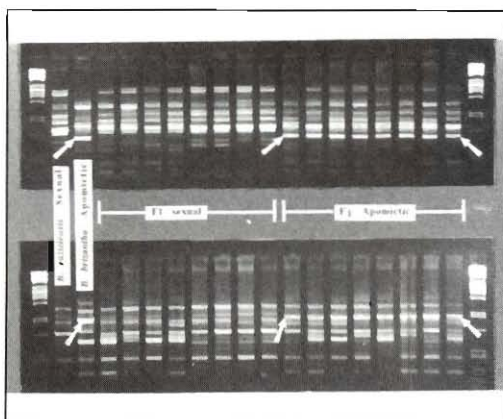
APOMIXIS IN *BRACHIARIA*: NEW HOPE FOR HYBRIDS?

Brachiaria and other tropical forage grasses are among the few economically important species able to reproduce by means of *apomixis*, that is, from seeds whose embryo arises through an asexual process (see photo). Plants grown from this seed are genetically identical to their female progenitor and to one another. If apomixis could be introduced into crops that are normally propagated sexually, it would ensure that vigorous

hybrids breed true from one generation to the next.



Reproductive structures of sexual and apomictic plants (as seen with interference contrast microscopy) resulting from a cross between tetraploid sexual *B. ruziziensis* and tetraploid apomictic *B. brizantha*.



DNA amplification of two RAPD markers linked to the apomixis gene in a segregating *Brachiaria* population.

At CIAT we've recently identified molecular markers (RAPDs) linked to a single, dominant gene that appears to control apomixis in *Brachiaria* (see photo). By allowing rapid identification of apomixis among progeny of crosses between apomictic and sexual types, the markers should help breeders at CIAT and in Brazil combine desirable traits from different *Brachiaria* species in apomictic cultivars.

Even if the work went no further, the importance of *Brachiaria* for tropical America would justify the use of molecular markers to tag apomixis genes in these species. In Brazil alone more than 50 million hectares are sown to *Brachiaria* pastures. And in the region as a whole, these are by far the most widely grown commercial forages.

But rather than stop with *Brachiaria*, why not start there? Assuming that an apomixis gene could be transferred from this to other crops, we could gain a convenient, inexpensive way to multiply hybrids uniformly.

Hybrids are the vigorous progeny of crosses between genetically distinct parents. Since only the first sexual generation of this seed shows hybrid vigor, it makes no sense for farmers to adopt hybrids unless they buy new seed for each crop. In many developing countries, weak seed systems and rural poverty make this practically impossible. Another problem is that the reproductive biology of certain self-pollinating crops (characterized by deficient pollen production or transfer) greatly complicates large-scale production of conventional hybrid seed.

Getting around the obstacles to hybrid development is one goal of the various institutions already trying to introgress apomixis genes into major cereals. Eventually, it may be possible to engineer apomictic crops by using new biotechnology techniques to transfer apomixis genes between more genetically distant species.

Once farmers have their first supply of apomictic hybrids, they can produce one generation after another of hybrid seed.

Windows of cooperation: To genetically engineer apomictic crops, we must complete three main tasks: 1) densely map the *Brachiaria* chromosome region on which the apomixis gene is located, using molecular markers, 2) isolate and clone the apomixis gene, and 3) genetically transform target crops. Each step presents a major challenge and will require close collaboration with advanced labs. But by identifying a molecular marker linked to the apomixis gene, CIAT has begun the first task, and by regenerating *Brachiaria* plants from tissue culture, we've made progress in developing a transformation protocol as well. This technique will enable us to test the expression of the apomixis gene in different *Brachiaria* backgrounds.

CONTACT: J. Miles and J. Tohme

December 1994



Other useful applications of molecular markers are to facilitate the use of exotic germplasm in crop improvement, identify duplication in germplasm collections, determine evolutionary relationships among crops species and between them and their wild relatives, and measure genetic diversity.

Points of genetic intervention

In seeking genetic solutions to problems of plant production and utilization, it isn't always clear how or where we can intervene. To use germplasm efficiently, we must identify points of genetic intervention by elucidating the biochemical factors and genetic mechanisms involved in important physiological and quality traits (Figure 4) and in the interactions between plants and biotic stresses. We can then develop biochemical and molecular assays to detect those factors and eventually isolate and clone the genes responsible for them.

Genetic engineering

Using molecular techniques, we can next attempt to modify these genes, either enhancing their normal function (overexpression) or inhibiting it (downregulation). This procedure employs a transformation "cassette," comprising the complete coding sequence of the structural gene of interest, gene promoters, enhancers, gene markers, etc. (Figure 5). All these elements are needed for proper expression when the modified gene construct is returned to the plant or transferred to other species through genetic transformation.

The genes to be used must first be isolated, cloned, and modified. At the same time, we have to develop a workable system or protocol for genetic transformation of the crop. At CIAT we've employed two approaches successfully: *Agrobacterium*-mediated and particle bombardment-mediated genetic transformation (Figure 6). With the first approach, we've produced

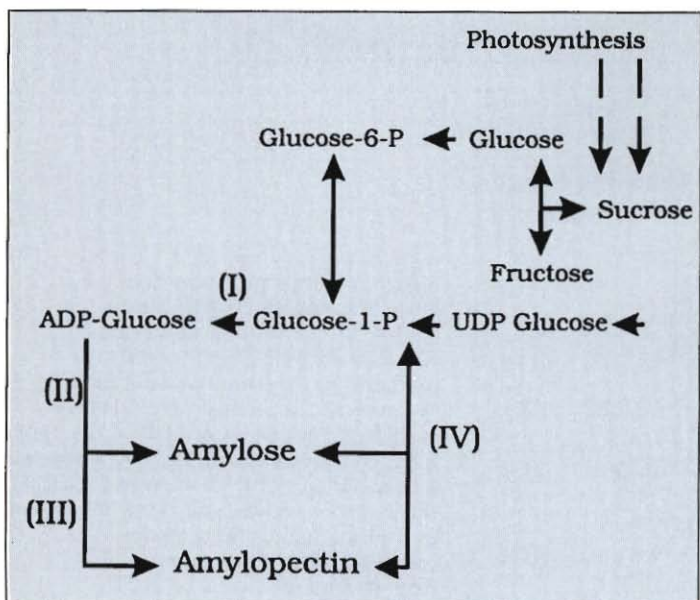


Figure 4. Four points of potential genetic intervention and manipulation in cassava starch biosynthesis: I) ADP-glucose pyrophosphorylase, II) granule-bound starch synthase, III) branching enzyme, and IV) starch phosphorylase.

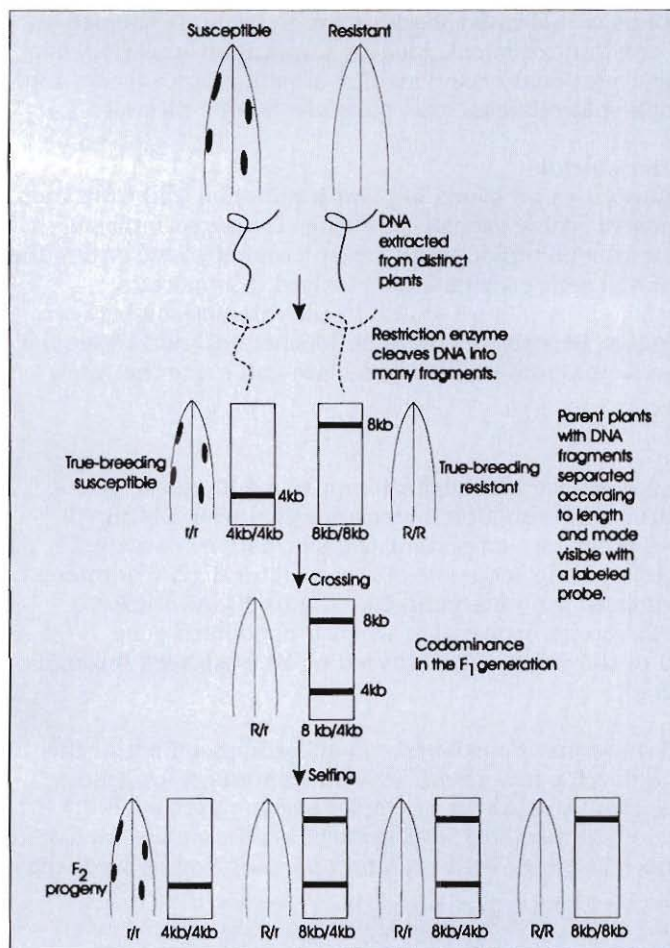


Figure 2. The pattern of inheritance of an RFLP marker related to a disease resistance gene. In this case resistance is dominant over susceptibility. Because the marker differs in size between resistant and susceptible parents, it is said to be "polymorphic." Three out of four individuals from a cross between these parents show the resistant phenotype. Yet, as is clear from the RFLP banding pattern, only one carries the resistance gene in a homozygous condition.

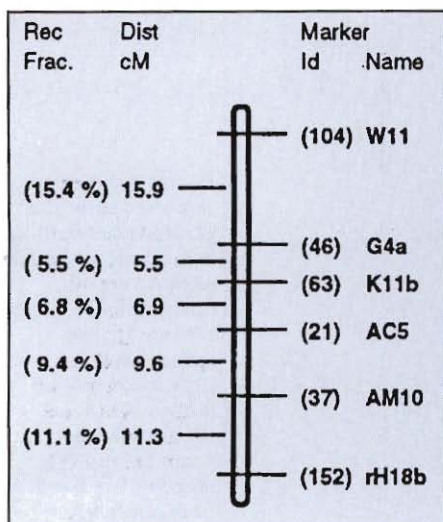


Figure 3. A linkage group from the molecular genetic map of cassava. It shows the linear order and relative distances between RFLP and RAPD markers, as determined using the Mapmaker computer program (with a statistical confidence of $LOD > 5.0$). Each mapped marker is identified by a number and name (at right). Also given (at left) is the distance between markers, measured in centimorgans (cM), and the corresponding percentage of recombination.

can analyze a much larger number of DNA fragments and thus distinguish genotypes more precisely at the molecular level.

Molecular genetic mapping

New recombinant DNA technology has greatly facilitated the development of genetic maps. Previously, researchers could detect only a few genetic recombination events by observing phenotypic markers in plants. With *molecular markers*, genetic differences can be detected more thoroughly and simply.

For example, an RFLP map of the plant genome can be developed by using numerous markers to determine the position of specific restriction fragments relative to one another. This is accomplished through a procedure termed *linkage analysis*, in which markers that segregate together after meiosis are said to be "linked."

Since the number of such markers is unlimited, it's possible to literally saturate the plant genome and thus obtain information about all regions of each chromosome of a plant species. Markers linked to particular genes enable us to follow the transfer of these tagged genes from one generation to another, as chromosomal segments are exchanged during meiosis (Figure 2).

Important advantages of RFLP markers are that they can be detected at any stage in plant development and in any tissue, are not affected by the environment, and are genetically codominant. Consequently, they provide a reliable, direct means to locate and monitor chromosome segments and the genes they contain.

CIAT is currently developing two molecular genetic maps, for cassava and tepary bean. We're also using common bean and rice molecular maps developed at the University of Florida and Cornell University, respectively, in the USA. Such maps comprise sets of molecular markers, referred to as *linkage groups* (Figure 3). Normally, the number of these groups equals the species' basic chromosome number ($n = x$).

Other uses of molecular markers

In addition to helping map plant genomes, RFLPs and RAPDs show great promise for facilitating plant selection for important traits. With a procedure referred to as *bulk segregant analysis*, we test many RAPDs to identify those lying closest to genes of interest. Though not yet widely practiced, *molecular marker-aided selection* should simplify particular tasks in plant breeding by reducing the need for extensive field evaluation of segregating progeny.

RFLP, RAPD, and AFLP analysis can also be applied to the DNA of crop pests and pathogens in a procedure called *DNA fingerprinting*. By revealing differences at the molecular level between races of a highly diverse pathogen, for example, this approach enables us to group the races into genetically distinct families or lineages. That in turn should help us determine genetic relationships and eventually deploy genetic resistance more effectively against the disease.

INSIDE BIOTECHNOLOGY'S TOOLBOX

Since establishing the Biotechnology Research Unit in 1985, CIAT has rapidly expanded its capacity to apply new technologies. This report gives an overview of techniques currently in use at the Center.

Molecular marker techniques at CIAT

One of these involves *restriction fragment length polymorphisms (RFLPs)*. These are the result of digestion of DNA by *restriction enzymes*. A given enzyme, which identifies a particular sequence of nucleotides or base pairs, cuts the DNA wherever that sequence occurs. The resulting DNA fragments are separated on an electrophoresis gel, transferred to a nylon membrane, and placed in a hybridization solution with a *probe*. This is a cloned DNA fragment generated through duplication of DNA in a vector organism. The probe may be radioactive or labeled with nonradioactive compounds. In the hybridization solution, it bonds to DNA fragments of complementary sequence. The probe's position is determined through autoradiography, in which it appears as a dark band.

More recent methods of detecting genetic differences at the molecular level are based on *polymerase chain reaction (PCR) amplification* (Figure 1). This technique synthesizes millions of copies of a given DNA fragment by means of relatively short DNA sequences called *oligonucleotide primers*. These may correspond to part of known genes or to specific locations on a chromosome or may be of unknown origin. A DNA fragment synthesized with the latter type of primer is referred to as *random amplified polymorphic DNA (RAPD)*.

The target DNA is split into two strands through heating. As the DNA cools, single oligonucleotide primers bind to both ends of each strand. The enzyme Taq polymerase adds nucleotides until a new copy of the original template has been produced. It takes some 30 cycles to produce a million copies of the original DNA fragment.

Such a large quantity of DNA is produced that one can easily observe it by means of electrophoresis. Two key advantages of this approach are that it's highly sensitive to genetic differences at the molecular level and avoids the use of potentially hazardous radioactive materials and costly restriction

enzymes. RAPDs are commonly used at CIAT in genome mapping, gene tagging, and genetic fingerprinting.



Figure 1. A thermocycler makes millions of copies of DNA from a small sample by means of PCR.

A new molecular technique, developed by researchers in The Netherlands and referred to as *amplified fragment length polymorphism (AFLP)* technology, combines features of RFLP- and RAPD-based techniques. The main advantage of this approach is that in a single electrophoresis run one

BUILDING BIOTECHNOLOGY CAPACITY

At CIAT biotechnology offers tangible outputs. Since 1988 we've developed a number of practical applications, which scientists at CIAT and partner institutions use today in the conservation and enhancement of agrobiodiversity and in crop improvement.

Application	CIAT user (program/unit)	Year
Isozyme fingerprinting	Cassava, Genetic Resources	1988
Protein (phaseolin) markers	Bean, Genetic Resources	1989
In vitro gene bank	Cassava, Genetic Resources	1989
In vitro germplasm exchange	Cassava, Genetic Resources	1989
Anther culture breeding	Rice	1990
Molecular marker techniques	Bean, Cassava, Rice, Tropical Forages	In progress

To make these technologies more widely available in developing countries, CIAT foments cooperation in biotechnology research through networks, training, and other activities.

Networks

To help build bridges between institutions and identify useful applications for biotechnology, CIAT promotes international research networks focusing on major crops. The networks provide a way to prioritize topics for biotechnology research and bring these to the attention of donors and the world scientific community.

CIAT has strongly supported the Cassava Biotechnology Network (CBN) since its establishment in 1988. The network's central objectives are to stimulate advanced research on cassava and ensure that this work is based



Mass propagation of CIAT cassava germplasm in tissue culture form at the South China Institute of Botany. A Chinese scientist shows here how micropropagated plantlets are packed in cardboard boxes for distribution to yield trial sites in farmers' fields (photo courtesy of Prof. Kuo Chun-Yen).

on the priorities of beneficiaries, particularly consumers and small-scale farmers. With funding from the Directorate General for International Cooperation (DGIS) in The Netherlands, CBN works toward these goals by fostering information exchange among farmer associations, NGOs, national research programs, other networks, advanced labs, international centers, and donor agencies.

The network organizes conferences and workshops, collaborative research projects, and short-term training for developing country scientists. The most recent



Multidisciplinary teams of scientists from the five countries of South America's Southern Cone perform DNA separation by gel electrophoresis in a course on the application of molecular markers to research on rice blast.

conference, held in August 1994 at Bogor, Indonesia, was attended by 150 scientists from 16 developing and 8 developed countries. Member institutions are engaged in 60 collaborative biotechnology projects (up from just 4 in 1988) aimed at solving problems in cassava production and utilization.

In addition, the Center is an active participant in the Rice Biotechnology Network supported by the Rockefeller Foundation. CIAT has also established the *Phaseolus* Beans Advanced Biotechnology Research Network (BARN). During a workshop held at the Center in 1993, 50 scientists from 16 countries identified a wide range of topics, around which cooperative research projects can be developed. This event was funded by the Germany Agency for Technical Cooperation (GTZ).

Training

Over the last five years, more than 100 scientists have taken part in biotechnology training at the Center. Some received on-the-job training for varying periods; others conducted their post- or undergraduate thesis research with us; and many participated in group training courses.

In 1994, with support from the Rockefeller Foundation, we began a series of courses on rice improvement using another culture. Participants form teams, consisting of a tissue culture specialist and a rice breeder from the same institution, to focus their thinking on integration of another culture with rice breeding. In October 1994, CIAT and the Programa Cooperativo para el Desarrollo Tecnológico Agropecuario (PROCISUR) organized a similar course for breeders and pathologists from South America's

Southern Cone countries. It dealt with molecular marker-aided analysis of pathogen diversity and identification and tagging of genes for host plant resistance to rice blast.

CIAT is also developing the capacity to train scientists in Latin America and the Caribbean in the application of biotechnology to research on agrobiodiversity. In November 1994, we offered our first international course on biotechnology for the conservation and use of agrobiodiversity in cooperation with the Organization of American States (OAS), Instituto Colombiano de Crédito Educativo y Estudios Técnicos en el Exterior (ICETEX), and the Fondo Colombiano de Investigaciones Científicas y Proyectos Especiales "Francisco José de Caldas" (COLCIENCIAS). Seventeen scientists from universities, national research institutes, and environmental agencies in 10 Latin American countries participated in this event.

In addition to providing training, CIAT is a source of first-rate students for other institutions. We're especially proud of the fact that over the last several years more than 15 of our Colombian research assistants have obtained scholarships to pursue advanced degrees at universities such as Cornell, Ohio State, Purdue, and Yale in the USA and the University of Strasbourg in France.

Issues in biotechnology

In 1991, CIAT established the Institutional Biosafety Committee to oversee all research at the Center involving recombinant DNA techniques and to monitor the release and testing of transgenic organisms. In addition, we co-organized a regional workshop on biosafety in Latin America with the Instituto Interamericano de Cooperación para la Agricultura (IICA). Participants in this event, which was funded by DGIS and the US Department of Agriculture (USDA), called for partnerships in the Andean countries to develop biosafety guidelines and cooperate in properly controlled experimental release of transgenic organisms.

The Center also contributes to the debate on intellectual property rights (IPR) and on policies governing the status and international movement of plant genetic resources. Under our current IPR policy, biotechnology products and methods developed at CIAT are within the public domain. The policy also allows for strategic alliances with institutions in developed and developing countries to facilitate the latter's access to information and technology. We're developing Material Transfer Agreements (MTAs) for all exchanges of biotechnology products and methodologies to guarantee their availability to national institutions in developing countries.

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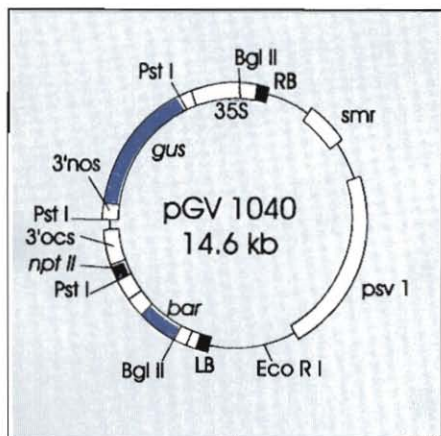


Figure 5. The plasmid pGV1040, a typical transformation cassette provided by PGS in Belgium, is one of the gene constructs used at CIAT in research on *Agrobacterium*-mediated transformation. Between its right and left borders (RB and LB), the plasmid contains two selectable marker genes: 1) the *bar* gene coding for phosphinotricin resistance and the *npt II* gene for kanamycin resistance. It also possesses a screenable marker, the *gus* gene, which encodes the enzyme β -glucuronidase.

transgenic plants of the tropical forage legume *Stylosanthes guianensis* and made good progress with cassava. With the second approach, we've generated transgenic rice plants (Figure 7).

Often, genes of interest are found in wild relatives, more genetically distant plant species, or microorganisms. An exciting prospect of biotechnology is that it will enable us to enrich gene pools by introducing well-characterized genes into the target genome from otherwise inaccessible sources, avoiding the *linkage drag* (introduction of undesirable genes along with desirable ones) that often occurs in conventional sexual crosses.



Figure 6. CIAT staff are using biolistics to transform *Brachiaria*, common bean, and rice. One such approach involves the use of a PDS-He/100 helium-driven particle accelerator, which bombards regenerative tissues with DNA-coated microprojectiles.

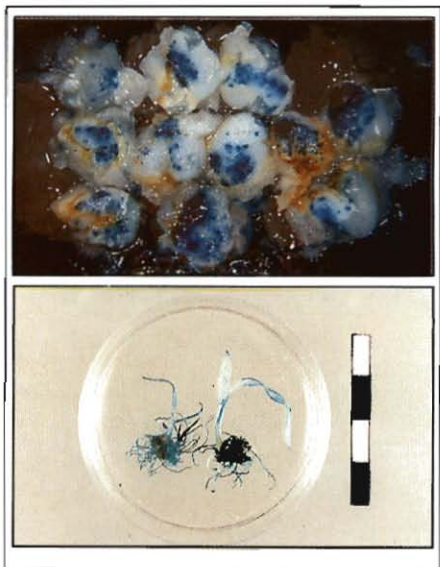


Figure 7. Transgenic rice: above, embryo-derived embryonic callus expresses the *gus* gene, as indicated by dark blue spots; below, transgenic plantlets (of the rice variety CICA 8), regenerated from the callus, express the *gus* gene in leaves and roots.

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