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# Executive Summary

## Project IP-4. Improved Rice Germplasm for Latin America and the Caribbean

**Project Description**

**Objective:** To increase rice genetic diversity and enhance gene pools for higher, more stable yields with lower unit production costs and which proportion lower prices to consumers and reduce environmental hazards.

**Outputs:**
1. Enhancing Gene Pools
2. Integrated Pest And Disease Management
3. Education and Rice Cultivation as a Vehicle to Alleviate Poverty

**Gains:** Robust high yielding rice varieties requiring lower inputs will be developed. We will provide well-characterized progenitors and advanced materials with an ample genetic base as well as training to our partners. The focus will be on developing the capability to increase the number of desirable traits in varieties. This will lower unit costs giving farmers higher profits as well as maintain rice as an affordable food for the consumers.

**Milestones:**

2003 Improved rice populations with high yields and high quality grain will be made available for their evaluation by national organizations. These populations will include advance materials from the interspecific breeding activities that are designed to broaden the genetic base of rice. Molecular markers for resistance to partial and dominant blast resistance genes, sheath blight, crinkling disease, RHBV, and *T. orizicolus* will be developed. Sources of improved progenitors with combinations of blast resistance genes will be distributed to national programs. Improved rice populations with broader genetic base enhanced by recurrent selection will be distributed to national programs. Studies to assure the safety of transgenic rice will be made and novel traits for hoja blanca and sheath blight will be tested. Participatory breeding will be used to reach the resource poor rice farmers.

2004 Genetic progress and gains for populations enhanced by recurrent selection for different traits will be assessed in several countries. Studies of the genetics of complex traits including yield that used interspecific crosses and molecular markers will be completed. Marker aided selection will be used to combine partial and complete resistant genes to produce rice that has durable blast resistance. Molecular and virulence characterization of other rice pathogens including brown spot and sheath rot. Advanced populations using wild rice genes and recurrent selection will continue to be developed. These populations will include characteristics additional traits such as resistant to crinkling disease and drought tolerance. The use of marker aided selection, as a breeding tool will be implemented selected characteristics.

2005 Marker aided selection for some traits will become a routine activity. Participatory rice selection and breeding will be releasing new rice varieties for resource poor farmers. Advanced lines with multiple traits from wild species of rice will be tested for national programs for their release as varieties. An interactive training for rice researchers through the Internet or available as CD-ROMs will be available. Many of the modules will be appropriate for farmers. The developing systematic selection methods for complex traits will increasingly become the focus of the genetic studies. Near isogenic lines for blast resistant genes will be used in regional studies to understand the dynamics of the pathogen and develop locally resistant varieties.

**Users:** Rice researchers especially in Latin America. Ultimate beneficiaries are the LA rice farmers most of whom are small farmers, and the resource poor consumer who are eating rice because it is available and affordable.

**Principal Collaborators:** France CIRAD & IRD, FLAR, IRRI, WARDA, Japan JIRCAS, Korea RDA, Brazil EMBRAPA, Colombia FEDEARROZ & CORPOICA, Peru INIA, Venezuela INIA & DANAC, Cuba IIA, Nicaragua, Bolivia CIAT, Chile INIA & U. La Plata, Uruguay INIA, Argentina U. Corrientes, China, US Universities: KSU, Cornell, Purdue, LSU, U. Arkansas, Texas A&M and Yale.

**CGIAR system linkages:** Enhancement and Breeding (50%); Protecting the Environment (20%); Saving Biodiversity (15%); Transfer of Technologies (10%); Crop Systems (5%). Linked to IRRI and WARDA.

**CIAT project linkages:** Germplasm conservation SB-1, genomics SB-2, participatory research SW-3 for upland in hillsides PE-3 and cropping systems SW-2 for the savannas. Provide improved germplasm to PE-1 and PE-2.

**Project IP-4 Log-Frame 2003**

**Improved Rice Germplasm for Latin America and the Caribbean**

| **Narrative Summary** | **Measurable Indicators** | **Means of Verification** | **Important Assumptions** |
|---|---|---|---|
| **Goal**<br>To add to the well being of the rice sector with emphasis on the resource poor rice farmers by increasing genetic diversity and the stability of high yielding varieties. | Increased rice production with farmers having more access to improved germplasm and information, and markets. | National production statistics | |
| **Purpose**<br>To produce robust high yielding rice varieties requiring lower inputs, we will provide well-characterized progenitors and advanced materials with an ample genetic base as well as training to our partners. | Monitoring of yields of new varieties that were developed using our improved germplasm.<br>Reductions in pesticide use and lower costs of production due to adoption of ICM practices leading to stable production and a cleaner environment. | Project, CIAT, FLAR and NARS annual reports.<br>Publications.<br>Impact assessment reports | Stability (internal and external)<br>National policies favor adoption of new technology. |
| **OUTPUT 1.**<br>Enhancing Gene Pools | Rice populations with improved tolerance to biotic and abiotic stresses with good grain quality and physiological traits.<br>Number of populations and lines selected as well as the distribution of these for line development.<br>Number of double haploid produced and used. | Project, CIAT, FLAR and NARS annual reports.<br>Publications.<br>Improved varieties released by partners. | Continued donor support.<br>Maintaining multidisciplinary team |
| **OUTPUT 2.**<br>Integrated Pest and Disease Management | Understanding components of resistance and virulence of rice blast, rhizoctonia, hoja blanca, crinkling disease, and other selected pathogens.<br>Molecular markers associated and number of resistance genes for rice pathogens and pests.<br>Crop management components developed.<br>Using novel genes resistance to rice pathogens including hoja blanca and rhizoctonia. | Project, CIAT, FLAR and NARS annual reports.<br>Publications.<br>Pest and disease resistant varieties released by partners. | Continued donor support.<br>Maintaining multidisciplinary team |
| **OUTPUT 3.**<br>Education and Rice Cultivation as a Vehicle to Alleviate Poverty | Number of communities participating<br>New varieties and small equipment for rice<br>Number of workshops and scientists trained.<br>Published reports of courses.<br>Development of web pages | Project, CIAT, FLAR and NARS annual reports.<br>Publications.<br>Impact assessment reports<br>CIAT's Rice Web page | Continued donor support.<br>Maintaining multidisciplinary team |

## Project IP-4: Improved Rice Germplasm for Latin America and the Caribbean

### Summary of Annual Report 2003

### Inputs

| Principal Staff | Allocation of time | Affiliations | Work Location |
|---|---|---|---|
| Dr. Lee Calvert | 70% | CIAT | CIAT HQ |
| Dr. Marc Chatel | 100% | CIRAD/CIAT | CIAT HQ |
| Dr. Fernando Correa | 80% | CIAT | CIAT HQ |
| Dr. Zaida Lentini | 20% | CIAT | CIAT HQ |
| Dr. Mathias Lorieux | 50% | IRD/CIAT | CIAT HQ |
| Dr. César Martínez | 51% | CIAT | CIAT HQ |
| Dr. Rafael Meneses | 50% | IIA Cuba/CIAT | CIAT/Cuba |
| Dr. Gilles Trouche | 50% | CIRAD/CIAT | Managua, Nicaragua |
| Dr. Michel Valès | 75% | CIRAD/CIAT | CIAT HQ |
| Total | 5.46 Principal Staff positions | | |
| Dr. Carlos Bruzzone worked as a consultant | 50% | CIAT | Chiclayo, Peru |
| Dr. Douglas White worked as a consultant | 5% | CIAT | CIAT/HQ |

There are 14 associates or assistants, 3 visiting scientists and 28 technical and support staff.

### Budget 2003

PROJECT IP4. Improved Rice Germplasm for Latin America and the Caribbean

| SOURCE | AMOUNT US$ | PROPORTION (%) |
|---|---|---|
| Unrestricted Core | 0 | 0 |
| Restricted Core | 618,220 | 54% |
| Carry over from 2002 | 23,231 | 2% |
| Sub-total | 641,450 | 56% |
| Special Projects | 513,972 | 44% |
| Total Project | 1,155,422 | 100% |

## The CIAT Rice Project Strategic Summary

### Our Project and its Supporters

The CIAT Rice Project IP-4 has nine senior scientists, which devote most of their time to rice. Some have part of their time in the Biotechnology, IPM, and Watershed projects. In addition, the project has fifteen junior staff and twenty-eight technical support staff. There is additional support staff in the Biotechnology project.

Since 2000, the CIAT Rice Project has received substantial contributions from the European Union. Their contribution has allowed the Rice Project to prosper and develop new initiatives that are directed improving the small rice farmer. This year, the Rice Project was reviewed by the EU, and their report is favorable to the strategy and actions of the project. The views and suggestions in this report are being critically evaluated to improve our strategy and tactics.

The CIAT Rice Project and CIRAD/IRD have a special relationship. The French institutions have maintained an average of three senior scientists and financial support during the last three years. Before that there were two senior scientists from CIRAD. We work together as a team with credit for our success going to all three organizations. A summary of the impact of some of our collaborative work is in the 2002 Annual Report. This is briefly covered this year in the section on Impact.

The CIAT Rice Project has received substantial contributions over the last decade from the Colombian Ministry of Agriculture. This contribution is for specific activities and assures that we attend to the priorities of the Colombian Ministry of Agriculture. An impact study over the impact of rice in the Llanos of Colombia demonstrated that this investment has paid off for Colombia. We maintain collaborative research with FEDEARROZ of Colombia and they post an average of two of their scientist in CIAT. Both organizations have benefited through this close collaboration and have increased our impact because of the contribution of Colombia. We also receive support through Pronatta and Colciencias for activities not covered by the funds from the Colombia Ministry of Agriculture.

The CIAT Rice Project has direct support from Peru. With these funds we partially support one rice breeder who recently was named as the Project Leader for Rice in INIA of Peru. These activities are in the third year and we expect new varieties will soon be released that are the fruits of this collaboration. We also have a collaborative agreement with IIA of Cuba. We fill our entomology position by contracting a scientist of IIA 50-60% of the time. This has allowed us to expand our efforts in integrated pest management and develop a more intensive set of activities with Cuba.

The Rice Project wishes to express our sincere gratitude to all financial supporters of the project. The Annual Report and this Summary helps inform our partners, donors and other interested partiers of some of our work including the major advances.

**The International Year of Rice**

For the first time, the United Nations has declared a year in honor of a crop, and 2004 will be The International Year of Rice (IYR). We are working with our partners and expect that there will be activities in many countries in the region that will be done use the IYR as the theme. We will be collaborating with our partners to develop new activities that will have lasting impact. An example is the network of rice breeders. We agreed to activate this network in workshop on Aerobic Rice in August with the goal of increasing regional collaboration. The network will be inaugurated next year in a combination of Workshop and Forum on the theme of Rice Breeding. This will be held in Brazil and will include both Irrigated and Aerobic Rice. Other activities will include a workshop in Cuba, a Forum in Colombia and rice biotechnology will be a major theme in a Congress on Molecular Biology that will be held in the Dominican Republic. We expect additional activities will be announced during the following months.

**Why We Use Wild Relatives of Rice in our Breeding Program?**

The genera Oryza consists of 21 wild rice and two cultivated species. After 6,000 years of continuous selection by man and intensive breeding efforts during the last 100 years, the genetic base of the crop, exemplified by the modern rice varieties that ushered in the green revolution and brought about dramatic increases in rice production worldwide, is narrower than ever. There are many reports of rice production in farmers' fields reaching a yield plateau, and the narrow base is contributing to instability of rice yields caused by biotic and abiotic factors. The Oryza wild species represent a potential source of new alleles for improving the yield, quality and stress resistance of cultivated rice. The narrow genetic diversity in the commercial varieties makes them susceptible to both biotic and abiotic stresses. The wild relatives of rice are resistant to many pests and diseases for which there is not sufficient genetic resistance in cultivated rice. There may even be components in the wild species that will increase the yield potential of rice. Interspecific crosses are being made for many traits including *O. rufipogon* as a source of aluminum tolerance and vigorous root growth. High levels of resistance to the rice stripe necrosis virus were found in *O. glaberrima*, and this resistance has been transferred through interspecific crosses to Bg90-2 and Caiapo. The results for disease resistance are also impressive. Advanced breeding lines with resistance to *Rhizoctonia solani* were derived from the interspecific crosses of Oryzica3/*O. rufipogon*. Advanced lines from the crosses of Lemont/*O. barthii* mature early having good yields and excellent grain quality. The diversity of the parents makes these interspecific crosses ideal in studies to develop molecular markers. Already, they have been used to mark regions of the chromosome of rice for important traits. The wild rice species are also an important source of traits for our efforts to develop rice that uses water more efficiently.

**Rice Blast and the Search for Durable Resistance**

Most commercial varieties remain resistant to rice blast for only one to three years. There is a tug of war between the pathogen and the host. While a host may be resistant to many of the rice blast isolates, there always seem to be a subset of the population that is not recognized by the plant's defenses and it soon becomes the predominant. When this happens the resistance is broken and the new variety becomes susceptible to rice blast. Hot spot selection under high disease pressure and pathogen diversity has been the principal method for breeding rice blast resistant lines and varieties. For example, the variety Fedearroz 50 is widely grown in Colombia and has remained highly resistant to rice blast for over three years. The genetic resistance profile of Fedearroz 50 is similar to that of Oryzica Llanos 5. This contrasts with many varieties that start to have problems one to two years after their release. Much work remains before we can declare that we have the knowledge and methods to consistently develop rice with durable resistance, but there is evidence that step-by-step, we are making progress. Oryzica Llanos 5 is a variety that was developed through hot spot breeding and is exceptional because it has remained resistant to rice blast for more than a decade. The genome of Oryzica Llanos 5 is being analyzed to identify its combination of resistance genes. And this is part of a larger effort to catalog both the resistance genes in the plant as well as the virulence genes in the fungus. Using near isogenic lines carrying individual resistant genes and biological testing with the known rice blast lineages, progress is being made. The search is on for molecular markers, and with the information from the rice genomic sequencing project, the rate of discovery of rice blast resistant genes is increasing. This will facilitate the isolation, characterization and utilization of these genes. Already, we are testing associations of these genes in order to develop rice varieties that have a series of resistant gene combinations that confer durable resistance.

**The New Rice Hoja Blanca Resistant Varieties are Better than their Parents**

Developing rice varieties with resistance to rice hoja blanca virus (RHBV) has been a research objective even before the CIAT came into existence. For many years, it seems like there was only marginal progress and most commercial varieties are not resistant to hoja blanca disease. In the mid-1990s, it appeared as if a new epidemic was imminent. CIAT, Fedearroz in Colombia and Danac in Venezuela made the development of RHBV resistant varieties a priority. In addition to the mass screen method that has been in place since the mid 1980's, we introduced an evaluation scheme using different levels of disease pressure. Since the capacity is more limited, only selected advanced lines can be evaluated by intensive screening. Nevertheless, this has led to the liberation of five varieties with resistance to hoja blanca disease in Venezuela and Colombia. Two varieties, Fedearroz 2000 and Fedearroz Victoria 1 have resistance to hoja blanca disease that is superior to any of their parents including the principal source of resistance Colombia 1. Fedearroz 2000 is the most resistant variety and is now considered the standard for high resistant to hoja blanca disease. The release of these varieties confirms the success of the two-step breeding strategy, and these new commercial varieties are recommended as parental sources for hoja blanca resistance.

**The Role of Biotechnology**

The CIAT rice project is positioned in the middle of another agricultural revolution. Both the entire sequences of the rice genome and the genome of the rice blast fungus are in the public domain. The challenge is to use the increasing knowledge to benefit all the rice farmers. Most rice farmers in Latin America and the Caribbean are small farmers with limited resources. The rice project at CIAT is working to broaden the genetic base of the rice crop by bringing in useful traits from the wild relatives of rice. Recurrent selection, anther culture and transgenesis are methods that we have been utilizing to increase the efficiency of our breeding efforts. We are working to understand the mechanisms and genetics of resistance to rice blast and rice hoja blanca virus to facilitate the development of rice cultivars with durable resistance to these diseases. Many agronomic traits such as drought resistance, earliness, yield and quality are essential for the success of new varieties. The entire process of breeding will soon change. For more than 100 years, it has been an empirical science and the breeder has needed to be both a scientist and an artist. By knowing the genetic basis of characteristics of agronomic importance and where they are located on the genome, we expect that our breeders will be able to juggle many more traits and produce rice varieties more efficiently.

To help facilitate the understanding of the rice genome, we are collaborating with advanced research centers. For example, in the framework of its work plan for functional analysis of cereal genomes, the Génoplante consortium constructed a rice T-DNA insertional mutagenesis collection. This T-DNA mutant collection is a library of 35,000 lines obtained from transformation by *Agrobacterium tumefaciens*, using a specific construct called the *T-DNA insert*. A T-DNA mutant collection is a powerful tool for discovering gene functions.

Génoplante in collaboration with CIAT initiated a program of seed multiplication and phenotypic analysis of a rice T-DNA mutant collection. This year, the first set of 5,000 lines were evaluated. An English-Spanish-French lexical of botanical and agronomic terms was established to facilitate phenotype identification. A phenotypic database was set up to serve the functional genomics studies. Numerous interesting phenotypes were observed, including modifications of size, tillering, lesion mimics, panicle development, general architecture, and chlorotic or albino leaves. The T2 seed was harvested and constitutes a rice T-DNA mutant stock center for future distribution and collaboration with Génoplante partners. It will be sent to Génoplante, France as well as stored at CIAT. CIAT is well positioned to be part of this basic effort of gene discovery.

**Rice for the Small Resource Poor Small Landholders**

Impact assessment has shown that both large and small farmers benefit equally from new technologies when they are in the same agroecosystem. In the Latin America and Caribbean region, most rice producers are small farmers living in marginal areas and they have not benefited from the technologies that are improving production in major rice growing zones. The CIAT rice project is increasing our efforts to reach these resource poor rice farmers. Very high rainfall agricultural systems have unique problems. These include flooding, low luminosity, high disease incidence as well as post harvest problems including the drying of the grain. Most small rice farmers produce their crop in areas with at least one season of adequate rain. They do not have irrigation infrastructure and they suffer when the rainfall is less than normal. In these environments drought, pests and diseases are all major problems. Breeding populations have begun for many of these difficult environments. Also there are several projects were we are working with farmers groups to select the best varieties for their needs. This is becoming an important in understanding the problems of the small farmers and being able to direct our efforts to better serve them.

**Rice, a Crop with Impact**

The combined effort of CIAT and our many partners has led to higher yielding rice varieties throughout the region. The successful use of these genetic resources as the parents of varieties was documented for upland rice. Over 90% of the approximately 40 upland varieties released in the last 20 years have at least one CIRAD parent. Germplasm that has been developed by CIAT has also been crucial as parents, and often the crosses for these varieties were made at CIAT. For the last decade, CIRAD and CIAT have had a strategic alliance that has worked synergistically by bring the critical human capital, resources and infrastructure together to help serve the Latin American and Caribbean rice sectors. Since the mid-1990s recurrent selection was emphasized as a new tool in the breeders arsenal, and the CIAT/CIRAD collaborative project in Brazil is now bearing fruit. In the State of St. Catarina Brazil in 20002, the release of “Tio Taka” was a milestone since it is first variety to be developed by recurrent selection in the region. There are currently populations that have been developed using recurrent selection in eleven countries, and there are many advanced lines that could soon be selected as new varieties. We expect that Tio Taka will be the first of many varieties that have their origins in recurrent selection populations, and this shows the importance of maintaining a consistent effort over a sufficient period of time.

An example, where CIAT, CIRAD, CORPOICA, and FEDEARROZ worked together, is documented in the impact study “Un negocio de amplios horizontes para el Llano”. It is estimated that the economic impact of CIAT rice projects contribution is $450 million between 1994-2001. The strategic alliance of CIRAD/CIAT with many local partners will continue to have lasting impact in the Llanos of Colombia and throughout Latin America.

In the last few years, FEDEARROZ has released 5 new varieties for Colombia. FEDEARROZ and CIAT have worked closely together in the development of these varieties. Traits including resistance to rice blast and rice hoja blanca virus and its vector as well as quality analysis were areas of collaboration. The success of Fedearroz 50 has surpassed expectations. Released late in 1998, more than 60% of the rice grown in Colombia is now Fedearroz 50. This variety is yielding on average 7 t/ha and this is the principal reason that Colombia returned to self-sufficiency in rice production after several years of deficits. More recently Fedearroz 2000, Victoria 1, Victoria 2, and Colombia XXI were released. FEDEARROZ will soon release this year two new commercial varieties: CT 11275-3F4-8P-2 and CT 11369. Several of these promise to become important varieties, but they must compete with Fedearroz 50.

Another example of varieties that fit the needs of small farmers is the release of an early variety in Pucallpa Peru. Although the early variety did not have higher yields than the local varieties, it did allow the farmers to grow more rice during the season. This is because of labor constraints at the time of harvest. We expect to continue to learn about the special needs of small farmers and attempt to help fill some of their needs using participatory activities.

Many other varieties, most of which have CIAT/CIRAD parentage, were released in countries throughout Latin America and the Caribbean region. We are reactivating international observational nurseries to assure the continued flow of diverse genetic resources. We are confident that our partners will continue to make good use of these genetic resources.

**Future Perspectives**

The rice project has the challenge of being part of the bridge between the rapid advances in the molecular characterization of rice and producing usable products for the rice farmers. The Rice project (IP-4) and Biotechnology (SB-2) are working together to develop the strategic alliances to bring the advances of molecular biology into practical applications. The Rice project is also strengthening partnerships to better meet the needs of rice farmers with emphasis on the resource poor small farmer. Water efficient rice and developing information systems will both be important to assure that the small farmers continue to receive their share of the benefits from the research done by CIAT and our partners.

## Problems Encountered and their Solutions

### Quarantine Restrictions

Quarantine restrictions are increasing for both the export and import of rice germplasm. In Colombia, we are having problems meeting the requirements of some countries. Better facilities are needed for the production of exceptional clean rice for export. This is not an issue for rice but all of the CIAT crops. CIAT should have a facility for the production of clean seed.

### Demand for Region Breeder's Workshops

This activities has a very high priority with our partners, and we had a very successful meeting this year in Villavicencio. This is in effect a renewal of the INGER workshops that were stopped in approximately 1992. In order to reduce costs, it was decided to join Irrigated and Aerobic rice workshops. A stable source of funding would allow the meeting to be better attended and have greater regional impact. The long term funding of these workshops is needed.

### Capital Equipment

The recommendations to purchase capital equipment are now made by the disciplinary groups. The projects are not consulted, but it is the projects not the disciplinary groups that are responsible for accomplishing their workplans and outputs. While the advice of the disciplinary groups is valuable to prevent undo redundancy of capital equipment, the needs of each project should be taken into account. Under the present system, disciplinary projects are getting most of the capital allocations and the needs of many projects are being ignored.

## Plans for Next Year

- Develop interspecific rice with characteristics that are hard to find with cultivated rice
- Release varieties that were developed using recurrent selection techniques
- Identify and implement marker assisted selection for incorporating rice blast resistance genes into commercial rice cultivars resistance especially for the genes Pi-ta[2], Pi-kh, and Pi-sh confering resistance to different genetic lineages of the pathogen
- Identify new blast resistance genes in wild species of rice and unutilized germplasm within *O. sativa*
- Identify molecular markers associated to tolerance to sheath blight
- Characterize molecular and pathogenicity diversity in populations of the sheath blight pathogen
- Develop germplasm nurseries with potential donors of resistance to different rice pathogens
- Develop molecular markers of for RHBV and *T. orizicolus*
- Increase activities with additional pests including *Spodoptera* and *Hydrellia*.
- Develop web based IPM information
- Research on biosafety and introduction of novel traits use transgenic approaches.

- Continue the characterization of the Genoplante T-DNA mutant collection
- Develop comprehensive research strategy for Water Use Efficiency in rice
- Develop rice varieties using participatory methods

## Project Performance Indicators

### 1. Technologies, Methods and Tools

#### 1.1 Released Varieties

**Conventional Breeding**

- **Brazil**

**CURINGA**
Origin: CIAT Line (CT13226-11-1-M-BR1)
Adaptation: Upland and Irrigated Rice Ecosystems (Cerrados and Varzea)
Launching: Second Semester 2003

- **Bolivia**

**JACUÚ**
Origin: CIRAD Line (IRAT 357)
Adaptation: Upland Small-farmer's Rice Ecosystem
Launching: February 2003

- **Colombia**

**"Línea 30"**
Origin: CIRAD/CIAT Line (CT 11891-2-2-7-M or CIRAD/CIAT 409)
Adaptation: Upland Savannas Rice Ecosystem
Launching: October 2003

FEDEARROZ is going to release two new varieties and will announce them during their national rice congress in December. They are the lines CT 11275-3F4-8P-2 and CT 11369.

**Composite population breeding**

- **Bolivia**

**PCT-4\0\0\1>S2-1584-4-M-5-M-6-M-M**
Origin: CIRAD/CIAT (Upland *japonica* composite population PCT-4)
Adaptation: Upland Small-farmer's and Mechanized Rice Ecosystems
Launching: February 2004
First Upland variety coming from the Composite Population PCT-4

## 1.2. Elite Materials

- **Colombia**

PCT-4\SA\1\1>975-M-2-M-3
Origin: CIRAD/CIAT (First cycle of recurrent selection of the Upland *japonica* composite population PCT-4)
Adaptation: Upland Savannas Rice Ecosystem
*Promising line coming from Recurrent Selection of the Composite Population PCT-4*

**Rice Blast RILs**

Development and distribution to Latin American partners (Brazil, Argentina, Uruguay, Venezuela) of near isogenic lines with combination of different blast resistance genes

**Aerobic and Irrigated CIAT ION**

These nurseries were developed during 2002 and 2003 and are composed of elite lines.

## 1.3. Genetic Materials Distributed

**Distribution of Germplasm:**

**Aerobic Rice CIAT-ION**

- 3 Upland Rice Nurseries (211 lines)

Seed Increase: November 2002-March 2003
Shipping: April-September 2003
Receptors:

| | |
|---|---|
| Bolivia | CIAT Santa Cruz |
| Brazil | Embrapa Rice and Beans Center |
| Colombia | CORPOICA Regional 8 |
| Costa Rica | National Rice Corporation |
| Cuba | IIA |
| Nicaragua | INTA |
| Honduras | DICTA |
| Venezuela | INIA-Guárico |

**Irrigated Rice CIAT-ION**

Three types of nurseries were distributed to 18 collaborators from Colombia (10), Brazil (2), and one each to collaborators in Argentina, Dominican Republic, El Salvador, Nicaragua, Surinam and Venezuela.

**Participatory Breeding**

- **Elite Materials Developed :**
  - 3 new site-specific and narrow genetic base populations for Nicaragua in creation
  - 71 $S_1$ lines from PCT-18 and PCT-17 populations using PPB schemes

- **Participatory Varietal Selection**

- 40 PVS on-farm trials carried out in Nicaragua and Honduras
- identification of 2-3 preferred varieties with better performances than commercial checks at each PVS site

### 1.4. Genetic Mechanisms Understood

- Resistance to blast in commercial cultivars with durable resistance is controlled by the action of both major and minor genes
- Resistance to sheath blight seems to be controlled by the action of minor genes which can be accumulated to improve the resistance over the parents
- Genetic studies of the inheritance of RHBV were made and fits a 2 or 3 gene model. These populations are being analyzed by molecular markers.

### 1.5. Methods and Tools

- Improved greenhouse methodology for evaluating tolerance to sheath blight (*Rhizoctonia solani*)
- Improved evaluating scale for differentiating tolerant and susceptible germplasm to sheath blight
- Identify molecular markers for improving blast resistance through marker assisted selection
- Developed field methodologies for selection of potential donors of stable blast resistance involved in genetic crosses yielding a higher number of resistant lines
- Implemented molecular markers for analysis of the genetic diversity of the sheath blight pathogen
- Developed quantitative method to determine “dosage” of RHBV in the vector and correlate that with field experience in the mass screening trials. This allows more precise screening for RHBV.

### 1.6. Sources Identified

- Sources of resistance to blast giving origin to stable blast resistant lines in different genetic crosses
- Sources of tolerance to sheath blight selected within interspecific crosses with the species *O. rufipogon* and identification of unknown tolerance within the cultivated species *O. sativa*
- Sources of resistance to crinkling disease selected within interspecific crosses with the species *O. glaberrima*
- Sources of resistance to grain discoloration and leaf scald selected over several years of field evaluations

## 2. Publications

### 2.1. Referred Journals

**Published**
Eleven articles were published in referred journals.

**Submitted**
One was submitted and accepted for publication.

### 2.2. Books

**Book Chapters**

Nine book chapters were published.

**Published Proceedings**

Five articles were published in Proceedings.

**Scientific Meetings or Publications**

Nineteen abstracts, posters and newsletters were published.

## 3. Strengthening NARs and NGOs

Workshops to help strengthen NARs and/or NGOs were carried out in five countries including Colombia, Cuba, Nicaragua, Peru and Honduras.

**Individual Training**

The Rice Project worked with 20 scientists for intensive specialized training.

### 3.1. PhDs, MS & BS

The Rice Project is involved in the thesis with 3 Bachelor of Sciences, 10 Masters of Sciences and 3 PhD Students.

### 3.2. Workshop and Meetings

The Rice Project sponsored or participated in 23 workshops.

### 3.3. Advanced Research Organizations Research Partnerships

CIRAD, IRD, JIRCAS, KSU, Cornell, Purdue, LSU, U. Arkansas, Texas A&M and Yale.

# 4. Resource Mobilization

## 4.1. Proposals Funded

1. Colciencias grant (3 year) for US$4,500
2. FONTAGRO project on characterization of rice blast populations for Argentina and Uruguay (US$5,000).
3. Génoplante. Project for phenotypic and characterization of a new series of T-DNA mutants, funded for US$ 66,000.
4. PERU and GTZ project on characterization of rice blast populations from Peru (US$2,000).
5. Peru STC-CGIAR was funded US$50,000 for 2003.
6. A project proposal on sorghum PPB was accepted by the French regional office of cooperation for Central America for US $ 12,000.
7. PRONATTA was funded US$20,000 for 2003

### a. Proposals and Concept Notes Submitted

- FONTAGRO Costa Rica-CIAT: Búsqueda de Fuentes de resistencia a *Magnaporthe grisea* a partir de la especie silvestre *O. glumepatula*, variedades comerciales, criollas, y arroz rojo.
- Genetic Resource Challenge Program "Unlocking Genetic Resources in Crops for the Resource-Poor". Three concept notes presented requesting US$ 50,000.
- Challenge Program on Water and Food "Developing Cereal and Pulse Cultivars for Sustainable Cropping Systems, Increased Food Security and Community Well-Being in the São Francisco Basin. Presented Full Proposal requesting US$ 125,000.
- Elaboration of two concept notes on participatory rice production improvement for Atlantic area of Nicaragua and High altitude hillsides in Honduras

### b. Resource Mobilization Activities

FAO/IAEA Developing a proposal on the Identification and Pyramiding of Genes Responsible for Crop Quality Characters and Resistance to Quality Affecting Stresses

### Impact Monitored

Impact of CIAT Upland Rice Varieties on Resource-Poor Farms in the Peruvian Amazon
Douglas White, Efraín Leguía, José Sánchez and Sam Fujisaka

## OUTPUT 1. ENHANCING GENE POOLS

### 1A. Rice Improvement using Conventional Breeding and Gene Pools and Populations with Recessive Male-Sterile Genes

- **Aerobic Rice Composite Population Breeding Leads to the Selection of Promising Lines for the Colombian Savannas Ecosystem**

M. Châtel, Y. Ospina, F. Rodríguez, V.H. Lozano


### Abstract

Since 1996, the CIAT/CIRAD activities have gradually phased out intra-specific tropical japonica conventional crossbreeding activities and concentrated on broadening the genetic base of rice by the development and enhancement by recurrent selection of aerobic rice composite populations. These breeding strategies use a recessive male-sterile gene (ms) to develop highly diverse rice populations based on multiple parents.

In Colombia, the basic aerobic composite populations were enhanced using two recurrent selection-breeding methods. At each enhancement cycle, fertile plants are selected for the development of segregating lines and progeny selection using the conventional pedigree methods. The most advanced lines are evaluated in yield trials in collaboration with the Colombian research organization CORPOICA Regional 8 in Villavicencio. Promising lines having good adaptation to acid soils were identified. One promising line extracted from the first cycle of recurrent selection of the composite population PCT-4 shows a higher yield potential with same earliness than the best commercial check (Línea 30) developed by conventional crossbreeding.


**Key words:** Aerobic rice, breeding, composite population, promising line.

### Introduction

Conventional crossbreeding projects of CIAT and CIAT/CIRAD have been and still are a source for the release of new varieties in Latin America including Bolivia, Brazil and Colombia (INGER, 1991). These varieties tend to have a narrow genetic base which needs to be broadened (Cuevas-Pérez et al., 1992; Rangel et al., 1996; Montalván et al., 1998). CIAT and CIRAD have joined forces to propose and implement new breeding tools for the creation and future release to the rice producers of germplasm with wider and different genetic background. The creation of populations with broad genetic base (Châtel and Guimarães, 1998), and their breeding through recurrent selection is a new breeding method proposed and implemented by the CIAT/CIRAD Rice Project.

Since 1996 (Châtel et al., 2001), the activities concentrate on the development and enhancement of aerobic rice gene pools (*Oryza sativa* L., Tropical Japonica type). Using a recessive male-sterile gene (ms) from a mutant of IR36 (Singh y Ikehashi, 1981). The basic populations were enhanced using two recurrent selection-breeding methods. The main purpose of a breeding project is the creation of breeding populations with broad genetic diversity that leads to the identification of promising lines and the development of new cultivars. To do so, fertile plants are selected from the basic composite populations and at each enhancement step by recurrent selection. They are the starting point for the selection of segregating progenies by conventional pedigree method and the identification of promising fixed lines. The number of progenies developed from population breeding has steadily increased from 1997. This report presents the outputs of the breeding strategy of the composite population PCT-4 and the identification of promising lines adapted to the Colombian savannas rice ecosystem.

**Materials and Methods**

**Composite Population Breeding**

Population breeding by recurrent selection is very efficient for trait improvement showing low heritability. Through short selection-recombination cycles, linkage blocks are broken down and favorable genes are accumulated. This is a smooth process of continuous improvement. Rice composite populations are highly segregating for numerous traits and are made of fertile (Msms) and male-sterile plants (msms) allowing natural cross-pollination. Grains produced by male-sterile plants are Msms and msms (pollen produced by fertile plants is ms or Ms and female organs of male-sterile plants are ms). To allow complete recombination between early and late flowering material, two to three sowing dates are made in the same plot. To avoid pollen contamination from other rice, a border of maize is planted around each population. Harvesting the male-sterile plants represents a new cycle of recombination as well as seed multiplication of the population.

**Composite Population Breeding Strategies**

Recurrent selection is a cyclic process involving three main steps: plant selection (selection unit), evaluation and recombination (recombination units) of the best performing selection units. Two recurrent selection strategies where used: mass recurrent selection and $S_2$ progenies evaluation. The first strategy is based on phenotype selection or mass selection on both sexes before flowering time. $S_0$ plants of each cycle of recurrent selection are at the same time the selection and recombination units. Each recurrent cycle is one year long. To select for traits such as acid soil tolerance and disease resistance, sufficient selection pressure is needed in the field trial. The second strategy involves progeny evaluation. $S_0$ fertile plants are selected during the normal cropping season (March-September) at LSE. The generation $S_1$ is advanced at the Experimental Station of Palmira (PES) during the period October-February. $S_2$ seeds are harvested at PES and planted at LES during the normal cropping season. $S_2$ lines are evaluated and compared to commercial checks in a statistical design (Federer, 1956). The best progenies are selected and then recombined from the remnant seed from $S_0$ plants. A recurrent cycle is completed in 2 years.

## Selection of Fertile Plants for Line Development

The selection of $S_0$ fertile plants (Msms) is the starting point for segregating line development. Throughout the selection process, selecting and harvesting only fertile plants allows the elimination of the male-sterile gene, and advanced progenies are 100% fertile (MsMs). Line development follows traditional evaluation and pedigree selection. The major characteristics bred for savanna conditions are early vigor, tolerance of soil acidity, resistance to rice blast (*Pyricularia grisea* Sacc.), good grain quality (translucent, long-slender grain) and early maturity (total cycle about 115 days). A total of 179 advanced $S_9$ lines were selected using the pedigree method. These materials come from different phases of enhancement of the population PCT-4 (Table 1) and pass through the overall process of selection and agronomic evaluations.

## Yield Trials

Promising lines from different breeding populations were selected during the last years. Some of them were evaluated in preliminary experimental yield trials in Colombia, at LES and on-farm, in collaboration with CORPOICA Regional 8. The experimental design was of randomized blocks with 3 replications. The yield trials were cultivated on acid soils (Table 2). The fertilization was 300 $kg/ha^{-1}$ of dolomite lime applied 30 days before sowing (calcium and magnesium supplements but not enough for acid soil correction), 177 $kg/ha^{-1}$ of nitrogen (59 $kg/ha^{-1}$ at 20, 35 and 45 days after sowing), 155 $kg/ha^{-1}$ of phosphorus at sowing and 116 $kg/ha^{-1}$ of potassium (58 $kg/ha^{-1}$ at sowing and 29 $kg/ha^{-1}$ at 20 and 35 days after sowing). No pesticide or insecticide was applied. The 24 advanced lines selected from the first recurrent selection cycle of the population PCT-4 and 3 commercial checks (Oryzica Sabana 6, Oryzica Sabana 10, and "Línea 30") were evaluated. During the cropping seasons 2000 and 2001 the trials were located at LES and in 2002, in collaboration with CORPOICA, they were conducted in five places, including two at LES, and three on-farm trials under savanna condition (see section 2 of this report).

## Results and Discussion

The combined analysis of the 3 years at LES (Table 3) shows that grain yields vary between 1550 and 3300 $kg/ha^{-1}$. The checks Oryzica Sabana 10, Oryzica Sabana 6 and Línea 30 yielded 1550, 2614 and 2038 $kg.ha^{-1}$, respectively. Línea 30 showed erratic yields between the years and the lowest yield was in 2002. This is mainly because of spicklets sterility due to low temperatures (Figure 1) as a consequence of cold fronts that came from Brazil. Cold susceptibility of the Línea 30 was formerly detected when it was cultivated in the coffee region of Colombia at 1450 masl where more than 60% of the spicklets were sterile. The year 2002 was also atypical with above normal precipitation and below normal solar radiation (Figure 2). The factors in association with cold temperature contributed to high level of physiologic sterility. All the lines from the population PCT-4 flower and mature as early as the Línea 30 and showed stable yields all three years. In 2002, these lines did not have high levels of sterility and that was an indirect measurement of their tolerance to cold. The analysis of the 3 years trials shows that the average yields of the line

PCT-4\SA\1\1>975-M-2-M-3 is 35, 50 and 113% more than Línea 30, Oryzica Sabana 6 and Oryzica Sabana 10, respectively, and is as early as the earliest check Línea 30. This is a confirmation of the last year results where we stated that it was possible to breakdown the negative correlation generally observed between earliness and grain yield.

The best performing line PCT-4\SA\1\1>975-M-2-M-3 at LES was also selected by Hernando Delgado in the trials conducted by CORPOICA Regional 8 (Table 1, Section 2). Twelve other lines showed similar yield potential as the best check, Línea 30. From these 12 lines, CORPOICA Regional 8, selected five (Table 3 and Table 1, Section 2). These lines represent a diversified option for aerobic rice in the Colombian savannas and can contribute to diversify the genetic material for the producers. Grain quality of three lines is presented in the Table 4. In 2002, the 24 lines of the Colombian trial were shipped to Bolivia (CIAT Santa Cruz), Nicaragua (Gilles Trouche CIRAD/CIAT) and Venezuela (Fundación DANAC and INIA Guárico) for local evaluation and selection.

**Seed Multiplication and Genetic Seed Production**

During 2003, the 13 best promising lines were cultivated at PES for seed increase and further evaluation of milling and grain quality. 50 individual panicles of each line were evaluated for the production of genetic seed. Depending of the results, the top best materials will be grown next year in demonstration plots at LES.

**Table 1. Segregating Lines from different Composite Populations Experimental Station La Libertad, Villavicencio-Colombia, 2002**

| Population | Generation and number of line | | | | | | |
|---|---|---|---|---|---|---|---|
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_7$ | $S_9$ |
| PCT-A\PHB\1\0, PHB\1, PHB\1, PHB\1<br>PCT-4\PHB\1\0, PHB\1, PHB\1, PHB\1<br>PCT-5\PHB\1\0, PHB\1, PHB\1, PHB\1<br>PCT-4\SA\1\1\, SA\2\1<br>PCT-4\SA\5\1<br>PCT-4 Bolivia<br>PCT-11 Bolivia<br>CNA-7 Bolivia | 394 | | | | | | |
| PCT-4\SA\4\1 | | 15 | | | | | |
| PCT-4\SA\1\1, SA\1\1<br>PCT-11\0\0\3<br>PCT-4\SA\4\1 | | | 23 | | | | |
| PCT-4\0\0\2<br>PCT-4\SA\2\1 | | | | 23 | | | |
| PCT-4\0\0\0 | | | | | 7 | | |
| PCT-11\0\0\1 | | | | | | 2 | |
| PCT-4\SA\1\1<br>PCT-4\PHB\1\1, PHB\1<br>PCT-A\0\0\0<br>PCT-4\0\0\1 | | | | | | | 178 |

**Table 2. Soil Analysis Experimental Station La Libertad, Villavicencio-Colombia 2002**

| Rep. | cm | M.O. (%) | P Bray II (ppm) | pH | Al | Ca | Mg | K | C.I.E. | B | Zn | Mn | Cu | Al Saturation (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | (meq / 100 g) | | | | | | | | | |
| 1 | 0-20 | 4.1 | 17.4 | 3.9 | 2.39 | 0.90 | 0.37 | 0.25 | 3.91 | 0.52 | 0.48 | 14.6 | 0.52 | 61.1 |
| | 20-40 | 3.5 | 4.7 | 3.8 | 2.96 | 0.45 | 0.17 | 0.11 | 3.69 | 0.66 | 0.40 | 9.0 | 0.56 | 80.2 |
| 2 | 0-20 | 5.9 | 22.5 | 4.1 | 2.70 | 0.69 | 0.34 | 0.22 | 3.73 | 0.23 | 0.41 | 11.1 | 0.49 | 72.4 |
| | 20-40 | 4.3 | 2.0 | 3.8 | 2.90 | 0.30 | 0.12 | 0.17 | 3.32 | 0.12 | 0.29 | 6.53 | 0.42 | 87.4 |

Clay = 43.4%, Sand = 39.1%, Loam = 17.5%.

**Table 3. Selected Lines from the Yield Trial Experimental Station La Libertad, Villavicencio-Colombia, 2000, 2001 and 2002**

| Line from the population PCT-4\SA\1\1* | Grain yield (Kg/Ha) | | | | | | |
|---|---|---|---|---|---|---|---|
| | • Year | | | Average | % O.Sabana 6 | % CIRAD 409 | % O. Sabana 10 |
| | 2000 | 2001 | 2002 | | | | |
| >975-M-2-M-3 | 3644 | 3333 | 2924 | 3300 | 1.35 | 1.50 | 2.13 |
| >975-M-2-M-2 | 3275 | 3480 | 2669 | 3141 | 1.28 | 1.42 | 2.03 |
| >975-M-3-M-2 | 3215 | 3439 | 2580 | 3078 | 1.26 | 1.40 | 1.98 |
| >975-M-3-M-3 | 3367 | 3081 | 2529 | 2992 | 1.22 | 1.36 | 1.93 |
| >982-M-3-M-5 | 3277 | 3240 | 2388 | 2968 | 1.21 | 1.35 | 1.91 |
| >975-M-3-M-4 | 3321 | 3179 | 2375 | 2958 | 1.21 | 1.34 | 1.91 |
| >1479-M-1-M-3 | 3028 | 3477 | 2271 | 2925 | 1.19 | 1.33 | 1.89 |
| >1479-M-1-M-5 | 3016 | 3444 | 2306 | 2922 | 1.19 | 1.32 | 1.88 |
| >1036-M-6-M-2 | 2868 | 3647 | 2206 | 2907 | 1.19 | 1.32 | 1.87 |
| >1479-M-1-M-6 | 3265 | 3300 | 2142 | 2902 | 1.18 | 1.32 | 1.87 |
| >1479-M-1-M-1 | 3240 | 3074 | 2356 | 2890 | 1.18 | 1.31 | 1.86 |
| >975-M-2-M-1 | 2947 | 2972 | 2640 | 2853 | 1.16 | 1.29 | 1.84 |
| >1044-M-3-M-4 | 3379 | 2890 | 2001 | 2757 | 1.13 | 1.25 | 1.78 |
| **Check** | | | | | | | |
| O. Sabana 6 | 2139 | 3226 | 1978 | 2448 | - | 0.90 | 0.63 |
| Línea 30-CIRAD 409- | 2332 | 3531 | 749 | 2204 | 1.11 | - | 0.70 |
| O. Sabana 10 | 1240 | 2770 | 641 | 1550 | 1.58 | 1.42 | - |

* PCT-4\SA\1\1: Nomenclature of the population PCT-4. One cycle of recurrent selection cycle for acid soil conditions.

**Table 4. Grain Quality of 3 Bets Promising Lines and Checks Years 2000, 2001 and 2002**

| Line | Year | | | % Amylase |
|---|---|---|---|---|
| | 2000 | 2001 | 2002 | |
| | White Belly | | | |
| PCT-4\SA\1\1>975-M-2-M-3 | 0.6 | 0.3 | 0.7 | 25.4 |
| PCT-4\SA\1\1>975-M-3-M-3 | 0.5 | 0.3 | 0.6 | 26.4 |
| PCT-4\SA\1\1>975-M-2-M-1 | 0.9 | 0.4 | 0.7 | 25.0 |
| **Check** | | | | |
| Línea 30 (CIRAD/CIAT 409) | 0.4 | 1.0 | 0.8 | 25.5 |
| Oryzica Sabana 6 | 0.6 | 0.7 | 0.4 | 25.2 |
| Oryzica Sabana 10 | 0.8 | 0.5 | 0.8 | 24.0 |

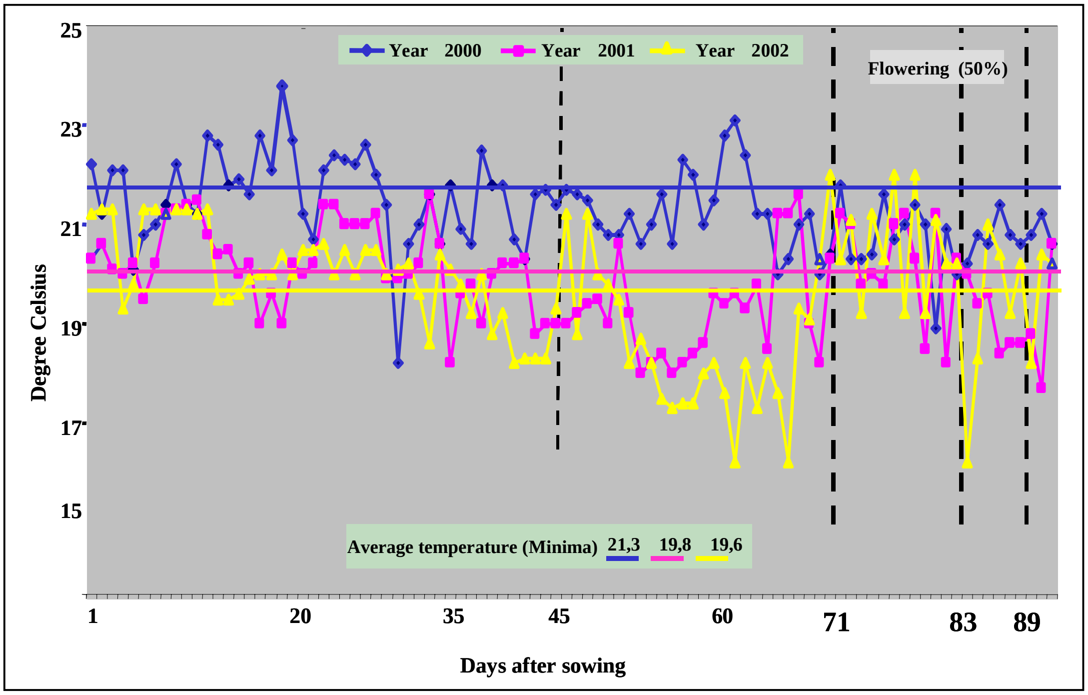


**Figure 1. Minimum Temperature during 3 Cropping Seasons Experimental Station La Libertad, Villavicencio-Colombia, 2000, 2001 and 2002**

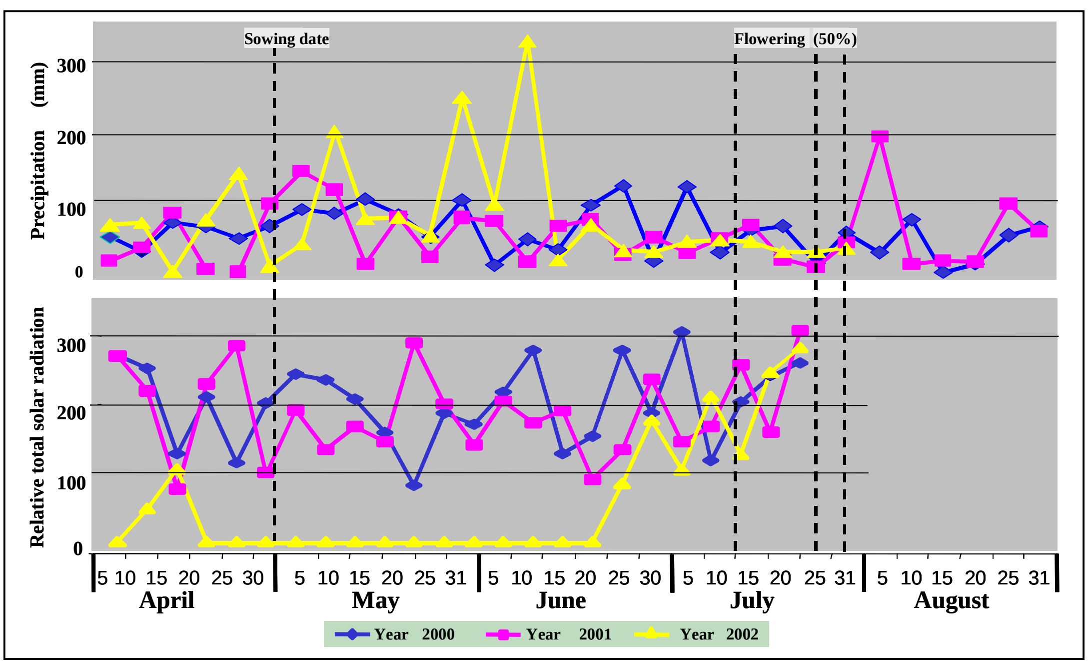


**Figure 2. Rain Precipitation and Total Relative Solar Radiation Experimental Station La Libertad, Villavicencio-Colombia, 2000, 2001 and 2002**

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- **Aerobic Rice Yield Trials in the Colombian Altillanura Savannas and the Research Center La Libertad. CIAT-CORPOICA, 2002**

H. Delgado (CORPOICA Regional 8) and M. Châtel

### Yield Trials

24 advanced lines and 3 commercial checks were evaluated. Location: Farms Santa Cruz, La Palomera and Lagos de Menegua in the Colombian Altillanura savannas and the Research Center La libertad (C.I. La Libertad).

### Farm la Palomera

The majority of the lines presented a high degree of physiological spicklets sterility as a consequence of low night temperature that coincided with the period of influence of the cold fronts coming from Brazil.

### Farm Lagos de Menegua and Santa Cruz

Because the trials were cultivated on soils that had been heavily amended for maize with 2 tons/ha of lime, the material presented high vigor. Stormy weather and strong winds during the grain filling stage lead to the lodging of some of the materials.

**C.I. La Libertad**
In this location the liming was normal for savannas rice cultivation (300 kg/ha). Nevertheless, some lines did not presented good plant type and were too tall with fragile straws and susceptible to lodging.

**Line Selection**
Base on the evaluations, observations and yield potential mainly at the farm Santa Cruz and C.I. La Libertad, 12 materials (Table 1) were selected for evaluation next year in native savannas conditions with the recommended lime application for savannas rice. One location will be the new experimental station of CORPOICA (Estación Experimental Sabanas).

**Recommendation**
More emphasis need to be the selection of promising lines with shorter plant height and/or strong straws for avoiding lodging problems. As the future varieties have to be well adapted to the specific conditions of the Colombian savannas and specifically to blast strains that are different from the ones present at C.I. La Libertad, it is important to select and evaluate the lines in the savannas area.

**Table 1. Aerobic Rice Lines Selected by CORPOICA for Next Year Evaluation**

| Entry | Pedigree |
|---|---|
| 104 | PCT-4\SA\1\1>540-M-3-M-5 |
| 105 | PCT-4\SA\1\1>975-M-2-M-1 |
| 107 | PCT-4\SA\1\1>975-M-2-M-3 |
| 109 | PCT-4\SA\1\1>975-M-3M-3 |
| 110 | PCT-4\SA\1\1>975-M-3-M-4 |
| 111 | PCT-4\SA\1\1>982-M-3-M-4 |
| 113 | PCT-4\SA\1\1>1036-M-6-M-2 |
| 114 | PCT-4\SA\1\1>1044-M-3-M-2 |
| 116 | PCT-4\SA\1\1>1260-M-6-M-6 |
| 120 | PCT-4\SA\1\1>1479-M-1-M-5 |
| 123 | PCT-4\SA\1\1>1837-M-2-M-2 |
| 124 | PCT-4\SA\1\1>1837-M-2-M-3 |

- **Aerobic Rice International Observation Nursery for Latin America and the Caribbean**

M. Châtel, Y. Ospina, F. Rodríguez, V.H. Lozano, D. Guzmán

**Abstract**

As IRRI was no more in condition to finance its association with FLAR which was in charged of the distribution of the INGER-LAC public nurseries to regional NARS, this activity was formally reactivated by the Rice Project in late 2002. The new nurseries are called CIAT-ION (International Observation Nursery) and for the specific case of aerobicrice, 3 CIAT-ION for a total of 211 materials, were dispatched to 8 national breeding programs of Latin America and the Caribbean.

**Key words:** Nurseries, public material, upland rice, national programs, Latin America and the Caribbean.

**Introduction**

CIAT rice germplasm sharing with regional partners was made through a mechanism known as INGER-LAC nurseries sponsored by IRRI. From 1996 to 2001, IRRI was a funding partner of FLAR, which in counterpart was managing and distributing the public INGER-LAC nurseries. At the end of 2001, IRRI took the decision to discontinue fundinig INGER-LAC and its partnership with FLAR. This was a worldwide decision to decrease the costs of INGER. In 2002, to continue attending the necessity of the rice national programs, the IP-4 Rice Project of CIAT took the initiative to formally reactivate the sharing of public genetic goods it produces through its own nurseries. Regional partners were informed of the continuity of public germplasm sharing and the new nurseries are called CIAT-ION (International Observation Nursery).

**Aerobic Rice Nurseries**

The Aerobic Rice Project CIAT/CIRAD is in charge of the preparation and dispatch of the three aerobic rice CIAT-ION nurseries.

1. CIAT-ION **SC** (Conventional crossbreeding), with 27 advanced CIAT/CIRAD lines.
2. CIAT-ION **SI** (Inter-specific crossbreeding) with lines from CIAT (Progreso/*O. barthii* and Caiapo/*O. glaberrima*) and 88 NERICA lines (**NE**w **RIC**e for **A**frica) developed by WARDA-Côte d'Ivoire and introduced and selected at CIAT in collaboration with Dr. César Martínez.
3. CIAT-ION **SSR** (Composite population breeding) with 66 advanced lines ($S_5$-$S_9$) from CIAT/CIRAD.

**Seed Multiplication**
All the lines were grown for seed production at PES during the period October 2002-February 2003.

**Seed Distribution**
To dispatch plant material from Colombia, we need two documents. One is the import permit from the receptor country, and the other one is the phytosanitary certificate from the Colombian quarantine service. Having these documents the material can be dispatched out of Colombia.

**Receptors**
The nurseries were sent with a MTA to 8 receptors of Latin America and the Caribbean (Table 1). In Colombia, the upland CIAT-ION was cultivated at LES, Villavicencio with 3 replications. During the International Aerobic Breeding Workshop held at Villavicencio, August 19-22, 2003, the participants had a chance to evaluate and select lines that had been already been shipped to their respective country.

**Follow-Up**
Sharing breeding material is important for our regional partners as a source of materials for their varietal development activities, and it is also very important for the CIAT/CIRAD breeding project to do the follow-up of the use of the material selected by each receptor. If lines are selected as promising material and/or parental lines for local breeding, this is a direct measurement of the impact of the breeding work done in Colombia for the region. For that reason, we asked each receptor to send back to the project any relevant information about the CIAT-ION. The results of the current trials will be in next year annual report.

**Table 1. Upland Rice CIAT-ION 2003**

| Country | Institution | Responsible |
|---|---|---|
| Argentina | Universidad de Tucumán | Alberto Villegas |
| Bolivia | CIAT Santa Cruz | Roger Taboada |
| Brazil | EMBRAPA Arroz e Feijão | Orlando Peixoto |
| Colombia | CORPOICA Regional 8 | Hernando Delgado |
| | CIAT/CIRAD | Marc Châtel |
| Cuba | IIA | Rubén Alfonso |
| Honduras | DICTA and CIAT/CIRAD | Napoleón Reyes |
| Nicaragua | INTA and CIAT/CIRAD | Lázaro Narváes |
| Venezuela | INIA Guárico | Gelis Torrealba |

**OUPUT 1. ENHANCING GENE POOLS**

## 1B. Broadening the Genetic Base of Irrigated Rice in Latin America

- **Utilization of New Alleles from Wild Rice Species to Improve Cultivated Rice in Latin America**

C.P. Martínez, J. Borrero, A. Almeida, M. C. Duque, O. Giraldo, J. Silva, J. Tohme
Funding: CIAT core funds , Ministerio de Agricultura Colombia

### Introduction

Although great impact was made in rice production in Latin America (LAC) during the last 30 years, there still is a need to increase it in a sustainable way to meet increasing demand. New alleles can provide genetic variability for crop enhancement. There is wide genetic variability available in rice, but limited use of this variability has been made. It has been shown (Xiao et al, 1998; Moncada et al, 2001; Tanksley and McCouch, 1997; Thompson et al, 2003) that the Oryza wild species represent a potential source of new alleles for improving the yield, quality and stress resistance of cultivated rice. These studies indicated that *O. rufipogon* possesses new alleles on chromosomes 1 and 2 with positive effects on yield and yield components. However, these studies were preliminary, since early segregating populations ($BC_2F_2$) and few replications (1-2 sites) were used. No data are available confirming that yield advantages detected in the $BC_2F_2$ generation were passed on through generations of selection in pedigree nurseries, nor over a wide range of environments. This report focuses on the performance and stability of advanced breeding lines derived from the cross Bg90-2/*O. rufipogon* across locations in LAC, as well as progress made in the evaluation and selection of segregating populations, and distribution of breeding nurseries to our collaborators.

### Materials and Methods

Twenty-five lines ($BC_2F_8$) from the cross Bg90-2/*O. rufipogon*, derived from the $BC_2F_2$ generation following the pedigree method were planted in replicated yield trials in eleven locations under irrigated conditions (seven sites in Colombia and one each in Argentina, Surinam, Uruguay and Venezuela). This work was done in close collaboration with key partners from the national rice programs and private sector. Transplanting was done in CIAT while direct seeding was done elsewhere. A completely randomized design with three replications was used and crop management was based on recommended local agronomic practices. Varieties grown locally were used as checks. Data on the main agronomic traits, including grain yield, were taken. A two-way analysis of variance was used for the analysis of grain yield, while a GEBEI package that implements appropriate clustering and ordination procedures and an AMMI model were used in the analysis of the GxE data. DNA of young leaves from the parental genotypes and their progenies was extracted by the Dellaporta method (McCouch et al. 1988) modified for PCR assay by the CIAT Biotechnology Unit. Subsequent molecular assays were performed using 76 SSRs.

Traits for yield and yield-related characters were associated with the 76 molecular markers using simple point analysis**.**

Breeding nurseries of segregating populations derived from crosses between elite lines and wild rice species were planted in Santa Rosa Experimental Station, Villavicencio using standard screening methodologies already established by the Rice Project.

## Results

### Grain Yield

Data are presented in Figure 1 and Table 1. Statistical analysis showed no significant difference in grain yield between Bg90-2 and its progeny over all locations. Although none of the interspecific lines out yielded Bg90-2 in all locations, several lines performed better than Bg90-2 in each location. Analysis of the GxE (data not shown) indicated that contrasting and distinct environments were included in these trials and that the GxE interaction was high (75%). This suggests that the performance of genotypes was dependent on the climatic/soil conditions in each location and that there was a good level of genetic variability present in this group of lines, which explains the better performance of some progenies under specific conditions. This is very important for breeding purposes since the genetic variability hidden in this population was only observed when the progenies were exposed to a diverse set of climatic/soil conditions found in different rice growing areas.

Analysis of molecular data from the bulked seed sample of the $BC_2F_8$ lines shows that all of them had introgressions derived from *O. rufipogon* (Table 1). The number of introgressions ranged from 2 to 18 and this is estimated to be from 2.6% to 23% of the genome from *O. rufipogon*. More introgressions were detected in chromosomes 2(14), 5(9) and 7, 12, 3(7); chromosomes 4 and 10 had two introgressions. There was no correlation between number of introgressions and grain yield (Figure 1). Markers RM5 and RM1, located on chromosome 1, were found in some of the highest yielding lines.

Molecular data from the $BC_2F_2$ generation (Annual Report 2001) were compared with that of $BC_2F_8$. Only 46% of the 76 SSRs were detected in both generations, suggesting that some markers were lost during the phenotypic selection carried out through the generation advance. This was expected. However, 52% of the markers detected in the $BC_2F_8$ lines were not detected earlier in the $BC_2F_2$ generation. This could be explained because plants sampled for molecular and agronomic characterization were different. On the other hand, only 48% of markers detected in $BC_2F_8$ lines are somewhat associated with yield or yield components.

**Table 1. Grain Yield (t/ha) and Percentage of Introgression from *O. rufipogon* of Advanced Lines from the Bg90-2/*O. rufipogon* Cross**

| Pedigree | Bulk sample *O. rufipogon* No. Loci | Bulk sample *O. rufipogon* % | Yield (t/ha) Aceituno | Armero | CIAT | Jamundí | Montería | Saldaña | Villavicencio | Argentina | Surinam | Uruguay | Venezuela | Overall | Bg90-2 % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01.CT13941-11-M-25-1-M-M | 11 | 14.3 | 11.2 | 4.8 | 5.9 | 7.5 | 11.2 | 6.5 | 6.5 | 10.2 | 4.3 | 4.3 | 6.2 | 7.1 | 118.4 |
| 02.CT13941-11-M-25-4-M-M | 11 | 14.3 | 10.6 | 5.1 | 5.2 | 7.8 | 9.8 | 7.1 | 5.6 | 9.3 | 3.7 | 3.8 | 6.0 | 6.7 | 111.2 |
| 03.CT13941-11-M-25-5-M-M | 10 | 13.0 | 11.5 | 5.1 | 6.0 | 6.8 | 11.0 | 7.5 | 6.6 | 10.1 | 3.5 | 2.6 | 5.4 | 6.9 | 114.7 |
| 07.CT13941-27-M-19-1-M-M | 9 | 11.7 | 10.3 | 4.2 | 4.9 | 6.9 | 9.3 | 5.9 | 4.9 | 11.2 | 3.2 | 1.4 | 6.3 | 6.2 | 103.1 |
| 08.CT13946-26-M-5-3-M-M | 9 | 11.7 | 11.7 | 4.9 | 5.5 | 5.3 | 9.9 | 7.9 | 6.1 | 12.0 | 3.2 | 6.7 | 8.4 | 7.5 | 124.0 |
| 09.CT13946-26-M-5-6-M-M | 8 | 10.4 | 11.4 | 4.5 | 4.4 | 7.6 | 10.5 | 6.4 | 5.4 | 11.3 | 3.7 | 4.9 | 6.7 | 7.0 | 115.6 |
| 10.CT13956-29-M-14-1-M-M | 18 | 23.4 | 10.9 | 4.2 | 5.5 | 7.0 | 10.4 | 5.8 | 6.0 | 11.5 | 3.8 | 3.7 | 7.5 | 6.9 | 114.7 |
| 11.CT13956-29-M-25-7-M-M | 9 | 11.7 | 10.7 | 3.3 | 4.6 | 4.4 | 10.3 | 5.9 | 5.5 | 9.9 | 3.9 | 4.4 | 7.0 | 6.4 | 105.2 |
| 12.CT13958-12-M-1-7-M-M | 2 | 2.6 | 12.2 | 5.3 | 4.2 | 4.6 | 9.6 | 8.0 | 5.9 | 12.5 | 4.1 | 5.9 | 7.0 | 7.2 | 119.2 |
| 13.CT13958-13-M-17-5-M-M | 6 | 7.8 | 11.3 | 5.0 | 5.0 | 7.1 | 11.9 | 7.7 | 6.1 | 10.2 | 3.7 | 6.2 | 6.6 | 7.3 | 121.6 |
| 14.CT13958-13-M-2-1-M-M | 13 | 16.9 | 11.2 | 4.6 | 5.5 | 4.3 | 9.8 | 6.6 | 4.7 | 10.7 | 3.9 | 3.9 | 7.7 | 6.7 | 111.2 |
| 15.CT13958-13-M-2-3-M-M | 10 | 13.0 | 11.4 | 3.9 | 5.8 | 6.8 | 10.9 | 6.4 | 4.4 | 10.6 | 3.6 | 5.9 | 7.5 | 7.0 | 116.4 |
| 16.CT13958-13-M-2-4-M-M | 9 | 11.7 | 11.3 | 3.7 | 5.6 | 6.0 | 9.7 | 6.2 | 4.1 | 10.3 | 4.0 | 4.5 | 6.2 | 6.5 | 107.7 |
| 17.CT13958-13-M-7-5-M-M | 12 | 15.6 | 11.9 | 4.8 | 5.3 | 4.9 | 10.0 | 7.7 | 5.1 | 8.8 | 4.2 | 3.1 | 8.0 | 6.8 | 112.2 |
| 18.CT13958-13-M-26-4-M-M | 15 | 19.5 | 11.3 | 3.5 | 4.9 | 5.3 | 9.7 | 6.5 | 4.9 | -- | 3.5 | 5.8 | 7.0 | 6.3 | 103.6 |
| 19.CT13958-13-M-26-5-M-M | 16 | 20.8 | 11.5 | 4.9 | 5.5 | 6.2 | 8.9 | 6.8 | 4.4 | 9.4 | 3.5 | 5.1 | 7.2 | 6.6 | 109.0 |
| 20.CT13958-13-M-33-1-M-M | 15 | 19.5 | 12.0 | 5.4 | 4.9 | 5.3 | 8.8 | 6.3 | 5.3 | 9.5 | 3.6 | 3.8 | 6.6 | 6.5 | 107.7 |
| 21.CT13956-29-M-29-2-M-M | 12 | 15.6 | 11.6 | 3.9 | 5.6 | 5.9 | 9.5 | 7.3 | 5.5 | 12.6 | 3.4 | 5.1 | 6.5 | 7.0 | 116.2 |
| 22.CT13956-29-M-8-3-M-M | 10 | 13.0 | 11.0 | 5.3 | 3.9 | 5.3 | 10.2 | 7.4 | 5.4 | 8.9 | 2.8 | 3.2 | 6.4 | 6.3 | 104.6 |
| 23.CT13959-3-M-10-4-M-M | 8 | 10.4 | 11.3 | 5.6 | 3.9 | 5.5 | 9.1 | 7.3 | 5.0 | 11.6 | 3.7 | 4.2 | 5.8 | 6.6 | 109.8 |
| 24.CT13959-3-M-10-5-M-M | 7 | 9.1 | 12.2 | 4.7 | 5.0 | 5.3 | 9.4 | 8.0 | 5.0 | -- | 4.2 | 5.1 | 7.4 | 6.6 | 109.8 |
| 25.CT13976-7-M-14-1-M-M | 9 | 11.7 | 11.1 | 4.6 | 4.8 | 4.7 | 8.8 | 7.6 | 4.5 | 10.8 | 3.5 | 5.6 | 4.9 | 6.5 | 107.4 |
| 26.Bg90-2 | -- | -- | 10.8 | 3.8 | 4.9 | 5.7 | 9.4 | 7.8 | 4.3 | -- | 3.5 | 3.2 | 7.0 | 6.0 | 100.0 |
| 27.Fedearroz50 | -- | -- | 10.5 | 6.5 | 5.4 | 8.6 | 10.0 | 7.8 | 5.0 | -- | -- | -- | -- | 7.7 | 127.0 |
| **Overall Means** | -- | -- | 11.3 | 4.7 | 5.1 | 6.1 | 9.9 | 7.0 | 5.3 | 10.6 | 3.7 | 4.4 | 6.8 | 6.8 | 112.0 |

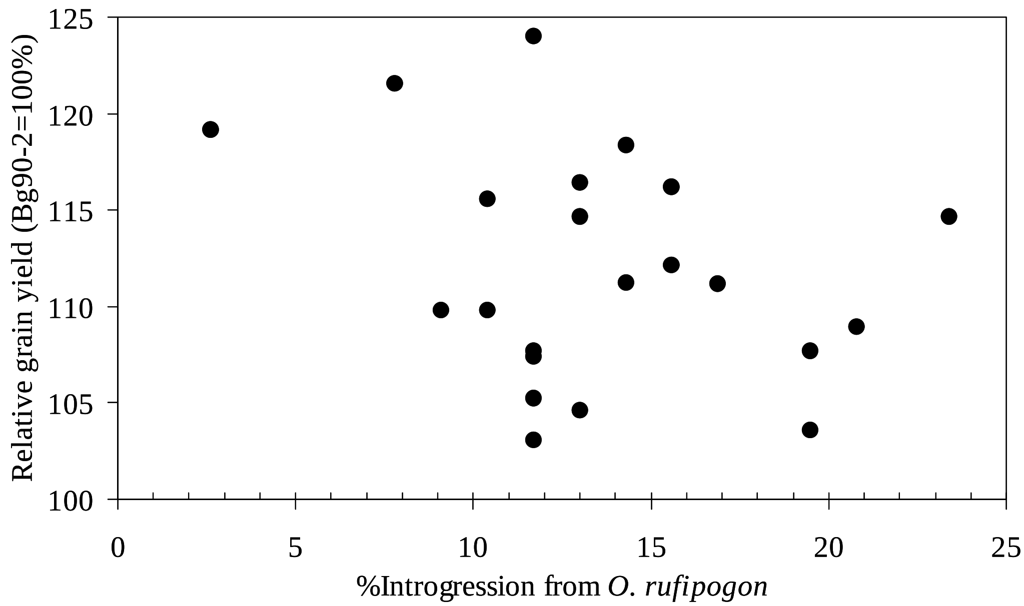


**Figure 1. Relation between relative yield and percentage of introgression from *O. Rufipogon* (all locations included)**

Molecular data confirmed that all breeding lines had introgressions from *O. rufipogon.* This wild species is known to have a high level of genetic variability and is adapted to diverse climatic/soil conditions. Statistical analysis on grain yield and performance through eleven locations in LAC indicated that there were contrasting differences among them.

Therefore, breeding lines were subjected to diverse biotic and abiotic conditions, including high disease incidence (Villavicencio, Saldaña and Jamundi), acid and infertile soils (Villavicencio and Jamundi), cold stress (Argentina and Uruguay) and good climatic conditions (Montería and Aceituno). These site represent a diversity of excellent and poor environments for rice production. However, all lines did better than Bg90-2, the improved/recurrent parent; some of the lines (8, 13, 12, 1, 15, 21, 9, 19, 3) yielded between 15-24% more than Bg90-2. The stability analysis, based on the method described by Eberhart and Russell indicated that all lines were stable across environments. Data suggest that the superior performance of inter specific breeding lines is due to favorable allele introgressions derived from *O. rufipogon*.

**Evaluation and Selection of Segregating Populations**

Genetic variability is an essential requirement to make progress in plant improvement programs, as well as the establishment of sound clearly-defined objectives, the use of appropriate breeding tools, and the availability of good breeding sites. CIAT's rice breeding project makes use of a range of alternatives aimed at broadening the genetic base of cultivated rice, mainly utilization of wild rice species, recurrent selection and introgression of the IRRI new plant type into the Latin American gene pool.

CIAT's Rice Project is fortunate to have access to an excellent breeding site such as Santa Rosa, where evaluation and selection of breeding populations is carried out under high disease pressure starting with the $F_2$ generation. A backcross scheme to diverse elite lines or varieties and top-crosses are used to introgression positive alleles from wild rice species into the Latin American gene pool. The main objective is to develop potential parents or improved inbred lines to be used by national rice breeding programs.

Nearly 400 crosses were made this year with emphasis on the recombination of positive traits exhibited by breeding lines derived from different inter specific crosses. A total of 3985 breeding lines ($F_2$-$F_8$) were evaluated during the first semester in Santa Rosa, under favorable upland conditions. A total of 3334 single plant selections were made for generation advance in CIAT-Palmira. The selection index varied from 8% in the recurrent selection nursery to 25% in more advanced generations.

Crosses between IRRI new plant type and elite lines from our gene pool have produced $F_6$ lines with thick and strong stems, long panicle, intermediate tillering and stay-green leaves. Although most of these lines have bold-grain type, they have a good level of resistance to several diseases.

Crosses between breeding lines derived from inter specific crosses and improved varieties or elite lines produced populations with excellent plant vigor and stems, good plant type, long and slender grains but high sterility. Selection for panicle fertility and tolerance to diseases were the main selection criteria. Sterility continues to be a problem is some combinations, especially with *O. glaberrima*.

About 531 breeding lines ($F_2$, $F_7$ and $F_8$) developed for the collaborative project CIAT/Peru were evaluated this year in Santa Rosa. Nearly 1327 plant selections were made, mainly in the $F_2$ generation. More advanced lines will be included in the CIAT-ION Nursery for 2004.

**Distribution of Breeding Nurseries**

The main objective of these nurseries is to make genetic variability generated in our breeding project available to our partners in NARIs (National Agricultural Research Institutes) as well as other interested organizations whose goals are to develop rice varieties. These nurseries are made available at our discretion and are accompanied by a MTA (Material Transfer Agreement). A major criteria for distribution is that the receiving party informs us of their evaluations so that further improvements to the nurseries can be made.

Three types of nurseries were distributed to 18 collaborators from Colombia (10), Brazil (2), and one each to collaborators in Argentina, Dominican Republic, El Salvador, Nicaragua, Surinam and Venezuela. One of the nurseries included 64 lines derived from wide crosses that are meant to be used as progenitors in breeding programs, while another set included 126 advanced lines derived from crosses among elite parents that could served as the basis for potential rice varieties. The last nursery included 84 lines that could be used for industrial purposes.

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## OUTPUT 1. ENHANCING GENE POOLS

### 1C. The Use of Anther Culture and In Vitro Culture for Enhancement of Gene Pools

- **The Use of Anther Culture and Embryo Rescue for Enhancement of Gene Pools**

A. Mora and Z. Lentini

### Abstract

The Anther Culture Laboratory (ACL) gives an active support to the various rice breeding efforts at CIAT by aiding the production of fixed lines through the generation of doubled haploids and embryo rescue from inter-specific hybrids. This task is accomplished by the coordinated planning and evaluation between the cell tissue culture specialists and the breeders. This year report summarizes the recovery of inter-specific hybrids and the production of doubled haploids lines from various crosses of the CIAT and FLAR breeding programs with their corresponding selection in the field at early and advanced generations in Colombia and abroad.

### Introduction

Homozygous doubled haploids (DH) lines derived from spontaneous chromosome doubling of the microspore haploid genome of rice can be obtained through anther culture (AC) in less than one year, saving time in evaluation trials (DH vs. $F_6$) and in building up pure stocks. It is also possible to gain efficiency with DH populations when selecting for qualitative traits because of the absence of dominance, and for quantitative traits due to a greater additive variance, no intra-family segregation, and no interplant competition (Snape and Simpson, 1981; and Snape, 1989). At CIAT, AC has proved to be useful in accelerating the development of germplasm tolerant to low temperatures and having excellent grain quality, increasing the recovery of useful recombinants from wide crosses for disease and pest resistance, drought tolerance; and facilitating the production of materials suitable for molecular markers gene tagging. The CIAT rice anther culture laboratory (ACL) currently focus on developing doubled haploid lines for the various breeding efforts stationed at CIAT. In the case of CIAT, the work has been mainly directed to advanced populations adapted to the irrigated and upland savanna ecosystems, as well as backcross populations derived from crosses between cultivated rice and wild rice species (Castaño, 2002). In the case of FLAR, CIAT has given a support service. The laboratory has generated lines from FLAR crosses targeting the sub-tropical and cold tolerant breeding lines pools for the Southern cone, and produced somaclone lines for Tropical Latin America. In addition to this support, the laboratory also aids the generation of broad crosses through the rescue of immature embryos from inter-specific hybridizations that otherwise abort few days after pollination.

**Materials and Methods**

For anther culture, plants are planted in the field, panicles harvested and cold pre-treated, and anthers dissected and cultured in vitro according to Lentini et al (1995). Regenerated plants are delivered to the breeders to continue with the selection process under field conditions. In the case of embryo rescue, embryos are cultured in vitro few days after pollination when the endosperm is still in milky stage (liquid). Rooted and elongated seedlings gown in vitro are transferred to the greenhouse and 25-day-old plants are grown in the field. Selected plants are then processed through anther culture.

- **Embryo Rescue from Inter-Specific Hybrids**

A. Mora (IP4), G. Delgado (IP4), S. Sánchez (IP4) J. Borrero (IP4),
C. Martínez (IP4, SB2), Z. Lentini (IP4, SB2)

A total of 336 plants were recovered from inter.-specific hybrids generated with wild Oryza species. About 59% were derived from crosses with the variety Fedearroz 2000, 19% with IRAT 13, 15% with Moroberekan and 3.5% with Fedearroz 50 and Wanda Wab 788, 54-1-1-2- B-4. The objective is to determine with which variety or advanced breeding line there is a higher compatibility, to be used as a first hybridization bridge with *O. sativa*, while generating genetic combinations carrying genes of interest such resistance to RHBV, Pyricularia, yield potential and grain quality. Hybrids recovered from embryo rescue between the variety Lemont and *Oryza barthii* were processed through anther culture. A total of 170 $R_2$ plants were selected and are being evaluated in yield trials. Of these, $R_3$ lines were selected with disease resistance in Santa Rosa Experimental Station. Three of these lines will be evaluated in replicated trials in first semester of 2004.

- **Doubled Haploids Lines Aiding Gene Mapping**

A. Mora (IP4), G. Delgado (IP4), T. Agrono (IP4), C. Ordóñez (IP4),
J. Carabalí (IP4) J. Borrero (IP4), C. Martínez (IP4, SB2), Z. Lentini (IP4, SB2)

Hybrids from the highly incompatible Indica/Japonica Line 93-11/Nipponbare were first rescued to then being processed through anther culture. These genotypes were used to generate the rice genome map in China and Japan. CIAT interest is to generate doubled haploids lines that could be used for the gene mapping of traits of interest. This activity is part of collaboration with Steve Delaporta, Yale University, aimed at generating transgenic rice carrying transposons for their use in functional genomics. A total of 417 hybrid plants were rescued from these crosses but plants are 100% sterile. Anther culture will be used to recovered fertile doubled haploids.

- **Anther Culture for Advancing Breeding Populations of FLAR**

A. Mora (IP4), G. Delgado (IP4), T. Agrono (IP4), C. Ordóñez (IP4), J. Ortega (FLAR), E. Torres (FLAR), M. Cruz (FLAR), E. Pulver (FLAR), Z. Lentini (IP4, SB2)

Doubled haploids are used as a tool to ease the development of lines high yielding with cold tolerance and commercial grain quality traits. Simple crosses generated in 2001 were processed through anther culture, generating 261 $R_2$ lines that were evaluated in Brazil and Uruguay in 2002-2003. Of these plants, about 46% were tolerant to cold, 22% intermediate and 32% susceptible. Three lines were selected as parents combining cold tolerance, good plant type, grain quality, and resistance to Pyricularia with score values between 0-3. The 72 selected plants will be evaluated in Santa Victoria do Palmar (Brazil) in 2003/04 for selection to lower temperatures. These lines combined cold tolerance from Japonica, and plant type and grain quality from Indica types.

Recent sets of $R_2$ lines are derived from triple crosses processed towards the end of 2002 and in 2003. The first set is composed of 185 $R_2$ lines derived from 69 triple crosses, generating 22% tolerant, 20% intermediate and 58% susceptible to cold, respectively. For the second set, 55 triple crosses were first selected by evaluation for cold tolerance at germination stage under cold room conditions. A total of 3,459 plants were generated. Of these plants, 185 lines were selected based on agronomic performance in the field, which 22% were tolerant to cold, 18% intermediate, and 60% susceptible. Only about 10% of the plants showed low amylose (<26%). Seeds of selected plants will be evaluated in the Southern Cone in 2003-2004.

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- **Temporary Immersion System (RITA) for Anther Culture of Rice**

M. Quintero[2], E. Tabares [1], R .Escobar [1], G. Delgado [2], Z. Lentini[1,2]
[1]SB2, [2]IP4

## Introduction

Plant in vitro culture using temporary immersion (RITA) offers all the advantages of a liquid medium system (automation, large scale production, easy changes of medium, filter sterilization, easy cleaning) without any of its drawbacks (reduce gas exchange, vitrification). Immersion time, i.e. duration or frequency, is the most decisive parameter for system efficiency. The optimization of the nutrient medium volume and the container volume also substantially improves efficacy, especially for shoot proliferation. Several reports confirmed large gains in efficacy from temporary immersion when using liquid medium for micro propagation. The main parameters involved reducing production costs are, firstly the drastic reduction of work labor, followed by a reduction in shelving area requirement and in the number of containers used. Scaling up the use of temporary immersions for embryogenesis and shoot proliferation procedures are currently taking place in order to commercialize this process (Berthouly and Etienne, 2002). This system has proved its efficacy for somatic embryogenesis of banana (Alvard et al, 1993; Escalant et al, 1994), coffee (Berthouly et al, 1995; Etienne et al, 1997), citrus (Cabasson et al, 1997), oil palm and rubber plant (Etienne et al, 1997), and at CIAT for cassava (Escobar and Roca, 1999). High efficiency has also been demonstrated for clonal propagation through micro-cuttings of coffee, and sugar cane (Lorenzo et al, 1998); for proliferation of meristems of banana, and pineapple, and for micro-tuberization of potato (Teisson and Alvarad, 1998). We have previously reported preliminary results using RITA for the induction of embryogenic callus derived rice from mature zygotic embryos (Tabares et al., CIAT SB2 Report 2000) and from anther culture (Tabares et al., CIAT SB2 Report 2001). This year we report a comparative analysis including various Indica and Japonica rice genotypes.

## Materials and Methods

Anther culture of the Indica rice Cica 8, Cimarron, Fedearroz 2000, and CT 11275, and of the Japonica breeding line CT 6241-17-1-5-1 were used. Donor plants were grown in the field, panicles harvested, and anthers cultured according to Lentini et al. (1995). Tissues were either culture in liquid medium contained in RITA vessels or in liquid medium in baby food jars (control). Induced callus from each culture system was then transfer onto solid plant regeneration medium according to Lentini et al. (1995).

The effect of different culture media was evaluated. The medium NL commonly used in the rice anther culture laboratory (Lentini et al, 1995) was used as control. This medium was supplemented with 2,4-D 2mg/L; picloram 0.07mg/L; kinetin 1mg/L; maltose 8%; with or without silver nitrate10mg/L. A modified medium was evaluated consisting of NL basal medium but replacing picloram with phenyl acetic acid (PAA) 10 mg/L, with or without silver nitrate (medium $M_1$). The rest of the culture procedure, including plant regeneration, was according to Lentini et al. (1995).

The optimal immersion frequency was determined by evaluating callus induction and embryogenesis at 3 different immersion frequencies. Treatments were conducted using immersions of 1 min every 4, 6, or 8 hr for a total of 4 weeks of culture. Three RITA vessels were used per genotype with 1,000 anthers per 200 ml medium; and 4 baby food jars (permanent immersion system, PIS) per genotype each with 250 anthers per 10 ml culture medium (control). Cultures were incubated at 24$^{o}$C a 26$^{o}$C.

Three different treatments were tested to increase plant regeneration. A water stress treatment was induced on callus prior culture by incubation on 1% agarose-containing medium in the dark at 27$^{o}$C to dehydrate callus. After two weeks of culture, stressed callus from 1% agarose-containing medium were transferred to 0.4% agarose solid medium for regeneration and incubated in light. Another set of callus was not treated with water stress, and was cultured directly on the medium semi-solidified with 0.4% agarose. The effect of osmotic stress was evaluated by sub-cultured on medium containing 3% sorbitol for 24 hr, after this partial desiccation treatment the callus were transferred on regular plant regeneration medium. Control consisted of callus transferred from callus induction medium without treatment to regular MS regeneration medium. A factorial experimental completely randomized design was used. At least 10 replicates of 10 callus each was evaluated per treatment.

**Results and Discussion**

A significant higher callus induction was obtained when immersion was conducted every 6 hr than every 4 hr and 8 hr independently of the genotype (Figure 1). The Japonica line CT 6241 showed about 7 fold more callus respect to the Indica varieties (Figure 1B). The maximum callus induction was noted at 40 days after culture on PIS and at 50 days after culture on RITA. But it seems the slower process of callus induction in RITA allows an optimal induction of embryogenesis. A significant higher number of embryogenic callus (95%) were obtained for both Indica and Japonica genotypes respect to the permanent immersion system (PIS, 45%) (Figure 2).

There was an interaction between the callus induction medium and the culture vessel used. No significant differences were seen between the different media on RITA, although there was a tendency of higher induction when using media $M_1$ with or without silver nitrate. However with PIS, the Indica genotypes with intermediate to low response such as CT11275, Cimarron and Cica 8 showed significant higher induction on $M_1$ medium with or without silver nitrate, but Fedearroz 2000 optimal induction was noted on NL medium with silver nitrate. It has been reported that PAA mode of action is similar to that of IAA, although higher levels of PAA are needed and it is more stable in culture inducing a larger number of organized structures. It seems PAA effect is related to the inhibition of ethylene production from the cultured tissues (Ziauddin et al., 1992). Silver nitrate has also been reported to inhibit the action of ethylene of tissues culture in vitro (Lentini et al., 1995). The beneficial effects of replacing sucrose by maltose increasing the callus induction from rice anther of recalcitrant genotypes has also been associated with a reduction of ethylene effects. These results jointly with the effects noted when using PAA and RITA in this work suggest that ethylene might be a critical factor determining the induction of androgenesis from microspores in rice.

Plant regeneration from embryogenic callus was not affected by the composition of the callus induction medium nor the callus induction culture vessel, indicating that the regeneration capacity depends on the level of embryogenesis. Once embryogenesis is obtained the capacity for plant differentiation is similar. Thus, the optimal callus induction medium and culture vessel should be selected based on the larger number of embryogenic callus produced per anther cultured.

Significant higher green plant regeneration was obtained when water stress treatment was applied using agarose 1% for 1 week followed by agarose 0.4% for the rest of the culture period (Figure 3). Twice as many green plants were obtained with this treatment respect to the control. Osmotic stress with sorbitol inhibited plant regeneration (Figure 2). Independently of the callus induction or plant regeneration treatments, about 50-60% of the green plants were doubled haploids, which is in the range previously reported (Lentini et al., 1995).

**Future Activities**

To study systematically different factors affecting the emission and action of ethylene on in vitro culture. To test different PAA concentrations and its interaction with maltose level for optimizing androgenesis in rice. To evaluate modifications of culture vessel allowing aeration and/or temporary immersion for reducing current cost for the implementation of the RITA system

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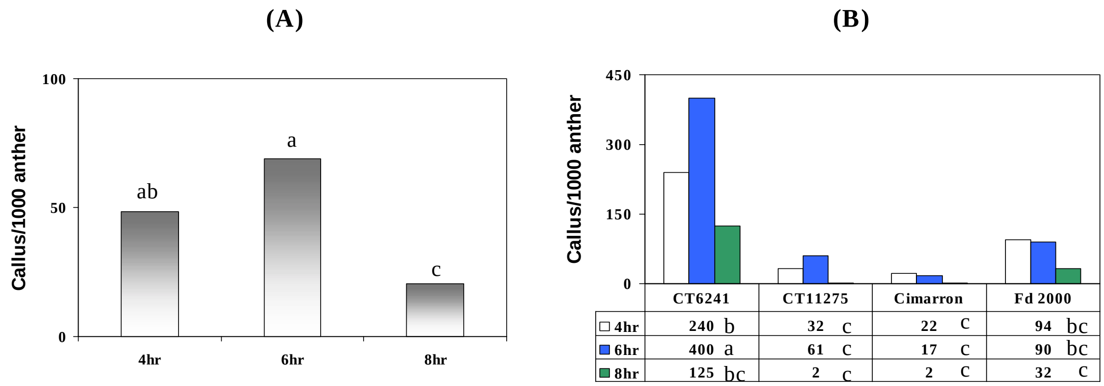
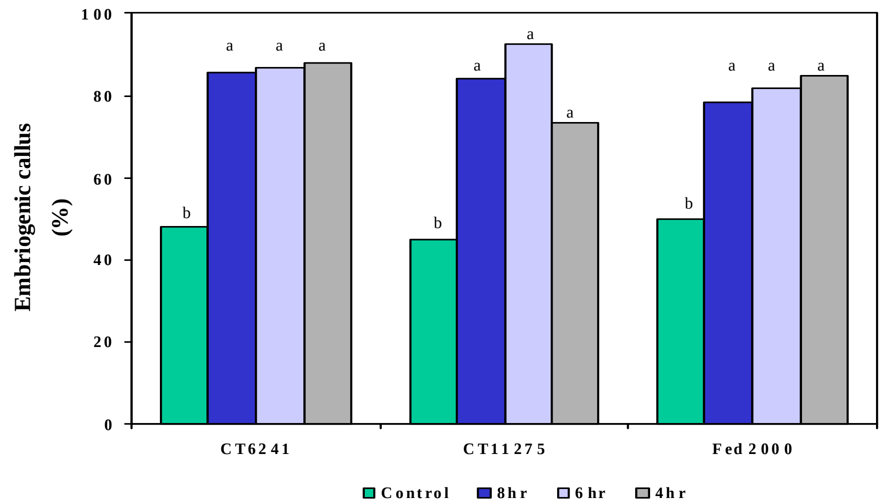


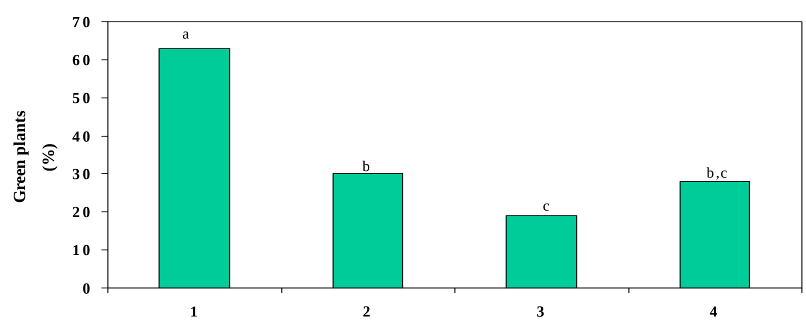

Different letter denote statistical significance ($P < 0.05$). Duncan's Multiple Range Test

**Figure 1. Callus induction in RITA system using 4 hr, 6 hr, and 8 hr temporary immersion frequencies. (A) Mean values of recalcitrant Indica varieties. (B) Comparison between different genotypes. Values refer to the mean number of callus induced per 1,000 cultured anthers**




Different letter denote statistical significance (P < 0.05). Ryan-Einot-Gabriel-Welsch Multiple Range Test

**Figure 2. Percentage of callus embryogenesis on permanent immersion system (PIS) and RITA at different immersion frequencies (8 hr, 6 hr, and 4 hr, respectively)**




Different letter denote statistical significance (P < 0.05). Ryan- Einot-Gabriel-Welsch Multiple Range Test

**Figure 3. Percentage of green plant regeneration on medium containing (1) agarose 1% for 1 week and then agarose 0.4%; (2) agarose 0.4%; (3) sorbitol; and (4) control**

- **Molecular Characterization of Rice and Red Rice using Microsatellites and its Relation with the Morphological Seed Traits**

E. González[1], L. F. Fory[1], J. J. Vásquez[1], P. Ruíz[1], A. Mora[2], J. Silva[2], M. C. Duque[1, 2], and Z. Lentini [1,2].
[1]SB2, [2]IP4 . GTZ, Germany. Project No. 99.7860.2-001.00

## Introduction

The genus Oryza (AA genome) contains two cultivated species of rice, *Oryza sativa* L. and *Oryza glaberrima* Steud and five wild species including *Oryza rufipogon* Griff., the ancestor of rice, and one of them (*O. glumaepatula*) native of Central and South America (Oka and Chang, 1961;Vaughan and Tomooka, 1999). Red rice (*Oryza sativa* f. *spontanea*) is a weedy rice with red pericarp and dark-colored grains, commonly found in rice fields. It is the same species as the cultivated, having similar morphologicals traits at vegetative phase that makes difficult to dintinguish them in the field at early life cycle, but at maturity they are taller, with profuse tillering, seed shattering and dormancy favoring its persistence in the field. Most published reports have classified red rice populations into two major groups based on hull color of mature seeds, which are strawhull and blackhull ecotypes (Smith et al., 1977; Sonnier, 1978 cited by Noldin, 1999). According to Langevin et al. (1990), the red rice can be grouped in ecotypes with characters alike cultivated rice or wild rice (Oka and Chang, 1961). Other researches indicate that red rice shows intermediate characteristics between wild rice *O. rufipogon* and cultivated Indica or Japonica varieties of *Oryza sativa* L. (Oka, 1988 cited by Bres-Patry et al. 2001). Another hypothesis is that weedy rice may evolve through the degeneration of domesticated rice, as weedy types of rice, where wild rice is not present (Vaughan et al. 2003).

Several population studies have focused on the genetic structure of red rice. These studies have related groups of individuals with morphological seed traits such as color of awn and hulls. A molecular analysis of 26 red rice accessions collected in Uruguay showed three groups: The first containing seeds with blackhulls, purple apiculus and long awn and the second showing seeds awnless or short awn and greyed yellow color apiculus and hulls. The third group included commercial varieties analyzed and some red rice accessions similar to the commercial varieties (Rodríguez et al., 2001). Avozani et al. (2001), analyzed 36 red rice accessions with RAPDs markers and found six groups, the most remarkable were the first with only red rice accessions, mainly with awned seeds, and the fifth group including commercial varieties and some individuals similar to varieties in seed traits. Gealy et al. (2002) used microsatellite markers to distinguish among red rice, rice cultivars, and red rice–cultivated rice hybrids derivatives (RC hybrids) and the cluster analysis suggested that there were three distinct genotypic groups. The first group consisted of awnless, strawhull red rice types. The second group had primarily awned, blackhull red rice. The third group was composed of RC hybrids and rice cultivars. Preliminary work conducted by our research group (González et al., 2002, and SB2 Annual Report 2002) using 148 accessions of red rice collected in farmers fields in Colombia, 9 commercial Latin American rice varieties, sixteen homozygous Cica 8 transgenic lines, four hand-made hybrids between these transgenic lines and three varieties, and the AA wild species *O. barthii, O. glaberrima, O. glumaepatula*, and *O. rufipogon*, and 50 microsatellites indicated that is possible to discriminate the diversity of red rice by microsatellites associated with plant morphological traits as found in other studies, but also associated with plant development (phenology) characteristics such flowering. Microsatellite cluster analyses discriminated awn from awnless red rice, and within those groups distinguished early to intermediate flowering types from late flowering. Some red rice variety types were clustered with commercial varieties, and some morphologically like wild species were clustered with *O. rufipogon*. This year we report results from a multiple correspondence analysis including five additional microsatellites markers.

## Materials and Methods

**Plant Material and Genetic Analysis using Microsatellite Markers.** The plant material used and procedures followed for the molecular characterization using microsatellites were the same as previously described in SB-02 Annual Report 2002 (González et al., 2002). The plant material was divided in four groups: Group 1 represented by the 148 red rice accesions, Group 2 included the rice commercial varieties (Coprosem, Oryzica 1, Cimarron and Fedearroz 50), Group 3 was composed by *O. rufipogon* accession from Malasia; and Group 4 by *Oryza glaberrima* and two wild species *Oryza barthii* and *O. glumaepatula* (accession from Costa Rica). The PCR products were resolved on silver-stained polyacrylamide gels and microsatellites alleles were sized by comparison to the 10 and 25 bp molecular weight standards (Promega).

**Statistic Analysis.** Allelic frequencies were calculated for all materials analyzed. Pearson chi square test was used to evaluate the association between specific microsatellites alleles with black brown awn, apiculus and hulls (BBAAH). Two multiple correspondence analyses (MCA) was conducted. The first analysis only included the molecular markers data (MCA-M), and the second analysis included both the molecular and seed morphological data (MCA-MSM). The Pearson chi-square and MCA are tests apply to establish the significance of associations between categorical variables. The Pearson chi-square test is based on expected frequencies in a two-entry data set, whereas MCA is a modeling technique to analyze associations in multi-entry data set. All analyses were conducted using SAS software (SAS, 1989).

## Results and Discusión

### Distribution of Alleles in Four Rice Groups

The number of alleles per locus ranged from 5 to 12 (average 10.4 alleles per locus). Figure 1 showed a total of 146 alleles obtained in this study. Group 1, composed by the red rice accessions, contained 110 alleles of which 56 alleles (51%) were specific to this group and were not present in the Groups. These results indicated that the red rice population is highly diverse and contained the highest number of specific alleles respect to the other

Groups. In contrast to Groups 2 and 4, it is interesting to note that *O. rufipogon* (Group 3) do not have specific alleles sharing all of them with red rice, and some of them with either the varieties (Group 2) or the other wild species (Group 4). However, *O. rufipogon* is represented in this analysis by just one accession. It will be important to determine if this pattern is obtained when a broader range of accessions are included (Figure 1).

**Association between Specific Microsatellites Alleles with Black Brown Awn, Apiculus and Hulls (BBAAH)**

Pearson chi-square test showed a highly significant association (Chi-squares from 18.4 to 61.2; p=0.0001) between 7 alleles (2, 7, 17, 23, 53, 90 and 115 derived from 7 microsatellites markers) and BBAAH traits (Table 2). These markers are distributed in chromosomes 1, 3, 5, 7 and 12 respectively (Table 2). These alleles were specific of red rice and/or *O. rufipogon* (Figure 1). A limited number of chromosomal regions (1, 3, 4, and 7) enclosing most of the genes/QTLs identified in a natural hybrid between a Japonica variety and red rice collected from a rice field was previously found to be associated with key morphological differences between red rice and rice (Bres-Patry et al. 2001). We found that 4 of the 7 alleles highly significantly associated with BBAAH traits were located in chromosomes 1, 3, 4 and 7, in addition of other two alleles present in chromosomes 5 and 6 not previously reported (Table 2). The Pearson test also showed a high association between the absence of allele number 95 and BBAAH traits (Chi-square 15.61, *p=0.0001*, Table 2). This allele was found in the red rice and rice varieties groups (Figure 1).

**Multiple Correspondence Analysis (MCA)**

The multiple correspondence analyses using molecular data **(**MCA-M) generated five groups of which three grouped all red rice accessions (Figure 2A). The wild species *O. barthii* and *O. glaberrima* clustered together in the fourth group, and *O. glumaepatula* in the fifth group (Figure 2A). The three groups that enclosed all red rice accessions were analyzed in more detail and the analysis was complemented with seed morphological traits (MAC-MSM) (Figure 2B). First group (Group V) clustered 86 red rice individuals with the rice varieties (Figure 2B). Group V was composed by 58% of red rice accessions, including awnless or greyed yellow-awn red rice (43 and 56% respectively), and 98% to 99% red rice with greyed yellow hulls and apiculus alike the varieties. Most of the red rice variety biotypes are in this group. Group OR clustered ten red rice (7%) with *O. rufipogon* (Figure 2B). This group included red rice with awn (91%) and with brown apiculus (91%) and brown hulls (82%). Group R (Figure 2B) was composed by the remaining 51 red rice accessions (35%) that did not fall in neither of the other two groups, and was characterized by individuals with awnless (41%), greyed yellow awn/hull (37%) or brown awn/hull (22%) seeds. Greyed yellow apiculus and hulls were registered in more than 63% of individuals, whereas the 22% of red rice showed brown hulls and apiculus.

In the MCA-M analysis, variability could be explained by 17 microsatellites alleles. Of these 15 alleles are specific to *O. barthii*, *O. glaberrima* and *O.* g*lumaepatula,* one allele is shared by *O. rufipogon* and red rice group, and the other allele is shared by red rice and rice varieties (Figure 1). In MCA-MSM analysis, variability was explained by the presence of 16 microsatellite alleles and four seed morphological traits (black brown awn, apiculus and hulls, absence of greyed yellow apiculus). Of these 16 alleles, 11 alleles were derived from the MCA-M analysis (Figure 1) and the other five alleles from the Pearson chi-square analysis (Table 1).

## Conclusions

Specific microsatellites alleles were identified distinguishing rice varieties, red rice accessions and wild species. These alleles could be useful for studying red rice genetics, dispersion of red rice genotypes, degree of hybridization between red rice and cultivated rice, and genetic introgression and persistence of domesticated genes in red rice and wild species populations. The addition of seed morphological traits complemented the molecular analysis facilitating the discrimination of three main groups within the red rice population. One group included red rice similar to commercial varieties, another group alike *O. rufipogon* accession, and the third group with intermediate traits.

## Future Activities

Red rice types similar to *O. rufipogon* based upon the morphological and molecular characterization should be subjected to taxonomic classification to elucidate if they are introduced accessions of the Asian wild species.

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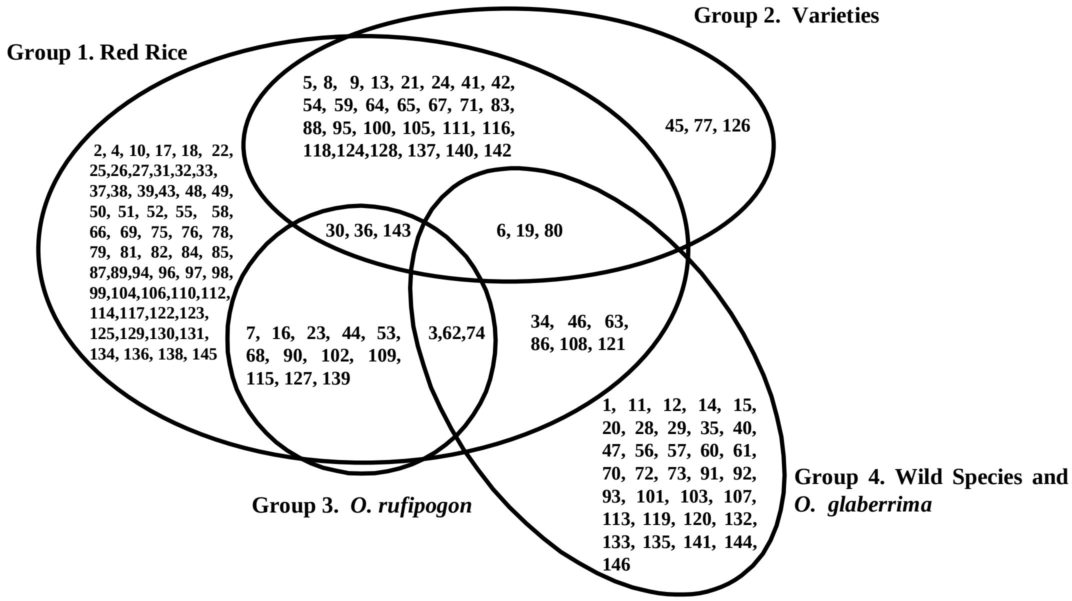


**Figure 1. Venn Diagram Showing the Microsatellites Alleles in each Rrice Group**

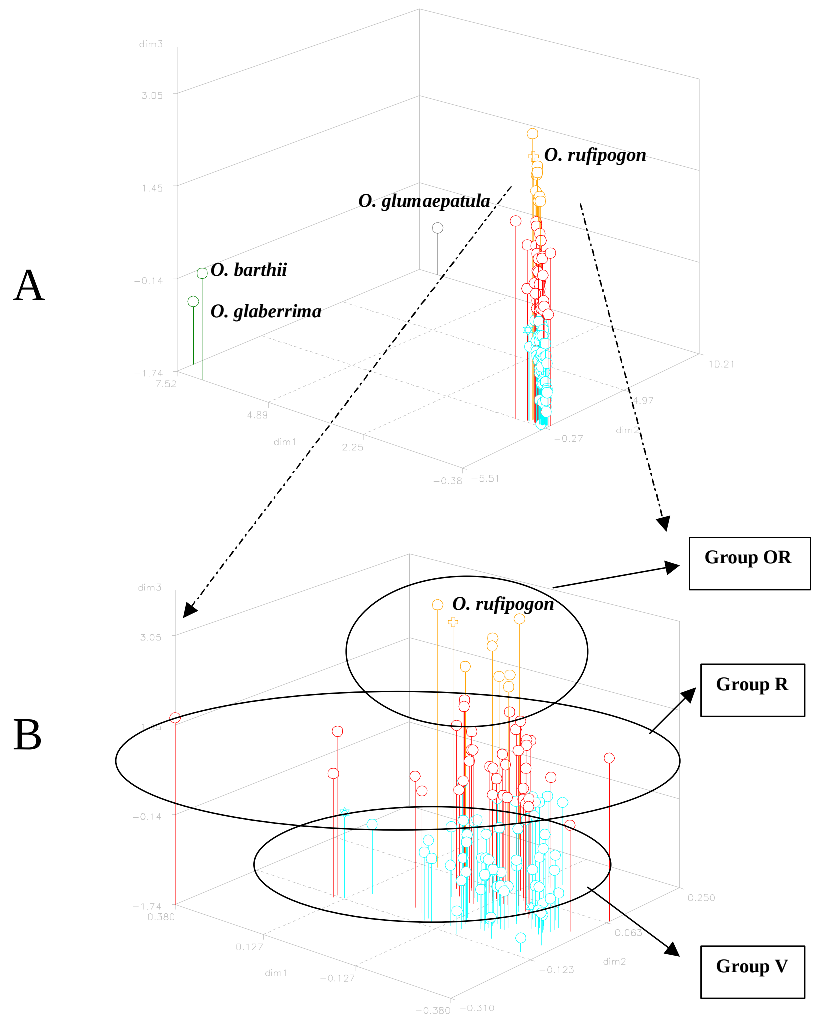


**Figure 2. MAC using Molecular Marker Data Set for All the ,aterials (A), and Combining Molecular Marker Data and Seed Morphological Traits for Red Rice (B)**

**Table 1. Association between Specific Microsatellites Alleles with Brown Awn, Apiculus, and Hulls (BBAAH)**

| Alleles | Chromosome | Chi-square | Probability | Presence BBAAH |
|---|---|---|---|---|
| 2 | 3 | 25.47 | 0.0001 | Yes |
| 7 | 5 | 38.98 | 0.0001 | Yes |
| 17 | 12 | 33.57 | 0.0001 | Yes |
| 23 | 12 | 61.26 | 0.0001 | Yes |
| 53 | 1 | 18.44 | 0.0001 | Yes |
| 90 | 7 | 44.79 | 0.0001 | Yes |
| 95 | 7 | 15.61 | 0.0010 | Not |
| 115 | 1 | 32.51 | 0.0001 | Yes |

- **Assessment of Combinatory Ability between Red Rice and Rice under Greenhouse Conditions**

R. Pineda[1], C. Ordóñez[2], J. Carabalí[2], L. Fory[1], S. Sánchez[2], P. Olaya[1], E. Tabares[1], and Z. Lentini[1, 2]

[1] SB2, [2]IP4. Funding from GTZ, Germany. Project No. 99.7860.2-001.00

## Introduction

Cultivated rice, *O. sativa* L., is an autogamous plant, with a low out crossing rate of 0-1% (Roberts et al. 1961). In contrast, red rice (*Oryza sativa* f. *spontanea*) a weedy relative of the crop, characterized by a red pericarp and dark-colored grains, shows out-crossing ranging from 1% to 52% hybridization rate (Langevin et al. 1990). Several biological, genetic and environmental factors affect the level of outcross compatibility. Among those the effects of temperature, humidity, genotype, flower morphology, stigma receptivity, pollen viability, pollen germination and development of pollen tube had been studied in detailed for most species (Jensen and Salisbury, 1988). Red rice seeds shatter readily and possess dormancy characteristics, which favors the persistence of the weed in rice field. These characteristics in addition to the vigorous growth and other plant traits make this weed highly competitive respect to rice, and a potential candidate as gene receptor from the cultivated species. This work is part of a project directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives in the Neotropics, and its effect(s) on the population genetic structure of the recipient species. Last year we reported on the morphological, phenological and molecular characterization of 152 plants collected from rice farmers fields in Tolima and Huila Departments in Colombia, and its corresponding first and second self-progeny. Based on that characterization 6 red rice types, including the scope of diversity present in the collection, were selected as candidates to conduct the studies on gene flow. As complementary step, the level of compatibility with transgenic and non-transgenic rice was determined using hand-made cross. Below is summarized results obtained from greenhouse and field grown plants. The hybrids generated will be used a controls in a parallel study optimizing the use of molecular marker to trace gene flow at large scale in the field.

## Materials and Methods

**Hand-Made Crosses.** Crosses were made following procedures as described by Sarkarung (1996) with some modifications (James Carabalí, personal communication, CIAT Project IP4). Six red rice biotypes (1-3-4, 1-21-3, 4-12-2-, 5-38-5, 5-36-4, and 5-48-2), the $F_3BC_1$ line derived from the Cica 8 transgenic line A3-49-0-12-3 (carrying the $NS_3$ gene for RHBV resistance and *uid-A* gene for gus expression) backcross to variety Cica 8, the non-transgenic variety Cica 8 (control), and rice line commonly known as Purple (IRRI accession) were used. The variety purple is characterized by having purple tillers and leaves, and grains with purple apiculus and pericarp. Preliminary inheritance analysis in crosses with other varieties had indicated that the $NS_3$ and *uid-A* gene are inherited following a simple Mendelian segregation. In rice, anthocyanins are encoded by few dominant genes (Reddy, A.R. 1996). The use of these traits would facilitate tracing the hybrids and inheritance in subsequent generations and ease the adaptation of molecular markers for assessing gene flow at large scale. These plants were grown to maturity under either greenhouse or field conditions. Reciprocal and self-crosses were made between the different materials. Un-emasculated, self-pollinated plants were included as controls. The percentage of seed setting, abortive crosses and dead flowers were evaluated. Pollen viability of pollen donor plants was determined by fluorescence microscopy.

**Floral Structure.** Spiklets of field grown plants were fixed with a solution of 3:1 ethanol: glacial acetic acid for 24 hr, and dissected under a dissecting scope. Ten spiklets per material from the central part of the panicles were evaluated by measuring the length of pistil (ovary, stile and stigma), anthers, and total length of the flowers.

## Results and Discussion

In general, higher out crossing rates were observed when rice was used as male parent (pollen donor) and red rice as a female parent (pollen recipient) (Table 1). A lower hybridization rate was noted in the reciprocal crosses (using red rice as a pollen donor), which was significant with the line Purple. Unexpectedly, higher crossing rates were noted in the hand-made crosses when using greenhouse-grown materials respect to those from the field in spite that the field pollen-donor plants showed more than 90% of pollen viable, and more than 80% fertility based on the seed setting from spontaneous self cross in the field. It seems that panicles from materials grown in the field may had been affected by stress during handling, in addition to the potential loss of pollen during the transportation of panicles from the field to the crossing house, which may explain these differences. The highest out crossing rates, between 40% and 59%, were noted with the red rice 1-21-3, 4-12-2-, 5-36-4, and 5-38-5 from the greenhouse (Table 1). Red rice 5-48-2 showed intermediate hybridization rates (12% to 20%) both from the greenhouse and from the field materials (Table 1). Unfortunately, several crosses could not be made with the line Purple in the greenhouse because of poor development due management problems. Crosses made with the transgenic line ranged from 5% to 12% with the field materials, and with Cica 8 from 17% to 60% with the greenhouse plants (Table 1). These results are in agreement with those reported by Langevin et al. (1990) on hand-made crosses using red rice collected from the Southern USA showing hybridization of 1% to 52% depending on the variety used. Flower or seed abortion was only noted when red rice was used as female parent, suggesting some level of incompatibility or higher susceptibility to hand manipulation.

Analyses of floral structures indicated that flower morphology of the transgenic line and Cica 8 is similar to those of red rice (Figure 1 and Table 2). The presence of anthocianins was noted on the stigma of the line Purple, and it was also more plumose and spongy respect to red rice, Cica 8 and the transgenic line. The ovary of Purple was shorter and the style was longer respect to the other materials (Figure 1). The pistil of Purple was below the anthers in contrast to the other materials that were at the same level, suggesting morphology more prompt to self-pollination in the case of Purple (Figure 1).

**Future Plans**

Due to the different hybridization rates obtained with field-grown materials respect to those of the greenhouse, new crosses will be made with plants grown in pots in the greenhouse and the field, in order to avoid the stress that panicles suffer during detachment from the tillers as previously done with field-grown plants. Additional crosses and floral morphology analyses will be done using other commercial rice varieties to evaluate potential effect of genotype on compatibility level with red rice and differences in flower morphology. This study is complementary to the assessment of gene flow currently conducted in the field under farm conditions.

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**Table 1. Hybridization Rates with Hand-Made Crosses between Six Red Rice Biotypes, the $F_3BC_1$ Transgenic Cica 8 A3-49-60-12-3/Cica 8 Line and the Line Purple Grown in the Field or Greenhouse [1].**

| | Panicles from the field | | | | Panicles from the greenhouse | | | |
|---|---|---|---|---|---|---|---|---|
| | Male Parent | | Female Parent | | Male Parent | | Female Parent | |
| Red rice | T | P | T | P | NT | P | NT | P |
| 1-3-4 | 0.00 | 10.8 | 0.00 | 0.81 | 17.1 | 37.5 | Nd | 1.4 |
| 4-12-2 | 4,97 | 0,52 | 3,78 | 0.26 | 37.2 | Nd | Nd | Nd |
| 1-21-3 | 12,40 | 7,50 | 15.00 | Nd | 58.8 | Nd | 4.8 | Nd |
| 5-38-5 | 0.00 | 3.48 | 0.00 | 2,38 | 51.9 | Nd | 29.7 | Nd |
| 5-36-4 | 1,23 | 0.00 | 8,17 | 0.00 | 41.7 | Nd | Nd | Nd |
| 5-48-2 | 11,60 | 0,74 | 14,80 | Nd | 19.9 | Nd | 15.1 | Nd |
| T | 0,39 | Nd | 0,39 | Nd | Nd | Nd | Nd | Nd |
| P | Nd | 0,48 | Nd | 0,48 | Nd | Nd | Nd | 1.7 |

[1]Total of two to eight panicles were used per cross. T: $F_3BC_1$ transgenic Cica 8 A3-49-60-12-3/Cica 8 line. P: Purple line. NT: Non-transgenic Cica 8. Nd: Not determined.

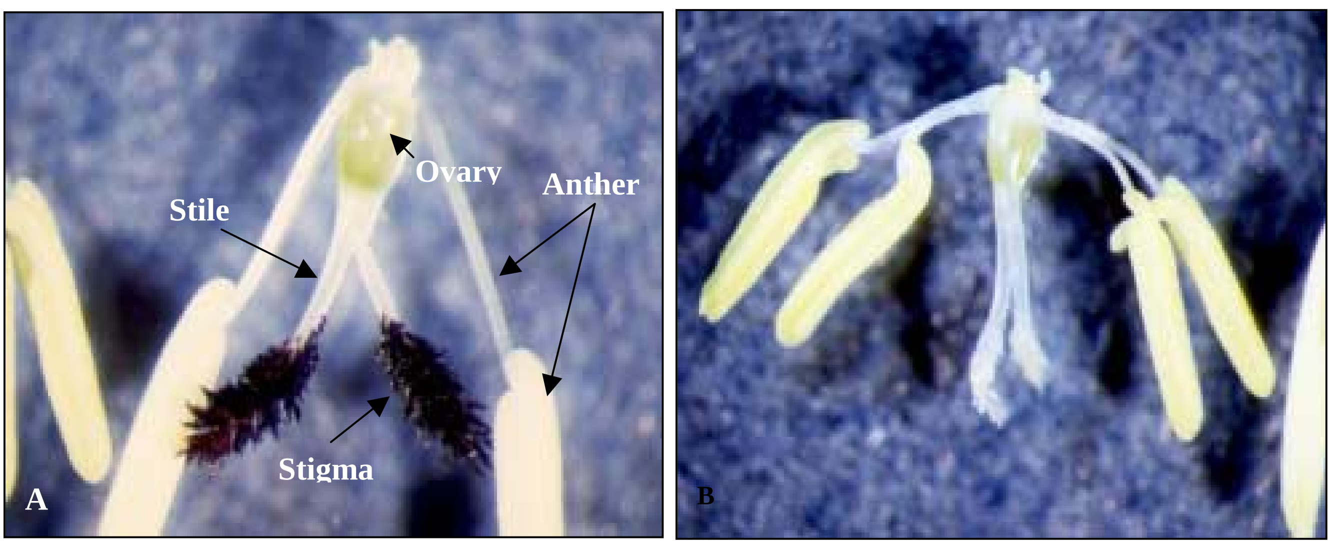


**Figure 1. Floral Morphology of Purple (A) and Transgenic Line (B)**

**Table 2. Length Mean Values of Pistil and Anthers from Flowers of Red Rice, Transgenic Line, Variety Cica 8 and Line Purple**

| Length[1] (mm) | Red rice | | | | Line transgenic | | Varieties | |
|---|---|---|---|---|---|---|---|---|
| | 4-12-2 | 5-36-4 | 1-21-3 | 5-48-2 | 60-4-5/ FB007-19[2] | T | Cica 8 | Purple |
| Ovary | 0.8 ± 0.2 | 0.8 ± 0.1 | 1.0 ± 0.2 | 0.9 ± 0.2 | 1.0 ± 0.2 | 1.0 ± 0.1 | 0.8 ± 0.1 | 0.5 ± 0.1 |
| Style | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.1 |
| Stigma | 1.1 ± 0.1 | 1.1 ± 0.1 | 1.0 ± 0.1 | 0.7 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.2 | 1.0 ± 0.1 | 0.9 ± 0.1 |
| Pistil | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.5 ± 0.2 | 2.0 ± 0.1 | 2.5 ± 0.1 | 2.6 ± 0.1 | 2.4 ± 0.1 | 2.2 ±0.1 |
| Anthers | 3.3 ± 0.3 | 3.9 ± 0.8 | 3.4 ± 0.2 | 3.2 ± 0.1 | 3.6 ± 0.2 | 3.5 ± 0.5 | 3.3 ± 0.2 | 4.0 ± 1.0 |
| Stigma respect anthers | 0.7 | 1.4 | 0.9 | 1.2 | 1.1 | 0.9 | 0.9 | 1.8 |

[1]Ten flowers (mm) per each material. Numbers refer to the mean ± Standard Error
[2] An additional transgenic line was included for comparison

- **Assessment of Gene Flow from Transgenic and Non-Transgenic Rice into Red Rice under Experimental Field Conditions**

R. Pineda[1], L. Fory[1], E. González[1], A. Mora[2], T. Agrono[2], C. Ordóñez[2], C. Cruz[2], E. Bolaños[1] , C.Dorado[1], A. Mina[1], M.C. Duque[1, 2], J. Silva[2] and Z. Lentini[1, 2]
[1] SB2, [2] IP4 . GTZ, Germany. Project No. 99.7860.2-001.00

## Introduction

A careful assessment of potential impacts of gene flow from transgenic plants on population genetics of natural crop plant biodiversity is needed in other to design strategies for the safe deployment and durable use of these crops in the tropics. Genes from rice varieties may transfer quickly into red rice (1% to 52% hybridization rate) (Langevin et al. 1990). However, most of the hybridization rate estimates have been done under temperate conditions. Gene flow rates lower than 1% was reported between herbicide-resistant transgenic line and a non-transgenic Mediterranean Japonica rice varieties (Messeguer et al., 2001). Similar rates were reported by Noldin et al. (2001) and Zhang et al. (2003) between red rice and resistant herbicide transgenic line which contains the *bar* gene. Current studies showed that gene flow rates reached about 3% when *O. rufipogon* and a commercial variety were sown together (Song et al., 2003). This rate is higher than those reported previously [Messeguer et al. (2001), Noldin et al. (2001), and Zhang et al. (2003)]. Last year we presented a detail morphological and molecular characterization of a red rice population collected from rice farmers field in Colombia. Based on these results some red rice accessions were selected to conduct gene flow analysis and identify indicators for easy tracing and monitoring of genetic introgression from rice into red rice, and persistence of domesticated genes in the weedy population under field conditions throughout subsequent generations. Here we present preliminary analyses of two field experimental designs to trace genetic introgression from transgenic and non-transgenic rice into red rice using the $NS_3$ (encoding for RHBV resistance in transgenic rice) and gus transgenes, the presence of anthocyanins in the leaves, tillers, and grain apiculus as morphological markers. This work is part of a project directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives in the Neotropics, and its effect(s) on the population genetic structure of the recipient species.

## Materials and Methods

### Comparison of Agronomic Traits for Red Rice, Transgenic Lines and Rice Varieties

Seedlings of $F_3BC_1$ Cica 8 transgenic line A3-49-60-12-3/Cica 8-2, $F_3$ Cica 8 transgenic lines A3-49-60-4-5/Fedearroz 50-19-1 and A3-49-60-4-5/Fedearroz 50-19-1-2, six red rice biotypes 1, and eight commercial rice varieties were transplanted in the field in three replicates of 42 plants per replicate. Morphological and phenological (days to flowering) characterization was conducted in order finalize the selection of the red rice candidates to be used in the gene flow studies. These red rice materials were pre-selected to include the phenotypic and genetic diversity present in the red rice population based on last year results on the analyses of qualitative and quantitative traits by principal coordinate and component tests respectively, agronomic traits, and molecular characterization by microsatellites markers at the same time some genotypes were included because they showed close overlap in flowering with rice varieties as well as similar height. These red rice types were also susceptible to rice hoja blanca virus (RHBV) as indicated by RHBV evaluations conducted in the field. The transgenic lines were chosen because they showed resistance encoded by the $NS_3$ transgene, and contained the *gus* and *hph* (hygromycin resistance) marker genes. The rice line commonly known as Purple (IRRI accession) characterized by having purple leaves, tillers, and grain apiculus, was used to trace the inheritance of anthocyanins as a morphological marker (control) facilitating the identification of hybrids since anthocyanin production in rice is encoded by dominant gene(s).

### Experimental Field Designs to Trace Gene Flow from Transgenic and Non-Transgenic (Purple Line) into Red Rice

Seeds of the experimental genotypes were sown in a nursery under the field conditions at different dates to ensure flowering overlap between rice (pollen donor) and red rice (pollen receptor). The expression of *gus* gene was assayed histochemical in seedlings after 15 days later. Plants with *gus* expression were selected for the gene flow study. Two experimental

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1 Some biotypes were progeny of second or third generation of self pollination from original material collected in farmers fields

field plots were used. The first design (multiple-square assay) consisted in square plots where rice and red rice were planted intermingled simulating farmers field conditions. The second design (concentric circles assay) was used to measure gene flow distance affected by wind.

For the multiple-square assay, seedlings of 25 to 30 day-old were transplanted in plots of 1.8 m X 1.8 m. Each plot contained 81 plants, 16 of which corresponded to one red rice type (20%). The plants were planted at a distance of 0.2 m between plants, and at 5 meters between plots. Each plot was surrounded with a biological barrier of sweet corn of 1.8 m of height planted at 2.5 m from the border of each plot. A complete randomized block design, with four replicates per red rice type and pollen donor source (transgenic line or Purple line) was used (Figure 1). In order to ensure flowering overlap between rice (pollen donor) and red rice, pollen donor plants from at least two different sowing dates were planted in each plot. Plants in each plot were scored throughout the life cycle to maturity. Average wind speed and wind direction during flowering were recorded.

For the concentric circle assay, plants from both transgenic lines and Purple line were inter-planted within a circle at the center of the plot. Total of 176 pollen donor plants were planted in concentric 7 circles respect to the center at a distance of 0.25 m between plants. Total of 262 red rice plants composed by a equal amount of four red rice types (5-38-5, 1-21-3, 5-36-4 and 1-3-4) were planted at 0.3 m between plants, in four concentric circles respect to the pollen source using a Statistic Latin Square Design (Figure 1). The first circle was 0.5 m from the pollen source, and the fourth circle was at 1 m. Likewise for the other assay, pollen donor plants of at least two different sowing dates wee used to ensure flowering overlap with red rice.

**Results and Discussion**

Red rice showed variation in stem (SC) and leaves (LC) color (Table1). Stem color ranged from light green (score 3) to green (score 6), and leaf color varied from green (score 6) to dark green (score 8). Color was defined using The Royal Horticultural Society color scale (1966). Red rice accessions 1-21-3, 1-3-3, 4-12-2 and 5-38-5 showed erect or intermediate growth habit (GH) likewise most rice commercial varieties (scores 3-5). In contrast red rice 5-36-4 and 5-48-2 showed decumbent habit (score 7-9). In relation to flowering, the Ryan-Einot-Gabriel-Welsch multiple range test ($p>0.005$) discriminated three groups: early flowering (red rice 1-21-3, 1-3-4, and 5-36-4, and the Purple line) with a mean value 92 to 95 day-after-sowing (DAS); intermediate flowering (red rice 5-38-5, 4-12-2, and 5-48-2, and all the rice varieties and transgenic lines A3-49-60-12-3/Cica 8-2, A3-49-60-4-5/FB007-19-1) with a mean value of 99 to 110 DAS); late flowering (the transgenic line A3-49-60-4-5/FB007-19-2) with a mean value of 123 DAS. About 71% red rice accessions flowered earlier than the variety Cica 8 and the transgenic line A3-49-60-12-3/Cica 8-2 (Table 1), and most red rice flowered earlier than and the transgenic line A3-49-60-4-5/FB007-19-2. No significant difference was noted in the number of tillers between the red rice, the transgenic lines and rice (Table 1). About 70% of the red rice was as tall as the transgenic line A3-49-60-12-3/Cica 8-2, and the Purple line, whereas the varieties Cica 8, Fedearroz 50 and Fedearroz Victoria 1 were taller than 70% of the red rice. Height differences seem not to be a critical point to prevent gene flow. Song et al. (2003) detected gene flow between species of *O. rufipogon* and the rice commercial variety Minghui - 63, which differed in 130 cm height (Table 1). Based on these results the transgenic line A3-49-60-12-3/Cica 8-2) was selected as one of the pollen donors for the gene flow studies.

About 60% of plants from the line A3-49-60-12-3/Cica 8-2 showed *gus* expression, indicating that the gene was still segregating in this $F_3BC_1$ generation. In order to have enough plants for the gene flow studies, a total of 4011 plants were evaluated, and 2246 plants showed *gus* expression (56%). Flowering was synchronous between the red rice; the Purple line and the transgenic line A3-49-60-12-3/Cica 8-2. Most treatments involving the Purple line and red rice 1-21-3, 1-3-4, 5-36-4 and 5-38-5 overlapped in flowering. The highest synchrony in flowering was noted between red rice 4-12-2 and the Purple line, with 87% of plants with flowering overlap. In the case of the transgenic line, flowering overlap with red rice of 56% to 77% plants was noted. Even though the red rice 5-38-5 flowered earlier than the transgenic line, there was overlap towards the end of the flowering cycle of the red rice. Wind velocity ranged from 0 to 7 m/s, the maximum mean value of 1.6 m/s was registered from 10:00 AM to 12:00 PM on cloudy days. Seeds from red rice, transgenic and purple line were harvested keeping record of the plant location within each experimental plot.

**Future Plans**

Progeny plants from the different treatments will be analyzed using specific microsatellites markers to identify hybrid plants, as well as by scoring *gus* expression and the presence of the $NS_3$ and *hph* genes by PCR (when using the transgenic line as pollen donor) or by the presence of anthocyanins in vegetative and reproductive tissues (when using the Purple line as pollen donor). These analysis will not only give an estimate of rate of hybridization between the different experimental types, but also the distance of gene flow, and will allow the optimization of an experimental approach to use molecular marker for tracing/and monitoring genetic introgression from rice at large scale suitable for risk assessment in farmers fields and natural environments. Hybrids plants will be used to study genetic introgression dynamics and persistence of domesticated genes I recipient population over time. The information generated will be used to define management practices allowing a safety deployment of transgenic rice in the tropics.

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**Figure 1. General View of Field Trials in (A) Square-Plots Assay, and (B) Concentric Circle Assay**

**Table 1. Mean Values of Trains Evaluated in the Field**

| Line | Genotype[1] | SC[2] | LC[3] | GH[4] | Fl [5] | Tiller /plant | Plant height (cm) | Hight to lower panicle (cm) |
|---|---|---|---|---|---|---|---|---|
| **Red rice** | 1-21-3 | 3 | 6 | 3 | 92f | 19 a | 102abcde | 96abcd |
| | 1-3-4 | 6 | 6 | 5 | 95ef | 19ab | 102abcde | 91abcd |
| | 4-12-2 | 3 | 8 | 3 | 105bcd | 10abcd | 95bcdef | 94abcd |
| | 5-36-4 | 4 | 8 | 9 | 100def | 15abcd | 94bcdef | 80d |
| | 5-36-4 | 4 | 8 | 9 | 95ef | 17abc | 97bcdef | 84cd |
| | 5-38-5 | 4 | 7 | 5 | 101cdef | 15abcd | 94cdef | 90abcd |
| | 5-48-2 | 6 | 7 | 7 | 107bcd | 16abc | 101bcdef | 98ab |
| **Transgenic** | 60-12-3/Cica 8-2 (GUS+) | 4 | 8 | 5 | 110b | 15abcd | 91ef | 85cd |
| | 60-12-3/Cica 8-2 (GUS-) | 4 | 8 | 5 | 111b | 13abcd | 86f | 80d |
| | 60-4-5/Fd50-19-1 | 7 | 8 | 1 | 98 def | 8.7cd | 104abcde | 96abcd |
| | 60-4-5/Fd50-19-2 | 7 | 8 | 1 | 124 a | 7.7d | 105abcd | 93abcd |
| **Rice varieties** | Cica 8 | 4 | 8 | 5 | 105bcd | 18ab | 105abc | 95abcd |
| | Purple | 10 | 9 | 3 | 92f | 12abcd | 93def | 87bcd |
| | Cimarron | 5 | 8 | 3 | 101cdef | 19ab | 95bcdef | 92abcd |
| | Coprosem | 3 | 7 | 3 | 100cdef | 10bcd | 103abcde | 95abcd |
| | Fedearroz 50 | 7 | 8 | 3 | 109bc | 12abcd | 111a | 103ab |
| | Fedearroz 2000 | 7 | 8 | 1 | 100cdef | 13abcd | 99bcdef | 98abc |
| | Fedearroz Victoria 1 | 5 | 7 | 1 | 104bcde | 14abcd | 107ab | 104a |
| | Oryzica 1 | 3 | 6 | 5 | 99def | 12abcd | 100abcde | 95abcd |

Values followed by the same letter are not significantly different (*p=0.05*) Ryan-Einot-Gabriel-Welsch multiple range test. [1]Genotypes used: 6 red rice types. Transgenic lines 60-12-3/Cica 8-2 lines with (+) or without (-) *gus* expression. [2] SC= Stem color, [3]LC = Leaf color. Stem and leaf color range from 3= light green color, 4 -6= green color, 7-8= dark green color, 9= purple color, 10= dark purple color. [4]GH = growth habit, scored as 1=Erect, 3=Semi erect, 5=Intermediate, 7= opened, 9=decumbent, [5] Days to 50% plants flowering

- **Use of Red Rice as Potential Trait Source for Commercial Rice Breeding**

L. F. Fory[1], E. Corredor[2], P. Ruíz[1], J. J. Vásquez[1], A. Mora[3], E. González[1], T. Agrono[3], C. Ordóñez[3], E. Bolañoz[3], J. Silva[1], and M. C. Duque[1], and Z. Lentini[1,3]
[1] SB2, [2] FEDEARROZ, [3]IP4

## Introduction

Recent studies on genetic diversity of Latin American rice commercial varieties had shown the need for broadening the genetic base of breeding materials. Main targets are increase productivity, stability across environments, increase weed competition, and durable resistance to various diseases and pests. Latin American wild Oryza species are new sources of genetic diversity not found in the cultigens (Buso et al., 1998). These species represent a rich, largely untapped source of resistance genes to biotic and abiotic stresses.

Work by CIAT rice breeding program has demonstrated increased evidence that *O. rufipogon* and *O. glaberrima* harbor genes of interest for the genetic improvement of cultivated rice (Martínez et al., 2002). However, other studies had shown that inter-specific hybrids in rice might be prompt to both $F_1$ sterility and later-generation hybrid breakdown (Oka, 1988: Burke and Arnold, 2001). Red rice (*Oryza sativa* f. *spontanea*) is a weedy rice with having similar morphologicals traits as cultivated rice at vegetative phase but generally they have profuse tillering, and vigorous growth and other plant traits make this weed highly competitive respect to rice. According to Langevin et al. (1990), the red rice can be grouped in ecotypes with characters alike cultivated rice or wild rice (Oka and Chang, 1961). Other researches indicate that red rice shows intermediate characteristics between wild rice *O. rufipogon* and cultivated Indica or Japonica varieties of *Oryza sativa* L. (Oka, 1988 cited by Bres-Patry et al. 2001). Another hypothesis is that weedy rice may evolve through the degeneration of domesticated rice, as weedy types of rice, where wild rice is not present (Vaughan et al. 2003). Our work using microsatellites markers had indicated that red rice accessions collected from farmer's field in Colombia (Huila and Tolima) showed a genetic diversity not present in either rice commercial varieties, and the accessions of *O. rufipogon*, *O. glumaepatula*, *O. barthii* and *O. glaberrima* analyzed (González et al., 2003 in this Report Output 1). Thus red rice could a potential source to broaden the genetic base of rice varieties.

## Materials and Methods

**Selection of Red Rice Accessions.** Red rice materials were pre-selected based on previous phenotypic, phenological and molecular characterization (Ruíz et al., 2002; and González et al., 2002). Priority selection was given to accessions with resistance to RHBV, higher tiller number, yield potential and high re-growth capacity upon harvest. Selection criteria also included materials genetically diverse identified using the principal coordinate and component analyses, and microsatellite molecular characterization.

**RHBV Resistance Assays.** Preliminary evaluations were conducted on a total of 141 accessions of red rice. Second generation of self-progeny seeds of the original plants collected in farmers fields were planted in a randomized plot design with 3 replications. Thirteen commercial rice varieties with known reaction to RHBV and one accession of *O. rufipogon* were used as control. Materials were inoculated at 15 days old with two insects per plant from a Togasodes colony of 80% virulence. Plants were evaluated following the IRRI scale at 15, 30 and 45 days after inoculation.

## Results and Discussion

Of the 141 red rice accessions evaluated 83% were susceptible, and 24 red rice materials showed intermediate resistance in the field evaluations, with scores ranging from 5 to 6. Except for Fedearroz 2000 (score 3) and the transgenic line A3-49-60-12-3-3-57-74 (score 1) that were resistant to the virus, all the other varieties and *O. rufipogon* were intermediate (Oryzica 1 and Fedearroz Victoria 1, scores 5-6) or susceptible (scores 7-9) to RHBV. Thirty seven percent of the red rice with intermediate level of resistance was derived from field plots where Oryzica 1 has been cropped for at least 2 years in a row, and the other 37% from plots with Fedearroz 50. The other accessions came from plots cultivated with either Coprosem 1 or Cimarron. At this point, it is not clear the source of resistance to RHBV present in these red rice materials, which needs more analysis.

A total of 54 red rice materials were selected by having a significant higher number of tillers respect the commercial varieties. These red rice materials showed a range in tiller number from 22 to 30 tillers per plant, whereas the varieties showed from 11 to 24 tillers per plant. Similarly the number of tillers that re-grew after harvest in red rice varied up to 29 tillers per plant, while the best variety (Fedearroz 50) showed a maximum of 29 tillers per plant. A total 59 red rice materials was selected based on its yield potential. These plants showed from 2-to 39-g/plant, while the commercial varieties (Cimarron, Coprosem, Oryzica 1 and Fedearroz 50) showed a production from 10 to 25 g per plant. Other materials were selected to include the diversity found in the red rice population based on the analysis of qualitative and quantitative traits, as well as on the microsatellite markers profile and resemblance with commercial varieties (17 materials) or wild species *O. rufipogon* (15 red rice). Selected materials will be planted in the field this semester for further characterization and selection, to initiate a breeding scheme (Figure 1). In addition to evaluations for agronomic traits, materials will be evaluated for RHBV resistance, milling and cooking quality traits. Selected plants will be crossed with Fedearroz 50, Fedearroz Victoria 1 and three FLAR lines, segregating populations will be evaluated and selected $F_2$ plants will be processed through anther culture to develop fix lines to initiate yield potential and regional evaluation earlier.

**Future Plans**

1. To initiate the field characterization of agronomic traits of the selected red rice.
2. To conduct molecular and taxonomic characterization of individuals similar to *O. rufipogon* by means of AFLP.
3. To initiate the crossing program between red rice and selected commercial varieties.
4. To advance backcrossed populations and select best candidates for the generation of doubled haploids through anther culture.
5. To identify quantitative traits loci (QTLs) associated with yield increase and/or out performance in populations derived from crosses with red rice and commercial varieties.

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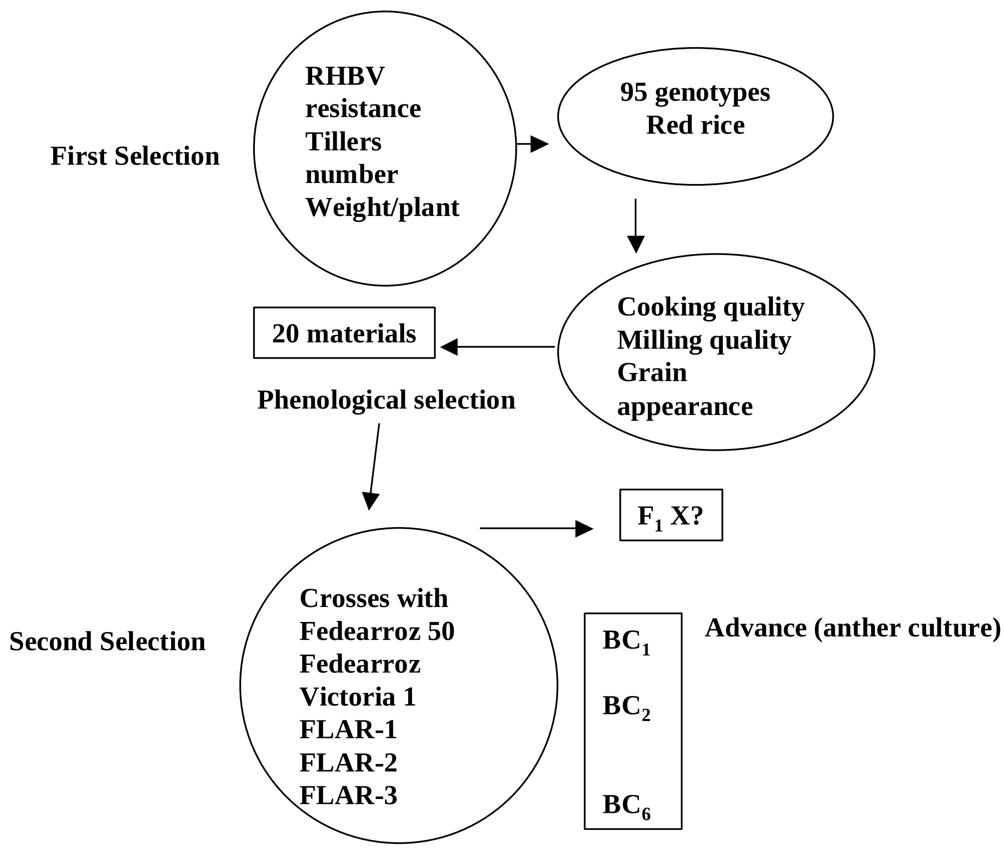



**Figure 1. Breeding scheme to incorporate red rice traits into commercial rice varieties**

## OUTPUT 1. ENHANCING GENE POOLS

### 1D. Functional Genomics

- **Phenotypic Characterization and Seed Multiplication of a Collection of Rice T-DNA Insertional Mutants**

M. Lorieux, J. Lozano
Project funded by the Génoplante consortium

### Introduction

In the framework of its work plan for functional analysis of cereal genomes, the Génoplante consortium decided to construct a rice T-DNA insertional mutagenesis collection. Rice was chosen because of its small genome and because of all the genomic resources available for this species (ESTs, genetic maps, complete sequence, etc.). The lines were produced in CIRAD laboratories, and grown in CIRAD and IRD greenhouses, in Montpellier, France. The present work carried out at CIAT as a collaboration with Génoplante consists in: (i) a systematical phenotypic evaluation of the mutant collection, with production of an associated phenotypic database, and (ii) the multiplication of seeds for the entire collection, for later distribution to all laboratories interested in rice functional genomics.

### What is a T-DNA Mutant Collection?

A T-DNA mutant collection is a library of lines obtained from transformation by *Agrobacterium tumefaciens*, using a specific construct called the T-DNA insert. If the T-DNA is inserted in a gene or its regulatory region, the insertion event results in alteration or disruption of the functionality of this gene, which can even be completely silenced. Each line contains one or several insertions, resulting in the silencing of one of several genes in the line. Typically, several thousand mutant lines are produced. As the transformation events can be considered to be uniformly dispersed along the genome, one may expect that an important fraction of the genes contain an insertion. Additionally, the insertion sites can be individually characterized in sequencing the genomic region flanking the T-DNA insertion site. We then make a Flanking Sequence Tags (FST) database. Hence, in the case of completely sequenced genomes like Arabidopsis or rice, the insertion(s) of each line can be placed on the annotated genome through sequence comparison and thus related to a specific gene or gene region.

### Utility of a T-DNA Mutant Collection

A T-DNA mutant collection is a powerful tool for discovering gene functions. Indeed, the disruption of a given gene or its promoter and/or transcription factors may result in alteration of the corresponding phenotype in relation to the wild phenotype. This phenotype can be observed at different levels including morphologic, developmental, physiologic, etc. As the entire collection allows genotypic-phenotypic association to be analyzed at the whole genome level, and the scientific studies based on such a material fall into the field of  functional genomics.

Basically, there are two main approaches for discovering gene functions using a such library:

1. The collection can be used for forward genetics screens through systematical evaluation of all available $T_1/T_2$ families for a given trait.  The mutant lines showing phenotypic variation in comparison to the wild phenotype can then be later analyzed for their T-DNA inserts (location on the genome, annotation on the sequence containing the insert) and association between the observed phenotype, and the affected gene may be possibly established.
2. The other approach is called reverse genetics.  This tests hypotheses about candidate sequences suspected of having a role in the control of the trait under study. Reverse genetics screens allow the identification of the mutant(s) disrupted in a given (gene) sequence based on:  (i) PCR based amplification product in 2D or 3D DNA pools,  (ii) hybridization signals on flanking regions spotted on medium density filters or (iii) by homology search in the FST database, supposing that the entire collection has been sequenced at least for one of the two flanking regions of each T-DNA insert.  The identified mutants can then be screened for the targeted trait to confirmgenotypic-phenotypic associations.  The main advantage of the reverse genetics approach is that it saves large-scale phenotypic screenings.  It is especially interesting for the evaluation of traits whose assays are   time-consuming and/or high-cost phenotypic screenings.

## Characteristics of the Génoplante T-DNA Rice Mutant Collection

The collection is based on the cultivar Nipponbare (*Oryza sativa* L. ssp. Japonica), because (i) it is relatively easy to transform, and (ii) its genome was used for the rice genome sequencing project.

The lines were generated through Agrobacterium-mediated transformation through an efficient protocol (Sallaud et al., 2003).  The objective was to ensure a primary coverage of the rice genome with enhancer trap T-DNA insertion sites ($n$ = 40,000 lines).  Individual and parallel characterization of insertion sites by systematic sequencing of the genomic region flanking the left border of the T-DNA was carried out.  Later, the enhancer trap T-DNA will be equipped with a non autonomous *Ds* element which can be further mobilized in trans for creating mutants alleles in genes in the vicinity of T-DNA insertion sites.  The lines are also being individually characterized by GUS assays.  All production and phenotypic (including GUS assays) data will be gathered from the different Génoplante partners (including the present work) using automated entry in a phenotype database.  A sequence database will integrate finished flanking sequence data, survey the data against known DNA sequences as well as a functionally oriented representation of their location in the rice genome.  All these data will be integrated in the future public database Oryza Tag Line.

General features of T-DNA integration in this collection include (i) 1 to 4 copies were integrated at 1 or 2 loci, (ii) we observed 30-40% of single-copy transformation events (TE), (iii) the T-DNA segregated according to a 3:1 ratio in 95% of single copy TE, as well as 50% of multiple copy TE, (iv) a preferential integration in the gene-rich fraction of the genome was observed.

**Phenotyping of the Rice Mutant Collection**

Here, we present one of the several projects carried out on the Génoplante rice mutant collection. In its first phase, it consisted the following main activities:

1. To carry out screenhouse and field phenotyping of a set of 5,000 lines,
2. To produce a mutant phenotypic database,
3. To produce $T_2$ entire panicles for further detailed analyzes of grain filling,
4. To multiply $T_2$ seeds to constitute a rice T-DNA mutant stock center for future distribution and collaboration with partners.

**Materials and Methods**

**Screenhouse**

Five thousand $T_0$ plants were produced at CIRAD and grown in CIRAD and IRD glasshouses in Montpellier, France. Twenty-five $T_1$ seeds per $T_0$ plant were received at CIAT and were sown in a screenhouse. Sowing was carried out in four batches of 1,250 lines, with about three weeks delay between the batches. Seeds were pre-treated by heat for three days at 50 °C to break dormancy, and planted in plastic trays with a mixture of CIAT (67%) and Santander de Quilichao (33%) soils. Germination was determined at ten days after sowing (DAS). The first phenotypic observations were carried out at 18-20 DAS, with counting of the number of individuals presenting the mutant phenotype. A list of possible phenotypic traits was established from data mining of several rice phenotypic databases (www.gramene.org, www.grs.nig.ac.jp/rice/oryzabase, www.irri.org/genomics), and was used as a guide for observations. An English-Spanish-French lexical of botanical and agronomic terms was established to facilitate phenotype identification.

**Field**

A two hectare field was set up following the requirements of the ICA (Instituto Colombiano Agropecuario). The entire field was covered by nets to avoid damage and seed dissemination by birds. Plantlets were transplanted at 25 DAS. A basic fertilization composed of Mono-Ammonium Phosphate, Iron Sulfate, Potassium Chloride and micro-elements was applied. The field was irrigated two times a week. Control lines of Nipponbare cv. were planted for each 10 T-DNA lines in order to facilitate the comparison with wild phenotype. Phenotypic analyses were carried out at different ages, using the list of possible traits as a guide. A first round of observation was done when the plants were approximately 45 days-old. A second evaluation was done at flowering, while the ultimate observation was done at maturity. This maximized the chances to detect phenotypic variations, as various traits could be observed at only one of these stages. Moreover, this permitted to follow the evolution of a suspected phenotype at early stage and possibly confirm or invalidate it.

**Harvest and Storage Conditions**

Harvest was organized as followed:

1. Three panicles per plant of each line were collected. This material is to be sent to Biogemma, a private partner of the Génoplante consortium. Biogemma will conduct phenotypic analyses on this material in the framework of a project on functional genomics of grain filling. Seeds from these panicles will later be sent to CIRAD to constitute the main mutant stock center.
2. The seeds from three to six panicles –depending on the level of sterility– were collected from each plant of each line. This will constitute the replicate of the mutant stock hosted by CIAT.
3. When possible, for each line showing a mutant phenotype, all remaining panicles from one plant with the phenotype were collected in order to facilitate segregation studies based on wild/mutant crosses.

**Results**

**Legal Aspects**
All issues relative to legal aspects, i.e. importation permit, quarantine and authorization for field trial of transgenic plants were followed.

**Germination Rates**
The germination rate of the controls (Nipponbare cv.) was close to 100%. For T-DNA lines, the mean germination rate was around 61%. A trial without heat treatment showed a mean germination rate of about 35% (Figure 1).

**Growth**
The rice plants generally grew normally. The Nipponbare variety showed a normal phenotype, however, short-day conditions induced a short cycle, and the plants showed slight reduction in height and tillering than under long-days conditions.

**Cycle**
Nipponbare is a photosensitive cultivar. Thus, under short-days conditions (about twelve hours of daylight at Cali), its cycle is reduced in length. At 60 DAS, 50% of the plants had flower, while the 90% of flowered plants was attained at 64 DAS. The complete cycle of Nipponbare (sowing to maturation) under our conditions was around 93 days.

**Diseases**
Plant health was generally very good and close to optimum. Only very localized attacks of Hydrelia and Rice Hoja Blanca Virus were observed. Also, zinc deficiency symptoms were observed in various locations of the field.

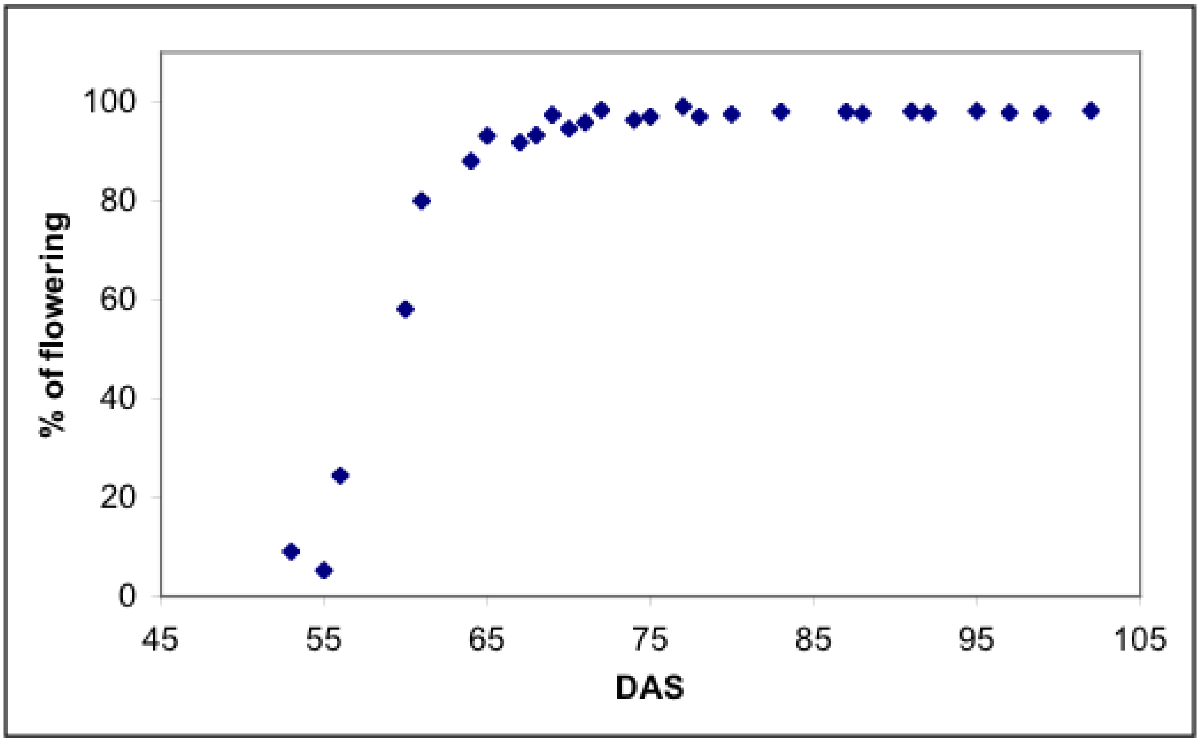



**Figure 1. Evolution of the % of Flowering in the Nipponbare Control**

**Fertility/Seed set**
Seed set of the controls was good. The mean fertility was around 81%.

**Mutant Phenotypes**
In the screenhouse, about 18% of the lines showed phenotype variation in comparison to the wild type. This is much more than currently observed in other mutant collections, where about 3 to 5% of mutants are identified by visual phenotypic screening. T-DNA insertion is probably not responsible for all the variation observed. Indeed, it is well known that other sources of mutation like the Tos 17 retrotransposon are positively activated by in vitro culture of rice. Moreover, discrepancies in germination dates and seed quality, mainly due to the growth conditions of the $T_0$ plants, may be responsible for apparent mutations, notably Retarded Growth (RG), tillering, and height. Also, redundancy of phenotypes was frequently observed between two or more lines. We think that, for lines showing close serial numbers, this could result from the same transformation event. It will be necessary to verify if those lines proceed from the same callus, thus bearing the same transformation event.
The rate of lines which showed any type of mutant phenotype was 18%, which should be, as in the screenhouse and for the same reasons, an upper bound. Also, we chose to include

even doubtful data as it is preferable to eliminate false-positive data after more detailed analyses for a specific trait than to miss real data. As it is impossible to describe all the data here in details, we chose to present only a few examples. Numerous lines showed chlorotic or albino plantlets, with associated deficiency in leaf development (Figure 2a, b). General abnormal development was also frequently observed (Figure 2c, d).

The most common phenotype included several types of dwarfism more or less pronounced (Figure 3a). Some lines also showed a mutation for increased size. Figure 3b shows an example combined with awn spikelets.

Several lines showed reduced or increased tillering. Notably, a phenotype with very high tillering was observed for several lines (Figure 4b). The retarded growth phenotype was frequent,and associated reduced tillering and height, and late flowering (Figure 4b).

**Database Set Up**

A local database of all data relative to growth conditions, germination, flowering, and phenotypic observations was set up. This database is used as a working tool to facilitate data entry and compilation. It also can be used for data browsing, because it permits the display of information by mutant bar code number or CIAT number. This database also displays photos of the mutants (Figure 5). From this database, a flat datafile will be extracted in order to fill the Génoplante database, which will be later made publicly available.

**Conclusion**

The overall process of seed multiplication and phenotypic analysis worked very well. The timetable was respected, and valuable phenotypic data were produced. This first trial should constitute a good basis for conducting a larger project. Future plans: to extend the phenotypic analysis and seed multiplication to the entire collection (35,000 lines).

**Reference**


1. C. Sallaud, D. Meynard, J. van Boxtel, C. Gay, M. Bès, J.P. Brizard, P. Larmande, D. Ortega, M. Raynal, M. Portefaix, P.B.F. Ouwerkerk, S. Rueb, M. Delseny, E. Guiderdoni. 2003. Highly efficient production and characterization of T-DNA plants for rice (*Oryza sativa* L.) functional genomics. Theoretical and Applied Genetics 106: 1396-1408.


**Figure 2. Examples of Mutant Phenotypes Observed in the Rice T-DNA Collection in the Screenhouse. a: Chlorotic, b: Albino, c and d: Development Defects**

a b

**Figure 3. Examples of Mutant Phenotypes Observed in the Rice T-DNA Collection. a: Dwarf, b: Increased Size Associated with Spikelet Awning**

a b

**Figure 4. Examples of Mutant Phenotypes Observed in the Rice T-DNA Collection. a: Growth Retardate, b: High Tillering**

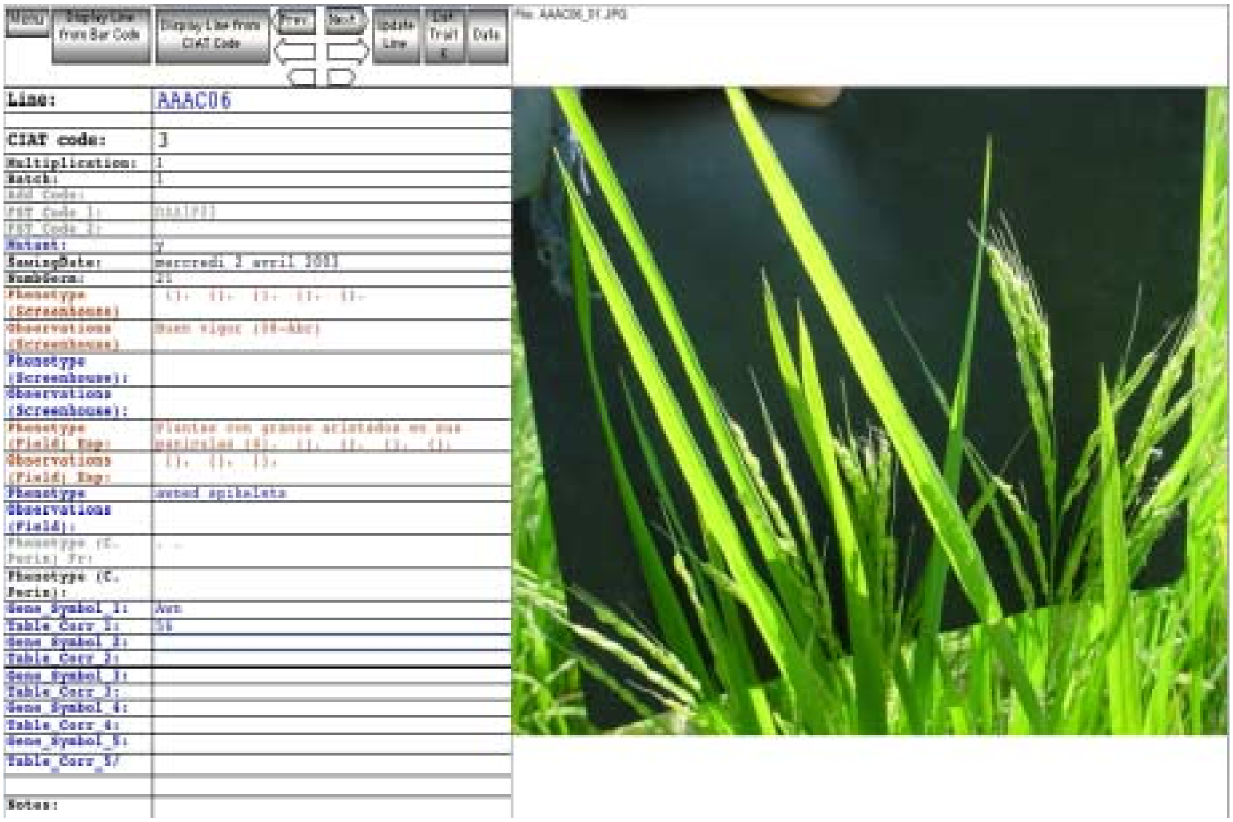



**Figure 5. Example of Request of the T-DNA Phenotypic Mutant Database**

- **International Rice Functional Genomic Consortium**

C.P. Martínez, J. Carabalí, J. Borrero and J. Tohme

Funding: USDA and CIAT core

Main Achievement: Development of a male-sterile Nipponbare population

**Background**

An international consortium of geneticists, molecular biologists and information scientists from Yale University, Cold Spring Harbor Laboratories, Brookhaven National Laboratory, and CIAT was assembled to address the following specific goals:

a To generate an extensive collection of rice lines, each containing an independent, dispersed insertion of a genetically-engineered Ds transposon;
b To determine the chromosomal position of each insertion by sequence of its flanking genomic DNA;
c To establish a database of that relates lines, sequences and phenotypic information;
d To publicly distribute mutant lines and associated informatics.

By making use of this public information, research scientists worldwide can rapidly identify mutant alleles in genes of agronomic importance for functional genomic studies and crop improvement.

**Materials and Methods**

A major CIAT involvement in this project is to produce foundation seed of stable rice lines containing the Ds insertions. All experiments are carried out in *Oryza sativa* sp. japonica cv Nipponbare. To produce $T_1$ seed, stock plants (provided by Yale University) will be crossed as males to wild type female plants in the CIAT nursery. Efficient out crossing can be achieved using a male-sterile female line.

Since male-sterility in Nipponbare was not presently available a backcross-breeding scheme shown in Figure 1 was used to introgression this trait into Nipponbare. A nuclear male-sterility allele (msms) found in IR36 (provided by GSKhush from IRRI) was used as the donor parent. The Nipponbare male-sterile version will be used for the production of foundation seed from each transposition selection ($T_1$ seed) produced in this project.

A simplified crossing method described by Sarkarung (1991) was used. A seed sample from the IR36 source segregating for male sterility was planted under field conditions in CIAT; male-sterile plants were phenotipically identified at flowering time and used as the female parent. $F_2$ seed was harvested from $F_1$ plants and grown in the field for the identification of male-sterile plants, which were backcrossed to Nipponbare to produce $BC_1F_1$ seed. A second BC to Nipponbare was done and the $BC_2F_2$ population was grown in the field to allow the identification of male-sterile plants, which were used to produce the $BC_3F_1$ seed, and subsequently the $BC_4F_1$. This seed was planted again to produce the $BC_4F_2$ generation to check for segregation of the sterility trait. Male-sterile plants very similar to Nipponbare were identified. However, some segregation in terms of plant height, tillering, flowering time, and presence of awns was observed. Therefore, another BC to Nipponbare was done to obtain a more uniform population. From here on, the male-sterile Nipponbare population will be maintained by growing it in isolation to allow open pollination. Just seed from the male-sterile plants will be harvested to maintain this population.

Seed increase of several genetic stocks was also done during the course of this project, and sent to Yale University for use in transformation experiments.

**Future plans**: Field/greenhouse evaluations of transformed lines carrying stable Ds insertions.

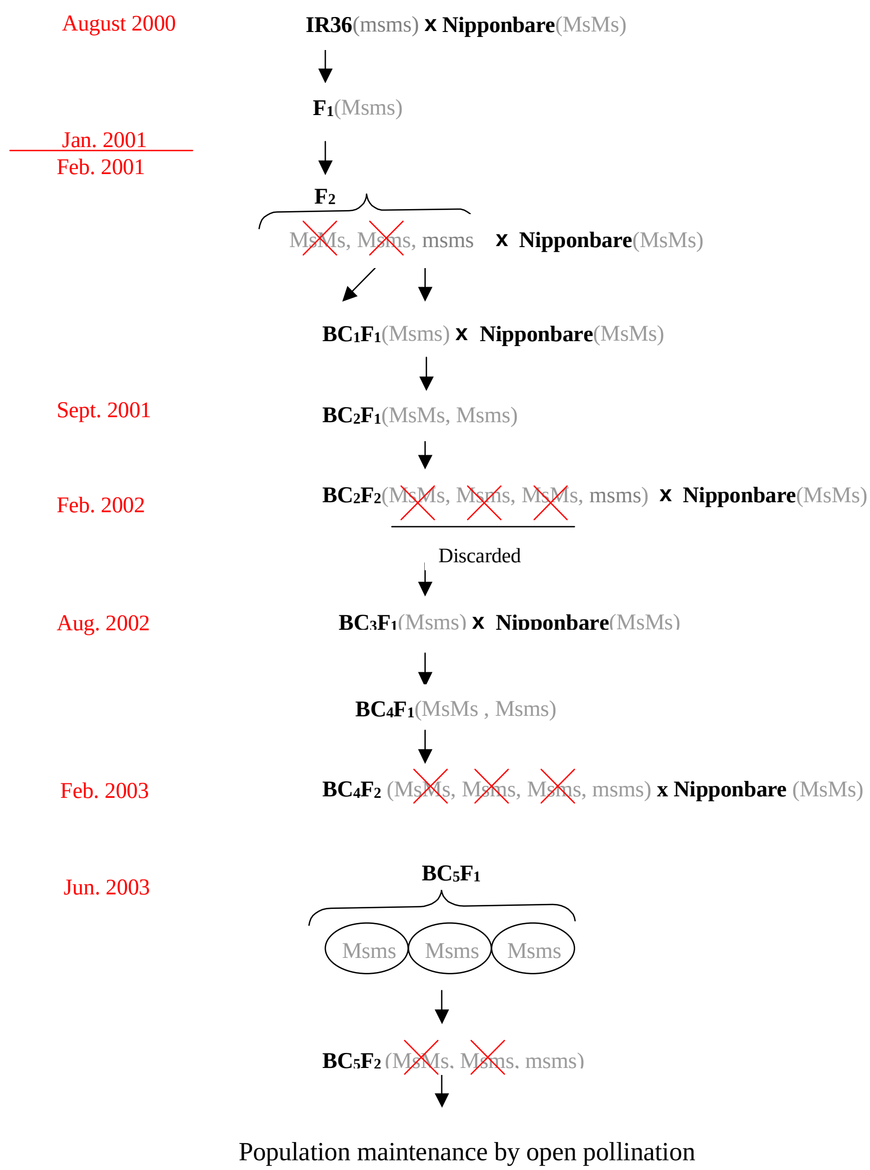



**Figure 1. Breeding Scheme to Introgress the Male Sterility Allele into Nipponbare**

## OUTPUT 1. ENHANCING GENE POOLS

### 1E. Preliminary Evaluation of the Response to *Hydrellia* spp. of the Main Commercial Rice Varieties Planted in Peru's Coastal Region

- **Preliminary Evaluation of the Response to *Hydrellia* spp. of the Main Commercial Rice Varieties Planted in Peru's Coastal Region**

C. Bruzzone[1] and A. Vigil[2]

[1]Consultant, CIAT/Peru Integrated Management for Coastal Areas (MIC) Rice Project
[2]Entomologist, Vista Florida Experiment Station, INIA-Peru

**Summary**

This year's evaluations carried out in plots forming part of two yield trials confirm the observations made in commercial fields that suggest that IR 43, currently the leading rice variety on Peru's northern coast, is more susceptible to the whorl maggot (*Hydrellia* spp.) than Viflor, the no. One variety planted during the 1990s. In one yield trial, the average number of larvae/m$^2$ in IR 43 was 4.25 compared with 0.8 in Viflor, and in the other yield trial these values were 5.85 and 3.25, respectively. This same trend was observed when the percentage of affected tillers was evaluated-in one yield trial, 32.6% in IR 43 compared with 21.5% in Viflor and, in the other, 38.6% and 16%, respectively. The response to Hydrellia of rice varieties Capirona, IDAL-2, Amor, and Pítipo was simultaneously evaluated in these trials. Vilfor and Pítipo proved to be the most tolerant, while the performance of IR 43, Capirona, and IDAL-2 indicated that these are the varieties most susceptible to *Hydrellia* spp.

**Introduction**

In recent years, the leaf miner or whorl maggot (*Hydrellia* spp.) has become the main phytosanitary problem of rice crops on Peru's northern coast, mainly in the rice-growing valleys of the Chancay (Department of Lambayeque) and the Jequetepeque rivers (Department of La Libertad). Most farmers apply pesticides two or three times during the crop cycle to control this insect and also increase fertilization doses to facilitate the fast recovery of the crop from pest attack. Word is that several producers have made more than four pesticide applications during the last growing seasons.

**Materials and Methods**

Varietal response to Hydrellia was evaluated in the plots of two yield trials established at the Vista Florida Experiment Station in Chiclayo, Lambayeque. Trial 1 included rice varieties IR 43 (currently the leading variety on the coast), Viflor (the former leading variety on the coast), Capirona (the leading variety in the upper jungle region), Pítipo (a variety recently released by INIA for the coastal region), and Amor (a variety recently released by a private company for the coastal region). Trial 2 evaluated six varieties: IR 43, Viflor, Capirona, Pitipo, and IDAL-2 (a variety in process of registration by a private company for the coastal region). A randomized complete block design with three replicates was used in both field trials.

For each variety and replicate, five groups of seedlings were selected at random and the total number of tillers, the number of Hydrellia-affected tillers on the still rolled leaf lamina, and the number of live larvae were counted. These observations were taken at 45 days after transplanting (80 days after planting) and served as basis for determining the percentage of tillers affected and the number of live larvae/$m^2$.

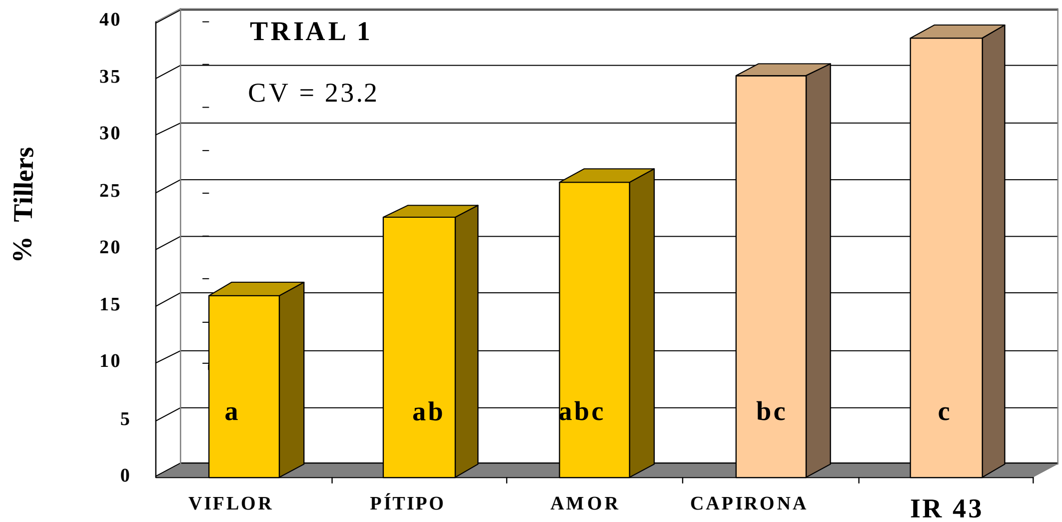
**Results and Discussion**

The levels of infestation of *Hydrellia* spp. in test fields of advanced rice lines at the Vista Florida Experiment Station were sufficiently high during the 2001-2002 growing season to allow the different varietal responses to be observed.

In Trial 1, there were no statistically significant differences in the number of live larvae among test varieties. However, significant differences were observed among varieties regarding the percentage of affected tillers. IR 43 was the variety most affected by *Hydrellia* spp., as compared with varieties Viflor and Pítipo (Figure 1).

In Trial 2, highly significant statistical differences were observed among test varieties regarding the number of live larvae found. Varieties IR 43, IDAL-2, and Capirona showed the highest number of larvae/$m^2$ and varieties Pítipo and Viflor, the lowest (Figure 1). No significant differences in the percentage of tillers affected were recorded in this trial.

Trial results are congruent and consistent with preliminary observations made in commercial fields. In both trials, the varieties showed the same trend regarding number of live larvae/$m^2$ and percentage of tillers affected. IR 43 showed the highest degree of susceptibility, followed by variety Capirona. Furthermore, Viflor and Pítipo showed the lowest incidence of all test varieties. This response is probably associated with its good initial vigor, a characteristic lacking in IR 43.



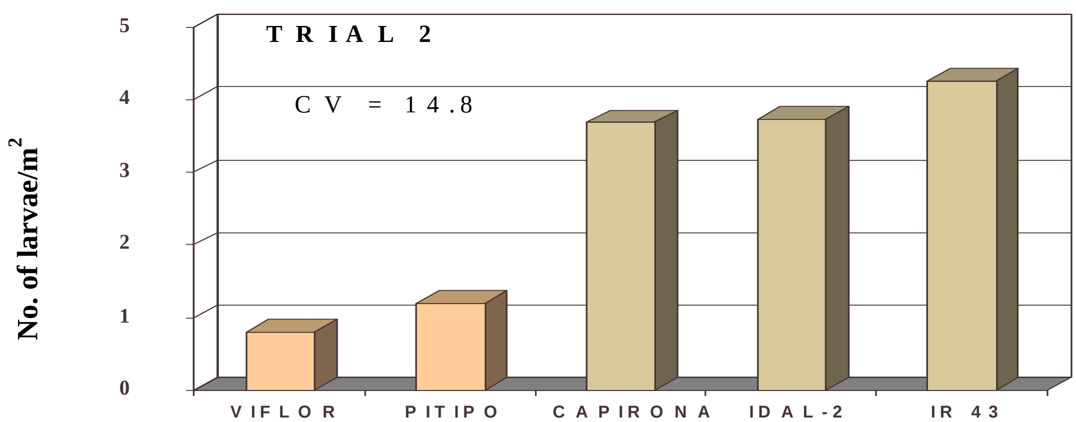




**Figure 1. Percentage of tillers affected by *Hydrellia* spp. and number of larvae/m$^2$, respectively in commercial rice varieties evaluated in two trials established at the Vista Florida Experiment Station located on Peru's northern coast during the 2001-2002 growing season. There were no statistically significant differences (P=0.05) between values marked with the same letters**

- **Economic Threshold for Controlling the Whorl Maggot (*Hydrellia* spp.) on Peru's Northern Coast: Preliminary Results**

C. Bruzzone[1] and A. Vigil[2]
[1]Consultant, CIAT/Peru Integrated Management for Coastal Areas (MIC) Rice Project
[2]Entomologist, Vista Florida Experiment Station, INIA-Peru

**Summary**

The correlation between the incidence of the whorl maggot (*Hydrellia* spp.) and rice yields and yield components was evaluated in a trial where plots planted to variety IR 43 were submitted to various levels of pest infestation. A pesticide was applied to control pest incidence. The correlation between the number of larvae per tiller 21 days after transplanting and grain yield was found to be negative.

**Introduction**

The whorl maggot or leaf miner is not considered a pest of economic importance for rice crops. Studies conducted so far in Latin America have not defined an action threshold for this pest, and it has not been possible to correlate the damage caused by the pest or its density with yield reduction. However, the importance of this insect has increased notably along Peru's northern coast, where most rice producers are applying pesticides two or three times during the crop cycle to control this insect, without using technical criteria or performing any type of sampling. This study therefore aimed to correlate the levels of crop damage and pest density with rice grain yield and yield components to help define an economic threshold for the pest.

**Materials and Methods**

To achieve various degrees of pest infestation, eight treatments were applied with four replicates. Seven treatments aimed to reach damage levels of 0, 5, 10, 15, 20, 25, and 30% affected tillers. Pest populations were monitored weekly and pesticides were applied every time a given plot reached the level of maximum damage predetermined for it. No pesticides were applied to plot No. 8 to allow the natural infestation of the pest.

The test variety used was IR 43, a variety widely planted in Peru's coastal region that is highly susceptible to this insect. The percentage of affected tillers and the number of live larvae were assessed on a weekly basis, over a 5-week period.

The number of panicles/m$^2$, paddy rice yield, number of filled grains per panicle, 1000-grain weight, and panicle length were determined at harvest. The correlations between the level of pest incidence (percentage of affected tillers and number of larvae/affected tiller) and paddy rice yield and yield components (number of filled grains/panicle, 1000-grain weight, number of panicles/m$^2$, and panicle length) were determined weekly. Those correlations that were statistically significant were submitted to regression analysis.

## Results and Discussion

Correlation analyses indicated statistically significant negative correlations between the percentage of affected tillers and number of larvae/tiller, at 14 and 21 days after transplanting (DAT) and 1000-grain weight. A significant negative correlation was observed between the number of filled grains/panicle and the percentage of affected tillers, 14 DAT. A statistically significant negative correlation was observed between the number of larvae/tiller at 21 DAT and both number of filled grains/panicle and paddy rice yield (Table 1).

**Table 1. Correlation Coefficients (r) and Probability Values (P Value of Statistically Significant Correlations between the Parameters of Hydrellia Incidence and Paddy Rice Yield and Yield Components in Variety IR 43. (Chiclayo, Peru, 2002-2003 Growing Season)**

| Time of Evaluation of Hydrellia Incidence | Yield and Yield Components | | Percentage affected Tillers | No. Larvae/ Tiller |
|---|---|---|---|---|
| 14 days after transplanting | 1000-grain weight | r | - 0.5353 | - 0.48952 |
| | | P value | 0.0016 | 0.0045 |
| | No. filled grains/ panicle | r | - 0.3523 | |
| | | P value | 0.0480 | |
| 21 days after transplanting | 1000-grain weight | r | - 0.3981 | - 0.44656 |
| | | P value | 0.0240 | 0.0104 |
| | No. filled grains/ panicle | r | | - 0.38154 |
| | | P value | | 0.0312 |
| | Yield | r | | - 0.37858 |
| | | P value | | 0.0326 |

**Table 2. Statistically Significant Parameters Estimated from Regression Analyses between the Parameters of Hydrellia Incidence and Paddy Rice Yield and Yield Components in Variety IR 43 (Chiclayo, Peru, 2002-2003 Growing Season)**

| Date of Evaluation of Hydrellia Incidence | Yield and Yield Components | Estimated Parameters* | Percentage affected Tillers | No. Larvae/ Tiller |
|---|---|---|---|---|
| 14 days after transplanting | 1000-grain weight | $R_2$ | 0.2865 | 0.2396 |
| | | Intersection | 29.26 | 29.29 |
| | | Coefficient | - 0,087 | - 2.17 |
| | | P value | 0.0016 | 0.0045 |
| | No. filled grains/ panicle | $R_2$ | 0.1241 | |
| | | Intersection | 162.34 | |
| | | Coefficient | - 1,122 | |
| | | P value | 0.048 | |
| 21 days after transplanting | 1000-grain weight | $R_2$ | 0.1585 | 0.1994 |
| | | Intersection | 28.8 | 29.0 |
| | | Coefficient | - 0,046 | - 1,094 |
| | | P value | 0.024 | 0.014 |
| | No. filled grains/ panicle | $R_2$ | | 0.1456 |
| | | Intersection | | 163.31 |
| | | Coefficient | | - 18,231 |
| | | P value | | 0.0312 |
| | Yield | $R_2$ | | 0.1433 |
| | | Intersection | | 10.723 |
| | | Coefficient | | - 1,089 |
| | | P value | | 0.0326 |

* R 2 = XXXXXX P value = probability value

## OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

### 2A. Rice Blast, Sheath Blight and Rice Stripe Necrosis Virus

- **Characterization on Blast Pathogen Populations. Monitoring the Evolution in the Genetic and Virulence Diversity of the Blast Pathogen over Time**

F. Correa, F. Escobar, G. Prado, G. Aricapa

**Abstract**

Rice blast, the most important rice disease worldwide can be managed through genetic resistance. Continuous monitoring of the evolution leading to important changes in the genetic structure and virulence spectrum of the pathogen is very important for the identification of resistance genes and their combinations to resist those pathogenic changes and preventing resistance breakdown. Understanding this pathogen-host interaction can attain development of suitable breeding strategies for a more stable blast resistance.

**Introduction**

Rice blast caused by *Pyricularia grisea* Sacc. is the most important disease worldwide. Genetic resistance is the most effective way to control the disease, but often resistance is defeated by the pathogen shortly after cultivar release. Two exceptions are the Colombian commercial cultivars Oryzica Llanos 5 and Fedearroz 50. This breakdown is mainly due to the continuous changes and evolution of the pathogen, which gives origin to new pathotypes compatible with the new rice cultivars. Continuous monitoring of blast pathogen populations in breeders fields is needed to detect recent changes in pathogen virulence. New pathotypes detected are used to identify resistance genes that can be introgressed into new genetic material before there is an increase in frequency of these new isolates, and therefore reducing the risks of resistance breakdown.

**Materials and Methods**

Rice leaves and panicles with typical blast symptoms are collected throughout the growing seasons from different rice lines in the pathology and breeder's plots at Santa Rosa experiment station. Blast isolates recovered from the infected samples in the laboratory are inoculated on a set of differential rice lines with different resistance genes to identify potential sources of resistance to new pathotypes. The same sample of isolates is used for determining their genetic structure using the Pot-2 PCR fingerprinting technique. More than 100 blast isolates recovered from several rice lines were analyzed in 2003 and new pathotypes are reported in this chapter.

**Results and Discussion**

All blast isolates analyzed belonged to the known genetic groups SRL-6, SRL-5, SRL-4 and SRL-2, which were previously identified in Colombia. The most common pathotypes found were #1 (SRL-4) and # 2 (SRL-5) presented in Table 1. These isolates were recovered from several cultivars. In 2003, pathotypes identified as #1 induced more typical blast lesion on cultivars Fedearroz 50 and Oryzica Llanos 5 than in past years. These susceptible reactions were also observed in greenhouse inoculations, although the disease severity observed was around 10% of leaf area affected. Resistance genes effective against this pathotype are Pi-1 and Pi-$K^h$ as had been reported in year 2002. The resistance gene Pi-1 is being incorporated in the commercial cultivar Fedearroz 50 through marker assisted selection, greenhouse inoculations and field evaluations.

In our efforts to detect new changes in virulence in the pathogen population, few blast lesions observed in the highly resistant line FL 00147-8P-6-15P were collected and analyzed in the laboratory. All four isolates retrieved turned out to be lineage SRL-4, however three of them belonged to a different pathotype identified as #3 in Table 1, and the fourth isolate as pathotype #1. Greenhouse inoculations of pathotype #3 indicated for the first time in many years, the ability of an isolate to potentially defeat the three resistance genes Pi-1, Pi-2, and Pi-33. This pathotype was recovered from only one cultivar, indicating that its frequency is still very low. Besides, the cultivar giving origin to this pathotype exhibits a highly resistant reaction to blast. We don't know at this point the relevance of this pathotype and the role it may play in breaking down the resistance conferred by the combination of these three genes. It is interesting to observe, however, that while losing the three avirulence genes avr-Pi-1, avr-Pi-2, and avr-Pi-33, this pathotype maintained the avirulence gene for Pi-$ta^2$ (Table 1). The corresponding resistance gene Pi-$ta^2$ present in the differential F 128-1 confers resistance to this isolate (Table 1). These results indicate that the resistance gene Pi-$ta^2$ will probably have to join the combination of the three resistance genes Pi-1, Pi-2, and Pi-33 to prevent a potential breakdown of the resistance genes. The presence of the Pi-$ta^2$ gene in the cultivars Oryzica Llanos 5 and Fedearroz 50 explains why this isolate did not infect severely these cultivars in greenhouse inoculations (Table 1). We are in the process of analyzing more blast samples collected in 2003, both, in terms of genetic structure and virulence spectrum, to determine if this new pathotype can be recovered from other cultivars, including samples isolated from the few lesions observed in the near isogenic lines from the cross CT 13432 carrying the three resistance genes Pi-1, Pi-2 and Pi-33.

Our results from the last several years, and of this particular year, suggest that in order to develop a more stable blast resistance, a combination of several resistance genes is needed to resist the potential changes in virulence of the rice blast pathogen. The combination of several major resistance genes will probably have to be accompanied by some important minor or quantitative trait loci, as will be discussed later in this chapter in the analysis of the stable resistance of the cultivar Oryzica Llanos 5.

As we can see from Table 1, few gene combinations would confer resistance to the blast population present in the upland environment of the Llanos Orientales from Colombia. We see the need to identify new genes, and wild rice species will probably be good sources of these resistant genes. We have tested for two years the resistance gene Pi-9 present in the line 75-1-127 that was derived from *Oryza minuta*. The gene confers complete resistance in greenhouse inoculations as well as field evaluations (Table 1). We have observed high levels of field resistance in the species *O. glaberrima* that deserve attention to identify potential new resistance genes. Once more, we see the importance of having a “hot spot” site with high blast pressure and pathogen diversity, to identify the best resistance gene combinations, and to detect in advance potential changes in genetic structure and virulence in the pathogen population that could threaten cultivar resistance.

**Future Activities**

Blast populations will continue to be analyzed for their genetic structure and virulence spectrum in order to determine the potential changes of the pathogen that would lead to resistance breakdown. New resistance genes and proper combinations will be identified in the cultivated as well as wild species of rice to be incorporated in our breeding program. We will analyze the importance and potential role of the new pathotypes identified in 2003 and determine the effectiveness of resistance genes effective against those isolates. The potential importance of the resistance gene Pi-9 will be evaluated again in 2003 under field conditions and greenhouse inoculations.

**Table 1. Virulence Spectrum and Frequency of Rice Blast Pathotypes detected at the Santa Rosa Experiment Station in 2003**

| Rice | Resistance | Pathotypes (frequency %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Line** | **Gene** | **1 (60)** | **2 (23)** | **3 (5)** | **4 (4)** | **5 (2)** | **6 (2)** | **7 (2)** | **8 (2)** |
| C 104 LAC | Pi-1 | | +++ | +++ | + | +++ | | | |
| C 101 A51 | Pi-2 | +++ | | +++ | | +++ | | +++ | +++ |
| C 101 LAC | Pi-1+Pi-33 | | +++ | +++ | | +++ | | | |
| CT 13432-33 | Pi-33 | +++ | +++ | +++ | + | +++ | +++ | | +++ |
| CT 13432-34 | Pi-1+Pi-2+Pi-33 | | | +++ | | ++ | | | |
| C 104 PKT | Pi-3 | +++ | +++ | +++ | | +++ | | | +++ |
| C 101 PKT | Pi-4a | +++ | +++ | +++ | +++ | +++ | | | +++ |
| C 105 TTP4 (L23) | Pi-4b | +++ | | +++ | +++ | +++ | | | +++ |
| F 124-1 | Pi-ta | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| F 128-1 | Pi-ta$^2$ | +++ | +++ | | ++ | | ++ | + | |
| F 80-1 | Pi-k | +++ | +++ | +++ | + | +++ | ++ | | + |
| F 98-7 | Pi-k$^m$ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ |
| F 129-1 | Pi-k$^p$ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| F 145-2 | Pi-b | +++ | +++ | +++ | | | +++ | + | + |
| Aichi Asahi | Pi-a | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| K 3 | Pi-k$^h$ | | +++ | +++ | | + | | | |
| K 59 | Pi-t | +++ | +++ | +++ | + | | +++ | | |
| Rico 1 | Pi-k$^s$ | +++ | + | +++ | +++ | ++ | +++ | | +++ |
| Norin 2 | Pi-sh | +++ | + | + | +++ | ++ | ++ | + | ++ |
| Nato | Pi-I | +++ | +++ | +++ | +++ | ++ | +++ | | +++ |
| Ou 244 | Pi-z | +++ | | +++ | | ++ | ++ | | +++ |
| Toride 1 | Pi-z$^t$ | +++ | | +++ | | ++ | | + | +++ |
| **Commercial Cultivars** | | | | | | | | | |
| Fanny | | +++ | +++ | +++ | +++ | +++ | +++ | + | +++ |
| Metica 1 | | +++ | | +++ | +++ | +++ | | | +++ |
| Oryzica 1 | | +++ | | +++ | +++ | ++ | | + | +++ |
| Oryzica 2 | | | | +++ | | + | | | |
| Oryzica 3 | | +++ | | +++ | | + | | | |
| Cica 7 | | +++ | | +++ | +++ | +++ | | | +++ |
| Cica 8 | | | | +++ | | + | | | |
| Cica 9 | | +++ | | +++ | | + | | +++ | |
| IR 22 | | +++ | ++ | +++ | +++ | ++ | +++ | | |
| Tetep | | | | +++ | | + | | | |
| Ceysvoni | | ++ | | +++ | | ++ | +++ | | |
| O. Llanos 5 | | + | | + | | | | | + |
| Línea 2 (Semillano) | | ++ | | +++ | | + | | +++ | |
| O. Llanos 4 | | + | -/+ | +++ | | | | | + |
| O. Caribe 8 | | +++ | | +++ | | +++ | | | +++ |
| O. Yacu 9 | | +++ | | +++ | | +++ | | | + |
| Fedearroz 50 | | ++ | | + | | + | | | |
| 75-1-127 | Pi-9 | | | | | | | | |

- **Selection of Rice Blast Resistance Sources to Different Genetic Lineages of the Blast Pathogen. Development of a Blast Nursery with Potential Sources of Resistance**

F. Correa, D. Delgado, G. Prado, G. Aricapa

**Abstract**

The frequency of blast resistant plants in $F_2$ populations is highly dependent on the blast reaction and stability of the parents used for the development of these populations. We initiated in year 2000, the development of a nursery with potential sources of durable blast resistance. Advanced rice lines are being evaluated for at least seven seasons under high disease pressure and only highly and durable resistant lines will be maintained into the nursery. This nursery will be tested under different conditions in several countries and used as a source of parents for breeding programs in Latin America.

**Introduction**

The frequency of blast resistant plants observed in $F_2$ populations in the field is highly dependent on the blast reaction and stability of this reaction of the parents used for the development of these populations. An increase in the number of susceptible $F_2$ plants and $F_4$ lines found in the past years in different breeding materials from CIAT and FLAR at the Santa Rosa experiment station has been observed and will be discussed later in this chapter. This has been related probably to the low stability of the blast resistance of the parents used in the corresponding breeding programs. We have initiated the blast evaluation over time in the field and greenhouse of several hundred advanced as well as segregating lines exhibiting desired agronomic traits to identify potential sources of blast resistance. We are developing a nursery of potential sources of blast resistance to be used as parents, and will distribute them to partners in Latin America for testing and use in their breeding programs. Materials and Methods were followed according to those described in the Annual Report of the Rice Project for 2001.

**Results**

A total of 418 advanced rice lines from different sources described in last year report were evaluated and selected at the Santa Rosa field experiment station in 2003. The most resistant lines over the last three years are shown in Table 2. Most of the resistant lines with a blast score 0-3 belong to the Germplasm Bank of CIAT-FLAR (Table 2). Several of these lines have already been used in different crosses and yielded rice lines with potential stability of their blast resistance in advance generations as will be shown later in this chapter. These results indicate the importance of evaluating the potential donors of blast resistance for several semesters before their inclusion in a breeding program. Selected lines with a blast score of 0-3 as well as those with an intermediate reaction with a score of 4 will be evaluated again in replicated trials in year 2004 for their inclusion in a nursery as potential donors of stable blast resistance. We had already reported in previous years, the highly resistant reaction of the japonica lines to the grain discoloration pathogens. It should also be noted the resistant reaction of the rice cultivars from Surinam such as Ciwini and Eloni, which can be incorporated in our breeding programs and help to broaden the genetic diversity of our rice germplasm.

**Discussion**

Durability of blast resistance is in general associated with the period of time that a cultivar remains as resistant after being exposed to a targeted pathogen. Field studies conducted by CIAT at Santa Rosa demonstrated that stable blast resistance could only be identified if the lines were evaluated through the $F_6$-$F_7$ generation. At the current time, the most effective resistance genes and their combinations can be identified only after several generations of exposure. These genes at the same time should correspond to those avirulence genes highly conserved in the pathogen population with lower rates of change or mutation. In order to identify resistance genes associated with durability, it is necessary to evaluate and confirm the stable resistance of the potential donors for at least seven generations. We are in the process of developing a nursery with potential donors of resistance to different pathogens. Therefore, these nurseries will be evaluated continuously for several seasons under high disease pressure in the field to assure that the resistance selected is not a escape to infection and that the lines retain their durable resistance.

**Future Activities**

The evaluations of advanced breeding lines will be an annual activity to assure that the selected sources retain their stable resistance to the different pathogens. The search for new blast resistance genes will continue. The pathogen population will be monitored on these resistant lines to identify changes leading to a potential breakdown of the resistance. An analysis of the parents used in the genetic crosses giving origin to rice lines with stable and durable resistance will be initiated. Genetic crosses giving origin to rice lines with potentially durable resistance will be developed on the basis of the information generated since year 2000.

**Table 2. Potential Progenitors for Stable Blast Resistance Exhibiting Blast Scores 1-3 in Santa Rosa Field Evaluations during Four Cycles, Santa Rosa 2000-2003**

| Pedigree | Pedigree |
|---|---|
| 1. FL 00478-29P-23-3P | 22. FL00478-29P-5-1P-M |
| 2. FL 00518-16P-8-2P | 23. FL00518-14P-15-3P-M |
| 3. CNAx5013-13-2-2-4-B | 24. FL00518-23P-11-2P-M |
| 4. CT11275-3-F4-8P-2 | 25. FL00530-29P-4-2P |
| 5. CT11280-2-F4-12P-5 | 26. FL00530-7P-7-1P-M |
| 6. CT11891-2-2-7-M | 27. FL00535-21P-4-3P-M |
| 7. CT13394-5-6-M-M-1 | 28. FL00542-45P-8-2P-M |
| 8. CT13449-M-3-1-M | 29. FL00585-26P-1-2P-M |
| 9. CT13449-M-8-2-M | 30. FL00595-12P-10-4P-M |
| 10. CT13458-M-3-2-M-M | 31. FL00595-25P-9-3P-M |
| 11. CT13458-M-3-3-M-M | 32. FL00837-8P-5-2P-M |
| 12. CT13458-M-3-4-M-M | 33. FL00871-1P-3-1P-M |
| 13. CT13464-M-10-1-M-M | 34. FL00871-1P-5-2P-M |
| 14. CT13937-16-1-M-M-2 | 35. HUALLAGA INIA |
| 15. CT13937-16-2-M-M-2 | 36. IRAK 13 |
| 16. CT13937-16-2-M-M-3 | 37. LINEA 30 |
| 17. CT13937-16-3-M-M-4 | 38. PROGRESO |
| 18. CT13941-11-1-M-M-4 | 39. PURG-2\0\0\1>27-1 |
| 19. CT13943-10-2-M-M-3 | 40. RIO PARAGUAY |
| 20. CT8222-7-6-2P-1X | 41. SAN MARTÍN 83 |
| 21. FL00447-35P-4-2P-M | 42. TRES MARIAS |

- **Identification of Molecular Markers Associated with the Blast Resistance Genes Pi-1, Pi-2, Pi-33 and their Incorporation into Commercial Rice Varieties Through Backcrossing and Marker Assisted Selection (MAS)**

F. Correa, D. Tharreau (CIRAD), C.P. Martínez, G. Prado, F. Escobar, J. Fuentes

**Abstract**

Farmers often choose blast susceptible varieties because they have high yields and quality grain. To sustain these characteristics, farmers have to spray fungicides, including several applications per planting season, increasing their production costs, the contamination the environment and the possibility of affecting human health. Incorporation of blast resistance genes to these varieties would make them more cost effective and ecologically sustainable. The combination of the blast resistance genes Pi-1, Pi-2, and Pi-33 confers resistance to all known blast pathogen populations in Colombia. We are identifying molecular markers associated with different resistance genes and initiated a backcrossing program assisted by these markers to introduce these resistance genes into several popular Latin American rice varieties.

**Introduction**

Farmers quickly adopt rice varieties that have high yields and excellent grain quality. The characteristic such as resistance to diseases is highly desirable but not enough to make a variety successful. Inconsistent yields because of diseases are enough to cause varieties to be discarded by farmers. Varieties without durable blast resistance tend to become more susceptible every year and need more applications of fungicides. In seasons favorable for rice blast, even fungicides may not be sufficient to prevent substantial losses. Farmers would like these varieties to be blast resistant.

We have initiated a backcrossing program in order to introduce blast resistance genes into some of those susceptible rice cultivars, which still play an important role in the economy of many rice farmers and regions of Latin America. The resistance genes being incorporated into the commercial varieties are Pi-1, Pi-2, and Pi-33 as they confer resistance to all the pathogen population in Colombia and probably the Latin America region based on their reaction to other blast populations of the region. The $BC_1F_1$ and $BC_1F_2$ breeding populations, derived from the crosses between several Latin American rice varieties and four rice lines used as sources of the three resistance genes, were developed to identify heterozygous lines carrying the three resistance genes. The materials and methods were described in the 2001 Annual Report of the Rice Project. Greenhouse inoculations with blast isolates carrying the appropriate avirulence genes were performed in 2003 to select the resistant plants to prepare the $BC_2$. Additionally, $BC_1F_2$ populations were planted at the Santa Rosa experiment station for selection of resistant plants and backcrossed to the corresponding recurrent parents. A total of 56 microsatellites or PCR based markers relatively close to the resistance genes Pi-1, Pi-2 and Pi-3 were identified from different publications or databases and tested for their association with these genes.

## Results

Near isogenic lines carrying each of the three resistance genes, combinations of any two of the genes as well as all three resistance genes Pi-1, Pi-2, and Pi-33 were developed based. These were selected by controlled inoculations in the greenhouse and evaluations under field conditions in 2002 in our Santa Rosa experiment station. The only isogenic lines exhibiting leaf and panicle blast resistance at Santa Rosa in 2002 and 2003 were the four lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33. However, some plants within these lines exhibited few blast lesions in 2003, which were brought to the laboratory for pathogen isolation and for further analysis of the genetic structure and virulence spectrum of the pathogen. Most of the lines carrying one or two genes died before panicle development both in 2002 and 2003.

The four isogenic lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33 with high leaf and panicle blast resistance were used for the development of the $BC_1F_1$ populations with fourteen Latin American rice varieties and the $BC_2F_1$ of four of them following the procedure described in the Rice Annual Report of year 2002. Greenhouse inoculations of the $BC_1F_1$ and the $BC_2F_1$ were performed with the blast isolates carrying the corresponding avirulence genes and resistant plants selected for the production of the $BC_2$ and $BC_3$ populations. Field evaluations of the $BC_1F_2$ at the Santa Rosa experiment station were also performed under field conditions and resistant plants selected to develop the $BC_2F_1$ population. A total of 56 molecular markers (Gene Bank web site) close to the three blast resistance genes (37 for Pi-1, 11 for Pi-2, and 8 for Pi-33) were identified and tested for their gene association in near isogenic lines carrying single or combinations of the three genes. Six markers were identified highly associated with the presence of the resistance gene Pi-1, while one marker was associated with the absence of Pi-1 exhibiting a band only in the susceptible lines. Three markers were associated with Pi-33 and one with Pi-2 (Table 3). Most of the other markers did not amplify at the conditions tested of $50^oC$ and $55^oC$ used for the annealing temperature and will be tested under different conditions. The eleven markers associated with the three resistance genes were tested in replicated trials on 38 isogenic lines carrying different combinations of the three resistance genes and on 19 rice cultivars including those Latin American varieties used in our backcrossing program. The markers associated with Pi-1 and Pi-2 seem to be suitable to follow the introgression of these genes into the commercial cultivars, but the markers for Pi-33 failed to discriminate the presence or absence of the gene in the lines rated as susceptible and resistant cultivars in greenhouse inoculations. Efforts continue to develop closer markers associated with the three genes. The number of backcrosses needed will depend on the recovery of the desired agronomic traits of the recurrent rice varieties.

## Discussion

The combination of the three resistance genes Pi-1, Pi-2, and Pi-33 in a single near isogenic line exhibited in years 2002 and 2003 high levels of resistance to leaf and neck blast when exposed to high blast pressure. Since all combinations of two genes and lines with one of the genes were susceptible to blast under these conditions, it seems then that the blast pathogen is able to lose any of the three avirulence genes or any combination of two avirulence genes, but not all three of them in a single isolate. It is possible that losing all of these avirulence genes might affect some fitness parameters. such as competitiveness among pathotypes with different avirulence gene composition, and therefore this parameter needs to be measure under natural conditions of infection by collecting blast samples from different near isogenic lines carrying different combinations of the resistance genes. Few blast samples collected from lines carrying the three resistance genes were collected in 2003 and will be analyzed for their genetic and virulence structure to determine its potential importance.

**Future Activities**

Evaluation and selection of the $BC_2F_1$ populations and development of the $BC_3F_1$ of several backcrosses between Latin American rice varieties and near isogenic lines carrying the resistance genes Pi-1, Pi-2, and Pi-33 continues. Molecular markers as well as inoculations with appropriate blast isolates will be used for the identification of the rice lines carrying the three resistance genes. Field studies will be carried out to determine the possible association of the lost of an avirulence gene with parameters of pathogenic fitness such as pathogen competitiveness. These activities will be carried out by collecting blast isolates from near isogenic lines with different combinations of the three blast resistance genes and comparing the frequencies of the different avirulence gene combinations present in the pathogen population with the expected frequencies based on the resistance genes of each line. Genetic distances between the molecular markers and the resistance genes will be determined using a set of 283 near isogenic lines.

**Table 3. Identification of Microsatellite Markers Associated with the Resistance Genes Pi-1, Pi-2, and Pi-33 using a Set of Near Isogenic Lines**

| Marker Identification | | Resistance Gene | Primer Sequence |
|---|---|---|---|
| RM 1233*I | Forward | Pi-1 | TTCGTTTTCCTTGGTTAGTG |
| | Reverse | | ATTGGCTCCTGAAGAAGG |
| RM 7654*A | Forward | Pi-1 | CAAAAGTCTGACCGTTTACC |
| | Reverse | | TAAGAGACGGAAGAGTGAGC |
| RM7654*H | Forward | Pi-1 | CTCATGGTTGTGTCGTGGTC |
| | Reverse | | GTGCAGTGCCAGTGGTACG |
| RM 7654-2 | Forward | Pi-1 | GTGTCGTGGTCGTAACTTG |
| | Reverse | | TAAGAGACGGAAGAGTGAGC |
| RM 6094 | Forward | Pi-1 | TGCTTGATCTGTGTTCGTCC |
| | Reverse | | TAGCAGCACCAGCATGAAAG |
| RM 5926 | Forward | Pi-1 | ATATACTGTAGGTCCATCCA |
| | Reverse | | AGATAGTATAGCGTAGCAGC |
| RM 224 | Forward | Pi-1 | GATCGATCGATCTTCACGAGG |
| | Reverse | | TGCTATAAAAGGCATTCGGG |
| RM 527 | Forward | Pi-2 | GGCTCGATCTAGAAAATCCG |
| | Reverse | | TTGCACAGGTTGCGATAGAG |
| RM 409 | Forward | Pi-33 | CCAATCATTAACCCCTGAGC |
| | Reverse | | GCCTTCATGCTTCAGAAGAC |
| RM 483 | Forward | Pi-33 | CTTCCACCATAAAACCGGAG |
| | Reverse | | ACACCGGTGATCTTGTAGCC |
| RM 72 | Forward | Pi-33 | CCGGCGATAAAACAATGAG |
| | Reverse | | GCATCGGTCCTAACTAAGGG |

- **Identification of Molecular Markers Associated with the Durable Blast Resistance Genes in the Commercial Rice Cultivar Oryzica Llanos 5**

J. López (KSU), J. Tohme (BRU), F. Correa, C. Martínez, S. Hulbert (KSU), R. Zeigler (KSU), G. Gallego (BRU), G. Prado, G. Aricapa

## Abstract

The genetic basis of the high level of durable resistance to rice blast in the cultivar Oryzica Llanos 5 is being characterized in $F_7$ Recombinant Inbred Lines (RILs) from a cross between the susceptible cultivar Fanny and O. Llanos 5. A linkage map was constructed using 250 molecular markers: SSR, RFLP and RGAs. Eleven loci, distributed on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 11, and 12, were associated with the resistance of the cultivar. As a whole, the observed durable resistance in Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes.

## Introduction

Blast resistance in the Colombian commercial rice varieties has been defeated in periods of 1-3 three years after cultivar release. However, the resistance of the cultivar Oryzica Llanos 5 has been durable and has remained stable under field conditions for more than 14 years. Genetic studies have indicated the presence of at least four major genes controlling the resistance to some blast isolates. Based on the presence of avirulence genes in our blast populations, we have inferred that the cultivar O. Llanos 5 carries at least 8 major genes. Studies retrieving blast isolates from the immediate parents giving origin to this cultivar and characterizing their genetic structure and virulence composition suggest that the durable resistance is associated with the pyramiding of complementary resistance genes to the different lineages of the pathogen present in those parents. Understand the basis of the durable resistance of this rice cultivar is being done in order to establish a breeding strategy based on the same principle. A study to identify and localize major and minor loci genes controlling the resistance in Oryzica Llanos 5 was initiated in year 2000 in collaboration with Kansas State University (KSU) using a QTL (quantitative trait loci) detection approach.

## Materials and Methods

Nearly 1000 recombinant inbred lines (RIL's) of the cross between the resistant indica cultivar Oryzica Llanos 5 and the japonica susceptible cultivar Fanny have been developed. An initial set of 120 lines was inoculated with different blast isolates representing the pathogen genetic lineages SRL-1 to SRL-6 from Colombia. Inoculations and evaluations were performed at the Rice Pathology greenhouse of CIAT according to the methodology described in other annual reports. Two evaluation methods, lesion type (LT) and disease leaf area (DLA) were used to score the blast resistance. One isolate named "killer", was recovered from O. Llanos 5 and observed to be highly aggressive and have a very broad virulence spectrum. This isolate has been used to detect minor resistance genes. DNA of each of the 120 lines was extracted at KSU for molecular analysis and microsatellites were used as potential markers for the identification of the resistance genes present in Oryzica Llanos 5. Blast resistance genes to each genetic lineage of the pathogen are being identified based on the phenotypic reaction and located on the different chromosomes of the rice genome. The genetic linkage map constructed from the RIL mapping population contains 250 markers including simple sequence repeats, and RFLPs. The chromosomal locations of the markers were determined using the Mapmaker program Version 2.0. Both composite interval mapping (CIM) and multiple interval mapping (MIM) techniques were used for QTL detection using QTL Cartographer package v2.0.

## Results

QTLs associated with LT and DLA were detected for 7 of the 8 isolates on rice chromosomes 1, 2, 3, 4, 5, 6, 8, 9, and 11. QTLs with the largest effects were on chromosomes 8, 6, and 11, which explained 28%, 72%, and 42% of the genetic variation in resistance to some isolates. Other QTL explained 2% to 16% of the genetic variance. In some of the QTL locations, there are blast resistance major genes that have been reported i.e. chromosome 4, 6, 5, 8, 9, and 11. However, some of the QTL have small effects, which indicates the presence of minor genes. A number of these genes are located in areas previously identified to be associated with QTL with large effects. Chromosomes 1 and 8 were found to carry important resistance factors that are not associated with previously identified resistance genes.

**Discussion**

The durable broad-spectrum resistance in O. Llanos 5 is associated with multiple major genes that induce resistance to different blast isolates. All of the QTLs detected in this study for isolates, except the isolate Killer, were for lesion type blast resistance. LT is typically associated with QTLs with large effects (major, or "R" genes). In contrast all QTL identified by killer were for DLA. None of the major genes detected in Oryzica Llanos 5 are effective against the killer isolate, but O. Llanos 5 is still highly resistant. This resistance appears to be controlled by genes with small main effects. The killer isolate apparently allows these genes to be identified. As a whole, the observed durable resistance in O. Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes.

**Future Activities**

Evaluate the reaction of more RIL's to the blast isolates already used in previous inoculations as well as new isolates exhibiting a compatible reaction with the cultivar O. Llanos 5. Continue analysis with microsatellite markers to identify and locate more blast resistance genes. Develop near isogenic lines with blast resistance genes present in Oryzica Llanos 5

- **Evaluation of Breeding Populations Incorporating Complementary Resistance Sources to Blast. Association of Selection for Blast Resistance in Early Generations and Stability of the Resistance**

F. Correa and D. Delgado

**Abstract**

In breeding lines blast resistance is frequently lost after the $F_4$ generation. The first selections for blast resistance are made by breeders in early generations ($F_2$), however, the association of stability of resistance in the advanced generations of the selected plants and the original blast reaction of an $F_2$ population is not known. A long-term study was initiated by selecting resistant plants in the $F_2$ populations of FLAR during 2000-01, and to associate the stability of blast resistance in advanced generations and the reaction of $F_2$ populations. We expect to generate information for selecting resistant plants in early generations and to identify potential donors of stable resistance that would potentially have

more chances of leading to the identification of rice lines with durable blast resistance.

## Introduction

It is commonly observed that blast resistance is frequently lost in breeding lines selected for resistance after four or more generations. Stability of blast resistance under severe blast pressure is the result of the action of many resistance genes. Many strategies and breeding methodologies for the selection of rice lines with stable resistance have been tried. Normally the first selections for blast resistance are made in the $F_2$ generation, but the stability of resistance selected at this early stage is not known. We have initiated a long-term study using two different FLAR populations to determine the association of stable blast resistance and the original blast reactions observed in the $F_2$ population. This is being done using advanced lines developed in a breeding program, where parents and crosses are selected on the basis of their reaction to blast lineages and/or field reaction. Studies of the stability of the blast resistant reaction will continue with the advanced generations in replicated trials until the number of lines losing the resistance reaches a plateau.

## Materials and Methods

A study to evaluate the stability of blast resistance was initiated in breeding $F_2$ populations from FLAR in 2000 and 2001. Many of the materials and methods were described in the Rice Project Annual Report for 2001. Populations selected for this study included crosses where parents exhibited a resistance, intermediate or susceptible reaction (Table 4) and triple crosses involved three resistant parents (greenhouse and field evaluations), or three susceptible parents. Populations selected in both years also included crosses where the predominant $F_2$ family field reaction was susceptible, segregating (equal amount of $F_2$ susceptible/resistant plants), or resistant (Table 4, Table 5). In the $F_2$ generation, resistant plants were selected both in 2000 and 2001. The $F_3$ lines derived from $F_2$ resistant plants selected in 2000 and 2001 were evaluated in the following years. In 2003, we evaluated the $F_5$ and $F_4$ advanced lines derived from the selections made in 2000 and 2001, respectively. In the $F_3$ generation, each line was planted in 10 rows that were 2m long. Two plants per row were randomly selected and were evaluated for leaf blast at 25, 32, and 39 days after planting, and 25 and 32 days after flowering for panicle blast. Three to five $F_3$ resistant plants were selected from each line for field evaluations in 2002 and 2003. Studies on the stability of the blast resistant reaction were initiated in replicated trials in the $F_4$ generation and continue over more advanced generations on a year basis until the number of lines losing the resistance reaches a plateau. The $F_4$ and $F_5$ generations were evaluated in 2003 in two replications. Leaf blast was evaluated at 30, 37, and 45 days after planting, and panicle blast at 25 and 32 days after flowering. Scores 0-3 are considered resistant, a score of 4 intermediate, and a score of 5 or more as susceptible. Parents used in the crosses under study were planted for detailed observation of their blast reaction and for collection of blast isolates to be used in greenhouse studies.

**Results**

A total of 1017 $F_5$ lines derived from $F_2$ resistant plants selected in 2000 were evaluated in 2003 in replicated trials (Table 4). We can observe a high decline in the number of resistant lines as compared with the evaluation of the $F_4$ generation in 2002 (Table 4). The largest proportion of resistant lines (10.2%) originated from those lines coming from $F_2$ populations where the number of resistant plants predominated. Only 20 lines out of 196 resistant $F_3$ plants selected in 2001 maintained their resistance. High levels of decline in the number of resistant lines were also observed for the other populations in this study (Table 4). Very few lines selected within $F_2$ populations with a predominant susceptible reaction were still resistant in the $F_5$ generation. A few plants within each one of the 1017 lines were harvested and will be planted as $F_6$ lines in 2004 together with the $F_5$ lines coming from the trials started in 2001. Evaluation of the stability of the resistance of these advanced lines will continue on a yearly basis in replicated trials.

A total of 292 $F_2$ blast resistant plants from 87 different crosses and 147 families were selected in year 2001 (Table 5). The resistant plants were selected from crosses where the predominant $F_2$ family field reaction was susceptible, segregating (equal amount of $F_2$ susceptible/resistant plants), or resistant $F_2$ plants (Table 5). Evaluation of the $F_3$ lines derived from the $F_2$ resistant plants selected in 2001 yielded more resistant lines (35.5%) when selections were made from the $F_2$ populations that were rated as resistant to blast compared to those coming from segregating or susceptible. There were apparently no differences between these two latter groups, which yielded 6.7 and 8.7 % of resistant $F_3$ lines (Table 5). A total of 1242 resistant $F_4$ plants selected among these three groups of lines were made in 2002 and evaluated in two replications as described above in 2003. We can observe the high decline in the number of resistant lines in the $F_4$ generation of this group (originating in 2001) compared to the same generation of lines originating in year 2000 (Table 4, Table 5). Only 6% and 7.8% for the resistant and segregating groups, respectively, maintained their resistant reaction. This corresponds to 27 and 33 lines out of 448 and 422 lines, respectively.

Rice lines $F_5$ (10 lines) with stable blast resistance selected within $F_2$ FLAR populations from crosses between three resistant parents selected in greenhouse inoculations (R/R//R) are presented in Table 6. These lines originated from four different crosses in which at least one parent (CT 10310-15-3-2P-4-3) is involved in all the four crosses (Table 6).

Rice lines $F_5$ (5 lines) with stable blast resistance selected within susceptible $F_2$ FLAR populations are listed in (Table 7). The lines originated from three different crosses where at least a common resistant parent was involved (CT 11256-5-$F_4$-28P-4P). Lines $F_5$ (17 lines) with stable blast resistance selected within segregating $F_2$ FLAR populations can be seen in (Table 8); the resistant lines originated from 10 different crosses, which involved several common resistant parents such as CANx1053-12-13-2-2-4-B, CT 10310-15-3-2P-4-3 and CT 11256-5-$F_4$-28P-4P. It is interesting to note that several crosses involving three susceptible parents have generated stable resistant lines. Lines $F_5$ (20 lines) with stable blast resistance selected within resistant $F_2$ FLAR populations are presented in Table 9;

these lines originated from seven different crosses, which involved the same resistant parents identified above (CNAx50-13-12-13-2-2-4-B, CT 10310-15-3-2P-4-3, and CT 11256-5-$F_4$-28P-4P). As mentioned before, some crosses between three susceptible parents have yielded rice lines with stable resistance in the $F_5$ generation.

The number of rice lines $F_4$ with stable blast resistance selected within $F_2$ populations in 2001 was much lower compared to the same $F_4$ generation from selections made in 2000. The reason for this could be that populations $F_2$ planted in 2001 were developed using a different set of parents; another reason could be a higher blast pressure during year 2003, and/or the appearance of new pathotypes with a high spectrum of virulence. The identification of the lines and crosses originating rice lines $F_4$ with stable blast resistance (2, 33, and 27 lines) selected within susceptible, segregating, and resistant $F_2$ FLAR populations, respectively, are presented in Table 10 and Table 11. New sources of stable resistance can also be identified from this population, including FL 00585-12P-2-2P-M, CT 8250-21-12-2P-1X, FL 00470-29P-7-3P-M, FL 00447-27P-3-1P-M, FL 00595-12P-1-1P-M, FL 00510-17P-3-2P-M, FL 00595-25P-9-3P-M, and FL 00470-29P-2-3P-M. Some of these parents were involved in several crosses (Table 10 and Table 11). The commercial rice cultivar Fedearroz 50 was also a parent involved in many crosses that yielded lines with potential stable resistance to blast. Contrary to the populations selected in 2000, stable resistant lines derived from crosses between three susceptible parents were not identified.

Considering both $F_2$ populations used in this study from years 2000 and 2001, 22 potential sources of stable blast resistance were selected on the basis of stable resistant lines identified in the $F_4$ and $F_5$ generations evaluated in 2003 (Table 12). Most of these lines have had a resistant reaction (score 0-3), while few of the lines had some intermediate reactions (score 4) in field evaluations during 2000-2003. Several of these potential donors of resistance were involved in the development of several of the resistant lines identified. These lines can be considered as progenitors for the development of future crosses in order to increase the possibilities of developing stable resistant lines. These lines can be crossed with other parents susceptible to blast but carrying good agronomic traits.

**Discussion**

Our hypothesis in this study was that those lines originating in crosses, where the $F_2$ families that show a higher percentage of blast resistant plants, and which hadd a higher number of resistant sister lines, would give origin to more stable resistant lines in the advanced generations. In addition, those advanced resistant lines originating from $F_2$ resistant plants selected within crosses where $F_2$ susceptible plants predominate, will be less stable.

Our reasoning behind this hypothesis was that $F_2$ populations exhibiting a predominant number of resistant plants carry a larger number of different resistant genes including both major and minor genes. Therefore, advanced resistant lines originating in these populations have a greater probability to carry a larger number of these resistance genes and to be more stable. Those families with few $F_2$ resistant plants would tend to have fewer resistance genes, and these would be easily defeated by the pathogen in early generations. If this hypothesis is correct, breeders should rate the $F_2$ populations and eliminate those crosses where the susceptible plants predominate. This would allow that breeder's efforts to be concentrated on those crosses where there is a greater probability of selecting stable blast resistant rice lines.

On the basis of our results in the $F_4$ and F5 generations, our hypothesis seems to be true in relation to the development of stable blast resistant lines originating from $F_2$ populations where there are a dominant number of resistant plants. This can also be considered true for the segregating population where there are approximately an equal number of resistant and susceptible plants. We are still concerned with the rapid decline in the number of resistant lines from one generation to another. The number of resistant lines dropped from 37% in the $F_4$ generation to 5.1% in the $F_5$ generation (Table 4) for the population study began in year 2000. A worst situation is observed for the population study that began in year 2001 as the number of resistant lines in the $F_4$ generation was only 5.0% (Table 5) compared to the 37% observed in the same generation for the population selected in year 2000 (Table 4). The reason for this rapid decline in the number of resistant lines could be a possible high blast pressure observed in 2003. However, if we look at the blast reaction of the parents participating in more than 10 crosses of the 2000 and 2001 $F_2$ populations (Table 13), while there were 6 resistant parents involved in more than 10 crosses for the 2000 population, there was only one resistant parent involved in more than 10 crosses for the 2001 population studied. It should be noted that the FLAR $F_2$ population in year 2000 represented 245 triple crosses for the tropics and we selected for our study 69 (28.1%) crosses involving 49 (53.2%) of a total of 92 parents. Of the 149 triple crosses for the tropics that formed the FLAR $F_2$ population in year 2001 and we selected for our study 87 (58%) crosses involving 46 (51%) of a total of 90 parents. Therefore, we can say that our studies included a good representation of the crosses and parents used for the $F_2$ populations planted by FLAR in years 2000 and 2001.

We can see that all parents with a resistant blast reaction presented in Table 13 have yielded rice lines, which still held their resistance in 2003. These lines are included in Table 12. There are also some parents with an intermediate blast reaction (Table 13), which seem to be good sources of resistance and were included in Table 12 to be used as potential donors. Based on the different tables presented of the rice lines exhibiting high levels of blast resistance in the $F_4$ and $F_5$ generation, it can be observed that there may be some susceptible parents such as CT 10825-1-2-1-3-M and CT 9748-13-2-1-M-M-1-1 among others, which seem to combine better with some resistant parents than others. This deserves attention to identify donors for desirable agronomic traits even though their susceptibility to blast.

Our results then suggest that there are some parents better than others for potentially giving origin to stable blast resistance. This potentiality seems to be associated with a stable blast resistance of the same parent observed over time at the Santa Rosa conditions of blast pressure. A list of the best parents for potentially giving origin to rice lines with stable blast resistance is presented in Table 12. We recommend that for incorporating new parents as sources of stable blast resistance in a breeding program, a careful evaluation over time and under high blast pressure be conducted before its consideration. Based on our results, we can observe that the number of resistant lines was considerably increased by the plant selection made in the $F_3$ generation, which was evaluated at Santa Rosa in our studies. Our breeding program is not presently evaluating this generation at Santa Rosa, running the risk of missing valuable resistant material, and/or selecting susceptible plants in the selections made at Palmira under no blast pressure. We have to consider that plant selection made in the $F_2$ generation is not an accurate selection in terms of resistance given that it is based on a single plant for which we normally have no information on its leaf blast reaction and therefore running the risk of selecting a susceptible plant that escaped panicle infection. Selections made within an $F_3$ population are based on the observation of several plants within a line, having therefore more precise information on the reaction of the segregation of the line and the original reaction of the $F_2$ original plant. We are in the process of analyzing the segregation of resistant/susceptible plants observed in the $F_3$ for both populations studied, in order to correlate this reaction with the stability of the resistance in the advanced lines.

It is also important to note that some crosses between three susceptible parents gave origin to some lines with blast resistance. Given that this case is not that common, we recommend analyzing the parents involved in those crosses. Probably this can be explained by the presence of complementary resistance genes, which would need to be identified if we want to use this type of crosses. In most cases, careful attention should be given to the reaction of the $F_2$ population selecting plants just within those families exhibiting a high number of resistant plants.

**Future Activities**

Analysis of the blast reaction of the parents, $F_2$ generation, and $F_3$ generation involved in all crosses that gave origin to the rice lines of this study will be carried out and correlated to the blast reaction and stability of the resistance of the lines being selected. Crosses involving one, two, and three resistant parents identified as potential sources of stable resistance will be developed for studying their effect in the development of stable blast resistance. Our objective will be to develop a breeding strategy with higher probabilities for developing stable blast resistant lines. This study is in progress and will continue for several years for determining the stability of the rice lines selected.

**Table 4. Long Term Study on the Stability of Blast Resistance based on $F_2$ Resistant Plants Selected from Different Rice Populations in 2000. Santa Rosa, Colombia 2000-2003**

| Population Cross/Family | Crosses No. | Families No. | Resistant $F_2$ Plants Selected in 2000 No. | Evaluation of $F_3$ Lines in 2001: Resistant | | Segregating | | Susceptible | | Resistant $F_3$ Plants Selected in 2001 No. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | |
| **Parents Selected** | | | | | | | | | | |
| R/R//R | 13 | 27 | 53 | 12 | (23) | 34 | (64) | 7 | (13) | 164 |
| S/S//S | 8 | 15 | 28 | 1 | (4) | 1 | (4) | 26 | (92) | 92 |
| **$F_2$ Family Field Reaction in 2000** | | | | | | | | | | |
| Susceptible | 27 | 50 | 89 | 32 | (36) | 37 | (42) | 20 | (22) | 289 |
| Segregating | 27 | 47 | 79 | 45 | (57) | 27 | (34) | 7 | (9) | 276 |
| Resistant | 18 | 30 | 60 | 46 | (77) | 12 | (20) | 2 | (3) | 196 |
| **Total** | **69** | **169** | **309** | | | | | | | **1017** |

**Table 4. Continued**

| Population Cross/Family | Resistant $F_4$ Lines No. | Evaluation of $F_4$ Lines in 2002: Resistant | | Susceptible | | $F_5$ Lines No. | Evaluation of $F_5$ Lines in 2003: Resistant | | Susceptible | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | | No. | % | No. | % |
| **Parents Selected** | | | | | | | | | | |
| R/R//R | 164 | 58 | (35.4) | 106 | (64.6) | 164 | 10 | (6.1) | 154 | (93.9) |
| S/S//S | 92 | 2 | (2.2) | 90 | (97.8) | 92 | 0 | (0) | 92 | (100) |
| **$F_2$ Family Field Reaction in 2000** | | | | | | | | | | |
| Susceptible | 289 | 87 | (30.1) | 202 | (69.9) | 289 | 5 | (1.7) | 284 | (98.3) |
| Segregating | 276 | 133 | (48.2) | 143 | (51.2) | 276 | 17 | (6.2) | 259 | (93.8) |
| Resistant | 196 | 99 | (50.5) | 97 | (49.5) | 196 | 20 | (10.2) | 176 | (89.8) |
| **Total** | **1017** | **379** | **(37.0)** | **638** | **(63.0)** | **1017** | **52** | **(5.1)** | **965** | **(94.9)** |

**Table 5. Long Term Study on the Stability at Blast Resistance based on $F_2$ Resistant Plants Selected from different Rice Populations in 2001. Santa Rosa, Colombia 2001-2003**

| Population | Crosses No. | Families No. | Resistant $F_2$ Plants Selected in 2001 | Evaluation of $F_3$ Lines in 2002: Resistant No. | Resistant % | Segregating No. | Segregating % | Susceptible No. | Susceptible % | Resistant $F_3$ Plants Selected in 2002 | Evaluation of $F_4$ Lines in 2003: Resistant No. | Resistant % | Susceptible No. | Susceptible % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$F_2$ Family Field Reaction** | | | | | | | | | | | | | | |
| Susceptible | 42 | 50 | 105 | 7 | (6.7) | 37 | (35.2) | 61 | (58.1) | 372 | 2 | (0.5) | 370 | (99.5) |
| Segregating | 45 | 49 | 92 | 8 | (8.7) | 48 | (52.2) | 36 | (39.1) | 422 | 33 | (7.8) | 389 | (93.2) |
| Resistant | 36 | 48 | 95 | 34 | (35.8) | 48 | (50.5) | 13 | (13.7) | 448 | 27 | (6.0) | 421 | (94.0) |
| **Total** | **87** | **147** | **292** | | | | | | | **1242** | **62** | **(5.0)** | **1180** | **(95.0)** |

**Table 6. Lines $F_5$ with Stable Blast Resistance Selected within $F_2$ FLAR Populations from Crosses R/R//R in 2000. Santa Rosa, Colombia 2003**

| Pedigree | Cross |
|---|---|
| 1. FL 02145-2P-1-2-1 | CT 11369-1-$F_4$-17P-4P/CT 9509-17-3-1-1-M-1-3P-M-3-3P//CT 10310-15-3-2P-4-3 |
| 2. FL 02145-6P-2-1-1 | |
| 3. FL 02145-6P-2-3-1 | |
| 4. FL 02386-3P-1-3-1 | CT 8222-7-6-2P-1X/CT 11008-12-3-1M-1P-4P//CT 10310-15-3-2P-4-3 |
| 5. FL 02386-3P-2-5-1 | |
| 6. FL 02390-1P-1-1-1 | CT 8222-7-6-2P-1X/CT 9682-M-10-2-M-1-1P//CT 10310-15-3-2P-4-3 |
| 7. FL 02752-6P-1-3-1 | CT 10310-15-3-2P-4-3/CT 11369-$F_4$-17P-4P//CT 11256-5-$F_4$-28P-4P |
| 8. FL 02752-9P-2-1-1 | |
| 9. FL 02752-9P-2-2-1 | |
| 10. FL 02752-9P-2-3-1 | |

**Table 7. Lines $F_5$ with Stable Blast Resistance Selected within SUSCEPTIBLE $F_2$ Populations in 2000. Santa Rosa, Colombia 2003**

| Pedigree | Cross | Parent Reaction |
|---|---|---|
| 1. FL 02784-10P-1-4-1 | CNARR 4955-7B-BM70A-45-5P/CT 10308-27-3-3P-3-1//CT 11256-5-$F_4$-28P-4P | S/I//R |
| 2. FL 02784-10P-2-2-1 | | |
| 3. FL 02801-4P-2-3-1 | CT 9868-3-2-3-1-4P-M-1-1P/CT 10308-27-3-3P-3-1//CT 11256-5-$F_4$-28P-4P | S/I//R |
| 4. FL 02801-4P-2-5-1 | | |
| 5. FL 02807-10P-1-4-1 | CAPI 93/CT 11026-3-9-1T-2P-5P//CT 11256-5-$F_4$-28P-4P | S/S//R |

**Table 8. Lines $F_5$ with Stable Blast Resistance Selected within SEGREGATING $F_2$ FLAR Populations in 2000. Santa Rosa, Colombia 2003**

| Pedigree | Cross | Parent Reaction |
|---|---|---|
| 1. FL 02764-1P-1-3-1 | CT 9145-4-21-5P-1-MI-F8-1P/CNARR4955-7B-BM70A-45-5P//CT 10310-15-3-2-P-4-3 | S/S//I |
| 2. FL 02769-7P-1-3-1 | CNAx5013-12-13-2-2-4/CT 11026-3-9-1T-2P-5P//CT 11256-5-$F_4$-28P-4P | R/S//R |
| 3. FL 02769-7P-2-1-1 | | |
| 4. FL 02769-7P-2-3-1 | | |
| 5. FL 02769-7P-2-4-1 | | |
| 6. FL 02770-10P-2-2-1 | CNAx5013-12-13-2-2-4/ CT 11026-3-9-1T-2P-5P//CT 11299-4-$F_4$-25P-3P | R/S//S |
| 7. FL 02771-1P-1-1-1 | CNAx5013-12-13-2-2-4/CT 11256-5-$F_4$-28P-4P//CT 9509-17-3-1-1-M-1-3P-M-1 | R/R//S |
| 8. FL 02776-2P-2-4-1 | CNARR4955-7B-BM70A-45-5P/CCT 9509-17-3-1-1-M-1-3P-M-1 //CT11299-4-$F_4$-25P-3P | S/S//S |
| 9. FL 02776-3P-1-2-1 | | |
| 10.FL 02776-3P-1-3-1 | | |
| 11. FL 02782-9P-2-3-1 | CNARR4955-7B-BM70A-45-5P/CT 10321-6-10-3P-1-1T-3P//CT 9509-17-3-1-1-M-1-3P-M-1 | S/I//S |
| 12. FL 02783-6P-1-3-1 | CNARR4955-7B-BM70A-45-5P/CT 10321-6-10-3P-1-1T-3P// CT 10310-15-3-2-P-4-3 | S/I//I |
| 13.FL 02784-8P-1-2-1 | CNARR4955-7B-BM70A-45-5P/CT 10308-27-3-3P-3-1// CT 11256-5-$F_4$-28P-4P | S/I//R |
| 14.FL 02784-13P-1-3-1 | | |
| 15.FL 02784-13P-1-4-1 | | |
| 16. FL 02804-4P-1-1-1 | CAPI 93/CIMARRON// CT 9509-17-3-1-1-M-1-3P-M-1 | S/S//S |
| 17. FL 02805-6P-1-4-1 | CAPI 93/CIMARRON// CT 11256-5-$F_4$-28P-4P | S/S//R |

**Table 9. Lines $F_5$ with Stable Blast Resistance Selected within RESISTANT $F_2$ FLAR Populations in 2000. Santa Rosa, Colombia 2003**

| Pedigree | Cross | Parent Reaction |
|---|---|---|
| 1. FL 02755-3P-2-3-1 | CT 11275-3-$F_4$-8P-2/CT 10323-29-4-1-1-1T-2P//CT 10310-15-3-2P-4-3 | I/S//I |
| 2. FL 02755-3P-2-4-1 | | |
| 3. FL 02763-5P-2-3-1 | CT 9145-4-21-5P-1-MI-$F_8$-1P/CT 9841-5-2-1P-25-2I-M//CT 11299-4-$F_4$-25P-3P | S/S//S |
| 4. FL 02764-2P-2-1-1 | CT 9145-4-21-5P-1-MI-$F_8$-1P/CANRR 4955-7B-BM70A-45-5P// CT 10310-15-3-2P-4-3 | S/S//I |
| 5. FL 02764-2P-2-4-1 | | |
| 6. FL 02767-1P-1-1 | CNAx5013-12-13-2-2-4-B/ CT 9841-5-2-1P-25-2I-M//CT 11256-5-$F_4$-28P-4P | R/S//R |
| 7. FL 02767-1P-2-2-1 | | |
| 8. FL 02767-11P-1-1-1 | | |
| 9. FL 02767-11P-1-2-1 | | |
| 10. FL 02767-11P-1-4-1 | | |
| 11. FL 02767-11P-2-3-1 | | |
| 12. FL 02768-5P-2-3-1 | CNAx5013-12-13-2-2-4-B/CT 10325-29-4-1-1-1T// CT 11299-4-$F_4$-25P-3P | R/S//S |
| 13. FL 02768-5P-2-4-1 | | |
| 14. FL 02768-16P-1-4-1 | | |
| 15. FL 02768-16P-2-1-1 | | |
| 16. FL 02770-16P-1-1-1 | CNAx5013-12-13-2-2-4-B/CT 11026-3-9-1T-2P-5P// CT 11299-4-$F_4$-25P-3P | R/S//S |
| 17. FL 02770-16P-1-2-1 | | |
| 18. FL 02770-16P-1-3-1 | | |
| 19. FL 02770-16P-2-2-1 | | |
| 20. FL 02776-14P-1-2-1 | CANRR 4955-7B-BM70A-45-5P/CT 9509-17-3-1-1-M-1-3P-M-1//CT 11299-4-$F_4$-25P-3P | S/S//S |

**Table 10. Lines $F_4$ with Blast Resistance Selected within SUSCEPTIBLE and SEGREGATING $F_2$ FLAR Populations in 2001. Santa Rosa, Colombia 2003**

| Population $F_2$/ Pedigree | Cross | Parent Reaction |
|---|---|---|
| **$F_2$ Susceptible** | | |
| 1. FL 03243-3P-1-1 | CT 10825-1-2-1-3-M/CIMARRON//FL 00585-12P-2-2P-M | S/S//R |
| 2. FL 03243-3P-1-2 | | |
| | | |
| **$F_2$ Segregating** | | |
| 1. FL 03225-2P-1-5 | CT 8250-21-12-2P-IX/CT 6543-28-6I-1I-2I//FL 00596-54P-3-2P-M | R/I//S |
| 2. FL 03233-4P-1-1 | CT 10825-1-2-1-3-M/FEDEARROZ 50//FL 00470-29P-7-3P-M | S/I//R |
| 3. FL 03233-4P-2-3 | | |
| 4. FL 03233-4P-2-4 | | |
| 5. FL 03233-4P-2-5 | | |
| 6. FL 03233-13P-1-1 | | |
| 7. FL 03233-13P-1-3 | | |
| 8. FL 03233-13P-1-4 | | |
| 9. FL 03233-13P-1-5 | | |
| 10. FL 03243-2P-1-1 | CT 10825-1-2-1-3-M/CIMARRON//FLM00585-12P-2-2P-M | S/S//R |
| 11. FL 03243-2P-1-3 | | |
| 12. FL 03243-2P-1-4 | | |
| 13. FL 03243-2P-1-5 | | |
| 14. FL 03302-3P-1-1 | CT 9748-13-2-1-M-M-1-1/CT 6543-28-6I-1I-2I//FL 00470-29P-7-3P-M | S/I//R |
| 15. FL 03346-9P-1-1 | CT 11032-2-4-3T-3P-3P-1/FEDEARROZ 50//FL 00447-27P-3-1P-M | S/R//R |
| 16. FL 03346-9P-1-2 | | |
| 17. FL 03346-9P-1-3 | | |
| 18. FL 03346-9P-1-4 | | |
| 19. FL 03346-9P-1-5 | | |
| 20. FL 03346-9P-1-6 | | |
| 21. FL 03346-9P-1-7 | | |
| 22. FL 03346-9P-1-8 | | |
| 23. FL 03346-9P-2-1 | | |
| 24. FL 03346-9P-2-2 | | |
| 25. FL 03346-9P-2-3 | | |
| 26. FL 03346-9P-2-4 | | |
| 27. FL 00361-3P-2-1 | CT 11408-6-$F_4$-1P-3/CT 6543-28-6I-1I-2I//FL 00470-29P-5-2P-M | S/I//R |
| 28. FL 00361-3P-2-2 | | |
| 29. FL 00361-3P-2-3 | | |
| 30. FL 00361-3P-2-4 | | |
| 31. FL 00361-3P-2-5 | | |
| 32. FL 00361-3P-2-6 | | |
| 33 FL 00361-3P-2-7 | | |

**Table 11. Lines $F_4$ with Blast Resistance Selected within RESISTANT $F_2$ FLAR Populations in 2001. Santa Rosa, Colombia 2003**

| Pedigree | Cross | Parent Reaction |
|---|---|---|
| 1. FL 03224-3P-1-1 | CT 8250-21-12-2P-1X/CT 6543-28-6I-1I-21//FL 00595-12P-1-1P-M | R/I//R |
| 2. FL 03224-3P-1-3 | | |
| 3. FL 03224-3P-1-4 | | |
| 4. FL 03230-5P-1-2 | CT 10825-1-2-1-3-M/O. Caribe 8//FL 00585-12P-7-3P-M | S/S//R |
| 5. FL 03230-5P-1-3 | | |
| 6. FL 03230-5P-1-4 | | |
| 7. FL 03230-5P-1-5 | | |
| 8. FL 03233-19P-2-3 | CT 10825-1-2-1-3-M/FEDEARRROZ 50//FL 00470-29P-7-3P-M | S/R//R |
| 9. FL 03233-19P-2-5 | | |
| 10. FL 03247-4P-1-1 | CT 10825-1-2-1-3-M/CT 8240-1-3-9P-M//FL 00470-29P-5-2P-M | S/S//R |
| 11. FL 03254-1P-2-1 | CT 10825-1-2-1-3-M/CT 10308-27-3-1P-4-3-2P//FL 00510-17P-3-2P-M | S/S//R |
| 12. FL 03254-1P-2-5 | | |
| 13. FL 03254-1P-2-6 | | |
| 14. FL 03254-1P-2-7 | | |
| 15. FL 03279-4P-1-4 | CT 9748-13-2-1-M-M-1-1/FEDEARROZ 50//FL 00595-25P-9-3P-M | S/I//R |
| 16. FL 03279-4P-1-5 | | |
| 17. FL 03279-4P-1-6 | | |
| 18. FL 03280-1P-1-4 | CT 9748-13-2-1-M-M-1-1/FEDEARROZ 50// FL 00470-29P-5-2P-M | S/R//R |
| 19. FL 03280-1P-2-2 | | |
| 20. FL 03302-4P-2-6 | CT 9748-13-2-1-M-M-1-1/CT 6543-28-6I-1I-2I//FL 00470-29P-2-3P-M | S/I//R |
| 21. FL 03309-18P-2-1 | CT 9748-13-2-1-M-M-1-1/ARAURE 4//FEDEARROZ 50 | S/S//I |
| 22. FL 03346-2P-2-1 | CT 11032-2-4-3T-3P-3P-1/FEDEARROZ 50//FL 00447-27P-3-1P-M | S/R//R |
| 23. FL 03346-2P-2-2 | | |
| 24. FL 03346-2P-2-3 | | |
| 25. FL 03346-2P-2-4 | | |
| 26. FL 03354-1P-1-4 | CT 11256-5-$F_4$-28P-5P/FEDEARROZ 50//PALMAR | I/I//S |
| 27. FL 03356-2P-2-6 | CT 11256-5-$F_4$-28P-5P/CIMARRON//CNARR 4955-7B-BM70A-45-5P | I/S//S |

**Table 12. Potential Sources of Blast Resistance Selected from Long Term Studies on Resistance Stability. Santa Rosa, Colombia 2000-2003**

| No. | Pedigree |
|---|---|
| 1 | CT 11369-1-$F_4$-17P-4P |
| 2 | CT 9509-17-3-1-1-M-1-3P-M-3-3P |
| 3 | CT 10310-15-3-2P-4-3 |
| 4 | CT 11256-5-$F_4$-28P-4P |
| 5 | CT 10308-27-3-3P-3-1 |
| 6 | CNAx5013-12-13-2-2-4 |
| 7 | CT 10321-6-10-3P-1-1T-3P |
| 8 | CT 11275-3-$F_4$-8P-2 |
| 9 | FL 00585-12P-2-2P-M |
| 10 | CT 8250-21-12-2P-1X |
| 11 | CT 6543-28-6I-1I-2I |
| 12 | FL 00470-29P-7-3P-M |
| 13 | FL 00447-27P-3-1P-M |
| 14 | FL 00470-29P-5-2P-M |
| 15 | FL 00595-12P-1-1P-M |
| 16 | FL 00585-12P-7-3P-M |
| 17 | FL 00510-17P-3-2P-M |
| 18 | FL 00595-25P-9-3P-M |
| 19 | FL 00470-29P-2-3P-M |
| 20 | CT 11256-5-$F_4$-28P-5P |
| 21 | CT 8222-7-6-2P-1X |
| 22 | FEDEARROZ 50 |

**Table 13. Identification of Parents Participating in More than 10 Crosses of FLAR $F_2$ Populations (Tropics) Evaluated in 2000 and 2001 and their Field Blast Reaction**

| Parent $F_2$ 2000 | Crosses No. | Blast Reaction | Parent $F_2$ 2001 | Crosses No. | Blast Reaction |
|---|---|---|---|---|---|
| CIMARRON I | 16 | S | ARAURE 4 | 19 | S |
| CIMARRON II | 17 | S | CIMARRON | 24 | S |
| CNARR4955-7B-BM70A-45-5P | 10 | S | CNARR4955-7B-BM70A-45-5P | 10 | S |
| CNAx5013-12-13-2-2-4-B | 12 | R | CT 10308-27-3-1P-4-3-2P | 38 | S |
| CNAx5013-13-2-2-4-B | 10 | R | CT 10310-15-3-2P-4-3 | 17 | I |
| CT 10310-15-3-2P-4-3 | 56 | I | CT 10825-1-2-1-3-M | 29 | S |
| CT 10491-12-4-2T-3P-2P-1 | 14 | S | CT 11256-5-$F_4$-28P-4P | 14 | R |
| CT 10992-3-4-1T-3P-2P-3 | 34 | S | CT 11408-6-$F_4$-1P-3 | 32 | S |
| CT 11032-2-4-3T-3P-3P-1-1X | 10 | S | CT 6543-28-6I-1I-2I | 17 | I |
| CT 11072-2-4-1T-1P-2P-2 | 14 | S | CT 8008-16-31-3P-M | 10 | S |
| CT 11256-5-$F_4$-28P-4P | 23 | R | CT 8163-9-4-4 | 28 | S |
| CT 11275-3-$F_4$-8P-2 | 12 | I | CT 8240-1-3-9P-M | 21 | S |
| CT 11299-4-$F_4$-25P-3P | 12 | S | CT 9509-17-3-1-1-M-1-3P-M-1 | 19 | S |
| CT 11369-1-$F_4$-17P-4P | 10 | R | CT 9748-13-2-1-M-M-1-1 | 58 | S |
| CT 5786-49-4-4-4-M | 28 | S | FEDEARROZ 50 | 67 | I |
| CT 8222-7-6-2P-1X | 25 | R | FL 00475-10P-5-3P-M | 12 | S |
| CT 8248-1-12-1P-M-P | 11 | S | FL 00585-12P-1-2P-M | 10 | I |
| CT 8447-5-6-3P-1X | 11 | I | FONAIAP 1 | 26 | S |
| CT 8753-6-8-7P-2-M | 10 | I | IR 21015-72-3-3-3-1 | 14 | S |
| CT 9509-17-3-1-1-M-1-3P-M-1 | 63 | S | ORYZICA 1 | 21 | S |
| CT 9509-17-3-1-1-M-1-3P-M-3-3P | 38 | R | ORYZICA CARIBE 8 | 10 | S |
| CT 9852-3-2-1-2-$F_7$ | 24 | S | P3050-$F_4$-52 | 17 | I |
| CT 9868-3-2-3-1-4P-M-1-1P | 26 | I | PALMAR | 10 | S |
| P 5590-4-11-1-1-3X | 10 | S | PSB RC-70 | 14 | S |

- **Characterization of the Genetic Structure of Rice Blast Populations from Peru**

F. Correa, F. Escobar, C. Bruzzone

## Abstract

Characterization of the genetic structure of blast populations is very important for determining the genetic diversity and virulence composition of the fungus. We have initiated analyzing 116 blast samples from Peru collected from different regions and cultivars. The results suggest that few genetic lineages represent this population. Twenty-five haplotypes were found grouped in six genetic groups at the 80% similarity. These haplotypes will be inoculated on rice cultivars with different resistance genes to identify potential donors of resistance to be incorporated into the local breeding program. More blast isolates need to be collected and analyzed.

## Introduction

Rice blast is the most destructive disease worldwide. This disease has increased in importance in several rice growing areas from Peru, where yields have been affected severely by several epidemics in the last years. Genetic resistance is the most economic way to manage this disease, however resistance breakdown in a short period of time mainly due to the appearance of new pathotypes of the fungus. Determining the genetic structure and virulence diversity of a blast population is needed to identify suitable resistance genes and the best gene combinations for the development of durable blast resistance. We have initiated at CIAT the molecular characterization of blast populations from different regions from Peru. Representative isolates of the different genetic groups and haplotypes found will be sent to Peru for characterization of their virulence spectrum and identification of resistance genes that should be incorporated in the local breeding program.

## Materials and Methods

Rice leaves or panicles infected with typical blast symptoms were collected in different rice growing areas of Peru from trap nurseries of different susceptible rice cultivars. Infected samples were incubated under high humidity in petri dishes to induce sporulation of the fungus. Monosporic cultures were obtained from fresh samples but not from too dry or old material. DNA fingerprints were determined for 116 blast isolates using the rep-PCR Pot-2 fingerprinting technique described in previous annual reports. The samples were collected at seven locations in Alto and Bajo Mayo (Moyabamba- La Conquista, Shica-Rioja, Bagua Grande, San Martin-Juan Guerra, Valle La Conquista, Bella Vista-San Martin, and Rioja-Patria Nueva) from 30 different breeding lines and rice cultivars.

## Results

Analysis of the different DNA-fingerprints generated showed the existence of 25 different haplotypes. Figure 2 shows some of the DNA-fingerprints found. Similar fingerprints exist in the blast pathogen population from Colombia. Some haplotypes were represented by many isolates from different regions and collected from several rice cultivars. Other haplotypes were represented by single isolates. The most common haplotype was represented by 39 isolates. A cluster analysis showing the coefficients of similarity for isolates representing most haplotypes found is shown in Figure 3. Six genetic groups were found with 80% similarity. Blast isolates representing different haplotypes within each one of these genetic groups needs to be inoculated on rice differentials carrying different blast resistance genes to characterize their virulence profile. There were two isolates, which exhibited only one band in their fingerprints. These isolates were collected from the rice cultivar Bg90-2 at San Martin-Juan Guerra in the Bajo Mayo. The two isolates should also be inoculated to determine if they are or not pathogenic on rice.

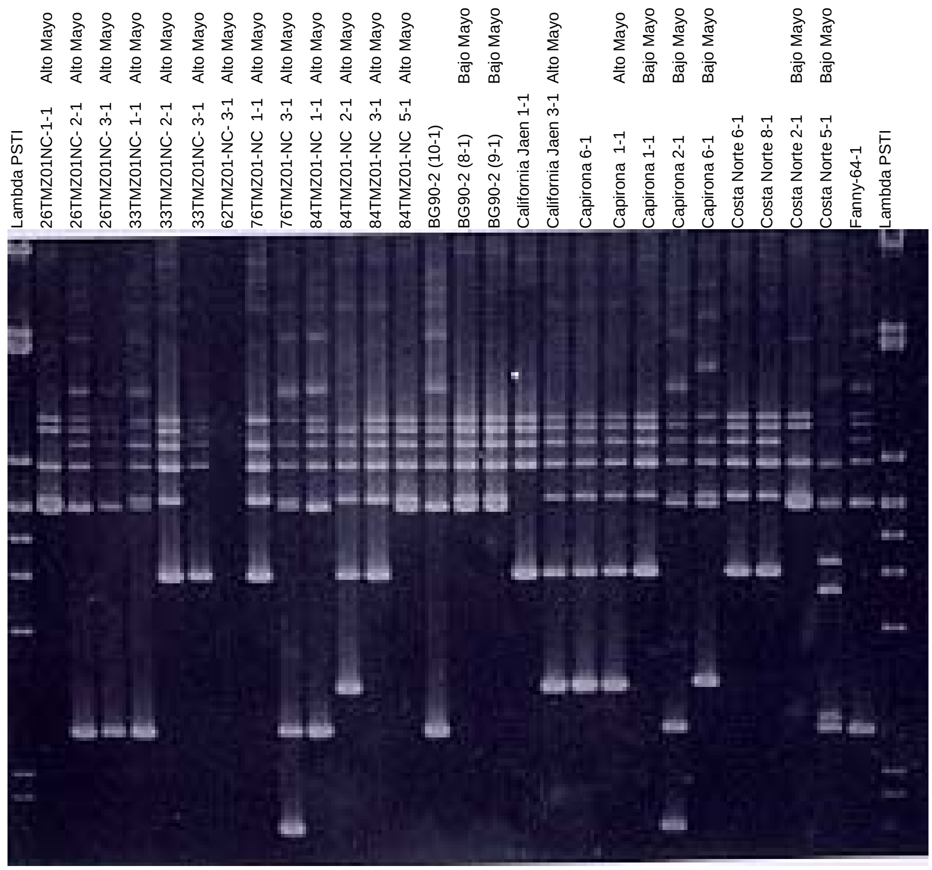



**Figure 2. DNA-fingerprints found in Rice Blast Populations from Peru**

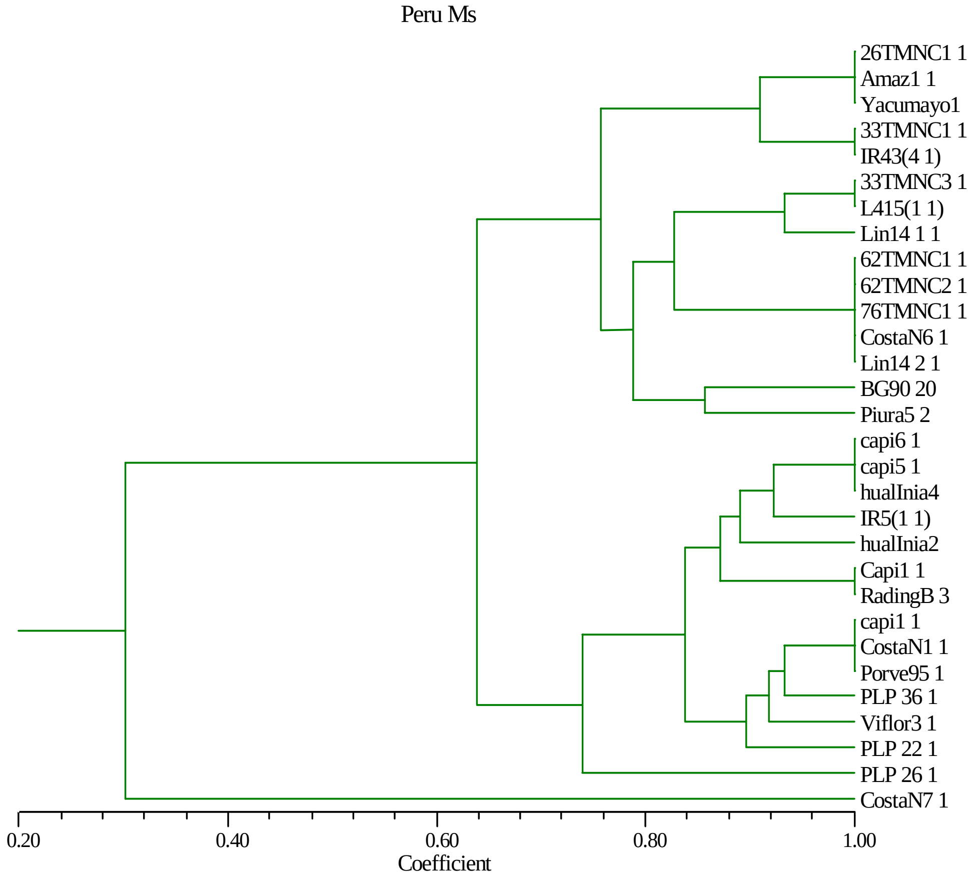



**Figure 3. Cluster Analysis of Rice Blast Haplotypes Representing the Genetic Diversity in Peru**

## Discussion

The blast pathogen population from Peru seems to be composed of a few different genetic groups. More isolates need to be collected and analyzed to determine the genetic diversity of the fungus and the partition of this diversity by site and cultivar. These results will guide us to determine the best sampling strategy for collecting a more representative pathogen population. Common haplotypes were found to be present at different locations from Peru probably as an effect of planting the same cultivar at different sites. Greenhouse inoculations need to be conducted with the different haplotypes found to identify the most suitable resistance genes that need to be incorporated in the local breeding programs.

## Future Work

More blast samples need to be collected from different sites and cultivars in Peru for determining their genetic structure. Greenhouse inoculations need to be performed for determining the virulence spectrum of the blast pathogen population found and for identifying effective blast resistance genes.

- **Introgression of RSNV Resistance from the Wild Species *Oryza glaberrima* into the Cultivated *Oryza sativa*. Studies on the Interaction of *Polymyxa graminis* on Rice**

F. Correa, C. Martínez, J. Echeverry, S. Valdéz, G. Prado

## Abstract

The RSNV disease has been disseminating in the rice growing areas of Colombia at a relatively rapid rate. All Colombian commercial rice cultivars are susceptible to the disease. High level of resistance to RSNV has been identified in the wild species *O. glaberima*. We are reporting the confirmation in transferring the resistance from the wild species into the rice cultivar Caiapo. Resistant lines to RSNV have identified and confirmed in several greenhouse trials and will be tested under natural infection in farmer's fields

## Introduction

The protozoario *Polymyxa graminis* transmits rice stripe necrosis virus (RSNV) or crinkling disease also known as entorchamiento. The disease has been disseminating in the rice growing areas of Colombia at a relatively rapid rate in the last few years, as a result of the movement of contaminated equipment to non-contaminated rice fields. Contaminated seed harvested in infected fields is another source of spread. Although the virus is not seed transmitted, the resting structures of the vector of the virus can be carried on the husk of rice seed in infected debris or soil particles. Most of the Colombian rice cultivars exhibit different levels of susceptibility to the virus under field and greenhouse conditions as reported in previous annual reports. We have reported in the last three years the identification of high levels of resistance to entorchamiento in the species *Oryza glaberrima* and the incorporation of this resistance into the rice cultivars Bg90-2 and Caiapo. We are reporting here the confirmation of the high levels of resistance in several lines of the Caiapo cross. These lines can be used in crosses with advanced lines of the program for the introgression of this resistance to the lines. A total of 97 lines from the Caiapo/*O. glaberrima* were well characterized for their RSNV resistance in 2003 and will be used for the identification of molecular markers associated with the resistance.

## Materials and Methods

We have followed the same methodology as described in the 2001 Annual Report of the Rice Project. Inoculum was infested soil collected from farmers' fields in the Cauca Valley, Tolima, or Meta. Three different experiments with two or three replications were performed in year 2003. Ten plants from each line were tested using three different sources of infested soil. The highly susceptible cultivar Oryzica 3 was used as a control in all the experiments. Incidence of all symptoms typical of entorchamiento including crinkling, yellowing or chlorosis, stunting and dead plants was evaluated on a weekly basis beginning 20 days after planting. The interaction between *P. graminis* and rice was examined in five highly resistant lines to the virus and the parents of the cross Bg90-2/*O. glaberrima* to determine if the resistance is against the virus or the vector. Ten plants per pot of each line were planted in several pots containing highly infested soil. Presence of resting spores (cystosori) was examined under the stereoscope in 100 roots of each line 10, 20, 30, 40, and 50 days after planting.

## Results

The species *O. glaberrima* exhibited high levels of resistance to entorchamiento and less than 1% of the plants had symptoms of the disease (Table 14). The susceptible control Oryzica 3 exhibited a high incidence of all symptoms of entorchamiento demonstrating the high inoculum pressure of the soil used for the inoculation (Table 14). The susceptible rice cultivar Caiapo used in the backcross with *O. glaberrima* exhibited lower levels of susceptibility than the susceptible control Oryzica 3 (Table 14). The resistance to entorchamiento present in the wild relative has been successfully transferred to the commercial cultivar used in the cross (Table 14). Four highly resistant lines (0% entorchamiento) were identified from three different experiments with several replications. Other resistant lines with less than 10% entorchamiento were identified and will be tested under field conditions (Table 14). Some lines were more susceptible than the susceptible parent Caiapo.

There were no significant differences in the number of resting spores in 100 roots between the five highly resistant lines and the resistant donor *O. glaberrima*. Three of the lines and the resistant donor had significantly less number of resting spores than the susceptible parent Bg90-2 and the susceptible check Oryzica 3, which had the highest number of cystosori. This cultivar exhibited a high number of cystosori since the first 20 days of the evaluation confirming its high susceptibility to the infection by *P. graminis.* These results suggest that the resistance to RSNV may be in part to resistance to infection to the vector, and further work is needed to clarify this pathogen-host interaction. The lowest number of cystosori was observed in the line CT 15150-M-149-1-1 (less than 10 in 100 roots), while the susceptible check Oryzica 3 had more than 600. The resistant wild parent had around 50 cystosori per 100 roots.

## Discussion

High levels of resistance to entorchamiento have not been detected in continuous evaluations of the cultivated species *O. sativa*. On the other hand, high levels of resistance have been detected in the wild species *O. glaberrima* in several independent trials. Transferring the resistance to entorchamiento observed in the wild species to the cultivated species seems to be an easy task according to greenhouse inoculations of two breeding populations evaluated for entorchamiento resistance. Rice lines with high levels of resistance to entorchamiento have been identified and confirmed in replicated trials during 2003. The resistance observed needs to be confirmed in additional trials under farmers' fields exposed to natural conditions of infection. Results of the experiments on the interaction of the vector and the rice host suggest that resistance may be against the vector and not to the virus. More specific studies are needed to elucidate the mechanisms of this resistance, and to determine what symptoms and damage is caused by the vector and by the virus. Breeding strategies will be affected by the mechanisms of this resistance and the damage caused by each organism.

## Future Activities

The evaluation and selection for entorchamiento resistance using interspecific progenies will continue by additional greenhouse screening. The results will be corroborated under natural field infection. Studies on the genetic control of entorchamiento resistance in the wild species *O. glaberrima* will be done using the segregating progeny of these crosses. Molecular markers associated with RSNV resistance will be identified and used in a marker assisted selection program. Studies need to be conducted to determine the mechanisms of resistance to the vector and to the virus and to determine the importance and symptoms caused by each organism.

**Table 14. Rice Lines from the Cross *Oryza glaberrima/*Caiapo with High and Intermediate Levels of Resistance to RSNV**

| | Rice Line |
|---|---|
| | **0 % Crinkling (entorchamiento)** |
| 1 | CT 16308-CA-8-M |
| 2 | CT 16329-CA-5-M |
| 3 | CT 16329-CA-10-M |
| 4 | CT 16353-CA-14-M |
| | **1-10 % Crinkling (entorchamiento)** |
| 1 | CT 16310(2)-CA-5-M |
| 2 | CT 16311(2)-CA-3-M |
| 3 | CT 16315(1)-CA-2-M |
| 4 | CT 16338-CA-10-M |
| 5 | CT 16353-CA-5-M |
| 6 | CT 16353-CA-11-M |
| 7 | CT 16356(2)-CA-8-M |
| | *O. glaberrima* 0% Crinkling |
| | Caiapo 15-23% Crinkling |
| | Oryzica 3 19-23% Crinkling |

- **Identification of Resistant Lines to *Rhizoctonia solani* (Sheath Blight) and Development of an Evaluating Methodology**

F. Correa, G. Aricapa, P. Guzmán

## Abstract

A suitable greenhouse screening method and evaluation scale has been developed for identification of tolerance to the sheath blight pathogen in rice. This method allows a better differentiation between tolerance and susceptibility. Tolerance to sheath blight present in the wild species *O. rufipogon* has been successfully transferred to the cultivated species. Tolerance to sheath blight has also been identified in advance rice lines of *O. sativa* with the new screening and evaluation method, and both sources of tolerance can be used in our breeding program. Tolerance to sheath blight identified in the greenhouse has been corroborated under field conditions in several rice lines. Tolerance seems to be controlled by the action of several minor genes. Increasing resistance to sheath blight seems to be possible by crossing parents with high levels of tolerance.

## Introduction

The filamentous basidiomycete *Thanatephorus cucumeris* (anamorph = *Rhizoctonia solani*) is the causal agent of sheath blight of rice. This disease has increased in economic importance in most Asian countries as well as in the USA in the last 10 years. The disease is also increasing in importance in most rice growing countries of tropical Latin America where the species *R. solani* AG-1 IA seems to be the most common while the species *R. oryza-sativae* seems to be the most common in the temperate areas of South America. The disease, which has increased in incidence and severity, is most frequently controlled with the use of fungicides, however this method of control has increased the production costs. There are not well known sources of resistance to the pathogen. We initiated in year 2000 the evaluation in the greenhouse of different rice lines in order to identify potential sources of resistance to this pathogen. The evaluated germplasm includes Colombian commercial cultivars, wild rice species, Asian and USA reported sources of resistance, and advanced breeding lines of CIAT and FLAR Rice Projects. The wild species *Oryza rufipogon* exhibited an intermediate resistant reaction to more than 14 different Rhizoctonia isolates in trials conducted between 2000 and 2002, while the species *O. barthii* and *O. glaberrima* were susceptible to most isolates.

## Materials and Methods

Several groups of rice lines including 415 advanced lines from the interspecific cross between *O. rufipogon/*Oryzica 3, advanced elite lines from FLAR, accessions from the rice germplasm bank, and a set of progenitors used by FEDEARROZ were evaluated in the greenhouse for their sheath blight tolerance in 2003. Tolerant lines were selected and their reaction corroborated in multiple replicated trials. The best lines identified were planted in the field in October in collaboration with FEDEARROZ (Saldaña) for determining the correlation between the two screening sites. The genetics of sheath blight tolerance to determine the possible number of genes was evaluated in the $F_2$ generation (70-80 plants) of six different crosses.

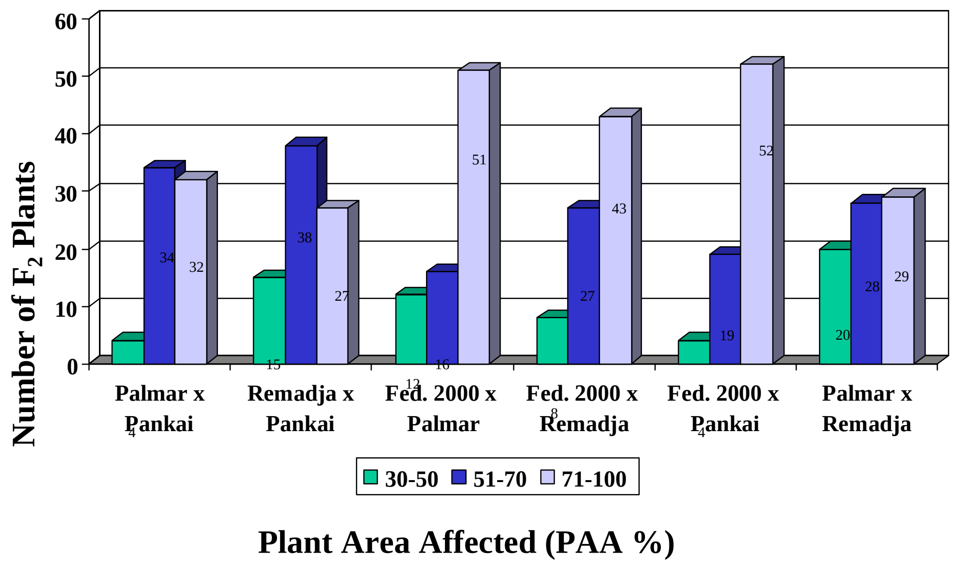
For greenhouse evaluations, each line is planted in three replications. Each replication consists of five plants sown in a pot. Disease development is favored by applying a high dose of nitrogen divided in several applications. Inoculation is performed when the plants are 50 days old by placing at the base of the main stem of each plant a 5 mm plug of agar + mycelium of a *R. solani* isolate grown for 4-8 days on rice polish agar. Inoculated plants are incubated under high relative humidity in a growth chamber for 12-15 days. After this period of time, the pots are removed from the chamber and placed onto greenhouse benches for five days to reduce the stress conditions and allow a period of time for recovery and expression of potential tolerance. The pots and then moved back to the high humidity conditions for another period of 10 days before evaluating their reaction.

For evaluating the reaction to sheath blight, we are considering the percentage of plant area affected, by evaluating the main tiller of each plant. The reaction score of a line is considered as the average of the 15 plants evaluated in the three replications. Percentage of plant area affected is calculated by given the following maximum values to the different leaves and stem evaluated: first leaf (flag leaf): 30%; second leaf: 15%; third leaf: 15%; fourth leaf: 10%; fifth leaf: 10%; stem: 20%. Since disease development and severity observed depends on the effect of the environmental conditions in the greenhouse, tolerant and susceptible checks are always included in each replication and used for determining the level of tolerance of the lines being evaluated.

**Results**

The greenhouse screening methodology including the two periods of incubation in the growth chamber under high humidity with an intermediate period of plant recovery has been suitable to detect better differences in tolerance among the rice lines evaluated. Tolerance to sheath blight observed in the wild species *O. rufipogon* has been successfully transferred to the cultivar Oryzica 3. The tolerant reaction identified was corroborated in several replicated trials (Table 15) and the best lines have been planted for evaluation under field conditions. Tolerance to sheath blight has also been detected in different rice lines including a set of progenitors being used by FEDEARROZ in its breeding program (Table 16). This tolerance has also been confirmed in several replicated trials in the greenhouse and is being confirmed under field evaluations. The tolerant reaction of several rice lines from the interspecific cross between *O. rufipogon/*Oryzica 3 and rice lines from the germplasm bank observed under greenhouse conditions has been corroborated under field conditions in evaluations performed by FEDEARROZ at the Saldaña experiment station (Table 17). These lines could be used as potential donors of tolerance to this pathogen. Tolerance to sheath blight has also been identified in several advanced elite lines from FLAR (Table 18). These lines are being used, as progenitors for other desired agronomic traits, or for potential release as commercial cultivars in different countries. The tolerance to sheath blight observed in these lines is a plus since they have never been selected for this trait.

Evaluation of the $F_2$ generation in six different crosses (Figure 1) do not suggest the presence of major dominant gene resistance as most $F_2$ plants exhibited a susceptible or intermediate reaction to sheath blight. Tolerance seems to be controlled by the effect and accumulation of several minor genes. In all crosses, rice lines with better tolerance than the two parents were identified, suggesting the effect of transgressive segregation or accumulation of several genes (Figure 1). These lines were present in the group of 30-50% plant area affected. The highest number of susceptible $F_2$ plants was observed in those crosses where the commercial cultivar Fedearroz 2000 was involved. However, tolerant lines better than the two parents were identified even in the cross between Fedearroz 2000/Remadja (Figure 1). Although the cultivar Pankai exhibited a higher tolerance to sheath blight than the cultivar Remadja, the tolerance of this last cultivar seems to be better inherited. Those crosses involving Remadja yielded more tolerant lines than those crosses with Pankai.




**Pankai (44 % PAA) Palmar (49 % PAA) Remadja (68 %PAA) Fedearroz 2000 (83 % PAA)**

**Figure 1. Evaluation to *Rhizoctonia solani* of the $F_2$ in six Rice Crosses**

## Future Activities

Characterization of the genetic and virulence structure of the sheath blight pathogen in Colombia using PCR based molecular markers and greenhouse inoculations on different sources of resistance identified in our recent studies. Determine the possible existence of races or pathogenic variability to identify sources of tolerance to different pathogenic groups. Correlate field and greenhouse reactions to sheath blight and develop a field screening method for massive evaluations of breeding lines. Develop a breeding strategy for incorporating tolerance to sheath blight in our breeding populations of the rice project.

**Table 15. Selection of the Most Tolerant Lines to *Rhizoctonia solani* in the Greenhouse Evaluation of 117 Advanced Lines from the Cross *Oryza rufipogon/*Oryzica 3**

| Rice Line | Plant Area Affected % | Rice Line | Plant Area Affected % |
|---|---|---|---|
| CT 14527-21-M-5-1 | 47.7 | CT 14547-28-M-4-2 | 49.3 |
| Ct 14532-18-M-3-2 | 36.8 | CT 14549-22-M-8-1 | 49.3 |
| CT 14534-2-M-3-1 | 45.7 | CT 14554-20-M-7-1 | 45.0 |
| CT 14534-23-M-5-3 | 44.3 | CT 14562-11-M-3-4 | 47.3 |
| CT 14534-35-M-2-1 | 45.0 | CT 14563-6-M-3-5 | 47.0 |
| CT 14537-9-M-1-1 | 45.3 | CT 14563-8-M-6-1 | 44.7 |
| CT 14537-9-M-1-3 | 40.3 | | |
| CT 14543-9-M-3-3 | 48.7 | **Susceptible check** | |
| CT 14546-5-M-1-1 | 49.3 | CT 14549-22-M-5-2 | 80.3 |
| CT 14556-6-M-6-1 | 49.3 | | |
| CT 14547-9-M-2-1 | 47.3 | | |

**Table 16. Selection of the Most Tolerant Parents to *Rhizoctonia solani* in the Greenhouse Evaluation of 20 Progenitors from FEDEARROZ**

| Identification | Pedigree | Plant Area Affected % |
|---|---|---|
| Prog RHZ 21 | FL 00984-10P-5-1P-M | 45.0 |
| Prog RHZ 23 | LV 636-1-7-4-1 | 42.0 |
| Prog RHZ 25 | LV 636-1-7-4 | 43.3 |
| Prog RHZ 32 | FSR 1310-1-1-1 | 44.3 |
| Prog RHZ 59 | FOA-16-9 | 44.0 |
| **Susceptible Parent** | | |
| Prog RHZ 29 | FSR 1185-6-2-M | 80.5 |

**Table 17. Rice Lines Tolerant to *Rhizoctonia solani* in Greenhouse and Field Evaluations**

| Identification | Plant Area Affected % | Identification | Plant Area Affected % |
|---|---|---|---|
| ***O. rufipogon/ Oryzica 3*** | | **Germplasm Bank** | 38 |
| CT 14551-3-M-M-1-2-M | 45 | CT 7201-16-5P | 40 |
| CT 14534-12-M-4-1-2 | 31 | AMAZONAS | 48 |
| CT 14537-21-M-6-4-2 | 43 | CT 8455-1-24-1P-1X | 47 |
| CT 14546-6-M-M-M-8-M | 43 | CT 11014-10-1-2 | |
| **Resistant Check** | | **Susceptible Check** | |
| *Oryza rufipogon* | 43 | PN-1 | 67 |
| Oryzica 3 | 47 | Colombia 1 | 100 |

**Table 18. Elite Rice Lines from FLAR Tolerant to *Rhizoctonia solani* in Greenhouse Evaluations**

| Rice Line | Plant Area Affected % | Rice Line | Plant Area Affected % |
|---|---|---|---|
| FL 03197-22PO-4-1P-2P-M | 38 | FL 03191-5P-13-3P-1P-M | 47 |
| FL 03188-7P-5-4P-1P-M | 39 | FL 03191-5P-13-3P-3P-M | 48 |
| FL 03199-26P-2-3P-2P-M | 42 | FL 03191-4P-58-3P-1P-M | 49 |
| FL 03191-5P-8-2P-2P-M | 44 | FL 03191-4P-58-3P-2P-M | 52 |
| FL 03188-7P-5-3P-3P-M | 47 | | |
| **Tolerant Check** | | **Tolerant Check** | |
| Oryzica 3 | 60 | CT 14534-12M-3-4M | 60 |

## OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

### 2B. Characterization of the Complex of Rice Hoja Blanca Virus and *T. orizicolus*

- **Characterizing the Interactions of Host Plant Rice Hoja Blanca Virus and *T. orizicolus* Complex**

L. Calvert, R. Meneses, M. Triana, I. Lozano,
L. Reyes, M. Cruz, R. Sedano, C. Pardey

### Introduction

This report is a synthesis of the research to develop rice with resistance to RHBV and the insect vector *Tagosodes orizicolus* (Muir). We often refer to research reported in previous annual reports in order to show the logic in the sequence of the research. The emphasis is on the important findings and how they are being used to develop solutions to the losses caused by RHBV and *T. orizicolus.*

### Developing Rice with Resistance to Rice Hoja Blanca Virus and its Vector *Tagosodes orizicolus*

### The Evolution of the Evaluation of Resistance to Rice Hoja Blanca Virus

Rice hoja blanca virus (RHBV) cannot be transmitted mechanically and propagates within the vector *T. orizicolus* (Muir). The insect does have genetic resistance to the virus making the maintenance of RHBV viruliferous colonies difficult. Since the mid 1980s, CIAT has maintained virulent colonies of *T. orizicolus* and a field method of evaluation of RHBV was developed. Methods for evaluation of resistance to direct damage by *T. orizicolus* were also developed, and with minor modifications are being used to develop rice with resistance to this pest. CIAT, FLAR and national programs have materials to be evaluated every year using these methods (Table 1). Although several attempts have been made by national programs to develop viruliferous colonies, but these are costly to maintain and no NAR is currently screening for RHBV using these methods. Recently, private companies have requested that their lines be evaluated in the field for RHBV and in the greenhouse for resistance to *T. orizicolus*. These services are available as a service to NARs and a fee structure has been implemented for private companies. These are preliminary evaluations and additional testing is needed to effectively select for resistant rice lines. This is especially true for the evaluation of RHBV, because the breeders wish the disease pressure to be moderate. To maintain moderate pressure is more of a challenge than high disease pressure and allows many materials without adequate resistance to RHBV to pass through the massive field evaluation.

Since 1996, there have been trails using different levels of viruliferous vectors and infestations are different dates. These trials were done in randomized replicated plots and confirmed that the infection rates in resistant varieties depended on the age of the plant and the number of the insects used to infest the plots. The resistant varieties have lower infection rates when the infestation is made at more than 15 days after planting and higher infection rates as the number of viruliferous vectors is increased. These results confirmed the earlier studies on the interaction of RHBV and its vector. The novel part of this research is that we started using the differences to more accurately rate the level of resistance of varieties and advanced lines. There were few resistance commercial varieties available when these studies were started and included Oryzica 1, Oryzica 3 and Llanos 5 from Colombia and Capirona from Peru. With the exception of Llanos 5, these were varieties that had been selected during epidemics of RHBV. As these screening experiments were repeated, there was a notable increase of disease in the resistant varieties that were used as controls. The conditions were the same, but the colonies were becoming more virulent. This was a much more rigorous screening and several advances lines had better resistance than the known source of resistance, Colombia 1. This led us to develop a new rating scheme: Susceptible, Intermediate Resistant, and Resistant.

**Looking at the Complex of RHBV and *T. orizicolus***

In the mid 1990s, the incidence of RHBV in the field started to increase. One of the observations was that the variety O. Llanos 5 that was supposed to have resistance to RHBV was one of the first varieties to become infected with RHBV in the field. In the first experiments using a CIAT colony that had not been renewed in many years, O. Llanos 5 appeared to have good resistance to RHBV. Various sources of seed to O. Llanos 5 were tested to make sure that the field reaction was not due to a field mixture of the seed. The reports from regions, where disease pressure was increasing, continued to demonstrate that O. Llanos 5 did not have good field resistance. In the archives, O. Llanos 5 was rated as susceptible to *T. orizicolus.* This led us to develop a series of experiments to test the resistance of O. Llanos 5 and a promising new line that would later become Fedearroz 50 for their resistance to both the insect and the virus.

One type of experiment was force feeding of the insects on a variety and then determining their longevity. The results indicated that Fedearroz 50 has an antibiotic type of resistance. Additional experiments were done and it was determined that Fedearroz 50 has both antibiotic and antixenosis resistance factors. Longevity was normal for O. Llanos 5. In preference experiments, plants of five varieties were grown in a randomized design, and the insects were liberated. The number of insects on each plant was determined at daily intervals. In these experiments, the insects tended not to settle on Fedearroz 50 and preferred O. Llanos 5 over most lines tested including the parents of Fedearroz 50. This was also true on the number of eggs deposited.

Similar results were obtained, in experiments that are described in the 2001 Rice Annual Report. In this group of varieties, the insects had less preference for Llanos 5 than the other experiments. Still in force feeding experiments, which more closely approximate field conditions, O. Llanos 5 had one of the highest rates of reproduction. It is notable that the insect had lower reproductive rates on both Oryzica 1 and Fedearroz 2000. The least preference and lowest reproduction rates were on Fedearroz 50. These results were interpreted to mean that developing RHBV resistant varieties depends on resistance to both the virus and its vector.

**The Development of Varieties Resistant to RHBV**

By 1997 (reported in 1998), we were conducting resistant trials using a randomize block design and different levels of disease pressure. Fedearroz 50 (FB0007) was in its second cycle of intensive testing for its resistance to RHBV. In that trial, it had a better rating than Oryzica 1, Oryzica 3 and O. Llanos 5, although it was statistically better only to Oryzica 1. By the second semester of 1998 (reported in 1999), it was statistically better than all three in the high infestation treatment. In this year, the colony was becoming more virulent and all varieties had higher incidence of RHBV infection. For example in 1997, the level of RHBV infected plants for Fedearroz 50 were rate as 3 and in 1998 as 4 on the scale of 1-9 with 1 being no infection and 9 being more than 70% of the plants affected. By the following year, there was a dramatic increase in disease pressure in all the varieties tested. In 1999 (reported in 2000 Annual Report), there was less incidence of disease in Fedearroz 2000 (CT10323) than Fedearroz 50, and it was statistically significant difference for both the high and low level of infestation. Other lines that were to become Fedearroz Victoria 1 (CT10240) and Fundarroz PN1 (PNA97004) also had comparatively low levels of disease incidence. This was the first time that Fedearroz 50 had an alarmingly high incidence of infection with a disease rating of 6.6 in the high level treatment and a 4.7 in the low infestation treatment. In the 2001 Annual Report, the disease pressure was even higher and Fedearroz 2000 had superior resistance than the other 9 varieties including Fedearroz 50, Llanos 5 and the known source of RHBV resistance; Colombia 1. The higher levels of disease pressure were allowing the selection of advanced lines that become varieties including Fedearroz Victoria 1 and Fedearroz 2000. This is notable because they have better resistance than the known source used by the breeders to incorporate RHBV resistance.

**Developing Consistent Methodologies for Low Disease Pressure Massive Screening of RHBV**

Each year the disease pressure was getting higher and at this level of disease pressure, the lines with intermediate resistance to RHBV were difficult to select. Although the highly virulent colonies were being used to successfully develop highly RHBV resistant lines, it was clear that lower disease pressure would be needed in the earlier mass screening or many promising materials would be eliminated. Over the last three years, we have had some massive field evaluation with lower pressure and others with higher pressure. Here we describe the process and experiments that are increasing our ability to predict and control the levels of disease pressure.

We can consistently repeat conditions of high disease pressure. The inconsistency season to season occurs when we try to repeat moderate disease pressure. As the colonies were becoming more viruliferous, our first response was to lower the disease pressure by reducing the number of insects per plant. For the field trials in 1995-1997 and average of two insects per plant were released. This was then reduced to one insect per plant and finally to 0.5 insect per plant. It is not feasible to further reduce the infestation level because the coverage by the insects becomes erratic and many plants escape infection because they never are inoculated.

It was suggested that biotypes of RHBV could be the reason for the variation in virulence. As reported in the Annual Report 2002 and additional experiments done this year, there were no differences in the virulence of the colonies based upon the source of the virus. We report in a later section that there do not appear to be biotypes of *T. orizicolus* in the Colombian populations that we have tested. These are the same populations that are the basis for the current viruliferous colonies. The conclusion is that the variation the aggressiveness between the colonies is not due to biotypes of the insect nor virus.

Different colonies do have different virulence levels, and we wanted to know why and how this affects the ability of the colony to transmit RHBV. During the period of 1996-1999, thousands of field insects were tested by serological methods that are quantitative. The great majority of insects collected from the field, that were positive for RHBV, had virus titers that were relatively low. From the results of serological testing in colonies of different ages, it was demonstrated that the insects normally begin with lower virus titer, but that after several generations in captivity, the average virus load per insect increases. Finally, most insects in the colony develop the ability to tolerate higher virus loads and reproduce efficiently in these controlled conditions (see later section). Studies have shown that the virus levels in the insects correlated with probability to cause infection (Annual Report 2002). These experiments were repeated this year and similar results were obtained. Until recently, dosage has been considered to be the number of viruliferous insect per plant, but it is more complex. Each insect has a different level of virus and dosage is an interaction between the number of viruliferous insects and the virus titer level in each of them.

This implies that one can use a serological method to predict the ability of a colony to transmit RHBV. Although it is not precise and more control will be needed, a brief review of the last five massive field screening and a comparison of the virus titer of 90 insects demonstrate the this is one indicator that is useful to predicting how aggressive the colony will be in transmitting RHBV. Further, we are testing the hypothesis that recently developed colonies of less than six months in captivity will be more stable for assuring moderate to low disease pressure for the massive RHBV screening.

**Evaluation of Advance Lines and Varieties**

The massive screening is not enough especially under conditions of moderate to low disease pressure to select for resistant lines of RHBV. The massive evaluation using moderate disease pressure is most useful to eliminate the most susceptible lines. The successful development of RHBV resistant varieties occurred using more rigorous evaluation with randomize block designs that have different levels and dates of planthopper infestation. The major limitation with those types of trials is the inability to test sufficient number of materials. The trials normally contained 16 varieties or advanced lines. We are testing a method of evaluation that is a hybrid of the blocks and the massive field trials. This should allow hundreds of lines to be evaluated using different level of disease pressure and at different plant ages. We will report these results in the next annual report. Using these methodologies, we expect that more rice breeding programs will be able to develop lines with RHBV. These will continue to prevent epidemics and lead to a cleaner environment because the farmers will not have to use pesticides to attempt to control the disease.

- **Interactions Between *T. orizicolus* and RHBV**

R. Meneses, M. Triana

The biological study of the insects is one of the principal aspects for correct Integrated Pest Management. The principal objective, of these experiments, was to determine of some biological parameters of *T. orizicolus* in fundamental relationship of the population increase of the insects as affected by infection with RHBV. Four possible combinations were tested: virulent male X virulent female; healthy male X virulent female; virulent male X healthy female; healthy male X healthy female. Thirty pairs of insects were selected for each group. Two colonies (CIAT and Costa) of *T. orizicolus*, which had been maintained in the greenhouse for more than two years, were used for these experiments.

In all crosses, there was no trend between the healthy or RHBV affected insects for either longevity or the number of eggs laid. The males in the colony Costa had a statistically significant greater longevity than the males in the colony CIAT. Although the females in the colony Costa lived longer and laid a greater number of eggs than those in the colony CIAT, the differences were not statistically significant.

Similarly for both colonies, there were no significant differences for the number of eggs/day/female either when the virus was present or absent. The percentage of winged female in the colony Costa was much higher than the colony CIAT. This may be due to the colony CIAT being maintained for a longer period than the colony Costa. The duration of 15 to 16 days for the nymphal stages were similar between all the crosses for both colonies, and there were no significant differences between treatments.

- **The Studies to Determine the Genetics of Resistance to RHBV and *T. orizicolus***

M. Triana, I. Lozano, C. Martínez, L. Calvert

To determine the genetics of inherence for RHBV, the $F_1$, $F_2$ y $F_3$ populations of crosses between the resistant variety Fedearroz 2000 (CT10323-29-4-1-1T-20) and the susceptible line WC 366 (IR65598-27-3-1) were evaluated for their susceptibility to the virus. The controls were the parental lines and the variety Oryzica 1 which is rated as intermediate resistant to RHBV.

Ten rows each with 20 plants for a population of 200 were used for the evaluation of the $F_2$

generation as well as for 235 lines of the $F_3$ generation. Twenty-five days after planting, the rice lines were infested with a dosage of 1.5 insects per plant using viruliferous insects from the colony "Costa-CIAT". Five days after the infestation, the 200 insects were collected and tested using ELISA in order to determine the virulence of the insects used in the evaluation. The remaining insects were eliminated and each week for four weeks the plants were evaluated for symptoms of RHBV.

For each of the $F_2$ plants and each $F_3$ line, DNA was extracted for analysis with molecular markers. To date, 131 micro-satellites have been tested on the eleven rice lines. These include the RHBV resistant varieties Fedearroz 2000, Fedearroz Victoria 1, and Fedearroz 50 as well as the susceptible parents used in these crosses WC 352 and WC 366. A total of 48 micro-satellite markers have been identified and are being used in the studies to mark the genes involved in RHBV and *T. orizicolus* resistance.

The evaluation of 235 families $F_3$ lines were made and analysis of fitness was done. The controls were used to determine the rating scale for the $F_3$ lines. Fedearroz 2000, the resistant variety, had less than 10% of the plants infected, WC 366 the susceptible variety consistently had more than 45% of the plants infected and Oryzica 1 the intermediate resistant variety had most replications with 10-40% of the plants infected. The lines with 0-9% infection were rated as resistant, the line with 9.1-45% infection were rated as intermediate resistant, and the lines with more than 45% plants with RHBV were rated as susceptible. A chi square analysis of goodness of fit was made and there are two models which could explain the genetic inheritance (Table 2). The best fit is a two gene model with one dominant and one recessive gene controlling RHBV resistance. To have high levels of resistance, a plant needs to be in the homozygotic state for both genes. There is also a three gene model with one dominant and two recessive genes for RHBV resistance. These experiments will be repeated and the $F_3$ lines are being tested with molecular markers. In the next report, we should be able to confirm which model best correlates with the biological and micro-satellite analyses. We also expect to report markers for RHBV resistance.

- **Testing to Determine if there are Biotypes of *Tagosodes orizicolus* in Different Rice Growing Regions of Colombia**

R. Meneses, L. Reyes, L. Calvert, M. Triana, M. Cuervo, M.C. Duque

Since the mid 1990's, there has been a concern that RHBV and *T. orizicolus* were increasing in several countries of Latin America including Colombia, and this stimulated many grows to attempt different control strategies for *T. orizicolus*. The main changes have been the use of resistant varieties and the type and number of applications insecticides.

This investigation consisted of five types of experiment. 1). The feeding of *T. orizicolus* on indicator varieties of rice and rating them for mechanical damage. 2) The evaluation of varieties for their resistance to RHBV. 3) Determining the biological characteristic of three

*T. orizicolus* colonies (north and south Tolima and Valle del Cauca). 4) Determine the effectiveness of insecticides in the control of *T. orizicolus* from these colonies. 5) The evaluation of *T. orizicolus* using the technique of random amplified polymorphism diversity (RAPD).

**Conclusions**

1) There were no significant differences in the aggressiveness of the three colonies of *T. orizicolus*.
2) There were no significant differences between the colonies for their ability to transmit RHBV.
3) There were no significant differences in either sex, in the duration of the nymphal stages or in the life cycle of the insects of the three colonies.
4) The percentage of control as determined using the time needed to achieve LD50 and LD90 depend on the insecticide used but there were no significant difference between the colonies.

None of the colonies appeared to represent a different biotype and the same control strategies should be effective in different areas of Colombia. The biological studies can only be done using insects from Colombia, but the RAPD data suggests that the insect is fairly homogeneous throughout the region.

- **Losses Due to Different Level of Infestation of *Tagosodes orizicolus* and RHBV on Selected Rice Varieties**

R. Meneses, M. Triana

During 2002 and 2003, these experiments were made using in a randomize block design using three replication per treatment. Each variety was planted at a density of 3 g/row in blocks of 12 rows that were 1.5 metros in length. At 15 days after planting, each block was infested with adult *T. orizicolus* and covered with a nylon cage. The varieties Fedearroz 50, Fedearroz 2000, Oryzica 1 and O. Caribe 8 were infested with different levels of *T. orizicolus*. The treatments were 6 insects per plant with an average virulence vector of 25% (for every 100 insects 25 were from the RHBV viruliferous colony and 75 were the rest from the planthopper colony used to screen for insect resistance and the controls had no infestation of planthoppers. After five days, the cages were removed and the experiment was sprayed with a pesticide to eliminate the planthoppers. In order to accurately determine the percentage of plants infected both the total number of plants and those with symptoms were counted. Starting at twenty days after the plants were infested, they were evaluated for RHBV. The yield of each treatment each block was harvested dried to 14% humidity and weighed. To determine the significance of these treatments an analysis of variance and tests of multiple comparisons were done.

**Results**

The reaction of the varieties in both years of the trial followed the same trend, in the latest trial, although there was a decrease in the yield of the variety Fedearroz 2000 by 13% in the non-viruliferous insect treatment and 18% in the RHBV treatment, these differences were not significantly at the 5% level (Table 3). There were decreases in the yield of the intermediate resistant variety Fedearroz 50 of 16% in the non-viruliferous insect treatment and 44% (significant difference at the 5% level) in the RHBV treatment. There were decreases in the yield of the intermediate resistant variety Oryzica 1 of 2% in the non-viruliferous insect treatment and 38% (significant difference at the 5% level) in the RHBV treatment. There were decreases in the yield of the susceptible variety Caribe 8 of 21% in the non-viruliferous insect treatment and 80% (significant difference at the 5% level) in the RHBV treatment.

Using the results of these experiments, thresholds of economic damage were calculated (Table 4). This is important for rice growers because they can use these values to determine if interventions with insecticides are needed. As stated in a previous section, IPM is information management intensive, and this is the type of guidelines that are needed for effective management of the crop. Clearly when using resistant varieties, normally no insecticides are needed to protect the crop from RHBV.

- **Integrated Pest Management is an Information Intensive Activity**

L. Reyes, R. Meneses, L. Calvert

A survey of 147 farmers in five rice areas was made to understand farmers' use of pesticides. One hundred forty-seven rice growers, randomly chosen from the five areas were survey using a questionnaire between February and June of 1999. It was found that 72% of the farmers used certified seed while 28% used their own seed. Most farmers used insecticides to control pest problems with 81% using chemical insecticides and 5% use biological pesticides. Only 4% reported using no control strategies and 10% reported using other methods. The survey had information on the pest problem and the control strategy including the names and frequency of the insecticides (see Report 1999). During this time RHBV was a strong preoccupation with many of the farmers.

This monitoring was part of the shift from concentrating on RHBV to the emphasis on integrated pest management (IPM). Expert studies on the insect pests and data on the sale of certified seeds are needed to complete the analysis. Knowing the framer’s perception of the problems are essential to a successful IPM programs and these surveys will become a standard part of the monitoring for RHBV.

Surveys are time intensive and expensive to repeat at every rice growing cycle which is needed to gather a greater understanding of farmers’ problems and practices. A web based survey is being developed that should be widely applicable to rice growing regions throughout Latin America. Because of the local terms give to pesticides, pests, disease, and weeds, the survey is being tailored to major rice growing regions. We will report on the success of this method of collecting information in the following reports.

**The Impact of the Research to Develop RHBV Resistant Varieties**

In 1999, the Federación Nacional de Arroceros de Colombia (FEDEARROZ) released the variety Fedearroz 50', and it has intermediate resistance to RHBV. In 2000, Colombia XXI, that has intermediate resistance, and Fedearroz 2000, that is the most RHBV resistant variety that we have tested to date, was released by FEDEARROZ. In Venezuela FUNDARROZ released the variety Fundarroz 2000, and it is highly resistant to RHBV. In 2001, Fedearroz Victoria 1 with high resistance and Fedearroz Victoria 2 with intermediate resistance were released by FEDEARROZ. Of these varieties, the most popular is Fedearroz 50, which accounts for about 60% of the rice grown in Colombia. It is also being tested and released in other countries including Venezuela.

The benefits not only come from the absence of epidemics of RHBV and the yield losses associated with it, but also from the lower use of pesticides to control the vector. These are both economic and environmental benefits. Once a disease is at a very low level, it is difficult to keep enough focus and funding for the research and the need to have resistance in the new varieties. The challenge is to continue to develop RHBV resistant varieties throughout the region where the disease is endemic.

**Table 1. The Number of Rice Lines Evaluated for Resistant to *T. orizicolus* in the Greenhouse and to RHBV in the Field Between 1999-2003***

| Pest being Evaluated | Year | Number of Lines Evaluated |
|---|---|---|
| *T. orizicolus* | 1999 | 3106 |
| *T. orizicolus* | 2000 | 5658 |
| *T. orizicolus* | 2001 | 6316 |
| *T. orizicolus* | 2002 | 1589 |
| *T. orizicolus* | 2003 | 1982 |
| RHBV | 1999 | 20952 |
| RHBV | 2000 | 13153 |
| RHBV | 2001 | 13545 |
| RHBV | 2002 | 20055 |
| RHBV | 2003 | 19255 |

* The reporting year starts on October 1

**Table 2. Chi Square Goodness of Fit Model for 2 and 3 Resistant Genes for RHBV**

| 1 Dominant Gene 2 Recessive Genes | Predicted Value | Observed Value | $\chi^2$ |
|---|---|---|---|
| P(R) = 3/64 | 10.8 | 13 | 0.4 |
| P(I) = 52/64 | 187.7 | 172 | 1.3 |
| P(S) = 9/64 | 32.5 | 46 | 5.6 |
| Total lines | 231 | 231 | 7.4 |

p = 0.025

| 1 Dominant Gene 1 Recessive Genes | Predicted Value | Observed Value | $\chi^2$ |
|---|---|---|---|
| P(R) = 1/16 | 14.4 | 13 | 0.1 |
| P(I) = 12/16 | 173.3 | 172 | 0.0 |
| P(S) = 3/16 | 43.3 | 46 | 0.2 |
| Total lines | 231 | 231 | 0.3 |

p = 0.853

**Table 3. Comparison of Yields of Four Rice Varieties that were Infested with Different Treatments of *T. orizicolus***

| | Yield (t/ha) | | |
|---|---|---|---|
| **Variety** | **No infestation** | **6 insects non-vectors** | **6 insects with RHBV**[a] |
| Fedearroz 2000 | 5.42 a[b] | 4.76 a | 4.42 a |
| Fedearroz 50 | 5.69 a | 4.79 a | 3.21 b |
| Oryzica 1 | 5.22 a | 5.16 a | 3.28 b |
| Oryzica Caribe 8 | 4.32 ab | 3.43 b | 0.84 c |

a Only 25% of the planthopper used in this treatment were viruliferous.
b The letters represent significant difference at the 5% level in the Duncan multiple range test.

**Table 4. Determining the Economic Threshold for RHBV/*T. orizicolus* Complex for Four Varieties of Rice**

| Variety | *T. orizicolus* | *T. orizicolus* with 25% virulence for RHBV |
|---|---|---|
| Fedearroz 2000 | 227[a] | 125 |
| Fedearroz 50 | 230 | 42 |
| Oryzica 1 | 235 | 53 |
| Oryzica Caribe 8 | 110 | 6 |

a. This is the number of *T. orizicolus* capture in 10 passes of an entomology sweep net.

**Determination of Damage Level to *Spodoptera frugiperda***

Seedlings of the varieties Fedearroz 50, Tailandia 3 y Tailandia 4 were planted at a density of 3 g/row in field plot of 1.05 x 1.20 m with 4 replications for each treatment. At 15 and 25 days after planting, the seedlings were infested with larvae of third instars of *S. frugiperda*, (0, 100 y 400 larvae/$m^2$) and covered using nylon cages. After feeding for 5 days, the cages were removed and the insects were eliminated by an insecticide application.

**Results**

There were no significant differences between treatments (0; 100 y 400 third instars larvae). This is true for both the yield and the foliar area affected (Table 5). When the control to made at the first four instars, the Spodoptera's damage is not important, even through there are high populations. Meneses et al., (1995) reported that the larvae of fifth instars of *S. frugiperda* only consume 11.2% of foliar area that in total cycle larvae will be 152.84 $cm^2$. The larvae's feeding was 5 days, and they not have time to reach the sixth instars, which is the most destructive phase of their lifecycle. This research needs to be repeated using longer feeding periods in order to determinate the economic threshold of *S. frugiperda* larvae.

**Table 5. Yield with Different *Spodoptera frugiperda* Populations**

| Variety | Infestation at 15 days of seedling | | | Infestation at 25 days of seedling | | |
|---|---|---|---|---|---|---|
| | 0 | 100 | 400 larvaes/$m^2$ | 0 | 100 | 400 larvaes/$m^2$ |
| Fedearroz 50 | 7.3* | 6.6 | 6.5 | 7.0 | 6.5 | 7.0 |
| Tailandia 3 | 7.2 | 6.6 | 6.1 | 7.1 | 7.0 | 7.1 |
| Tailandia 4 | 7.0 | 6.8 | 6.5 | 6.7 | 7.4 | 7.3 |

= Yield (t/ha)

## OUTPUT 2. CHARACTERIZING RICE PEST AND GENETICS RESISTANCE

### 2C. Foreign Genes as Novel Sources of Resistance to Rice Hoja Blanca Virus and *Rhizoctonia solani*

- **RHBV (Rice Hoja Blanca Virus) Resistance mediated by RNA-Cross Protection in Transgenic Rice**

L. Fory[1], A. Mora[2], J. Borrero[3], T. Agrono[2], E. Tabares[1],
E. González[1], C.P. Martinez[1,2], and Z. Lentini[1,2]
[1]SB2, [2]IP4

### Introduction

In the past decade there has been great progress in genetic transformation methods for the incorporation of genes conferring important agronomic traits in rice using both particle bombardment and Agrobacterium. Transgenic plants expressing resistance to herbicide, disease, insects, nematodes and tolerance to drought and salt stress have been obtained (Jiang et al., 2003). At CIAT, our earlier work was mainly using particle bombardment, but current work uses Agrobacterium mediated transformation as the method of choice because the simpler integration patterns without or fewer rearrangements, and stable inheritance and gene expression throughout generations. Our research has resulted in an improved transformation protocol for the production of large number of transgenic fertile rice with different genes including resistance to RHBV mediated by the viral nucleoprotein (N) or the non-structural protein 4 ($NS_4$) genes, fungal resistance by the PAP gene (Pokeweed Antiviral Protein a plant origin ribosome inhibitor protein) and regulation of expression of lignin synthesis by the OMT gene (O-metiltransferase). The most advanced project had generated transgenic rice with resistance to RHBV encoded by the viral N protein gene. This project has served as a learning experience to scale up the use of genetic transformation and to implement the required biosafety regulations from the laboratory to the field. Transgenic Cica 8 variety carrying the RHBV protein N gene shows resistance to the virus. This resistance is RNA-mediated and some plants show hypersensitive reaction when challenged with the RHBV virus (Lentini et al., 2002). Some of the transgenic lines outperform most commercial rice varieties in the field. Attempts to incorporate this resistance into other materials through regular crossing indicate that the RHBV-N transgene is inherited and expressed independently of the genotype background. Thus this transgenic resistance could be used to complement the breeding resistance that has been deployed so far and does not protect plants when younger than 25 day-old. Last year we reported the studies directed to understand the resistance mechanism underlying the transgenic RHBV resistance. This year we report the evaluation of two events of transformation using microsatellites markers and selection of advanced breeding lines in the field.

## Materials and Methods

### Evaluation of Transformation Events in the Field

Field evaluations were conducted using 18 progeny plants of the $T_3$-$T_6$ generation from self-cross of the Cica 8 transgenic line A3-49-60 and 4 progeny plants of the line A3-49-56, both resistant to RHBV. Twenty-five day-old plants were transplanted in the field using an experimental complete randomized block design with three replications, each of 102 plants per replicate. Agronomic traits were evaluated through the cycle up to maturity. Tiller number, plant height, fertility and plant vigor were evaluated. DNA was extracted from young leaves using leaf discs of 5 mm diameter according to Klimyuk et al. (1993). One or two highly polymorphic microsatellites markers that clearly distinguish Cica 8 from other rice varieties were used to evaluate the molecular profile. A total of eight plants per line were analyzed.

### Selection of Crossing Lines

A total of 82 crosses derived from backcross to Cica 8 or crosses between resistant transgenic Cica 8 plants and the commercial varieties Fedearroz 50, Oryzica 1, and Iniap 12 were evaluated in the field. Each line consisted of 51 plants, planted in three rows per line. Individual plants were selected and seeds harvested. Segregation analysis was done using microsatellites markers.

## Results and Discussion

Microsatellites marker analysis of advanced generations of the transgenic RHBV resistant lines indicated that there was no polymorphism between the non-transgenic Cica 8 variety and transgenic Cica 8 lines (Figure 1). It appears that the genetic change incorporated by transformation is minor and is not resolved by microsatellite analysis (González et al., 2001). Some polymorphism was noted for some lines that correlated with phenotypic segregation. This segregation was noted on progenies derived from seeds collected from un-bagged panicles of field-grown plants of previous evaluations suggesting spontaneous outcross. For some lines in spite of the genetic uniformity as indicated by the molecular marker analysis, segregation was noted for some agronomic traits indicating differential gene expression or the need to use a larger number of molecular markers to detect genetic differences. Based on agronomic performance a total of 202 individual plants were selected and harvested (highlighted in Table 1). These plants were alike the Cica 8 in most agronomic traits but some of them out-yielded Cica 8 since a natural infestation of RHBV was present in the field. Grains from only the inner part of each plant were harvested, to prevent the collection of seeds derived from the outcross with neighbor lines. The total yield per plant of the selected material ranged from 11g/plant to 123g/plant whereas the variation among different non-transgenic control Cica 8 plant was from 27g/plant to 35 g/plant (Table 1).

Plants derived from line A3-49-60-12-3 were more resistant to RHBV than Fedearroz 2000 over two field evaluations (Fory et al., 2001). Sterility was noted in about half of the lines derived from this transgenic event (Table 1). Fertility in these lines ranged from 0% to 90% (as in non-transgenic Cica 8). The source of sterility in these plants seems not to be due to male sterility, since pollen viability in these plants was 85% ± 1.5% respect to 90 ± 1.2% in Cica 8. No polymorphism was noted between fertile and sterile plants either (Figure 1). However, other genetic self-incompatibility mechanisms cannot be discarded at this point. The recovery of fertile as well as sterile plants from genetic transformation mediated by particle bombardment has been reported in other works. Wakita et al. (1998) reported that about half of the transgenic plants carrying the herbicide resistance *pat* gene, and the 3-fatty acid desaturase gene from tobacco generated by particle bombardment were sterile. In this work, fertility was not correlated with the number of gene inserts. It seems also that the low fertility cannot be attributed either to the insertion of the foreign DNA fragments into genes related to fecundity or key for normal plant growth. Different integration sites, copy numbers and transgenic locus configurations, as well as epigenetic silencing mechanisms can all contribute to the expression of low fertility. Other works have also shown that the complex insertion of the transgene(s) may be responsible for phenotypic abnormalities in transgenic rice, including high sterility (Jiang et al., 2003).

Of the 82 crosses derived from backcross to Cica 8 or crosses with the other varieties a total of 11 (13%) were selected based on their resistance to RHBV, high fertility, vigor, and yield potential (Table 2). These crosses included 38 lines with a total of 226 individual plant-selections (Tables 2). The mean yield per plant in these selected lines ranged from 12 g/plant to 146 g/plant respect to Cica 8 that varied from 27 g/plant to 35 g/plant (Table 2). The highest yield potential was noted in lines A3-49-60-4-5/Oryzica 1-15, A3-49-60-4-5/ Fedearroz 50-19 and A3-49-60-12-3/Iniap 12-18 with 146 g/plant, 104 g/plant and 104 g/plant, respectively. The lines A3-49-60-4-5/Oryzica 1-15, A3-49-60-4-5/ Fedearroz 50-19 showed resistance to RHBV (scores 3-5). In addition, the line A3-49-60-4-5/ Fedearroz 50-19 was not only resistant to RHBV but also showed tolerance to *R. solani* under greenhouse conditions. These lines are still segregating since they are in $F_3$ generation. Thus, individual plant selections are being evaluated first for resistance to RHBV for subsequently advance breeding for other traits. The selected plants will be subjected to anther culture for the production of doubled haploid (DH) lines to reduce breeding cycle by rapid fixation of homozygocity.

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**Table 1. Agronomic and Molecular Evaluation of Traits of 22 Transgenic Lines**

| Lines | Progeny | G[1] | P[2] | AS[3] | MS[4] | H[5] | DF[6] | F[7] | P[8] | W[9] |
|---|---|---|---|---|---|---|---|---|---|---|
| A3-49-56 | A3-49-56-22-M | $T_4$ | Y | S | NS | 85 | 139 | 75 | 13 | 18-56 |
| | A3-49-56-24-M | $T_4$ | Y | NS | S | 92 | 128 | 84 | 26 | 34-73 |
| | A3-49-56-34-M | $T_4$ | N | NS | NS | 112 | Nd | 73 | 3 | 40-45 |
| | A3-49-56-66-M | $T_4$ | N | NS | NS | 85 | Nd | 0 | 0 | Nd |
| A3-49-60-4 | A3-49-60-4-13-18-M-M | $T_6$ | N | S | S | 96 | 132 | 80 | 23 | 17-123 |
| | A3-49-60-4-5-8-M-M | $T_6$ | Y | NS | NS | 109 | 139 | 64 | 16 | 11-53 |
| A3-49-60-10 | A3-49-60-10-31-M | $T_4$ | Y | S | S | 81 | 132 | 59 | 22 | 12-64 |
| A3-49-60-12 | A3-49-60-12-3-20-M | $T_5$ | N | S | S | 104 | 143 | 86 | 14 | 5-98 |
| | A3-49-60-12-3-3-21-M | $T_6$ | N | S | NS | 92 | 134 | 83 | 15 | 17-81 |
| | A3-49-60-12-3-3-10-M-M | $T_7$ | Y | NS | NS | 80 | 141 | 0 | Nd | Nd |
| | A3-49-60-12-3-3-31-M-M | $T_7$ | Y | S | NS | 107 | 139 | 90 | 25 | 15-83 |
| | A3-49-60-12-3-3-58-M-M | $T_7$ | N | S | NS | 92 | 132 | 87 | 16 | 38-48 |
| | A3-49-60-12-3-3-59-M-M | $T_7$ | N | S | S | 102 | 140 | 86 | 16 | 23-85 |
| | A3-49-60-12-3-3-60-M-M | $T_7$ | Y | NS | NS | 75 | Nd | 0 | 0 | Nd |
| | A3-49-60-12-3-3-62-M-M | $T_7$ | N | NS | NS | 78 | Nd | 0 | 0 | Nd |
| | A3-49-60-12-3-3-65-M-M | $T_7$ | Y | NS | NS | 73 | 148 | 0 | 0 | Nd |
| | A3-49-60-12-3-3-79-M-M | $T_7$ | Y | NS | NS | 75 | Nd | 0 | 0 | Nd |
| | A3-49-60-12-4-5-13-M-M | $T_7$ | Y | NS | NS | 77 | Nd | 0 | 0 | Nd |
| A3-49-60-13 | A3-49-60-13-28-M | $T_4$ | N | NS | S | 78 | Nd | 0 | 0 | Nd |
| | A3-49-60-13-40-M | $T_4$ | N | S | S | 100 | 134 | 85 | 13 | 17-62 |
| | A3-49-60-13-7-M-M | $T_5$ | Y | NS | NS | 77 | Nd | 0 | 0 | Nd |
| | A3-49-60-13-9-M-M | $T_5$ | N | S | NS | 75 | Nd | 0 | 0 | Nd |
| A3-78-24-4 | A3-78-24-4* | $T_3$ | N | NS | NS | 100 | 128 | Nd | 0 | Nd |
| Non transgenic Cica 8 | | NA | N | NS | NS | 105 | 132 | 90 | NA | 27-35 |

[1]G, generation; [2]P, bagged panicle (Y) and un-bagged (N); [3]AS, agronomic segregation; [4]MS, molecular segregation; [5]H, Plant height; [5]DF, days to 100 % plants flowering; [6]F, Mean percentage of fertility per line; [7]P, number of individual plants selected; [8]W, range of grain weight (g)/plant of total selected plants; NA, not applicable. * This transgenic only contains hygromycin resistance gene

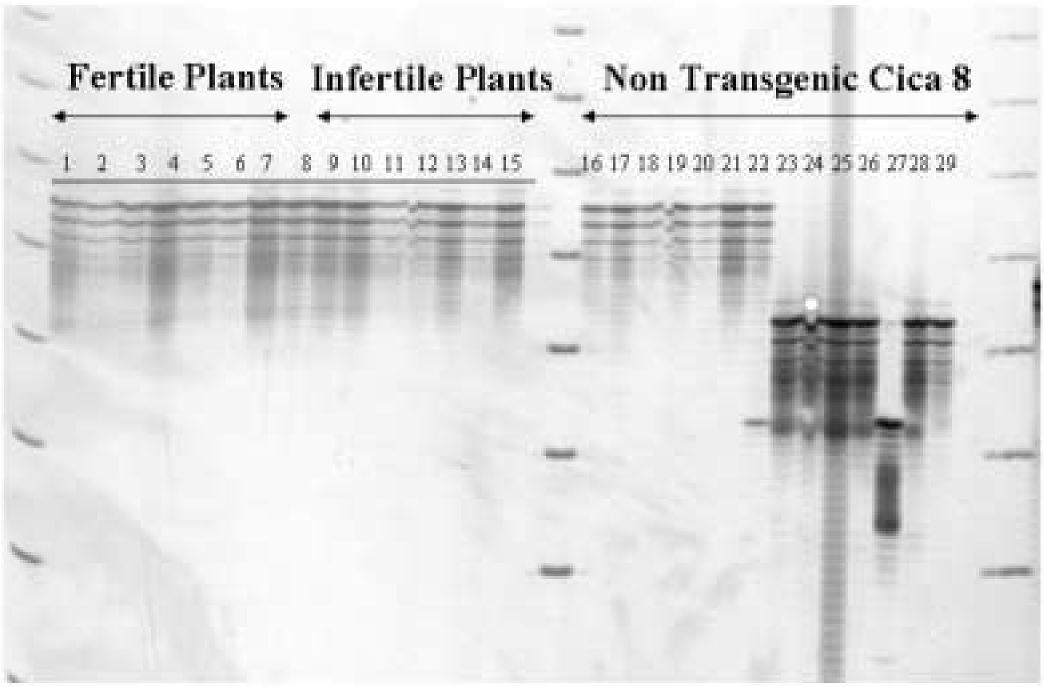


**Figure 1. Genetic profile using RM 211 microsatellites marker. Lines 1-7 (fertile plants A3-49-60-12-3-1-31). Lines 8-15 (sterile plants A3-49-60-12-3-3-57). Lines 16-22 (non-transgenic Cica 8, control). Lines 24-29 (correspond to Fedearroz 2000, Coprosem 1, Cimarron, Oryzica 1, Fedearroz Victoria 1, Oryzica Llanos 5 respectably**

**Table 2. Agronomic Evaluation of $F_3$ Lines Derived from Backcross to Cica 8 or Crosses to Oryzica 1, Fedearroz 50, and Iniap 12 of Resistant Transgenic Lines**

| Transgenic Line (Female parent) | Variety (Male parent) | $F_3$ line-No | P[1] | DF[2] | F[3] | W[4] |
|---|---|---|---|---|---|---|
| 60-12-3 | Cica 8 | -40, -2, -7, -9 | 30 | 134-141 | 40-90 | 12-77 |
| 60-4-13 | | -6 | 2 | 130 | 90 | 33-34 |
| 60-4-5 | | -7 | 3 | 139 | 90 | 38-70 |
| 60-12-3 | Oryzica 1 | -13, -17, -21 | 20 | 132-132 | 80-90 | 32-73 |
| 60-4-13 | | -4, -13III, -15A, -17 | 19 | 131-139 | 70-90 | 35-96 |
| 60-4-5 | | -13, -223, -66, -232, -15* | 22 | 125-143 | 70-80 | 26-146 |
| 101-18-19 | | -14M, -15, -64 | 24 | 130-139 | 80-90 | 37-83 |
| 60-4-13 | Fedearroz 50 | -14, -15AM, -18, -18C, -20, -20A | 32 | 127-142 | 90 | 24-105 |
| 60-4-5 | | -12A, -19*, -65, -66, -68, -60, -61B, -16A | 45 | 127-139 | 70-90 | 33-104 |
| 101-18-19 | | -15, -62,-67B | 24 | 131-129 | 90 | 34-84 |
| 60-12-3 | Iniap 12 | -18 | 5 | 134 | 90 | 62-104 |
| Cica 8 | NA | NA | NA | 132 | 90 | 27-35 |

[1] Number of individual plants selected; [2] Days to 100% plants flowering; [6]F, Mean percentage of fertility per line; [4]W, range of grain weight (g)/plant of total selected plants. NA, not applicable

- **Foreign Genes as Novel Sources of Resistance for Fungal Resistance**

L. Fory[1], E. Tabares[1], T. Agrono[2], G. Delgado[2], C. Ordóñez [2], F. Correa[2], M.A. Santana[3], N. Tumer[4], and Z. Lentini [1,2]. ([1]SB2 project, [2] IP4 project, [3]USB, Caracas, Venezuela, [4]Biotechology Center, Rutgers University

## Introduction

*Rhizoctonia solani* (causal agent of sheath blight), Helminthosporium, Rhynchosporium, and Sarocladium are diseases causing important high yield losses of rice in Latin America. All rice varieties are susceptible. CIAT's Rice Project has recently incorporated breeding efforts to develop strategies for controlling these diseases, aiming to reduce losses and the use of pesticides associated with their control. The characterization of different sources of tolerance to *Rhizoctonia solani* is in progress (Correa et al., 2000). The evaluation of wild species as a potential alternative to incorporate resistance is also underway (Martínez et al., 2002). Intermediate tolerance has been found in inter-specific crosses between Oryzica 3 and *O. rufipogon* (Martínez et al., 2002). Efforts are being placed to adapt and improve a reproducible methodology for evaluation of this resistance in the greenhouse (Correa et al., 2000) and more recently in the field. Molecular characterization of field strains of the pathogen has also initiated (Correa et al., 2000). However, no known sources of stable genetic resistance for these rice diseases have been identified yet. Thus alternative approaches are also needed.

The genus Phytolacca is the source of a number of proteins whose antiviral and anti-fungal properties have been analyzed. The PAP protein (pokeweed antiviral protein), derived from *Phytolacca americana* a weedy species naturally found throughout the Americas, inhibit the infection of a wide range of RNA and DNA viruses each representing a different plant virus group, such tobacco mosaic virus in plants (Chen et al., 1991), animal viruses, including poliovirus (Ussery et al., 1977), herpes simplex virus in animals (Aron and Irvin, 1980) and human immunodeficiency virus (HIV) (Zarling et al., 1990). Mutated versions of PAP gene have potent antifungal activity (Zoubenko et al., 1997). The work by the Dr.Tumer Group Rutgers University, USA shown homozygous progeny of transgenic tobacco and potato plants expressing these PAP genes displayed resistance to the fungal pathogen as *Rhizoctonia solani* and *Phytophtora infestans* (Wang, 1998), and transgenic PAP turf grass are resistant to various fungal pathogens (N. Tumer, personal communication). These results suggest the possibility of designing molecular strategies for incorporating fungal and/or viral resistance into rice.

Of the mutated versions of PAP, the non-toxic version PAPy123 is the one showing the most potent protection against fungal infection. We are interested in constitutively expressing PAPy123 gene in transgenic plants in order to obtain sheath blight resistance in rice. Last year we reported the selection of transgenic rice lines derived from the commercial varieties Palmar (Venezuela) and Cica 8 containing the PAPy123 gene. Last year we reported the evaluation and selection of transgenic lines with stable integration, inheritance and expression of the PAPy123 gene, as well as with desirable agronomic traits. Here we report the performance of $T_2$ and $T_3$ plants derived from these lines when challenge with the pathogen under greenhouse conditions. Also we report the generation of new transgenic lines using the indica varieties Cimarron, and Fedearroz 2000, the breeding line CT11275, and the japonica variety Nipponbare, widely used in rice functional genomic.

## Materials and Methods

**Genetic Transformation and Plant Regeneration.** Mature embryos-derived callus of the indica materials Cimarron, Fedearroz 2000, and CT11275, and the japonica variety, Nipponbare, were used. Transgenic plants were generated using the *Agrobacterium tumefaciens* strain Agl1 (Wang et al., 1997) containing the plasmid NT446 that carries the PAPy123 gene (Tabares et al., 2002).

**Molecular analyses of PAPy123 transgenic rice plants.** Genomic DNA was extracted from 1 g of rice leaves according to McCouch (1988). DNA was digested accordingly to analyze number of gene insertions, copy number, and rearrangements. Gels were denatured and neutralized by standard procedures. Membranes were hybridized using labeled specific- PAPy123 probe at 60°C (Sambrook et al., 1989).

**Evaluation for Rhizoctonia Resistance in the Greenhouse.** $T_2$ and $T_3$ progeny plants from transgenic lines were inoculated at 50-day-old with two Rhizoctonia strains (intermediate and hyper virulent). These strains were collected from rice farmer's fields in Tolima Department (Colombia), and characterized by its virulence using a susceptibility-differential reference. The strain 1953-2 shows intermediate virulence, and the strain 2399-1 is hyper virulent. The inoculated plants were placed in a humid chamber (>80% of relative humidity and 30°C) to simulate symptom development. Plants were evaluated 15 days and 25 days after inoculation according to IRRI evaluation scale and modified by the CIAT Rice Project (F. Correa, personal communication, Project IP4). The non-transgenic Cica 8 and Palmar varieties were used as susceptible control, and breeding line CT14534-12M-3-4M, derived from the inter-specific cross Oryzica 3/*O. rufipogon* was used as intermediate resistance control. In addition to the PAP-transgenic rice lines, transgenic lines A3-49-60-12-3-1-31, and derived crosses A3-49-60-12-3/Cica 8-2 and A3-49-60-4-5/FB007-19 containing the N-protein gene and with hypersensitive reaction as the mechanism underlined viral resistance were also evaluated. Five plants of each line were analyzed for the presence of the transgene by PCR.

**RHBV Resistance Assays.** Independent transgenic events containing the PAPy123 gene were evaluated for resistance to RHBV in the greenhouse. At least, 10 $T_2$ progeny per each of 22 transgenic events were inoculated with RHBV at 10 days or 15 days after planting using 4 viruliferous insects per plant. Disease evaluations were conducted using scale from 0 to 9, were 0 refers to no disease symptoms and 9 indicates more than 90% leaf area affected by the RHBV disease.

## Results and Discussion

From 83% to 100% of plants analyzed showed integration of the PAPy123 gene according to Southern blot analysis, yielding a total of 70 transgenic plants obtained from 371 callus originally Agro-infected (Table 1). The transformation efficiency varied from 11% for Fedearroz 2000 to 54% for Cimarron respectively (Table 1). The molecular analyses showed the integration of single and multiple copies of the PAPy123. All the Fedearroz 2000 plants showed single and non-rearranged gene copies (Figure 1). Most plants from Cimarron and CT11275 also showed simple integration patterns. But Nipponbare showed multiple copies, some of them of higher or lower molecular weight than the expected size indicating rearrangements in some of these copies, including possible gene fragmentation and deletion (Figure 1). These plants will be evaluated for agronomic traits, and selected lines will be advanced to $T_2$-$T_3$ generations and plants with stable inheritance and expression of PAP gene will be screened for sheath blight resistance.

Transgenic plants of Cica 8 and Palmar were evaluated for resistance to Rhizoctonia for two consecutive generations ($T_2$ and $T_3$) using intermediate and hyper virulent strains (Table 2). About 50 $T_2$ lines were identified showing intermediate level of resistance, sister plants of these lines were also evaluated for RHBV, and $T_3$ self cross-derived progeny plants from the resistant ones were evaluated for sheath blight in the following year. $T_2$ plants derived from transgenic Cica 8-446 8-1, Cica 8-446-14-6, Palmar-446 4-1, Palmar-446 23-1, and Palmar-446-39-4 showed intermediate resistance to Rhizoctonia. This resistance was inherited into $T_3$ plants from the Cica 8-446-14-6-18 and Palmar-446-39-4-7 lines showing intermediate resistance to both strains of fungi (Table 2). These plants showed a significant reduction in disease reaction respect to non-transgenic control. In addition, line Palmar-446-39-4 showed resistance to RHBV and the derived $T_3$ line Palmar-446-39-4-7 showed a reduced leaf area affected by the pathogen respect to the cross Oryzica 3/*O. rufipogon* when inoculated with the hyper virulent strain 2399-1 (Table 2, and Figure 2A). Southern blot analysis of genomic DNA indicated that 100% of these plants contained simple integration pattern of non-rearranged copy of the PAPy123 gene (Figure 2B). These plants showed a fertility of 93-95%.

PAP protein seems to activate the protein expression of host genes associated with hypersensitive response (HR) and defense-related signal transduction pathway in the absence of pathogen infection and elevated levels of salicylic acid (Zoubenko et al., 1997; Wang et al., 1998; Smirnov et al., 1997). Additionally, PAP inhibits local lesion formation to a number of different viruses, *including* both DNA and RNA viruses (Chen et al., 1993), conferring a resistant mechanism against both mechanically and aphid-transmitted virus. For this reason besides fungal resistance, it is interesting to evaluate the activity of the PAPy123 gene against RHBV and other rice viruses. Because PAP also confers resistance to a broad spectrum of fungal pathogen, it will be interesting to evaluate these transgenic plants for resistance to other pathogens as well. The transgenic Cica 8 line A3-49-60-4-5/FB007-19 containing the RHBV-N gene for RHBV resistance, showed highest level of tolerance to Rhizoctonia. The RHBV-N gene appears to confer resistance to this virus by RNA mediated cross protection where hypersensitive reaction mechanism is involved (Lentini et al., 2002). It could be possible that RNA transcripts from the RHBV-N gene could function as elicitors for the plant defense system before or during pathogen infection. This hypothesis requires more systematic research in order to determine the reproducibility of this phenomena and elucidate the resistance mechanism involve.

**Future Plans**

1. To study the mechanism of action of the gene PAPy123 in resistant transgenic Cica 8 and Palmar.
2. To evaluate the possible effect of the gene PAPy123 on *Tagosodes orizicolus*, the planthopper vector of RHBV.
3. To explain why some lines are resistant only to one strain of the fungus.
4. To study protein expression and gene integration in the resistant lines.
5. To evaluate the tolerance to Rice Stripe Necrosis Virus under greenhouse condition.

6. To multiply seed ($T_3$) resistant to *R. solani* and evaluate resistance for Rhizoctonia, and other pathogens such as Sarocladium, and Helminthosporium under field conditions.

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**Table 1. Transformation Efficiency of Three Indica Varieties and one Japonica Variety using *Agrobacterium tumefaciens* (NT446)**

| Genotype | [1]R | Callus | Plants | [2]RP (%) | Plants Analyzed by Southern | [3]Plants $S^+$ | Plants S+ (%) | [4]TE (%) |
|---|---|---|---|---|---|---|---|---|
| Indica | | | | | | | | |
| Cimarron | 3 | 52 | 28 | 53.8 | 27 | 27 | 100 | 53.8 |
| CT11275 | 2 | 166 | 27 | 16.2 | 17 | 17 | 100 | 16.3 |
| Fedearroz 2000 | 2 | 44 | 6 | 13.6 | 6 | 5 | 83 | 11.4 |
| ***Japonica*** | | | | | | | | |
| Nipponbare | 3 | 109 | 23 | 21.1 | 23 | 21 | 91 | 19.3 |

[1] Number of replicates. [2]Regenerated plants. [3]$S^+$ = Southern positive. [4]TE = Transformation efficiency

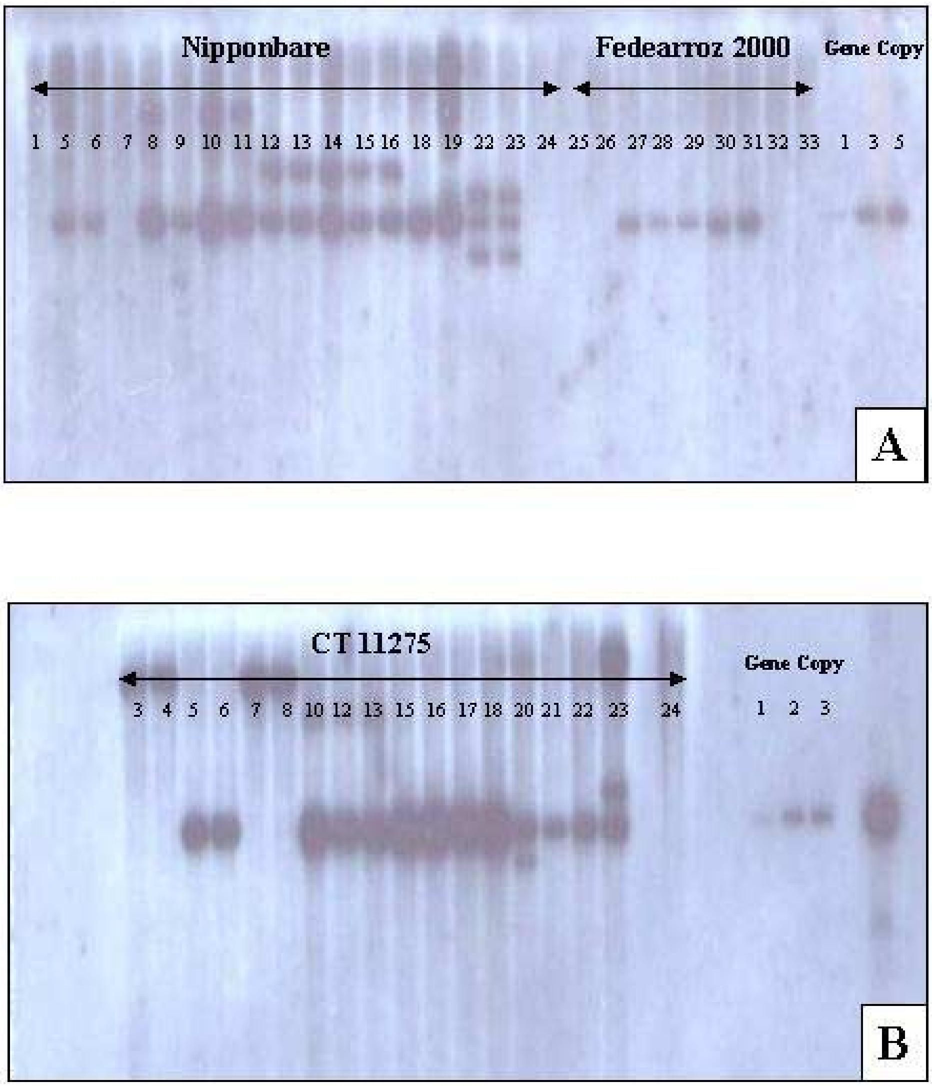


**Figure 1. Southern blots analysis of *PAPy123* gene in $T_0$ plants. (A) Lanes 1-23, DNA from Nipponbare, Line 24 non-transgenic control. Lines 25-31, DNA of Fedearroz 2000. Lines 32-33, non-transgenic control. (B) Lanes 3-23 DNA of CT1275. Line 24 non-transgenic control**

**Table 2. Evaluations for Resistance to Rhizoctonia and RHBV under Glasshouse Conditions of $T_2$ and $T_3$ Progeny Plants Derived from Six Transgenic Lines that Carrying PAPy123 Gene**

| | | | | % LAF Rhizoctonia (2002)[2] | | % LAF Rhizoctonia (2003)[3] | | |
|---|---|---|---|---|---|---|---|---|
| **Line** | **Gene** | **$T_2$ Plant** | **RHBV [1]** | **1953-2 I<26** | **2399-1 I<31** | **$T_3$ Plant** | **1953-2 I<40** | **2399-1 I<60** |
| Cica 8-446- | PAPy123 | 8-1 | 9 | 17 | 88 | 11 | 52 | 57 |
| | | | | | | 12 | 42 | 72 |
| | | | | | | 13 | 53 | 55 |
| | | 14-6 | Nd | 29 | 22 | 16 | 63 | 55 |
| | | | | | | 17 | 53 | 65 |
| | | | | | | 18 | 38 | 52 |
| | | | | | | 19 | 46 | 67 |
| Palmar-446- | | 4-1 | 5 | 23 | 60 | 11 | 45 | 50 |
| | | | | | | 12 | 51 | 68 |
| | | 23-2 | 5 | 26 | 26 | 6 | 41 | 73 |
| | | | | | | 12 | 55 | 58 |
| | | | | | | 16 | 53 | 58 |
| | | | | | | 17 | 50 | 81 |
| | | | | | | 18 | 38 | 77 |
| | | 39-4 | 3 | 23 | 46 | 7 | 48 | 48 |
| | | | | | | 14 | 58 | 68 |
| Non- transgenic Cica 8 | None | NA | 9 | 37 | 46 | NA | 57 | 64 |
| Non-transgenic Palmar | | | 7 | 26 | 39 | | 51 | 92 |
| Non-transgenic Fedearroz 2000 | | | 2 | 41 | 56 | | Nd | Nd |
| Non-transgenic CT14534-12-M-3-4-M | | | . | 26 | 23 | | 48 | 60 |
| A3-49-60-12-3-57-3 | RHBV-N | NA | 1 | 63 | 95 | | Nd | Nd |
| A3-49-60-12-3-1-31-9 | | | 6 | 29 | 25 | | 62 | 73 |
| A3-49-60-12 3/ Cica 8-2 | | | 5 | Nd | Nd | | 61 | 57 |
| A3-49-60-4-5/ Fedearroz 50-19 | | | 4 | Nd | Nd | | 33 | 55 |

[1] Mean score value of ten plants evaluated for RHBV per each $T_2$ line.
[2 and 3] Mean values of percentage leaf area affected (LAF). Reactions to two strains of Rhizoctonia (1953-2, intermediate virulence; 2999-1, hyper virulent). Two evaluations on two consecutive generations. (I) Values refer to the percentage of LAF indicating intermediate resistance. [2]Means of five plants per each $T_2$ line and [3]Means of fifteen plants per each $T_3$


**Figure 2. (A) Disease reaction of plants inoculated with hyper virulent Rhizoctonia strain 2399-1. Left non-transgenic CT14534-12-M-3-4-M derived from interspecific cross Oryzica 3/ *O. rufipogon*. Right, transgenic Palmar 39-4 plants carrying the PAPy123 gene. (B) Southern blot of transgenic plants carrying the PAP123y gene. Lanes 1-14, transgenic plants from lines 39-4, 39-9 and 39-10 respectively. NT, non-transgenic control**

## OUTPUT 3. ENHANCING REGIONAL RICE RESEARCH CAPACITIES AND PRIORITIZING NEEDS WITH EMPHASIS ON THE SMALL FARMERS

### 3A. Rice and Sorghum Participatory Plant Breeding in Central America

- **Rice and Sorghum Participatory Plant Breeding in Central America**

G. Trouche, C. Zildghean, M. Châtel

### Introduction

This collaborative research project between CIAT and CIRAD began in April 2002 with the signature of a Memorandum Of Understanding between CIAT and CIRAD-Ca and further outposting of Gilles Trouche in Managua, Nicaragua. The project proposes to develop and to apply participatory varietal selection (PVS) and participatory plant breeding (PPB) approaches for two « model plants » -- i.e., rice and sorghum – for small and medium-scale farmers undergoing progressive crop intensification and increased access to markets.

Upland rice, otherwise known as aerobic rice, is a re-developing crop in several Central American and Caribbean countries, mainly in plains with good rainfall (1300-2000 mm per year) but also on hillsides as a component of diversification of cropping systems and food security. The total area of rice, upland and irrigated, in Central America and the Caribbean totalizes 630,000 ha with a global paddy production of approx. 2400,000 tons (FAO, 2002). In Nicaragua, aerobic rice covers 55,000 of the 83,000 ha which makes up the average total rice area (MAGFOR, 2002).

Because of its superior drought tolerance, sorghum provides an alternative production to maize in the semi-arid areas, under dry farming cropping systems of Central America. In the four countries Honduras, El Salvador, Nicaragua and Guatemala, sorghum-planted areas comprise approximately 255,000 ha, half of which is cultivated by small-scale farmers. In this area, sorghum grain is either used for human consumption (particularly replacing corn for "tortillas" preparation) or for animal feeding (poultry, pigs) while straw is an important fodder for cattle during the dry season.

This research project has three principal objectives:

1. To develop and apply new PVS (Participatory Variety Selection) and PPB (Participatory Plant Breeding) methods including population enhancement and approaches
2. To identify and develop new germplasm matching the needs of small and medium farmers' cropping systems
3. To enhance partners' capacity:
   - Capacity of NARS in conventional and participatory breeding of rice and sorghum
   - Capacity of NGOs and farmers' groups to manage PVS and PPB activities and for local seed production

To develop the PPB work on both crops, two main guidelines will be followed:

The PPB work will be implemented in partnership with existing farmer organizations and other relevant actors like local NGOs or extension agencies. It is through these organizations that the activities of the project will be organized.

The PPB will be based on the use of a broad genetic base through introduction of diversified new germplasm with different genetic and geographic background and through population improvement and recurrent selection.

For rice, segregating material, fixed lines or varieties derived from conventional breeding, inter-specific crosses and improved populations developed by CIAT and CIRAD Rice Projects are being used.

For sorghum, CIRAD Collaborative Breeding Programs in West Africa are the first source of genetic resources and new improved germplasm. Other available improved breeding materials from INTSORMIL and regional NARS is also being used. Special emphasis will be placed on environmental adaptation (response to photoperiod, drought and pest tolerance) and on grain and forage quality (double purpose types). Existing composite populations from different sources will be further enhanced using materials identified to match the local constraints and production objectives.

## General Problem

The most important constraints to upland rice production in Central America identified in Nicaragua and Honduras are: a lack of improved varieties with good adaptation to the marginal environments or no mechanized cropping systems, deficient weed control, drought or water excess, diseases, particularly blast rice, and pest problems, unsatisfactory grain quality for industry requirements, problems for transformation and commercialization and competitiveness with imported rice. Constraints for sorghum production include low improved varieties offer, drought, low soil fertility, pests (midge, head bugs, fall armyworm) and diseases, as well as low fodder quality. For both crops, the current low human and financial inputs of the Central America NARS help to explain the lack of new varieties and level of research effort.

## Materials and Methods

### Follow-Up of the Identification of Sites and Partners

This first step, necessary for implementing the project and for ensuring its sustainability, was mainly done in Nicaragua and Honduras during the first six months, and efforts to strengthen the research framework continued in 2003. Emphasize was placed on Nicaragua, the current project location, because of its greater diversity of agro climatic, socioeconomic and institutional environments for both rice and sorghum. In 2003, we focused on identifing new partners and to select the sites with greater possibility of impact. For this purpose, literature and a data-base were reviewed (National Cense of agriculture and livestock in Nicaragua, regional farming systems characterizations…). Additional interviews and meetings were conducted with other key informants from extension agencies and NGOs and with farmer's leaders, in order to verify and complete the information. Fields visits during the cropping season and preliminary tests of varieties realized in the sites gave important information to determine the more relevant sites for impact.

### Diagnostic Work

This activity comprises diagnostic activities to characterize the rice and sorghum cropping systems as well as the main production constraints with a special focus on farmers' needs for new varieties. For this activity, workshops were organized with farmers' groups in each new site of interest. During these workshops a rapid diagnostic work was done using PRA tools. More complete diagnostics were executed in two sorghum sites.

Regarding rice, workshops or meetings, organized jointly with local partners, were held with rice growers in the sites of Belen (Rivas department), Posoltega (Chinandega department), Quilali ([1]Nueva Segovia department), Bocay and Wiwili (Jinotega department) and Siuna (RAAN) in Nicaragua, and in Tocoa (Bajo Aguan area) in Honduras (see map). Moreover, as a collaboration between UCA/ADAA[1] program and the project, UCA students are making an agronomic diagnostic of upland rice plots in Masaya region. This is particularly interesting, because it is the only site with a long-term experience of planting rice at the beginning of the second rice season ("postrera"). This could be a new option extendable to other rice areas for the new very early varieties derived from inter-specific and recurrent breeding activities.

For sorghum, little information was available about diversity of cropping systems, constraints and farmers' needs for Nicaragua. Therefore, it was considered necessary to conduct more complete diagnostic studies at the beginning of the project. The first diagnostic work was carried out by a Nicaraguan student, Felipe Martínez, as part of his requirements to obtain a Master of Science in rural development of the [2]CNEARC Tropical Agronomy School (France). The site in the Madriz departmentis one of the most representative sorghum areas with small and medium-scale farmers in Nicaragua. The thesis work comprises a participatory diagnostic of existing sorghum cropping systems, variety diversity and production and utilization constraints. It is also aimed to understand the farmers' perspective and priorities for sorghum varieties. This study have been carried out in collaboration with the [3]NGO UNICAM in four farmer communities representing different conditions of sorghum production in this area of semi-arid hillsides, in relation to climate, soils, topography, ethnic skills, social organization and institutional environment. The thesis was defended with success in December 2002 in Montpellier. during this study seeds and local knowledge of 35 local sorghum varieties was collected.

1 Universidad Centro Americana Managua, Programa de desarrollo agrario
2 Centre National d'Etudes en Agronomie des Régions Chaudes, Montpellier, France
3 Universidad Campesina, Esteli, Nicaragua

A second thesis work, carried out in 2002-2003 by a student in agronomy sciences of the UCA University, tried to gather information of the cultural practices, main constraints of production (a biotic and biotic) and yield potentials for different sorghum cropping systems (including photoperiod-sensitive and insensitive sorghum types) in three farmer communities of the same region. About 50 sorghum plots were followed during the two growing seasons , primera and postrera, in these communities  Because of the student's resignation, the thesis will not be concluded, but the available data will be analyzed in collaboration with UCA scientists and the results will be ready for the next annual report.

At the conclusion of the first work, it appeared interesting to investigate more  regarding the mechanisms and the existing informal channels used by farmers for seed exchanges, testing, adoption and diffusion of new sorghum varieties in the North of Nicaragua. During the last 20 years, there was a high level of adoption of several improved varieties from Honduras and El Salvador by small farmers, which did not follow the official release process of varieties. The understanding of these mechanisms and channels involving farmers and other local actors (NGO personal, traders, etc) will be useful  for the diffusion of the new varieties developed by the PPB work in this area. Two students working together, one from ISTOM[4] school, France and one from UCATSE[5] school of agriculture, Nicaragua, began in 2003 a study on this theme for 6 months in seven communities of the area. They will use PRA tools such as historical mapping, semi-structured  and focus group interviews.  In another sorghum site of Nicaragua, Ciudad Dario-Terra Bona, UCA students are making a diagnostic study of sorghum cropping systems, constraints and farmer's needs using the methodology implemented by Felipe Martinez for his work in Madriz.

## Conventional and Participatory Breeding

### Plant Materials

#### Rice

The following advanced rice lines were evaluated in various trails described in this report.

#### Observation Nurseries CIAT-ION

1. 210 $F_7$ lines from conventional breeding developed by the CIAT/Peru breeding program for irrigated conditions or favorable upland conditions.
2. 211 $F_6$-$F_7$ upland lines from conventional and composite pop. breeding (CIAT-CIRAD), inter-specific crosses (CIAT) between *O. sativa*, *O. glaberrima* and *O. barthii*.
3. 40 $BC_2F_3$ lines with high and stable resistance to rice blast developed by the CIAT rice pathology program for upland conditions with their two recurrent parents.
4. 12 varieties or lines with high resistance to rice blast, *Rhynchosporium oryzae* and grain discoloration from the CIAT Rice Pathology Program.
5. 31 $F_7$ lines from the CIAT-CIRAD RHICO program for high altitude areas.

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4 Institut pour les Sciences et Technologies d'Outre Mer, Cergy, France
5 Universidad Católica de Agricultura en el Trópico Seco de Esteli, Nicaragua

**Development of New Lines using Participatory Breeding**

To start this activity in 2002, two narrow genetic base populations from CIAT-CIRAD program were used: PCT-18 for upland conditions with high resistance to rice blast and grain quality and PCT-17 for high altitude hillsides upland conditions with tolerance to cool temperatures and grain quality. From these two populations, respectively 61 and 11 fertile plants were selected with farmers from the PCT-18 population in San Dionisio and in the PCT-17 population in Yorito. The derived $S_1$ lines were planted in 2003 in the same sites to continue the selection process with farmers.Thirty $S_1$ lines derived from PCT-18 were also planted in the Chinandega site to see the adaptation of these materials in this area. In 2003, three broad genetic base populations for upland conditions, CNA- 7 from Brazil and PCT-4 and PCT-11 from CIAT-CIRAD Colombia, were introduced and planted in two sites to verify their adaptability and to start participatory recurrent schemes with farmers. Finally, three new site-specific composite populations for Nicaragua are in the creation phase by incorporating into the population PCT-18 locally well adapted varieties and new materials selected for specific traits.

**On- Station Yields Trials**

Twenty-two (22) advanced lines from the PCT-4 population were evaluated with three (3) variety checks (INTA Malacatoya, CIRAD 409 and IRAT 366) in a preliminary trial with INTA in the CEO station in Posoltega, Nicaragua. Fifty-two (52) lines selected from 2002 nursery for irrigated conditions, including 35 lines from inter-specific crosses with *O. rufipogon* and *O. glaberrima* were evaluated again in Sebaco station during the 2003 rainy season.

**On-Farm PVS Trials**

The same twenty-two (22) advanced lines from PCT-4 populations were tested in a on-farm trial with two planting dates (primera y postrera) in comparison with the same checks in Rivas area, the driest rice site in Nicaragua. Eighteen (18) advanced lines and commercial varieties from CIRAD, CIAT, CORPOICA and INTA were evaluated in PVS trials in 5 sites in Nicaragua and 2 sites in Honduras.

**Sorghum**

**Observation Nurseries**

1. 18 photoperiod sensitive landraces and improved lines from CIRAD germplasm collection (late-flowering core from Cameroon, Chad, and Sudan).
2. 11 photosensitive landraces collected in Nicaragua.
3. 75 fixed lines and varieties from CIRAD-INERA program in Burkina Faso with drought tolerance, leaf diseases, midge and bugs resistance or for dual purpose use.
4. 28 early and/or R lines and varieties from the CIRAD sorghum collection.
5. 3 double purpose hybrids from CIRAD program for template conditions.

**Development of New Lines using Conventional or Participatory Breeding**
Two $F_2$ populations derived from simple crosses made for breeding objectives decided with farmers were planted in a site to begin the PPB work during the "postrera" 2003. Other crosses made in 2003, following results of 2002 PVS trials, will give $F_2$ seeds to develop new PPB schemes in 2004. The composite population PP 34 from CENTA El Salvador was planted on station for observation and for a first recombination cycle.

**On-Station Yield Trials**
28 CIRAD lines or varieties and 3 INTA commercial varieties

**On- Farm PVS Trials**
- For photosensitive sorghum, 22 advanced lines or varieties from INTSORMIL-INTA (13), CENTA (5) y CIRAD (4) were included in a regional on farm yield trial planted in Nicaragua (7 sites), El Salvador and Honduras.
- For insensitive sorghum, 10 CIRAD improved varieties and 11 INTA lines or varieties were used for a regional on-farm yield trial in Nicaragua and for other specific-site trials.

**Experimental Sites**

Observation nurseries and on-station yield trials were planted on INTA experimental stations in Managua (sorghum), Posoltega (upland rice) and Sebaco (irrigated rice) and at the CIAT reference sites for agriculture in hillsides, in San Dionisio (upland rice and sorghum) and Yorito (rice for high altitude). PPB work on rice was conducted with local partners at the same reference sites of San Dionisio and Yorito and at the CEO station of Posoltega.

During the 2003 rainy season, 40 on-farm PVS trials were conducted on rice in five sites of Nicaragua and two sites of Honduras: Chinandega, Rivas, Siuna, San Dionisio and Bocay-Wiwili in Nicaragua, Yorito and Bajo Aguan in Honduras. For sorghum, 20 on-farm PVS trials were planted in three sites in Nicaragua (Madriz, Ciudad Dario-San Dionisio and Villa Nueva) and in one site in Honduras (Lempira sur).

**Experimental Designs**

For the observation nurseries, the observation plots were consisted of the lines interspersed with the checks. For the on-station yield trials, a randomized complete block design with two to four replications or rectangular lattice design with three replications are used. The designs for on-farm PVS trials are randomized complete blocks with two o three replications. The conditions of the experiment are discussed with farmers during the planning meetings, and trials are normally managed following usual farmers practices, unless the farmers expressed to receive some specific input.

## Methodology for PVS and PPB Work

At this stage of the project, on-farm PVS trials with a pertinent set of diverse varieties (diversity of cycle, morphological traits, yield potential and grain quality) have three main objectives:

1. To complete and to verify the information obtained from the diagnostic work.
2. To understand better the diverse criteria that farmers consider to select a new variety and their ranking depending of the social category, geographic locality and gender and to understand how the farmers evaluate these criteria.
3. To identify the best adapted and most preferred varieties or genetic groups for a rapid adoption by farmers and/or to use in further breeding programs

Including the diagnostic, the general approach applied for this participatory work is presented in Diagram 1.

For the participatory evaluation and selection of fixed lines or varieties, we adapted a methodology developed by CIRAD and partners for sorghum in Burkina Faso (vom Brocke et al, 2003). The basic principle applied arethe following: varieties are to be evaluated according to the criteria that farmers consider as the most important and that, in discussion with research team, are the most relevant for the specific conditions of the trial. This information will be obtained during a workshop with farmers during the day of evaluation. Evaluation of varieties is achieved mostly at maturity phase, and if necessary, a post-harvest evaluation focused on grain quality for auto consumption is also carried out. For field evaluation, farmers groups are assembled considering geographic, social or gender criteria. Each variety is evaluated by each farmers' group in two replications of the trial, for the first three or four most important criteria and for an overall general value, using a scoring method with a four levels scale (bad, intermediate, good and excellent). At the end of the evaluation, farmers are asked to select the 3-5 best varieties according to their evaluation.

If the number of varieties to evaluate is high (more of 50 varieties), we used a simplified methodology where farmers groups are asked to choose the most preferred varieties and to discard the worst varieties, explaining the reasons of their decision. For the PPB work using composite or $F_2$ populations, it is usual to give to the farmers a small course to explain some basic elements of methods to create genetic variability, of genetics and breeding concepts before realizing the selection of individual plants. In this case, both the farmers and the breeders, participate in the plant selection because farmers objectives are mainly site-specific while the breeders objectives may consider various sites and conditions.

## Results

### Cooperators of the Project (Partners and Sites)

In 2003, the new identified partners in Nicaragua are the following:

1. Programa Campesino a campesino (Pcac) of the Union Nacional de Agricultores y Ganaderos (UNAG) for the site of Siuna.
2. Servitec, which is an private extension agency for the area of Bocay-Wiwili.
3. The UCA University with its Agrarian Science Department.
4. The INTSORMIL program for Central America for sorghum activities.
5. The technical cooperation of Republic of China Taiwan for rice activities in Nicaragua.

In Honduras, a stronger collaboration has been established with the DICTA rice program. For sorghum, another new partner is the FAO project of Lempira Sur. Moreover, in 2003, new collaborative activities were built with ICTA Guatemala and IIA Cuba based on exchange of rice germplasm. Exchanges of germplasm and collaborative variety trials are also done on sorghum with CENTA of El Salvador.

For rice, two new sites were selected in Nicaragua and one site in Honduras. In Nicaragua, the project now works on five sites representing diverse agro climatic, socioeconomic and institutional environments (see Map and Table 1). In Honduras research activities are focused on the area of Bajo Aguan in the Atlantic coast, which is the actual priority for government and for DICTA for increasing national rice production, and on the higher altitude hillsides of the Yorito area.

For sorghum, the main site is the Madriz department with UNICAM as a partner to reinforced our activities in four communities. The second site is a semi-arid zone including Ciudad Dario, Terra Bonna and the South of San Dionisio, with a strong collaboration in this area with an UCA research project. The third site is still the Villanueva-Somotillo area with NITLAPAN as partner (see Map and Table 2).

### Diagnostic of Cropping Systems, Production Constraints and Farmers' Needs

### Rice

For the five sites in Nicaragua, information about the cropping systems and main agronomic and socioeconomic constraints of the rice production, obtained from the diagnostic work with farmers, interviews with partners and revision of bibliography is summarized in the Table 3. This information, complemented with the results gotten from the first PVS trials done on each site, was used to determine the priority breeding objectives for each site (Table 4).

## Sorghum

The diagnostic works realized in Madriz department and in Ciudad Dario revealed a great diversity of cropping systems where sorghum is the basic component for food security (Diagram 2). Photoperiod-sensitive sorghum landraces, generally referred to as "millón" in Nicaragua and "maicillo" in Honduras, are planted in combination with maize, bean or insensitive sorghum with different dates of planting and geometric arrangements depending on soils, slopes or climatic constraints as well as farmers' strategies and goals. Because of its rusticity and ability to adapt to poor, unfertilized soils, as well as its very long cycle (May to December), which allows it to withstand middle cycle droughts, "millón" sorghum is a low-risk crop that guarantees family subsistence production when maize and/or bean production fails. Since the mid-1980s, short cycle and insensitive white grain sorghum have been gradually replacing maize and "millon" on the flattest and the most fertile lands and "millon" sorghum is now more concentrated in the hillsides. During the diagnostic work, 30 sorghum varieties representing insensitive and photoperiod-sensitive types were collected in the Madriz department. The collected "millon" varieties are being characterized for genetic diversity in comparison with a core collection of sorghum by CIRAD biotechnology team in Montpellier; they are also characterized in situ for their photoperiodic susceptibility and for basic morphological and agronomic traits.

The general priorities for the breeding objectives on sorghum on the working sites were determined from the diagnostic work and complemented by the analysis of the results of first PVS trials. They are summarized in the Table 5. Other identified objectivesare more site-specific and include the improvement of broom sorghum local varieties for fibers' length and quality, disease and lodging resistance, and the identification of dual purpose red-grain varieties for animal feeding. Finally, as a scientific support to the INTA national sorghum program, we are testing different new materials to contribute to the development of dual purpose and ensilage hybrids for great stock-breeders.

## Farmers' Selection Criteria

The determination of the farmers' criteria to evaluate and to select new varieties and their ranking is one of the important results of the applied methodology for farmer evaluation in the on-farm PVS trials. The evaluation of the information from multiple sites reveals the similarities or the divergences of the importance of each criterion among the sites.

For rice, at this date, the ranking of farmers' criteria was only done for Chinandega, San Dionisio and Bocay sites (Table 6). In Chinandega area, the three most important criteria to select a new variety are improved yields, a high level of resistance to leaf diseases and a good industrial quality of the grains. Earliness of varieties was not considered an important criteria until farmers evaluated during the 2003 season precocious lines with less than 95 days from sowing to harvest. They are now very interested in these new types, because it gives the option to plant rice at the beginning of the second rainy season (final of August) after the "canicula" dry period, with less risks of drought than the usual date of planting in July. Other important criteria for the farmers are resistance to lodging, short or intermediate plant height (less than 80 cm), uniformity of flowering and maturity and stay green trait to give cleaner paddy with a mechanized harvest.

In San Dionisio, the two most important criteria are  better production, with a preference to improve panicle weight than tillers number because of the manual harvest, and good grain quality for family consumption and  to obtain better yields and prices when they sell a part. The also rate resistance to lodging associated with intermediate height (80-100 cm) and tolerance to shattering (Table 6) as important traits.

For insensitive sorghum "tortillero", the farmers' criteria to choose a new variety are generally shared in all the sites but their ranking may be fairly different according to the climatic constraints and the utilization of grains and stover in each site (Table 7).  Improved grain yields, grain quality for human consumption (Tortilla and other) and fodder quality and yield are the top three criteria for all thesites.

## Crop Improvement

This chapter presents the  results from the 2002 rainy season and the 2003 off-season, as well as preliminary results of the current 2003 rainy season.

## Rice

### Creation of New Composite Populations

Oriented open crosses between the PCT-18 population and ten well adapted varieties selected for a specific traits (i.e. INTA N-1 for industrial grain quality, IRAT 301 for drought tolerance…) have been carried out in the Sebaco station during the 2003 off-season in order to create three new site-specific composite populations for Nicaragua.  For each cross, $F_2$ seeds will be obtained in December 2003 and a first recombination cycle will be done during the 2004 off-season.

### Upland Rice for Low Altitude Hillsides

Among the RHICO lines for hillsides conditions tested in San Dionisio in 2002, none yielded better than the locally improved variety IRAT 364/90 (a 90 days variant type from IRAT 364) (Table 8).  Several of these lines showed high susceptibility to lodge because of their weak stems.  Nevertheless during the participatory evaluation exercise, farmers selected three of them, CIRAD 447, PRA 522-2-6-66-2-1-M and PRA 522-7-24-5-1-M for their earliness, panicle weight, tolerance to lodging and satisfactory grain quality (Table 8).These lines were also selected in 2002.  The interest of these very early but limited yielding materials in Matagalpa area may be to use them in early September plantings following a leguminous crop.  On-farm PVS trials established in 2003 will confirm the interest and performances of these materials.

## Upland Rice for High Altitude Hillsides

In collaboration with the NGO IPCA and the local CIALs, the same set of RHICO lines was evaluated at 1,600 m in the La Patastera community in Yorito. Despite the cold temperatures for the site, PRA 565-46-34-3-1-M obtained respectable yields and gave a significant increase in comparison with the former best adapted variety, CIRAD 392 (Table 9). Moreover, it was very well appreciated by farmers. PRA 565-46-34-3-1-M and CIRAD 447 are being evaluated in 2003 in advanced on-farm trials in several sites located up to 1,000 m in Yoro and Francisco Morazan departments.

## Irrigated Rice or Upland Rice in very Favorable Climatic Conditions

Among the 323 lines evaluated under transplanting and irrigation conditions in the Sebaco valley during the January-May season, 52 were selected for their good agronomic performances and will be evaluated in the same site during the 2003 rainy season(Table 10). The lines derived from Bg90-2/*O. rufipogon* cross were particularly interesting with excellent yields until up to 9 t/ha with a great number of grains per panicle, high level of resistance to lodging and *Rhizoctonia solani*.Some of them also have fairly high 100 grain weights and long grains.

## Upland Rice for Diverse Conditions

In the course of a recent participatory evaluation of new upland lines from CIAT-CIRAD program (ION-CIAT nursery and advanced trials) in the Posoltega station (Chinandega area), four groups of farmers from four different localities have selected in total 63 early lines among which 13 lines are derived from conventional breeding, 26 from crosses with *O. glaberrima* and 24 from composite population breeding. Six lines were selected by all of the farmer groups. Earliness, good productivity, resistance to leaf and grain diseases, stay green, resistance to lodging and vegetative vigor were the main criteria of selection applied by farmers.

# Sorghum

## Short-Cycle dual Purpose Sorghum

Improved CIRAD varieties from West Africa in general adapted well to the agronomic and climatic conditions in San Dionisio, Villa Nueva and Madriz sites in Nicaragua. In these sites, their notable traits are earliness (less than 100 days to maturity), stay green, good grain productivity and adequate grain quality (color and size) (Table 11). However the tall types (more than 2 m) are less accepted by farmers because of the risk of lodging and difficulty for chemical pest control. In the on-farm PVS trials, conducted in five sites, farmers selected 24 different varieties according to their local preferences and objectives of production; among them, CEF 322/36-1-1 was chosen in all four sites and proved an excellent grain quality for making tortilla. All selected varieties in 2002 are being tested again in 2003 in on-farm PVS trials in the same sites in order to confirm these preliminary results.

**Photosensitive Sorghum**

The African varieties matured 8 to 15 days earlier than local landraces, but three of them are too early and were not adapted for the area because of birds and mold damages. The variety Souroukoukou, a caudatum landrace from Mali, got the higher yields and was also the preferred one by farmers during the participatory evaluation (Table 11). Farmers considered that this variety has adequate height and cycle, good fodder at maturity stage and gives a high grain production with pretty white grains. They think it will be interesting to plant it in associated with either corn or beans. However, we noted during tortilla-tasting test that its grain quality is not adequate and we started to correct this defectby crossing it with local landraces. The varieties 1581, 1600 and 1587 have notable 1000 grain weight, two to three times more than the local landraces. Considering agronomic results and farmers' appreciation in two sites, we can hope to find the more adaptable materials for the "millon" cropping system into the durra and durra-caudatum groups from Cameroon and Chad.

## Conclusion

### PPB Methodology

Methods adapted for participatory evaluation and selection of fixed lines, and for selection of segregating materials were developed and applied to conduct this PPB work. In general, the efficiency and the farmer were very motived.Nevertheless, additional training is needed to get the farmers used to the idea of selecting greater diversity.

### Rice Improvement

Germplasm introduction from Colombia leads to new opportunities to improve and diversify rice production in Central America. Preliminary results obtained in the 2002 season and overall in 2003 are very promising to identify better performing varieties for existing upland cropping systems and to give to farmers new varietal options to reduce climatic risks (Rivas area) and/or to enhance their revenues. In particular, new very early materials from population breeding are promising for these later purposes.

Considering the 2002 season and preliminary results of the 2003 season, the most relevant outputs on variety enhancement are:

1. Good performances and high farmers' praise for 3 RHICO varieties in the highland areas of Nicaragua and Honduras: CIRAD 447, PRA 522-2-66-2-1-M-M and PRA 565-46-34-3-1.
2. Promising results of new early lines derived from composite population breeding and from inter-specific crosses to reduce production risks in less favorable climatic areas and to give new cropping systems options for more favorable areas.
3. Start of PPB schemes with CIALs farmers in Nicaragua and Honduras, using PCT-18 and PCT-17 populations.
4. Creation of 3 new site-specific narrow genetic base populations for Nicaragua.
5. Validation Trials (Pre-launching) in Nicaragua with IRAT 301 (2003-04).

**Sorghum Improvement**

Germplasm introduction from CIRAD programs in West Africa has lead to new opportunities to improve and diversify sorghum production in Central America. The first outstanding results of the 2002 season are the following:

1. Photosensitive sorghum from West and Central Africa give semi-dwarf phenotypes with interesting gain in earliness and grain yields and huge increment in grain size.
2. Improved short-cycle sorghum from West Africa CIRAD Program offer very early varieties (85-90 days to harvest), improvement in grain yield, better fodder quality, good grain quality for human consumption.
3. Some short-cycle varieties gave excellent agronomic results and received good acceptation from farmers among them the most promising are CEF 322/36-1-1, CIRAD 437 and BF 89-12/1-1-1.

**Futures Activities**

PVS trials with the best identified materials and PPB activities using population breeding have to be strengthened on rice in Nicaragua and Honduras. A priority for the project will be to strengthen the participatory process with farmers' groups and other partners in the current sites in Nicaragua and Honduras. With the experiences and fields results of the second year of the project, it will be possible to determine better the priorities among sites and the way to have the best impact. In the selected sites, it will be also necessary to contract new alliances to handle research in agronomy in order to resolve specific problems identified with farmers during the PPB process.

At regional level, collaborations with other Mesoamerican countries like Guatemala, Costa Rica and Cuba have to be strengthened on rice.

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**First workshop with farmers groups and partners to explain the objectives and the methodology of the PR work**

↓

**Diagnostic of constraints and farmers needs of new varieties**

↓

**First on-farm PVS trials:**

- **Measurement of agronomic data by scientists**
- **Workshop for participatory evaluation of varieties with farmers**

↓

**Workshop of restitution and discussion of final results of the trials and planning of further activities**

**Diagram 1. Process and Steps of the PVS Work**


**Diagram 2. Inventory of Cropping systems Existing in Madriz Department, Center North of Nicaragua (Martínez F., 2003)**

## Table 1. Sites and Co-Operators for Rice Improvement in Nicaragua and Honduras

| Systems | Sites/Country | Research Partners | NGO and Extension Agency Partners | Farmers Organizations |
|---|---|---|---|---|
| Mechanized upland rice in favourable conditions | Chinandega (Nicaragua) | INTA | NITLAPAN, INTA, Mision Taiwan | "Informal rice growers groups" |
| | Bajo Aguan (Honduras) | DICTA | | |
| No mechanized upland rice in climatic favourable conditions | Siuna + Bocay (Nicaragua) | INTA | SERVITEC | PCAC/UNAG, UCA Siuna |
| | San Dionisio (Nicaragua) | | Campo Verde | CIALs |
| Semi-mechanized upland rice in less favourable climatic conditions | Rivas + Masaya (Nicaragua) | INTA, UCA | NITLAPAN | "Informal rice growers groups" |
| Manual upland rice in high hillsides | Yorito (Honduras) | | IPCA | CIALs |

## Table 2. Sites and Co-Operators for Sorghum in Nicaragua

| Agro Ecological Zone | Sites | Research Partners | NGO and Extension Agency Partners | Farmers Organizations |
|---|---|---|---|---|
| Semi-arid hillsides | Madriz | INTA, INTSORMIL | UNICAM | CPEC |
| | Ciudad Dario-San Dionisio | INTA, UCA, INTSORMIL, | | CIALs + informal sorghum growers groups |
| Semi-arid plains | Villa Nueva | INTA, INTSORMIL | NITLAPAN | Informal sorghum growers groups |

**Table 3. Description of the Cropping Systems and Main Production Constraints for the 5 Rice Sites in Nicaragua**

| Sites | Types of Farmers | Period of Planting | Annual Rainfall | Cropping Practices | Mean Yields (t/ha) | Main Varieties | Agronomic Constraints | Economic Constraints |
|---|---|---|---|---|---|---|---|---|
| Chinandega | Intermediate and big growers (2–400 ha) | Primera (July) | 1400 mm | Mechanized land preparation and sowing. High use of inputs (fertilizers, herbicides, fungicides) Mechanized harvest | 4.5-6 t/ha | INTA N-1, INTA Dorado, ANAR 97 | High incidence of rice blast and *Rhizoctonia solani*. Effective weed control. Drought. Lack of early varieties. | Excessive Application of fungicides and herbicides. Increasing costs of production. |
| Belén (Rivas) | Small and intermediate producers (1–5 ha). | Primera (June–July) | 1000 mm | Land preparation with animal plowing. Manual sowing in row. Usually no application of NPK but only urea. Weed control with herbicides. Mechanized harvest in lowlands or manual harvest in hillsides. | Carbonet: 2.5 t/ha INTA N°1: 4 t/ha | Carbonet, INTA N°1 | Lack of early varieties. Incidence of pests and weeds (red rice). Drought Low soil fertility. | Lack of credit to buy inputs. |
| Bocay–Wiwilí | Small producers (0.5–2 ha) | Primera (June) | 1800 mm | No plowing with manual planting in seed hole with “espeque” (Bocay) o animal ploughing (Wiwili). No application of chemical fertilizers Manual weed control or use of 2-4 D. Manual harvest. | 0.5-2 t/ha (Wiwilí) and 1–3 t/ha (Bocay) | “Arroz Blanco” “Arroz Amarillo” y Caturra | High incidence of soils and leaves pests and diseases. | Low prices, bad roads and no access to credit. |

| Sites | Types of Farmers | Period of Planting | Annual Rainfall | Cropping Practices | Mean Yields (t/ha) | Main Varieties | Agronomic Constraints | Economic Constraints |
|---|---|---|---|---|---|---|---|---|
| Siuna | Small and intermediate producers (1-20 ha). | Primera (May–June) | 2000 mm | No plowing, manual planting with espeque.<br>No application of fertilizers.<br>Manual weed control.<br>Application of chemicals limited to pest control.<br>Manual harvest. | Raizora: 3 t/ha<br>INTA Dorada: 4-4.5 t/ha | "Raizora Amarillo" y "R. Blanco", Carolina y Arroz Pujagua.<br>INTA Dorada | Incidence of diseases (blast + *Rhynchosporium oryzae*) and bugs (*Oebalus* sp).<br>Erosion and degradation of low soil fertility. | Low prices, bad roads and no access to credit. |
| San Dionisio (Matagalpa) | Small producers (0.5–2 ha) | Primera (June) | 1500 mm | Animal plowing. Manual sowing in row. Usually no application of NPK but only urea. Manual weed control or use of herbicides (2-4 D, propanyl).<br>Manual harvest | IRAT 301: 3 t/ha | Fortuna, IRAT 301 | Weed control | No access to credit, bad roads. |

**Table 4. Identified Priorities for Rice Improvement**

| Cropping Systems | Countries and Sites | Breeding Objectives | Variety Improvement Strategy |
|---|---|---|---|
| Mechanized and high inputs upland rice in climatic favourable conditions | Nicaragua: Chinandega area<br><br>Honduras: Bajo Aguan area | Stable resistance to rice blast and *Rhizoctonia solani* to reduce the utilization of fungicides<br>High yielding potential and low plant height<br>Short and medium cycles<br>Industrial grain quality<br>Adaptation to specific climatic conditions (Bajo Aguan) | PVS using available materials with high and stable resistance to diseases |
| No mechanized and low inputs upland rice in climatic favourable conditions | Nicaragua:<br>- Siuna and Bocay<br>- San Dionisio | Increase of yield in comparison with present varieties Medium cycles<br>Stable resistance to main diseases (rice blast, "escaldado")<br>Adaptation to low inputs and to manual harvest (high vigorous and intermediate height plant types)<br>Grain quality for family consumption and local market | PVS y PPB using broad and narrow genetic base populations |
| Semi-mechanized upland rice in less climatic favourable conditions | Nicaragua:<br>- Rivas | Vigor for initial growth<br>Tolerant to drought and short cycles<br>Yield Increase<br>Grain quality for family consumption and national market<br>Good quality and yield of fodder for cattle feeding during the dry season | PVS y PPB using broad and narrow genetic base populations |
| Manual upland rice in high hillsides | Honduras:<br>- Yorito: communities over 1,000 m | Tolerance o escape to cool temperatures<br>Cycles of 5 months<br>Objectives of yields: 2-3 t/ha<br>Grain quality for family consumption and local market | PVS y PPB using existing narrow genetic base populations |

**Table 5. Breeding Objectives Priorities on Sorghum**

| Sorghum Type | Breeding Objectives Priorities |
|---|---|
| Photoperiod-sensitive sorghum (in intercropped systems with corn or bean) | - Reducing plant height: easier control of post-flowering pests, less competence for intercrop with bean, to reduce lodging and to facilitate manual harvest<br>- Improving grain yields<br>- Reducing plant cycle for harvesting in December (earlier flowering and reducing flowering-maturity duration)<br>- Improving straw quality (more green leaves at harvest, improved stem quality) |
| Short-cycle insensitive sorghum | - Increasing grain yields<br>- Better resistance to head bugs, sorghum midge and stocks insects<br>- Higher grain size in order to get good prices<br>- Early cycle and drought resistance<br>- Grain quality to make tortillas and other local dishes<br>- Improving Stover yields and quality for cattle feeding |

**Table 6. Ranking of Selection Criteria for Rice in 3 Sites in Nicaragua**

| Criteria | Chinandega | San Dionisio | Bocay |
|---|---|---|---|
| 1. Improved grain yields | 1 | 1 | 1 |
| 2. Resistance to leaf diseases | 2 | 8 | 2 |
| 3. Grain quality for sale | 3 | 2 | 5 |
| 4. Resistance to lodging | 5 | 3 | 3 |
| 5. Adequate plant height [1] | 6 | 4 | 4 |
| 6. Short-cycle [2] | 8 | 6 | / |
| 7. Tolerance to drought | 4 | 5 | / |
| 8. Grain quality for family consumption | 7 | 2 | 5 |
| 9. Resistance to grain shattering | / | 3 | 6 |

[1] Adequate plant height is 60-80 cm for Chinandega, 80-100 cm for San Dionisio and for Bocay
[2] A shorter maturity cycle was not a priority for farmers in Chinandega until they know the availability of new very early varieties (less than 95 days to maturity) that may permit to plant rice at the beginning of the postrera season instead of using the entire rain season

**Table 7. Ranking of the Selection Criteria for Sorghum "Tortillero" in 5 Villages of the Sorghum Area in Nicaragua**

| Criteria | San Dionisio | El Mamel-Totogalpa | Santo Domingo-Totogalpa | Unile-San Lucas | Villa Nueva | Mean * |
|---|---|---|---|---|---|---|
| 1. Good grain quality for making | 1 | 2 | 2 | 5 | 1 | 2.2 |
| tortillas | 2 | 1 | 3 | 2 | 1 | 1.8 |
| 2. Improved grain yields | 3 | 3 | | 1 | 2 | 3 |
| 3. Good fodder yield and quality | | | | | | |
| for cattle feeding | 3 | 4 | 1 | 3 | | 3.4 |
| 4. Early maturing (3 months) | 4 | 4 | | 4 | 3 | 4.2 |
| 5. Resistance to lodging | 4 | 3 | | 4 | | 4.6 |
| 6. Drought resistant | 3 | 5 | | 4 | | 4.8 |
| 7. Short Plant height (1.4 -1.8 m) | 4 | 4 | | 5 | 4 | 4.8 |
| 8. Diseases and pests resistant | | | | | | |

* mean calculated attributing a rank 6 in each site for the not mentioned criteria

**Table 8. Agronomic Results and Farmers' Appreciation of 10 New Upland Rice Lines in San Dionisio 2002**

| Variety name | Days to flowering | Plant Height (cm) | Lodging (%) | Panicle length (cm) | Grain yield (kg/ha) | Grain length (cm) | 100 grains weight (g) | Farmers' Scoring [2] |
|---|---|---|---|---|---|---|---|---|
| IRAT 364/90 [1] | 65 | 79 | 70 | 18.5 | 5532 s | 8.4 | 3.3 | 2.2 |
| CIRAD 447 (LMV-5) | 53 | 70 | 23 | 19.1 | 4745 s | 8.3 | 3.5 | 2.7 |
| PRA 522-2-66-2-1-M (LMV-2) | 65 | 73 | 53 | 22.1 | 4660 s | 9.8 | 3.9 | 2.2 |
| PRA 546-38-71-3-1-M (LMV-4) | 87 | 90 | 30 | 18.8 | 4485 s | 7.9 | 3.7 | 2 |
| PRA 522-7-24-5-1-M (LMV-3) | 65 | 73 | 100 | 22.5 | 3889 | 8.3 | 3.2 | 2.2 |
| PRA 553-45-8-4-1-M (LMV-7) | 65 | 77 | 100 | 18.8 | 3475 | 7.3 | 3.2 | 1.3 |
| PRA 553-45-8-5-1-M (LMV-8) | 65 | 70 | 58 | 17.6 | 3475 | 7.2 | 3.2 | 1.2 |
| CIRAD 446 (LMV-11) | 60 | 71 | 100 | 16.9 | 3435 | 6.9 | 3.1 | 1 |
| INTA 2000 | 87 | 62 | 0 | 23.1 | 2888 | 9.0 | 3.0 | 2 |
| CT 122-49 | 87 | 58 | 0 | 23.6 | 2748 | 8.6 | 2.8 | 1.5 |
| IRAT 301 San Dionisio | 87 | 101 | 40 | 24.5 | 2561 | 9.3 | 3.4 | 2.5 |
| Fortuna (Local check) | 97 | 116 | 43 | 26.2 | 1174 | 8.5 | 2.6 | 2.5 |
| Mean | | 78 | 54 | 20.9 | 3590 | 8.3 | | |
| CV (%) | | 5.8 | 8.4 | 6.1 | 18.7 | 3.9 | | |
| F test variety | | *** | *** | *** | *** | *** | | |
| SE | | 2.6 | 2.6 | 0.7 | 388 | 0.2 | | |

[1] variety selected by farmers
[2] average score for general acceptability appreciated by 3 gender diversified farmer groups using a 1-3 scale: 1=bad; 2=intermediate; 3=good

**Table 9. Agronomic Results and Farmers' Appreciation of 12 new Rice Lines for High Altitude in Yorito, 2002**

| Variety Name | Days to flowering | Days to maturity | Plant height (cm) | Grain yield (kg/ha) | Farmers'Agronomic Score [1] |
|---|---|---|---|---|---|
| PRA 565-46-34-3-1-M (LMV-12) | 80 | 149 | 69 | 1688 | 4.5 |
| PRA 553-45-8-4-1-M (LMV-7) | 82 | 145 | 65 | 1298 | 3.5 |
| PRA 553-45-8-5-1-M (LMV-8) | 99 | 148 | 63 | 909 | 5 |
| PRA 565-46-42-1-1-M-M (LMV-13) | 101 | 150 | 80 | 584 | 4 |
| PRA 553-45-8-6-1-M-M (LMV-9) | 98 | 148 | 66 | 552 | 3.2 |
| CIRAD 447 (LMV-5) | 83 | 146 | 69 | 519 | 2.7 |
| PRA 565-46-64-1-1-M-M (LMV-16) CIRAD 446 | 101 | 154 | 83 | 390 | 2 |
| (LMV-11) | 89 | 145 | 74 | 324 | 5 |
| LMV-x | 101 | 148 | 65 | 259 | 4 |
| CIRAD 392 (Check) | 86 | 148 | 65 | 227 | 2.7 |
| PRA 553-45-8-7-1-M-M (LMV-10) | 90 | 147 | 81 | 195 | 4.7 |
| IRAT 364/90 | not flowered | - | 79 | | 1 |

[1] average score for general agronomic value appreciated by 8 farmers (4 men and 4 women) using the following scale: 1=bad; 3=intermediate; 5=good

Source: IPCA Honduras

**Table 10. Agronomic Performances of 51 New Lines from Conventional and Inter-Specific Crosses under Irrigated Conditions in Sebaco Valley, Nicaragua**

| Genetic origin | Pedigree | Days to flowering | Plant height (cm) | Lodge | Number of tillers/plant | Number of grains /panicle | 100 grains weight (g) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| 1 | CT 15672-12-1-7-1-2 | 85 | 94 | 1 | 23 | 81 | 17 | 8968 |
| 1 | CT 15691-4-3-1-1-4 | 83 | 96 | 1 | 22 | 85 | 17 | 8902 |
| 1 | CT 15696-3-4-2-1-1 | 77 | 90 | 3 | 19 | 82 | 18 | 6447 |
| 1 | CT 15716-6-1-2-1-1 | 81 | 87 | 1 | 16 | 96 | 13 | 8071 |
| 1 | CT 15727-14-3-2-4-1 | 82 | 88 | 1 | 23 | 110 | 22 | 9476 |
| 1 | CT 15727-14-3-2-4-2 | 81 | 91 | 1 | 22 | 67 | 30 | 8514 |
| 1 | CT 15765-2-1-2-1-1 | 86 | 99 | 1 | 28 | 63 | 23 | 8494 |
| 1 | CT 15765-2-1-2-3-2 | 86 | 96 | 1 | 21 | 63 | 26 | 8549 |
| 1 | CT 15765-2-1-2-3-3 | 83 | 96 | 1 | 19 | 94 | 22 | 7621 |
| 1 | CT 15765-2-1-4-2-2 | 83 | 98 | 1 | 19 | 78 | 21 | 8699 |
| 1 | CT 15765-2-1-4-2-4 | 82 | 92 | 5 | 16 | 71 | 24 | 7969 |
| 1 | CT 15765-2-1-4-2-6 | 81 | 89 | 1 | 19 | 45 | 29 | 6469 |
| 1 | CT 15765-12-1-2-2-1 | 82 | 93 | 1 | 18 | 77 | 19 | 6300 |
| 1 | CT 15765-12-1-2-2-2 | 83 | 93 | 1 | 22 | 78 | 25 | 7238 |
| 1 | CT 15765-12-1-2-2-3 | 78 | 89 | 1 | 19 | 69 | 30 | 6624 |
| 1 | CT 15765-14-2-3-1-3 | 83 | 81 | 1 | 18 | 78 | 20 | 5519 |
| 1 | CT 15765-14-2-3-2-1 | 78 | 82 | 1 | 19 | 78 | 25 | 5952 |
| 2 | CT 14551-3-M-M-1-2 | 83 | 95 | 1 | 20 | 100 | 24 | 6917 |
| 2 | CT 14555-7-M-M-1-3 | 84 | 109 | 1 | 18 | 76 | 19 | 7028 |
| 3 | CT 13941-27-M-5-4-M-M | 88 | 110 | 1 | 21 | 80 | 26 | 5915 |
| 3 | CT 13941-27-M-15-3-M-M | 84 | 93 | 1 | 35 | 47 | 22 | 6108 |
| 3 | CT 13941-27-M-19-1-M-M | 81 | 97 | 1 | 31 | 55 | 22 | 6176 |
| 3 | CT 13946-26-M-5-3-M-M | 80 | 96 | 3 | 29 | 60 | 33 | 8293 |
| 3 | CT 13946-26-M-5-6-M-M | 83 | 97 | 1 | 34 | 85 | 26 | 5996 |
| 3 | CT 13956-29-M-25-7-M-M | 80 | 99 | 1 | 19 | 70 | 19 | 6850 |
| 3 | CT 13958-12-M-1-7-M-M | 78 | 93 | 1 | 15 | 56 | 24 | 6950 |

| Genetic origin | Pedigree | Days to flowering | Plant height (cm) | Lodge | Number of tillers/plant | Number of grains /panicle | 100 grains weight (g) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| 4 | CT 14938-28-M-M-5-1-2 | 81 | 92 | 1 | 17 | 95 | 21 | 7094 |
| 4 | CT 14938-28-M-M-5-1-3 | 82 | 97 | 1 | 19 | 120 | 22 | 6700 |
| 5 | CT 15150-M-25-M-3-1 | 81 | 86 | 1 | 22 | 74 | 16 | 7555 |
| 5 | CT 15150-M-25-M-4-1 | 86 | 88 | 1 | 21 | 79 | 21 | 7643 |
| 5 | CT 15150-M-102-M-1-2 | 77 | 91 | 1 | 23 | 110 | 29 | 6294 |
| 5 | CT 15150-M-102-M-1-4 | 80 | 90 | 1 | 23 | 55 | 22 | 6503 |
| 5 | CT 15150-M-102-M-2-1 | 77 | 93 | 1 | 26 | 62 | 23 | 6406 |
| 4 | CT 14936-8-M-M-3-M-M | 81 | 104 | 1 | 20 | 58 | 30 | 5219 |
| 6 | CT 16049A-7-3-1-1-M-M | 81 | 94 | 1 | 26 | 75 | 23 | 8884 |
| 6 | CT 16049A-7-3-1-2-M-M | 79 | 95 | 1 | 27 | 114 | 25 | 8822 |
| 3 | CT 13937-16-2-M-M-2-M | 84 | 95 | 1 | 23 | 65 | 30 | 8353 |
| 3 | CT 13941-11-1-M-M-2-M | 77 | 102 | 1 | 24 | 63 | 20 | 9287 |
| 3 | CT 13941-11-1-M-M-10-M | 77 | 102 | 1 | 23 | 93 | 25 | 9627 |
| 3 | CT 13941-11-4-M-M-4-M | 77 | 101 | 1 | 28 | 81 | 26 | 9615 |
| 3 | CT 13941-11-4-M-M-8-M | 78 | 94 | 1 | 24 | 82 | 22 | 9535 |
| 3 | CT 13941-23-2-M-M-11-M | 97 | 97 | 1 | 35 | 127 | 25 | 7580 |
| 3 | CT 13943-10-2-M-M-6-M | 94 | 94 | 1 | 46 | 95 | 25 | 8536 |
| 3 | CT 13943-10-2-M-M-7-M | 97 | 93 | 1 | 43 | 86 | 25 | 7535 |
| 2 | CT 14539-26-M-3-M-2-M-M | 74 | 81 | 1 | 32 | 85 | 21 | 8803 |
| 2 | CT 14539-26-M-3-M-4-M-M | 81 | 81 | 1 | 24 | 111 | 22 | 8031 |
| 2 | CT 14539-26-M-3-M-7-M-M | 81 | 79 | 1 | 28 | 86 | 23 | 7490 |
| 2 | CT 14545-5-M-M-M-2-M-M | 80 | 78 | 1 | 32 | 68 | 22 | 7722 |
| 2 | CT 14545-5-M-M-M-3-M-M | 80 | 79 | 1 | 31 | 62 | 20 | 7953 |
| 2 | CT 14545-5-M-M-M-6-M-M | 81 | 85 | 1 | 19 | 91 | 28 | 7799 |
| 2 | CT 14546-6-M-M-M-9-M-M | 75 | 79 | 1 | 28 | 73 | 22 | 7722 |
| | Mean of selected lines | 82 | 92 | 1 | 24 | 79 | 23 | 7583 |
| | Local checks | | | | | | | |
| | ANAR 97 | 82 | 93 | 1 | 30 | 80 | 23 | 8463 |
| | INTA DORADA | 91 | 93 | 1 | 35 | 83 | 25 | 8149 |

| Genetic origin | Pedigree | Days to flowering | Plant height (cm) | Lodge | Number of tillers/plant | Number of grains /panicle | 100 grains weight (g) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| | INTA N-1 | 82 | 104 | 1 | 33 | 80 | 26 | 8511 |
| | International checks | | | | | | | |
| | FEDEARROZ 2000 | 80 | 95 | 1 | 23 | 75 | 28 | 7166 |
| | FEDEARROZ 50 | 80 | 94 | 1 | 20 | 78 | 30 | 8685 |
| | ORYZICA 1 | 84 | 96 | 1 | 30 | 70 | 25 | 8026 |

**Source: INTA/CIAT**

**Genetic origin:**
**1: $F_6$ CIAT/Peru**
**2: $BC_2$ *O. rufipogon/* Oryzica 3**
**3: Bg 90-2/*O. rufipogon***
**4: $BC_3$ Lemont*4/*O. barthii***
**5: $BC_2$ Bg 90-2/*O. glaberrima***
**6: $BC_3$ Bg 90-2/*O. glaberrima***

**Lodge: 1-9 scale where 1= no lodge and 9= 100 % lodging plants**

**Table 11. Agronomic Results and Farmers' Appreciation of 18 New Double Purpose Sorghum Lines in San Dionisio, 2002**

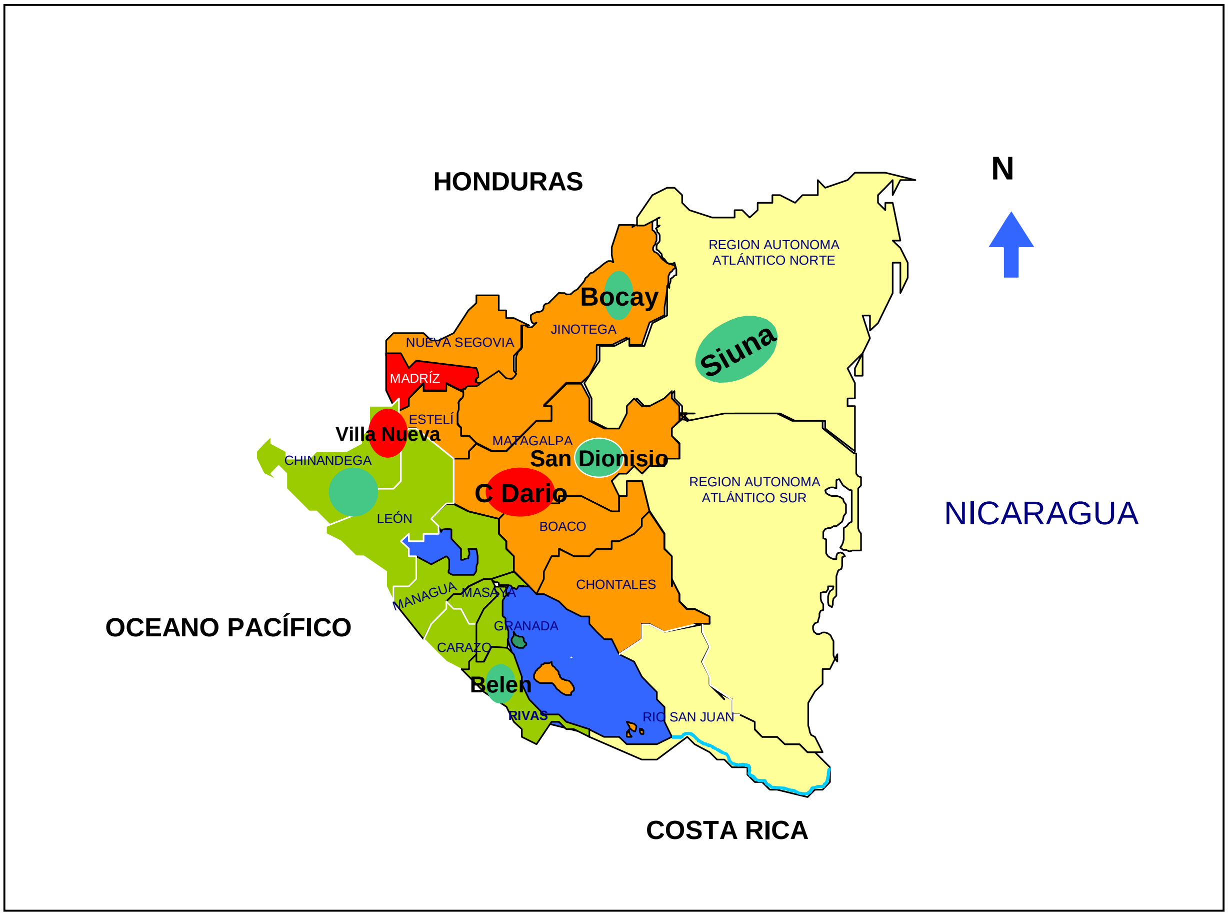
| Variety Name | Days to Flowering | Plant Height (cm) | Grain Yield (kg/ha) | Threshing Yield (%) | 1000 Grains Weight (g) | Kernel Color | Farmers' Scoring [3] |
|---|---|---|---|---|---|---|---|
| BF 89-18/133-2-1 | 60 | 222 | 3970 a[2] | 75 | 34 | White | 2.5 |
| IRAT 9[1] | 55 | 175 | 3310 ab | 79 | 27 | Red | 2.5 |
| CEF 322/36-1-1 | 59 | 175 | 3110 ab | 78 | 24 | White | 3.0 |
| CIRAD 437 | 59 | 192 | 3090 ab | 84 | 25 | White | 2.5 |
| CIRAD 438 | 64 | 169 | 3000 ab | 72 | 24 | White | 2.6 |
| Carta blanca (Check) | 64 | 216 | 2990 ab | 79 | 32 | White | 2.6 |
| CEF 418/1-3-2-1 | 59 | 198 | 2910 ab | 81 | 29 | White | 2.2 |
| IRAT 204 | 61 | 158 | 2810 ab | 77 | 31 | White | 2.7 |
| CIRAD 440 | 59 | 182 | 2770 ab | 80 | 27 | White | 2.7 |
| BF 89-12/1-1-1 | 61 | 204 | 2760 ab | 71 | 35 | White | 2.3 |
| CG 27/32-3-1 | 60 | 214 | 2510 ab | 75 | 26 | White | 1.4 |
| BF 88-2/34-1 | 65 | 168 | 2510 ab | 73 | 25 | White | 2.3 |
| Tortillero precoz (Check) | 62 | 157 | 2100 b | 73 | 32 | White | 2.5 |
| IS 20583 | 61 | 143 | 1740 b | 77 | 28 | White | 2.1 |
| CIRAD 406 | 60 | 214 | nc | nc | nc | White | 1.8 |
| CIRAD 436 | 57 | 198 | nc | nc | nc | Yellow | 1.1 |
| BF 94-6/11-1K-1K | 68 | 159 | nc | nc | nc | White | 2.8 |
| Pinolero 1 | 73 | 207 | nc | nc | nc | White | 2.6 |
| | | | | | | | |
| Mean | 61 | 186 | 2827 | 77 | 28 | | |
| VC (%) | 1.9 | 4.3 | 15.9 | | 3.1 | | |
| F test | *** | *** | * | | *** | | |
| SE | 0.84 | 5.62 | 318 | | 0.6 | | |

[1] Variety selected by farmers.
[2] Newman-Keuls Test: Treatments means with the same letter are not significant different at 0.05.
[3] Average score for general agronomic valor appreciated by 3 gender diversified farmers groups using a 1-4 scale: 1=bad; 2=intermediate; 3=good; 4=excellent.

**Table 12. Agronomic Results and Farmers' Appreciation of 17 New Photosensitive Sorghum Varieties in Somoto, 2002**

| Variety | Origin | Date of Flowering[2] | Plant Height (cm) | Lodge (%) | Hervested Heads | Grain Weight / Panicle (g) | Grain Yield (kg/ha) | 1000 Grains Weight (g) |
|---|---|---|---|---|---|---|---|---|
| Souroukoukou[1] | Mali | 30 Oct | 180 | 0 | 63 | 45 | 3050 | 34 s |
| 1825 | Camerun | 29 Oct | 364 | 0 | 66 | 37 | 2700 | 40 s |
| Millón Santa Cruz (Check 1) | Nicaragua | 16 Nov | 286 | 30 | 60 | 31 | 2020 | 26 |
| Millón Somoto (Check 2) | Nicaragua | 8 Nov | 304 | 10 | 53 | 34 | 1990 | 26 |
| PI 570072 | Sudan | 4 Nov | 343 | 0 | 61 | 28 | 1890 | 31 |
| PI 569465[1] | Sudan | 9 Nov | 317 | 0 | 41 | 40 | 1860 | 35 s[3] |
| PI 570078 | Sudan | 6 Nov | 340 | 0 | 59 | 28 | 1810 | 34 s |
| Wassoulou | Mali | 3 Nov | 454 | 0 | 34 | 46 | 1690 | 36 s |
| Millón Tomabu (Check 3) | Nicaragua | 10 Nov | 258 | 10 | 60 | 23 | 1530 | 28 |
| 1520 | Cam | 25 Oct | 304 | 0 | 30 | 41 | 1320 | 41 s |
| Kende 63 | Mali | | 290 | 0 | 59 | 19 | 1240 | 13 i |
| PI 570374 | Sudan | 6 Nov | 320 | 20 | 35 | 31 | 1180 | 21 |
| PI 569438[1] | Sudan | 4 Nov | 282 | 0 | 41 | 26 | 1180 | 40 s |
| 1401 | Chad | 20 Oct | 250 | 0 | 28 | 29 | 920 | 36 s |
| 1827 | Togo | 30 Oct | 305 | 50 | 71 | 8 | 620 | 8 i |
| 1581[1] | Camerun | 28 Oct | 250 | 0 | 9 | 72 | 600 | 72 s |
| 1423 | Burkina | 22 Oct | 227 | 20 | 56 | 8 | 555 i | 33 s |
| 1587[1] | Camerun | 30 Oct | 180 | 0 | 11 | 43 | 480 i | 49 s |
| W311 | Mali | 20 Oct | 173 | 0 | 22 | 19 | 430 i | 34 s |
| 1600[1] | Chad | 2 Nov | 230 | 0 | 4 | 51 | 230 i | 53 s |
| | | | | | | | | |
| Mean | | | 285 | | 43 | 33 | 1364 | 34 |
| CV (%) | | / | | | | 36 | 32.5 | 5.7 |
| F Test | | | | | | ** | *** | *** |
| SE | | | | | | 8.4 | | 1.4 |

[1] Variety selected by farmers
[2] Date of planting of June 4
[3] s, i = Significant superior or inferior to local checks mean according to Dunnett Test with probability of 0.05
[4] Average score for general desiderability appreciated by 3 farmers groups using a 1-4 scale: 1=bad; 2=intermediate; 3=good; 4=excellent


HONDURAS
N
REGION AUTONOMA ATLÁNTICO NORTE
Bocay
JINOTEGA
NUEVA SEGOVIA
Siuna
MADRÍZ
ESTELÍ
Villa Nueva
MATAGALPA
San Dionisio
CHINANDEGA
REGION AUTONOMA ATLÁNTICO SUR
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NICARAGUA
LEÓN
BOACO
CHONTALES
MANAGUA
MASAYA
OCEANO PACÍFICO
GRANADA
CARAZO
Belen
RIVAS
RIO SAN JUAN
COSTA RICA

**Map**

## OUTPUT 3. ENHANCING REGIONAL RICE RESEARCH CAPACITIES AND PRIORITIZING NEEDS WITH EMPHASIS ON THE SMALL FARMERS

### 3B. Education and Rice Cultivation as a Vehicle to Alleviate Poverty. Measurable Indicator: New Varieties and Germplasm

- **Brazil - Launches a New Variety Adapted to Upland Aerobic and Lowland Rice Ecosystems**

Orlando Peixoto de Morais, EMBRAPA Arroz e Feijão
Marc Châtel, CIAT/CIRAD-Colombia

The new variety named CURINGA originates from the conventional upland crossbreeding line CT13226-11-1-M-BR1. Crossing was made at CIAT in 1993 and involved 10 parents from different origins adapted to upland aerobic and lowland rice ecosystems (Table 1).

**Table 1. Genetic Constitution CURINGA**

| Parental line | Contribution (%) | Origin | Rice Ecosystem |
|---|---|---|---|
| IRAT 216 | 29.0 | CIRAD-Côte d'Ivoire | Upland |
| Tox 1780 | 17.6 | IITA-Nigeria | Upland |
| IAC 47 | 11.8 | IAC-Brazil | Upland |
| IAC 165 | 5.9 | IAC-Brazil | Upland |
| IRAT 124 | 5.9 | CIRAD Madagascar | Tropical Lowland |
| Camponi | 5.9 | Surinam | Tropical Lowland |
| Tox 1785 | 5.9 | IITA-Nigeria | Upland |
| Taipei 309 | 5.9 | Taiwan | Tropical Lowland |
| Ngovie | 5.9 | Liberia (Ex WARDA HQ) | Tropical Lowland |
| Quilla 65101 | 5.9 | INIA-Chile | Temperate Climate Lowland |

In 1996, breeding lines ($F_4$ generation) were shipped to Brazil and the EMBRAPA Rice and Beans Center followed-up the selection. The lines passed through the different agronomic evaluation trials and were identified as promising lines showing a broad adaptation to aerobic ("Ecosistema de Terras altas") and lowland ("Ecosistema de Várzeas") rice ecosystems.

The official launching of the variety is scheduled in late 2003 or early 2004.

- **Colombia - Launches the "Línea 30" as a New Commercial Variety for the Upland Savannas Ecosystem**

H. Delgado - CORPOICA Regional 8-Colombia
M. Châtel, Y. Ospina, F. Rodríguez, V.H. Lozano - CIAT/CIRAD-Colombia
J. Gómez, F. Correa, L. Calvert - CIAT-Colombia

Five years ago, a rice line adapted to the Colombian savannas ecosystem was identified as promising. The line comes from the CIAT/CIRAD upland conventional crossbreeding project. A number of yield trials conducted in experimental station and on-farm have demonstrated that this lines has good yield potential and earliness (3 months seed to seed). This material can be a component of the agronomic systems for the Colombian savannas, in rotation with other annual crops or in association with young perennial species. During 2002, and the first semester of 2003, CIAT and CORPOICA Regional 8 in Villavicencio jointly set-up efficiency trials that are the Colombian Institution ICA perquisites for the official launching of any plant material as commercial variety. The promising line is known "Línea 30" (CIAT), "Línea 60189" (CORPOICA) or CIRAD/CIAT 409 after it registration in the rice breeding catalogue of CIRAD

## Efficiency Trials

The efficiency trials were set-up with the funding support of the CIAT Rice Project at the La Libertad Experimental Station (LES) and on-farm in the Altillanura plain (Santa Cruz, La Palomera and Lagos de Menegua).

## Results

### Santa Cruz and La Palomera Farms

The line presented sterility on the secondary ramifications of 45.8 and 40.3% in average respectively (Table1) that affected grain production (2611 y 2451 Kg./ha respectively). Low temperature registered during the cropping season 2002, as it was reported before as well as the development of saprophytes (Table 2) was the main factor that reduced the grain yield. Nevertheless, the commercial plot sowed one month later in the season in the Santa Cruz farm, the Línea 30 yielded 3155 Kg./ha (Table 3). This indicates that the difference in yield on the same farm was because of low temperature during the flowering time of the first sowing date.

### Lagos de Menegua Farm

On this farm, the sowing date was on May 9, 2002 and cold temperatures did not affect the grain yield of the line during the flowering period. The line yielded 3806 Kg./ha or 300, and 558 Kg./ha the checks Oryzica Sabana 6 and Oryzica Sabana 10.

### La Libertad

The sowing date was May 17, 2002. The line yielded 3339 Kg./ha similar to Oryzica Sabana 6 with 3402 Kg./ha but lower than Oryzica Sabana 10 with 4350 Kg./ha.

## Disease Incidence

This year, the Linea 30 continues showing very low levels of leaf blast scoring (0-1) but some higher susceptibility to rhynchosporuim (leaf scald), helmintosporium (brown spot) and grain discoloration. As it was reported above, the line presented some level of grain sterility caused by cold temperature but not by neck blast. After 7 years of experimentation in different locations of the Colombian Altillanura plain the Línea 30 shows a high degree of tolerance on leaves and panicles. The plant pathology laboratory of CIAT assessed the pathogens present on the panicle collected at the Santa Cruz farm, and the incidence of rice blast is 8% (Table 2).

## Official Recommendation and Launching

The results of the efficiency trials during the cropping season 2002 and the anterior results of regional trials conducted since 1995 were submitted to the Colombian Institute ICA for consideration and recommendation of the Línea 30 as a new commercial variety for the Colombian savannas of the Altillanura plain. During the first semester of 2003, CORPOICA Regional 8 cultivated a demonstration plot and ICA recommended the line as variety. The official notification was in August 2003.

## Seed Multiplication and Distribution

Seed multiplication was made during the second semester of 2003 at the Experimental Station Santa Rosa, Villavicencio and 3.5 Tons of seed were harvested in March 2004. One ton of seed was given to CORPOICA Regional 8 and the rest was stored in the installations of FEDEARROZ of Villavicencio. The private company ARGEAGRO officially registered by the commercial legal office of Villavicencio showed interest in buying seed and CIAT responded with the selling of one ton of seed with MTA and a specific agreement for royalties. Mister Julio Roberto Camacho, who owns a farm in the Altillanura plain of Colombia and is a pioneer in annual crop production in the region also showed interest in buying seeds. CIAT sold one ton under the same terms as for ARGEAGRO. We are confident that the distribution of seed to the private sector is an appropriate manner to launch for the new variety.

**Table 1. Sterility (%) of the Line 60189. Efficiency Trials in the Altillanura Plain, 2002**

| Replication | "La Palomera" Sowing date: May 1 | "Santa Cruz" Sowing date: April, 29 | Commercial plot "Santa Cruz" Sowing date: June, 6 |
|---|---|---|---|
| I | 30 | 60 | 20 |
| II | 28 | 45 | - |
| III | 35 | 40 | - |
| IV | 68 | 38 | - |
| Average | 40.3 | 45.8 | - |

**Table 2. Diagnosis of Fungus Presence Line 60189. Efficiency Trial: Farm Santa Cruz 2002**

| Pathogen | Incidence (%) |
|---|---|
| Fusarium | 38 |
| Rhynchosporium | 35 |
| Curvularia | 35 |
| Penicillium | 13 |
| **Pyricularia** | **8** |
| Helminthosporium | 5 |
| Nigrospora | 5 |
| Cladosporium | 3 |

**Table 3. Agronomic Data of the Line 60189. Efficiency Trials in the Altillanura Plain, 2002**

| Location | Area (ha) | Vigor | Bl | NBl | LSc | Bs | Gd | Flowering (Days) | Average yield (Kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| La Libertad | 1 | 1-3 | 3 | 1-3 | 5 | 3 | 1 | 63 | 3632 |
| Lagos de Menegua | 0.2 | 1-3 | 1 | 1-3 | 5 | 1 | 1 | 62 | 3806 |
| Santa Cruz | 3 | 3 | 1 | 3 | 3 | 5 | 3 | 62 | 3155 |

Bl =Leaf blast NBl = Neck blast LSc = Leaf scald Bs = Brown spot Gd = Crain discoloration

**Table 4. General Behavior of the Line 60189 and Commercial Checks. Efficiency Trials: Santa Cruz, La Palomera and Lagos de Menegua Farms and La Libertad, 2002**

| Material | Vigor | Bl | NBl | LSc | Bs | Flowering (days) |
|---|---|---|---|---|---|---|
| O. Sabana 6 | 3-5 | 3-5 | 1-3 | 5 | 3 | 79 |
| O. Sabana 10 | 3 | 3 | 1-3 | 5 | 1-3 | 83 |
| Selecta 3-20 | 5 | 7 | 5 | 5-7 | 3-5 | 96 |
| Línea 30 | 1-3 | 1 | 3 | 5 | 3-5 | 60 |

Bl =Leaf blast NBl = Neck blast LSc = Leaf scald Bs = Brown spot

- **Bolivia - Launches Two New Upland Rice Varieties from Conventional Crossbreeding and Composite Population Breeding. CIRAD Upland Variety for the Small-Farmers Rice Ecosystem**

Roger Taboada, René Guzmán, Juana Viruez, V. Hugo Callaú, CIAT Santa Cruz-Bolivia
Marc Châtel, CIAT/CIRAD-Colombia

The new upland rice variety named JACUÚ (Sacia-9) is the CIRAD variety IRAT 357 that originates from conventional crossbreeding. The cross was made in Côte d'Ivoire and the variety was released in that country in 1989. It involves 2 parents, E 425 and IRAT 257. IRAT 357 was introduced in Bolivia through the upland INGER-LAC nursery managed by the former collaborative project between IRRI and CIAT.

The line passed through the local different agronomic evaluation trials and was identified as a promising variety well adapted to the small-farmers manual rice ecosystem. The main characteristics the farmers' praise are:

1. Grain yield (3600 Kg./ha)
2. Good initial vigor
3. Long and wide leaves that rapidly covers the soil (natural weed control)
4. Strong straws and good resistance to lodging
5. Tolerant to major diseases
6. Tolerant to drought
7. Medium duration cycle (130 days from seed to seed)
8. Plant height for manual harvesting (110-120 cm.)
9. Medium grain shape (popular grain type) and high seed weight (35.7 grams)

In 2002, the Rice Program of the Tropical Research Agriculture Center (Centro de Investigación Agrícola Tropical) of Santa Cruz de la Sierra (CIAT-Santa Cruz) produced the genetic seed. The official launching of the variety JACUÚ was in January 2003.

- **First Upland Rice Variety from Composite Population Breeding**

Roger Taboada, René Guzmán, Juana Viruez, V. Hugo Callaú. - CIAT Santa Cruz-Bolivia
M. Châtel, Y. Ospina, F. Rodríguez, V.H. Lozano. - CIAT/CIRAD-Colombia

In Bolivia, rice is a very important staple food. Consumption is steadily increasing reaching in the recent years 35 Kg. per capita. In Bolivia, the two main rice production ecosystems are (1) upland mechanize where the Province of Santa Cruz represents 75% of the total Bolivian rice area and produces 80% of the total production of the country and (2) the manual ecosystem is represented in all the Bolivian Provinces and the production is by small farmers.

The overall production is for the domestic market and auto-consumption. One of the main factors limiting the development of the rice sector is the lack of adapted varieties for each ecosystem. This has lead the rice program of CIAT Santa Cruz to invest in breeding with the objective of releasing new varieties better adapted. One of the outputs of the project is the release scheduled in early 2004 of a new germplasm that is the result of the close collaboration with the CIAT/CIARD upland rice composite population breeding project

## New Variety for Small Farmers and Mechanize Rice Ecosystem

The new upland rice variety with the code name SR 99343 is a CIAT/CIRAD line that originates from composite population breeding. The line was selected at La Libertad Experimental Station, Villavicencio-Colombia from the first cycle of recombination of the composite population PCT-4. The genetic composition of the composite population PCT-4 developed in Colombia is presented in Table 1. Segregating generations were advanced using the conventional pedigree method. The advanced line PCT-4\0\0\1>S2-1584-4-M-5-M-6-M-M was shipped along with many other materials to Bolivia for local evaluation.

At CIAT Santa Cruz-Bolivia, the line passed through the local different agronomic selection and evaluation trials and was identified as a promising variety well adapted to both manual smallfarmers and mechanize upland rice ecosystems. The agronomic characteristics and reaction to disease of the line are presented in the Tables 2 and 3. Yield potential data from the efficiency trial in 2003, for manual and mechanize rice ecosystem are presented in the Table 4 and the characteristics of the new variety is presented in the Table 5.

For the small farmers, the earliness of the variety and good yield potential are important qualities, allowing early harvest and easing crop rotation. Furthermore it permits the commercialization at a better price of the surplus production at a time when there is little rice in the market place. For the mechanize system, the variety shows good plant type and long grain shape which is the type preferred by the rice industry and market.

## Launching the Variety

CIAT Santa Cruz informed the Rice Project of CIAT-Colombia of the good behavior of the new germplasm recognizing the Intellectual Property Rights (IPR) of CIAT and CIRAD and invited the rice project participation to the launching ceremony scheduled for late February 2004.

## Table 1. Genetic Constitution of the Original Composite Population PCT-4

| Parent | Origin | Frequency (%) |
|---|---|---|
| CT6196-33-11-1-3-M | Upland line from CIAT | 8.33 |
| CT11231-2-2-1-4-M | Upland line from CIAT | 4.17 |
| CT11231-2-2-3-1-M | Upland line from CIAT | 4.17 |
| CT11231-2-2-2-1-2-M | Upland line from CIAT | 8.33 |
| CT11608-8-6-M-2-M | Upland line from CIAT | 8.33 |
| IR53167-3-M | Upland line from IRRI | 8.33 |
| A 8-394-M | Upland line from Brazil | 8.33 |
| CNA-IRAT A | Japonica composite population | 50.0 |
| **Genetic Constitution of the Japonica Composite Population CNA-IRAT A** | | |
| IRAT 104 | IRAT 13/Moroberekan | 6.25 |
| 53/2 | IRAT 2/IAC 25 | 12.5 |
| IRAT 257 | Mutante of Makuta | 6.25 |
| Batatais | Brazil | 6.25 |
| Batatais 1 | Brazil | 6.25 |
| IRAT 199 | Cuttack 4/IRAT 104 | 6.25 |
| Ligero | Brazil | 6.25 |
| CNA-IRAT 5 | Japonica gene pool | 50.0 |

## Table 2. Agronomic Characteristics. Regional Adaptation Trial, CIAT Santa Cruz. Summer, 2002-2003

| | Flowering (Days) | Plant height (cm) | Vigor | Grain length (mm) | 1000 seed weight (g) | Head rice (%) | White Belly | Temp. Gel. |
|---|---|---|---|---|---|---|---|---|
| **Line** | | | | | | | | |
| SR 99343 | 85 | 96 | 1 | 7,05 | 32,3 | 49,4 | 1,7 | High |
| **Check** | | | | | | | | |
| Jasayé | 100 | 108 | 1 | 6,82 | 36,1 | 56,4 | 4,1 | Interm. |
| Tutuma | 93 | 104 | 3 | 6,73 | 26,6 | 47,7 | 1,8 | High |

## Table 3. Disease Incidence. Regional Adaptation Trial, CIAT Santa Cruz. Summer, 2002-2003

| Location | Saavedra II | | | | | CRI-Yapacaní | | | | | San Pedro | | | | | Canandoa | | | | | Peta Grande | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bl | Lsc | Bs | NBl | Gd | Bl | Lsc | Bs | NBl | Gd | Bl | Lsc | Bs | NBl | Gd | Bl | Lsc | Bs | NBl | Gd | Bl | Lsc | Bs | NBl | Gd |
| **Line** | | | | | | | | | | | | | | | | | | | | | | | | | |
| SR 99343 | 3 | 3 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 |
| **Check** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Jasayé | 3 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 3 | 3 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 |
| Tutuma | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 5 | 1 | 3 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 |

Bl =Leaf blast NBl = Neck blast LSc = Leaf scald Bs = Brown spot Gd = Crain discoloration

## Table 4. Efficiency Trials. Manual and Mechanized Rice Ecosystems

| Grain yield. Upland Manual Ecosystem (Kg./ha) | |
|---|---|
| **Line** | |
| SR 99343 | 4706 |
| **Check** | |
| Jacuú | 4861 |
| Jisunú | 4115 |
| Cheruje | 3948 |
| Jasayé | 3713 |
| Tapeque | 3102 |

| Grain yield. Mechanized Ecosystem (Kg./ha) | |
|---|---|
| **Line** | |
| SR - 99343 | 4662 |
| **Check** | |
| Tari | 5380 |
| Epagri - 109 | 4633 |

## Table 5. Characteristics of the New Variety

| Agronomic Characteristics | |
|---|---|
| Average yield (Kg./ha) | 4091 |
| Vigor | Good |
| Flowering (Days) | 90 |
| Growing duration (days) | 120 |
| Plant height (cm.) | 112 |
| Lodging | Resistant |
| Panicle exertion | Good |
| Panicle length (cm.) | 22.8 |
| Grain/panicle | 161 |
| Shattering | Intermediate |
| **Diseases** | |
| Leaf blast | Resistant |
| Neck blast | Resistant |
| Brown spot | Intermediate |
| Leaf scald | Intermediate |
| Grain discoloration | Resistant |
| **Grain Characteristics** | |
| Porosity | No |
| 1000 grain weight (g) | 32.2 |
| Grain length -White rice (mm) | 7.60 |
| Grain width (mm) | 2.75 |
| Longitude/width | 2.76 |
| Temperature of gelatinization | High |
| Grain type | Long |
| White belly | 1.8 |
| Visual appearance | Good |

- **Chile - Develops a New Rice Site-Specific Composite Population with Cold Tolerance for the Temperate Climate Lowland Ecosystem**

Santiago Hernaiz L, José Alvarado A. - INIA Quilamapu, Chile
M. Châtel, Y. Ospina. - CIAT/CIRAD, Colombia

Conventional crossbreeding has permitted the selection of the commercial varieties actually planted in Chile with greater yield potential and grain quality. Data collected showed that they reached a yield plateau. The best Chilean rice producers are getting between 8.5 to 10 tons $ha^{-1}$ that seems very difficult to overpass. New breeding methods like composite population breeding are new tools that can help break the yield plateau.

A new site-specific composite population was developed in collaboration with the CIAT/CIRAD rice collaborative project. The parental lines used to set-up the population and its genetic constitution is presented in the Tables 1 and 2. The population named PQUI-2 was set-up by INIA Quilamapu Center - Chile and recombined at CIAT-Colombia. PQUI-2 is composed of japonica parental lines with cold tolerance from different geographic and genetic background. This is a guarantee that population breeding by recurrent selection and line selection could result in promising lines showing cold tolerance and high yield potential.

**Table 1. Parent of the Composite Population PQUI - 2**

| Parental line | Origin | Pedigree |
|---|---|---|
| CH 410-2 | INIA-Chile | B581-A6-545-2/Peta IR 276-1-6-9 / Kuatsu |
| Quila 121304 | INIA-Chile | Diamante / CT 6746 |
| Quila 68405 | INIA-Chile | Delta / Quila 29101* |
| Tuc 25 | INIA-Chile | - |
| CH 530-14 | INIA-Chile | Dw/T(N)1IR 151-4-19/Ch 101 (RR/B138-1-1/Oro) |
| Cinia 1014 | CIAT/INIA-Chile | CT 10809 |
| IR 13155-4-1 | IRRI-Filipinas | Bg90-2/KN-1B-214-1-4-3//IR28 |
| PRA 767-5CH | CIRAD-Madagascar | PRA 523/CIRAD 403 |
| PRA 775-1CH | CIRAD-Madagascar | PRA 622/Luluwini 22-M |
| PRA 741-1CH | CIRAD-Madagascar | Estrela/Long sweet glutinous rice |
| PRA 737-1CH | CIRAD-Madagascar | Cuiabana/Long sweet glutinous rice |
| PRA 760-1CH | CIRAD-Madagascar | Long sweet glutinous rice/Progreso |

* Krasnodarskj 3352//Gallardo/Kuatsut

**Table 2. Relative Participation of the Different Parents used for the Creation of the Composite Population PQUI-2**

| Parents | Relative participation (%) |
|---|---|
| PRA 760-1CH | 13,15 |
| PRA 767- 5CH | 8,20 |
| IRRI 13155-4-1 | 5,75 |
| PRA 775-1CH | 4,78 |
| CH 530-14 | 3,70 |
| QUILA 68405 | 3,40 |
| CH 410-2 | 3,10 |
| TUC 25 | 2,41 |
| QUILA 121304 | 2,15 |
| PRA 741-1 CH | 1,55 |
| CINIA 1014 | 1,30 |
| PRA 737 –1CH | 0,51 |
| **PQUI – 1 \CH\3\1** | **50,00** |

Measurable indicator: Workshops

- **International Upland Rice Breeding Seminar-Workshop for Latin America and the Caribbean (LAC). Villavicencio, Meta-Colombia, August 19-22, 2003**

M. Châtel, Y. Ospina, F. Rodríguez, V. Hugo Lozano, J. Gómez, L. Calvert

The CIAT/CIRAD rice collaborative project has regional responsibility and has created throughout the year's strong links with National Rice Breeding Programs of LAC. In late 1999, CIAT/CIRAD, FAO and LAC NARS established a Working Group on Advanced Rice Breeding (GRUMEGA in Spanish) managed by CIAT/CIRAD. More information about GRUMEGA is found at:
**http://www.fao.org/ag/AGP/AGPC/doc/riceinfo/America/AmCont.htm**

Within the networking activities are the offer of workshops in Colombia and helping NARS in doing the same at local level. The first International workshop took place in 2000 in Colombia; the second was in 2001 in Bolivia co organized with CIAT Santa Cruz and the Japanese cooperation in this country. The third workshop was organized by CIAT/CIRAD in August of this year. The main objective is the regional integration of upland rice breeders and to create a forum for sharing ideas and experiences as well as to have the opportunity to do participatory breeding of genetic material at field condition

**Workshop Funding**

Every invited participant was asked for fund raising within its country to support its participation but at the same time the CIAT rice project funded the organizing committee with US$ 4,000 in case of some participant could not raise sufficient funding. The estimated total coast of the event was US$ 10,000.

**Participants**

32 researchers from 7 countries (Bolivia; Brazil; Colombia; Cuba; Honduras; Nicaragua and Venezuela) attended the event (Table 1). As the programmed workshop coincided with the coming to CIAT of two people from the European Union for the review of the rice project they were invited to participate.

**Objectives of the Workshop**

1. Regional integration of the community of upland rice breeders
2. Creation of a forum for discussion and ideas sharing
3. Implementation of participatory breeding at field
4. Training of the less advanced rice breeders in composite population breeding (Honduras and Nicaragua)
5. To have the group concept about the outputs of the CIAT/CIRAD Rice Breeding Project
6. Selection of germplasm by the different breeders and follow-up of its use

**Duration: 4 Days**

1. Two days of technical presentations about the status and advances of upland rice research programs in LAC. 20 communications were presented: Argentina (1); Bolivia (2); Brazil (2); Colombia (10), Cuba (1); Honduras (1); Nicaragua (2) and Venezuela (1)

2. Two field days at the La Libertad and Santa Rosa Experimental Stations where the participants were able to observe and select the following genetic material:

   a) Segregating generations
   b) Composite populations
   c) Yield trials of promising lines
   d) Advanced lines from the upland CIAT-ION nurseries

All the lines selected by the different LAC breeders will be seed increased at Palmira Experimental Station and then shipped to the respective countries.

**Recompiling Information of the Germplasm Selected by the LAC Breeders' Panel**

The proceedings of the Seminar-Workshop are in preparation and will be published electronically and will also be available in the Web page of the Rice Project of CIAT.
**http://www.ciat.cgiar.org/riceweb/esp/inicio.htm**

Proceedings content:

- General information
- Abstracts of the communications
- Oral presentations slide show
- Results and analysis of germplasm selection by the LAC breeders panel

In this report we present the last point that we consider as the main output of the workshop.

**Line Selection: Segregating and Advanced Generations**

**General Comments**

The results of the selection by the panel of 11 LAC breeders are presented in the Table 2. In average, 71.8% of the observed lines were selected. Selection intensity varies depending of the status of the generation; it was of 63.3% in the early generation $S_1$ and 97.4% in the advanced generation $S_5$. This is in part because the more fixed is the line the easiest is the selection based on visual observation. For the CIAT/CIRAD breeding project, the high intensity of selection in the advanced generations by the breeders' panel is a very important data showing that the lines present good adaptation and are promising lines and future varieties for the Colombian savannas ecosystem.

**Specific Comments**

**Generation $S_1$**

The average selection intensity was 63.3% and the most selected lines (83.8%) were from the composite population PCT-11\0\0\2,Bo\2 coming from shuttle breeding with Bolivia (one cycle of selection in Colombia and one cycle of recurrent selection in Bolivia). This data justifies the implementation of this collaborative activity. The selection intensity of the lines coming from others composite population is about 50%.

**Generation $S_2$**

The average selection intensity was 80.3%. The highest selection intensity (92.8%) was in the composite populations PCT-4\SA\1\1\,SA\2\1 witch is the result of two cycles of recurrent selection in Colombia. This suggests that recurrent selection is an efficient breeding tool.

**Generation $S_3$**

The selection intensity of the unique population represented was 90.9% and is suggesting the overall good adaptation of the lines to the savannas rice ecosystem and the efficiency of the selection of the previous segregating generations. The composite population PCT-4\SA\4\1 comes from one cycle of recurrent selection followed by 3 recombination.

## Advanced Generations

The average selection intensity of the generations $S_4$ and $S_5$ was 83.3 and 97.4% respectively. These numbers clearly indicate that the selected advanced lines have a very good potential for the future development of varieties after passing through agronomic trials.

## Most Selected Material

30 lines (5.5% of the total number of observed lines) were the most selected ones by the breeders' panel (Table 3). They originate from different generations and composite populations. The following 5 lines were selected by more than 70% of the breeder's panel: PCT-4\SA\6\1>2; PCT-11\0\0\2,Bo\2>49; PCT-4\SA\5\1>1742-5; PCT-4\SA\4\1>330-1-2 and PCT-4\SA\2\1>10-2-3-1-1.

We will dedicate special attention in the follow-up of the selection of the most selected lines during next year cropping season as well as their adaptation in the breeding programs of LAC.

## Upland Rice Nurseries CIAT-ION

## General Comments

The results of the selection by the panel of 10 LAC breeders are presented in the Table 4. It is to notice that depending of the breeder the intensity of selection varies from 4.3 by INIA-Venezuela up-to 88.1% by DICTA-Honduras. The other breeders of the panel selected about 10% of the observed lines, except IIA-Cuba and Fundación DANAC-Venezuela with 29.4 and 19.4% respectively.

## Specific Comments

The result of the selection are presented in the table5, and are arranged within 3 ranking groups of selection intensity:

1. Lines selected by up-to 30% of the breeders panel
2. Lines selected by 30 to 50% of the panel
3. Lines selected by more than 50% of the panel

The most selected nurseries were CIAT-ION SC and CIAT-ION SSR.

## Most Selected Material

13 materials (6,2% of the total number observed) were the most selected ones by the breeders' panel (Table 6). This material are considered as highly promising and special attention will be put in future agronomic evaluation in Colombia as well as in the different

countries that received and evaluated the CIAT-ION nurseries.

The following 4 materials were selected by more than 70% of the breeder's panel:

1. CT13576-1-2-M-1-M from CIAT-ION SC
2. WAB901-7A1.1-1 from CIAT-ION SI WARDA
3. PCT-4\SA\1\1>721-M-2-M-4-M-6-M-2-M from CIAT-ION SSR
4. PCT-4\SA\1\1>721-M-4-M-1-M-5-M-1-M from CIAT-ION SSR

## Conclusion

Workshops with participatory selection by breeders is a very useful mechanism in different fronts: (1) every participant of the workshop is now well aware about the activities of the different upland rice breeding projects in LAC; (2) breeders know better what the offer of the CIAT/CIRAD upland rice project is; (3) in-site line selection by each national breeder make them better appropriate the genetic resource they selected and (4) as the CIAT/CIRAD upland breeding project is regional, the result of the selection is an important input. We know better what kind of breeding lines and associated traits they like and need.

## Table 1. List of Participants

| Country | Institution | Participant |
|---|---|---|
| **Bolivia** | CIAT Santa Cruz | Roger Taboada Paniagua |
| | | Juana Viruez |
| **Brazil** | EMBRAPA Arroz e Feijão | Beatriz Pinheiro |
| | | Orlando Peixoto |
| **Colombia** | CORPOICA Regional 8 | Diego Aristizábal |
| | | Hernando Delgado |
| | FEDEARROZ | Harold Bastidas |
| | | Alberto Dávalos |
| | Semillas El Aceituno | Roberto Simmonds |
| | CIAT | Joachim Voss |
| | | Lee Calvert |
| | | César Martínez |
| | | Fernando Correa |
| | | Diego Molina |
| | | Jaime Gómez |
| | | Diana Delgado |
| | | James Carabalí |
| | | Jaime Borrero |
| | | Mónica Triana |
| | CIRAD/CIAT | Francisco Rodríguez |
| | | Victor Hugo Lozano |
| | | Marc Châtel |
| | | Yolima Ospina |
| | | Joanna Dossmann |

| | | |
|---|---|---|
| **Cuba** | IIA | Rubén Alfonso |
| **Honduras** | DICTA | Napoleón Reyes |
| **Nicaragua** | INTA | Lázaro Narvaez Rojas<br>Marlon Ortega |
| | CIRAD/CIAT | Gilles Trouche |
| | Misión Técnica China - Taiwán | Victor Kuo |
| **Venezuela** | INIA | Gelis Torrealba |
| | Fundación DANAC | Carlos Gamboa<br>Yorman Jayaro |
| **European Union** | GTZ Germany | Andreas Springer-Heinze |
| | NRI United Kingdom | Tim Chancellor |

## Table 2. Selection of Segregating and Advanced Lines by the Breeders' Panel

| Material | | Nbr. of line | | Nbr. of selected lines (%) |
|---|---|---|---|---|
| | | Evaluated | Selected | |
| **Population Generation $S_1$** | | | | |
| PCT-4\SA\1\1,SA\3\1 | | 59 | 31 | 52,5 |
| PCT-4\SA\6\1 | | 42 | 21 | 50,0 |
| PCT-4\SA\1\1,Bo\2 | | 75 | 38 | 50,7 |
| PCT-11\0\0\2,Bo\2 | | 117 | 98 | 83,8 |
| CNA 7\Bo\2\1 | | 53 | 31 | 58,5 |
| | **Total** | 346 | 219 | 63,3 |
| **Population Generation $S_2$** | | | | |
| PCT-5\PHB\1\0,PHB\1,PHB\1,PHB\1 | | 4 | 3 | 75,0 |
| PCT-4\SA\1\1\, SA\2\1 | | 14 | 13 | 92,8 |
| PCT-4\SA\5\1 | | 27 | 23 | 85,2 |
| PCT-4\SA\1\1,Bo\1 | | 14 | 10 | 71,4 |
| PCT-11\0\0\2,Bo\1 | | 34 | 27 | 79,4 |
| CNA-7\Bo\1\1 | | 19 | 14 | 73,6 |
| | **Total** | 112 | 90 | 80,3 |
| **Population Generation $S_3$** | | | | |
| PCT-4\SA\4\1 | | 33 | 30 | 90,9 |
| | **Total** | 33 | 30 | 90,9 |
| **Population Generation $S_4$** | | | | |
| PCT-4\SA\1\1,SA\1\1 | | 6 | 4 | 66,7 |
| PCT-11\0\0\3 | | 6 | 6 | 100, |
| | **Total** | 12 | 10 | 83,3 |
| **Population Generation $S_5$** | | | | |
| PCT-4\0\0\2 | | 8 | 8 | 100 |
| PCT-4\SA\2\1 | | 24 | 24 | 100 |
| PCT-4\SA\4\1 | | 7 | 6 | 85,7 |
| | **Total** | 39 | 38 | 97,4 |
| **Cross Generation $F_7$** | | | | |
| CT10069 | | 7 | 7 | 100 |
| | **Total** | 7 | 7 | 100 |
| Grand Total | | 549 | 394 | 71,8 |

**Table 3. Most Selected Lines**

| | Most Selected Lines | |
|---|---|---|
| | **Nbr. of Selection** | **In % of the Panel** |
| **Generation $S_1$** | | |
| PCT-4\SA\1\1,SA\3\1>40 | 5 | 45.4 |
| PCT-4\SA\1\1,SA\3\1>18 | 5 | 45.4 |
| PCT-4\SA\6\1>2 | 9 | 81.8 |
| PCT-11\0\0\2,Bo\2>39 | 5 | 45.4 |
| PCT-11\0\0\2,Bo\2>47 | 5 | 45.4 |
| PCT-11\0\0\2,Bo\2>49 | 8 | 72.7 |
| PCT-11\0\0\2,Bo\2>51 | 7 | 63.6 |
| PCT-11\0\0\2,Bo\2>76 | 6 | 54.5 |
| PCT-11\0\0\2,Bo\2>81 | 5 | 45.4 |
| CNA 7\Bo\2\1>53 | 5 | 45.4 |
| **Generation $S_2$** | | |
| PCT-4\SA\1\1\,SA\2\1>164-2 | 6 | 54.5 |
| PCT-4\SA\1\1\,SA\2\1>1059-1 | 7 | 63.6 |
| PCT-4\SA\5\1>1360-2 | 6 | 54.5 |
| PCT-4\SA\5\1>1742-5 | 9 | 81.8 |
| PCT-11\0\0\2,Bo\1>19-1 | 5 | 45.4 |
| PCT-11\0\0\2,Bo\1>62-2 | 5 | 45.4 |
| **Generation $S_3$** | | |
| PCT-4\SA\4\1>302-2-5 | 5 | 45.4 |
| PCT-4\SA\4\1>330-1-2 | 10 | 90.9 |
| PCT-4\SA\4\1>330-2-2 | 7 | 63.6 |
| PCT-4\SA\4\1>330-2-4 | 6 | 54.5 |
| PCT-4\SA\4\1>330-3-2 | 5 | 45.4 |
| **Generation $S_4$** | | |
| PCT-11\0\0\3>1497-M-1-2 | 6 | 54.5 |
| **Generation $S_5$** | | |
| PCT-4\SA\2\1>10-2-1-2-4 | 5 | 4.4 |
| PCT-4\SA\2\1>10-2-1-2-5 | 5 | 4.4 |
| PCT-4\SA\2\1>10-2-1-3-1 | 6 | 54.5 |
| PCT-4\SA\2\1>10-2-1-3-2 | 5 | 45.4 |
| PCT-4\SA\2\1>10-2-3-1-1 | 8 | 72.7 |
| PCT-4\SA\2\1>10-2-3-2-3 | 5 | 45.4 |
| PCT-4\SA\2\1>10-2-5-1-4 | 6 | 54.5 |
| PCT-4\SA\2\1>44-3-1-1-1 | 7 | 63.6 |

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## Table 4. Result of the Selection of the CIAT-ION

| | | Nurseries CIAT-ION | | | | | |
|---|---|---|---|---|---|---|---|
| | | SC* | SI** | | SSR*** | Total of line | |
| | | Number of line evaluated in each nursery | | | | | |
| | | 27 | 118 | | 66 | 211 | |
| | | | (30 CIAT) | (88 WARDA) | | | |
| **Country** | **Institution** | **Number of line selected in each nursery** | | | | **Total** | **%** |
| Brazil | EMBRAPA Arroz e Feijão | 2 | 0 | 10 | 0 | 12 | 5.7 |
| Colombia | Corpoica | 1 | 0 | 6 | 8 | 15 | 7.1 |
| | Semillas El Aceituno | 4 | 4 | 21 | 11 | 40 | 18.9 |
| Cuba | IIA | 9 | 7 | 21 | 25 | 62 | 29.4 |
| Honduras | DICTA | 26 | 28 | 67 | 65 | 186 | 88.1 |
| Nicaragua | CIAT/CIRAD | 7 | 4 | 12 | 4 | 27 | 12.8 |
| | INTA | 3 | 0 | 9 | 7 | 19 | 9.0 |
| | Chinese Mission - Taiwan | 8 | 0 | 4 | 7 | 19 | 9.0 |
| Venezuela | Fundación DANAC | 3 | 0 | 22 | 16 | 41 | 1.4 |
| | INIA Guárico | 2 | 0 | 5 | 2 | 9 | 4.3 |

* SC= Conventional Crossbreeding ** SI= Inter-specific cross *** SSR= Recurrent selection breeding

## Table 5. CIAT-ION Nurseries. Selection Intensity

| | | Selected material | | | | | |
|---|---|---|---|---|---|---|---|
| | | Up to 30% of the panel | | 30 - 50% of the panel | | More than 50% of the panel | |
| | | Selected lines | | | | | |
| **CIAT-ION nursery** | **Evaluated lines** | **Selected** | **%** | **Selected** | **%** | **Selected** | **%** |
| SC | 27 | 21 | 77.8 | 3 | 11.1 | 3 | 11.1 |
| SI CIAT | 30 | 27 | 90.9 | 3 | 10.0 | 0 | 0.0 |
| SI WARDA | 88 | 55 | 62.5 | 20 | 22.7 | 4 | 4.5 |
| SSR | 66 | 44 | 66.7 | 15 | 22.7 | 7 | 10.6 |

## Table 6. CIAT-ION. Most selected lines

| Nursery and line | Most selected line | |
|---|---|---|
| | Number of selection | In % of the panel |
| **CIAT-ION SC** | | |
| CT13576-1-2-M-1-M | 9 | 90 |
| CT13576-1-4-M-1-M | 6 | 60 |
| CT13572-3-3-M-2-6-1-M | 6 | 60 |
| **CIAT-ION SI WARDA** | | |
| WAB759-54-2-3-HB-2 | 6 | 60 |
| WAB894-B-5A2.1-4 | 6 | 60 |
| WAB901-7A1.1-1 | 7 | 70 |
| WAB901-7A1.1-3 | 6 | 60 |
| **CIAT-ION SSR** | | |
| PCT-4\SA\1\1>721-M-2-M-4-M-2-M-3-M | 6 | 60 |
| PCT-4\SA\1\1>721-M-2-M-4-M-6-M-2-M | 7 | 70 |
| PCT-4\SA\1\1>721-M-4-M-1-M-4-M-1-M | 5 | 50 |
| PCT-4\SA\1\1>721-M-4-M-1-M-4-M-4-M | 6 | 60 |
| PCT-4\SA\1\1>721-M-4-M-1-M-5-M-1-M | 7 | 70 |
| PCT-4\SA\1\1>721-M-4-M-1-M-5-M-2-M | 6 | 60 |

## OUTPUT 3. ENHANCING REGIONAL ICE RESEARCH CAPACITIES AND PRIORITIZING NEEDS WITH EMPHASIS ON THE SMALL FARMERS

### 3C. Impact of CIAT Upland Rice Varieties on Resource-Poor Farms in the Peruvian Amazon

- **Impact of CIAT Upland Rice Varieties on Resource-Poor Farms in the Peruvian Amazon**

Douglas White, Efraín Leguía, José Sánchez, Sam Fujisaka

### Introduction

Upland rice farmers have few assets other than natural capital. Natural resources such as soils, however, are in most cases low quality; and financial resources are almost always in short supply, thereby limiting agricultural production. To meet household food consumption needs, farmers cultivate a variety of traditional crops such as rice, maize, plantain, and cassava. Excess production is sold in local markets.

Rice is an important crop of many upland farm families, though harvests remain meager. Many rice varieties developed over a decade ago are still cultivated and produce yields that are at best around 1.2 t $ha^{-1}$. Such yields match those of irrigated rice production forty years ago prior to the Green Revolution. But while upland rice yields stagnated, irrigated rice yields have boomed. The development of new irrigated varieties, improved infrastructure and the use of chemical inputs enable yields today of 7 to 10 t $ha^{-1}$ (Jennings et al. 2002).

Upland rice farmers have become marginalized. The continued low productivity of upland rice, in part, demonstrates an emphasis to realize large production gains. Such a research strategy led to substantial economic benefits for some producers but even more so for consumers as food prices declined. Yet the strategy came at a cost of systemic neglect for upland rice farmers who often have few options to earn a living.

A challenge remains of improving upland rice productivity without requiring the purchase of expensive inputs. CIAT and partners are developing new varieties that meet these needs. As in the case of modern irrigated rice varieties: newer upland rice varieties are N-responsive and out-perform traditional varieties even without additional chemical inputs. The objective of this paper is to review the impact of a research and development effort conducted in the Pucallpa region of the Peruvian Amazon where farmers tested and selected new upland rice varieties.

Field interviews reveal that farmers continue to plant two of the modern varieties first introduced in 1999 by CIAT and the national agricultural research institute (INIA). Approximately nine upland rice varieties are planted in the region. Average yields in both controlled farmer trials and farmers' fields of traditional (i.e. older) varieties are about 1.8 t $ha^{-1}$ while modern varieties produce 2.2 t $ha^{-1}$, a 20% increase. Despite the financial benefit realized with improved yields, the early maturity characteristic of new varieties enables farmers to earn even more. Rices with both increased yield and early maturity characteristics can raise household income by about 7% (White et al. 2003a). Early rices: 1) require less labor input (i.e. one less weeding ), 2) can be harvested when the household labor supply is not as scarce, 3) marketed earlier when market prices are higher, 4) permit farmer to cultivate rice two times per year, and 5) are subject to a shorter exposure period of field hazards.

Early maturing rices also have important beneficial social and environmental impacts. The lessened labor requirement enables farmers to choose between increasing rice production and diversifying into other activities. Smallholder livelihoods improve with greater and more stable earnings. Also, farmers can more successfully plant in fallows rather than only on plots of newly-converted forest. More intensive use of fallow land may lessen the need to convert new areas to agricultural use. Although these environmental and social benefits are likely to reduce the need for settlers to migrate to new areas (White et al. 2003b) and although such technology impacts upon the forest can be estimated in a shorter-term, on-farm context--the wider and longer-term implications of technology improvements are difficult to foresee clearly (Angelsen and Kaimowitz, eds. 2001, White et al. 2001).

Despite these benefits, relatively few farmers in Pucallpa plant new rices. Widespread adoption is slow. Farmers merely share new varieties with neighbors and family relations. In some instances, farmers keep the varieties a secret in order to maintain a competitive edge. A formal dissemination strategy does not exist in the region because government staffing changes and budget cutbacks during the past four years have hampered a consistent research and development strategy for rice and other crops. Only with a comprehensive strategy of varietal development, post-harvest storage, extension, fostering of farmer organizations and marketing information can resource-poor smallholder farmers earn more from rice.

### Research Site Context

Many Peruvians live in poverty. After Bolivia, Peru has the second highest incidence of poverty in South America. The dualistic agricultural sector in Peru is in a process of transition. Although an increasing number of farmers use modern agricultural practices, the vast majority cultivates crops on lands with low productivity using traditional methods. Scarce financial resources and restricted government funding make agricultural improvements difficult, so farmers make do with the few available resources available.

Many settlers migrate to and within the Amazon with the hope of earning a better living. Yet after clearing forested land for agricultural use, soil fertility and associated bountiful harvests are typically short-lived. Rapid nutrient depletion due to low levels of soil organic matter and leaching of surface nutrients due to heavy rainfall and invasive weeds restrict annual crop cultivation to a few seasons. Such slash-and-burn or shifting cultivation practices are sustainable when fallow periods are of sufficient duration. In many instances, 15-30 years are required for land to recuperate. In areas of higher population density, smallholder farmers are compelled to shorten fallow cycles, thereby reducing the ability of the land to recover (Nye and Greenland, 1960; Ruthenberg, 1976).

Pucallpa, Peru is situated in the western region of the Amazon basin. It is an evolving forest margins region with a variety of farmer types and associated land uses. Agricultural practices range from slash-and-burn methods employed by recently arrived settlers to cattle ranches of the wealthier absentee-owners. Large-scale settlement began in 1940 when a road linking Pucallpa to the capital, Lima, was completed (Figure 1). Pucallpa serves as the principle market for a majority of farm products. The city has a population of approximately 250,000 inhabitants and lies on the shores of the Ucayali River, a major tributary of the Amazon.

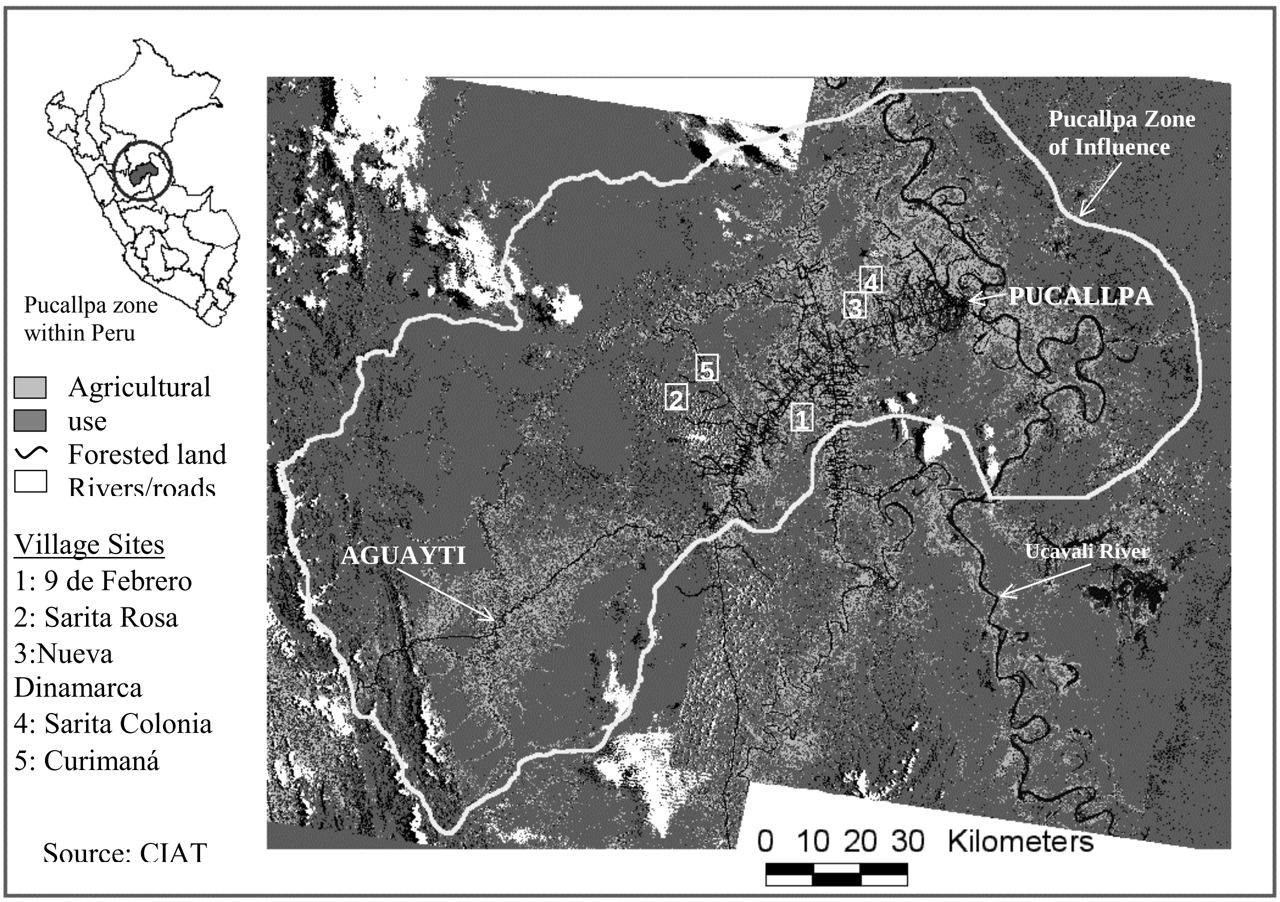


**Figure 1. Research Site of Pucallpa Peru**

Mean annual rainfall is 2300mm, ranging from 1700 in Pucallpa to 3000 mm in Aguaytía, a smaller city in the foothills, about 160 km to the west-south-west. Rainfall follows a bimodal pattern with wet months of February-April and October-December, and dry months June-August and December-February (Figure 2). The mean annual temperature is 25°C with mean daily high and low of 31°C and 20°C respectively. Soils include those found in alluvial, riverine systems, where pH is about 7.7 and available P is 15 ppm, and poorer upland soils that are acidic (pH 4.4), and have only 2 ppm of available P (Loker, 1993).

The resource-poor farms of this study are located in the well-drained lands away from the rivers. These farms are not situated along the highway to Lima where older, larger cattle ranches predominate, but are along the secondary roads and behind the ranches accessed by short tertiary links. Such farms are resource-poor in terms of financial capital: few have any relation with banks, public or private. Often small animals are sold when urgent cash needs arise. In terms of infrastructure, household dwellings are rustic, often with dirt floors. Electricity, potable water and adequate sanitation are rare. Road access is seasonally variable. While the Pucallpa-Lima road is paved, its deteriorated state and the bad quality of the secondary roads cause frequent service interruptions during the rainy season. Transportation costs are high due to the maintenance costs of rapidly depreciating vehicles being passed onto the fare-paying consumers.

## Seasonal Labor and Rice

Settlers in the Peruvian Amazon cultivate a mix of traditional subsistence crops such as rice, maize, cassava, beans, and plantain. Labor required for agricultural production varies over the year (Figure 2). Farmers prepare the land with slash-and-burn techniques before the onset of the wet season. Soon after, they plant their crops (August) and hand weed (September-October). At the end of this wet season, the high labor-demand period of the harvest occurs (January and February). The bars in their entirety represent the total amount of farm labor required for the cropping systems, while the lower darker section depicts the amount of labor needed to produce the minimum household food requirement. Although labor inputs for the food household requirement are extremely variable throughout the year, subsistence requirements never exceed more than half of the family labor availability in one month (White et al. 2003a).

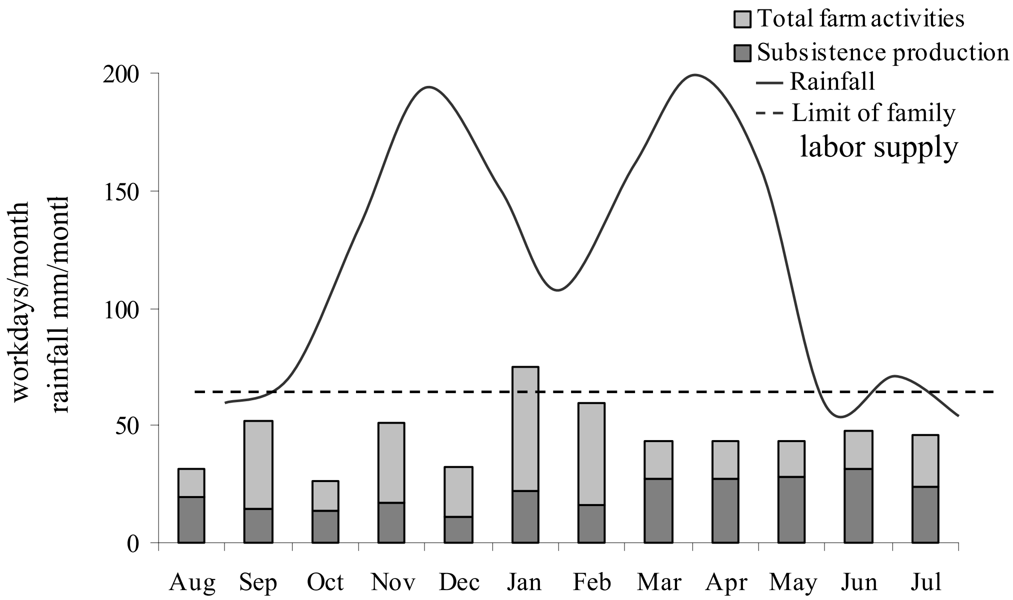


**Figure 2. Monthly Rainfall and Labor Requirements (Total and Subsistence Production Activities) of a Typical Established Settler Farm**

In such remote land-abundant areas, labor rather than land is typically the limiting input to farm production (Boserup, 1965; Binswanger and McIntire, 1987). Capital is almost always scarce for smallholder farmers and thereby restricts their ability to hire laborers. Especially in the forest margins, returns to labor better represent the criteria that smallholder farmers use to evaluate land use options (Collinson, 2000; Vosti et al. 2000). Technology innovations that require few labor inputs during high labor demand periods are more likely to be adopted. Some new rice varieties, described below, enable farmers to reduce labor bottlenecks (White et al. 2003a).

Labor requirements for upland rice production vary according to the agricultural calendar and plot location (Table 1). Rice cultivation requires distinct tasks throughout the year starting with land preparation and ending with harvest, threshing, drying and sales. Less overall labor is required for rice production in fallows due to easier land preparation. Weeding requirements in fallows, however, are greater than in previously forested plots.

**Table 1. Traditional Rice Labor Requirements in Forest and Fallow Lands per Ha**

| Activity | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rice in forest plot** | | | | | | | | | | | | | |
| Slash | - | - | - | - | - | - | - | - | - | - | - | 21 | 21 |
| Tree Felling | 21 | - | - | - | - | - | - | - | - | - | - | - | 21 |
| Planting | - | 10 | - | - | - | - | - | - | - | - | - | - | 10 |
| Weeding | - | - | 8 | - | - | - | - | - | - | - | - | - | 8 |
| Harvest | - | - | - | - | - | 20 | - | - | - | - | - | - | 20 |
| Other* | - | - | - | - | - | 3 | - | - | - | - | - | - | 3 |
| Total labor | 21 | 10 | 8 | - | - | 23 | - | - | - | - | - | 21 | 83 |
| **Rice in fallowed plot** | | | | | | | | | | | | | |
| Slash | 11 | - | - | - | - | - | - | - | - | - | - | 10 | 21 |
| Planting | - | 10 | - | - | - | - | - | - | - | - | - | - | 10 |
| Weeding | - | - | 11 | 11 | - | - | - | - | - | - | - | - | 22 |
| Harvest | - | - | - | - | - | 20 | - | - | - | - | - | - | 20 |
| Other* | - | - | - | - | - | 3 | - | - | - | - | - | - | 3 |
| Total labor | 11 | 10 | 11 | 11 | - | 23 | - | - | - | - | - | 10 | 76 |

Other includes: drying, sales.

## Diverse Farms of the Amazon

This study compares two types of smallholder slash-and-burn farmers: pioneer and established. Average farm size is approximately 25 ha. Pioneers convert high forest or older fallows (secondary forests) ranging from 6-20 years of age to obtain nutrients from the biomass and to take advantage of reduced pests and weed levels. Traditional long crop-fallow rotations typically start with upland rice in the first year followed by two years of maize, plantain or cassava. In contrast, established farmers practice bush fallow agriculture. These shorter crop-fallow rotations involve the same annual food crops, usually grown for three years, followed by 2-4 years of natural bush fallow. Weed invasions and lower soil fertility often lead to lower yields as compared to those of longer fallows. Approximately 2 ha of annual crops are planted with a smaller area dedicated to perennials such as citrus. Often kudzu (*Pueraria phaseoloides*), an aggressively growing legume, fixes nitrogen during the fallow period (Yanggen and Reardon, 2000) although much is volatilized. Small animals such as chickens and pigs are common. Although few of the resource-poor farmers have cattle, farmers often establish pastures in the hope of someday having them (White et al. 2001). On average, fallow areas of different ages cover about 1/3 of the farm and approximately 3 ha is still in high forest (Fujisaka, 1997).

The amount of remaining on-farm forest is associated with farm type and inversely related to the age of settlement. In the more recently inhabited areas, 59% of the farm remained forested, whereas in more mature settlements, forest coverage decreases to 40%. Conversely, the land area dedicated to pastures generally increases according to the age of the settlement. Pastures cover 60% of the larger cattle ranches. Recent smallholders settlers have about 5% in pasture, whereas established farmers have 19% (Fujisaka and White, 1998).

The Pucallpa region produces both irrigated and upland rice. Upland rice is typically planted in areas of newly-felled forest: 88% of respondents plant rice as the first crop. Rice is also the first crop planted in the converted fallows: 73% of farmers follow this practice. Maize, cassava or plantain typically follows in the cycle (Labarta, 1998). In the Ucayali department, only 200 ha of rice are irrigated using gravity systems. Average yield is 6 t $ha^{-1}$ ha with a range from 5 to 8 t $ha^{-1}$ (Manuel Vásquez, personal communication). In contrast, the average yield of traditional upland rice varieties is 1.8 t $ha^{-1}$, with a range of 0.7 - 3.0 t $ha^{-1}$. Similarly, average yields of other crops are often modest (Table 2).

**Table 2. Traditional Crop Yields of Pucallpa Slash-and-Burn farms (Source: White et al. 2003; Labarta 1998)**

| Crop | Mean | Minimum | Maximum |
|---|---|---|---|
| Rice (t/ha) | 1.8 | 0.7 | 3.0 |
| Maize (t/ha) | 2.0 | 1.2 | 3.0 |
| Bean (t/ha) | 1.0 | 0.3 | 1.2 |
| Cassava (t/ha) | 13.0 | 9.0 | 18.0 |
| Plantain (bunches/ha) | 741 | 300 | 900 |

Smallholder farmers face marketing challenges. Not only yields, but market prices are relatively low and variable. The relative isolation of the region from the rest of the country and the rudimentary local and regional infrastructure network exacerbate marketing costs. For products to reach international markets, they need to be transported down the longest river or across some of the tallest mountains in the world. Such conditions frustrate farmers' attempts to be competitive in a global marketplace. Prices received for traditional crops are not considered high; but most price variation is reasonably low (Table 3). For the years 1996-2000, plantain had the lowest standard deviation of price variability while that of beans was the highest.

**Table 3. Descriptive Statistics of Crop Prices ($/kg) in Pucallpa, Peru 1996-2000 (Source: Ministry of Agriculture, 2000)**

| Crop | Min | Max | Average | Std Dev |
|---|---|---|---|---|
| Plantain | 0.03 | 0.10 | 0.06 | 0.04 |
| Cassava | 0.02 | 0.08 | 0.05 | 0.05 |
| Rice | 0.08 | 0.24 | 0.14 | 0.10 |
| Maize | 0.05 | 0.20 | 0.12 | 0.11 |
| Citrus | 0.06 | 0.22 | 0.12 | 0.17 |
| Bean | 0.27 | 0.76 | 0.47 | 0.43 |

Rice prices vary during the year (3.6 Peruvian Sol ≈ US$1). Both the January/February harvest of the upland rice and the August/September harvest from the riverine area are met with lower market prices in the Pucallpa market (Figure 3). Prices are inversely related to regional and national production levels. Annual price differences are often caused by national policy. For example, in 1998 an agricultural credit program that promoted rice cultivation led to a marked production increase and depressed prices the following year (Figure 4). The negative impact of the el Niño year (1997) can also be seen rice decreased production in the riverine areas of Pucallpa.

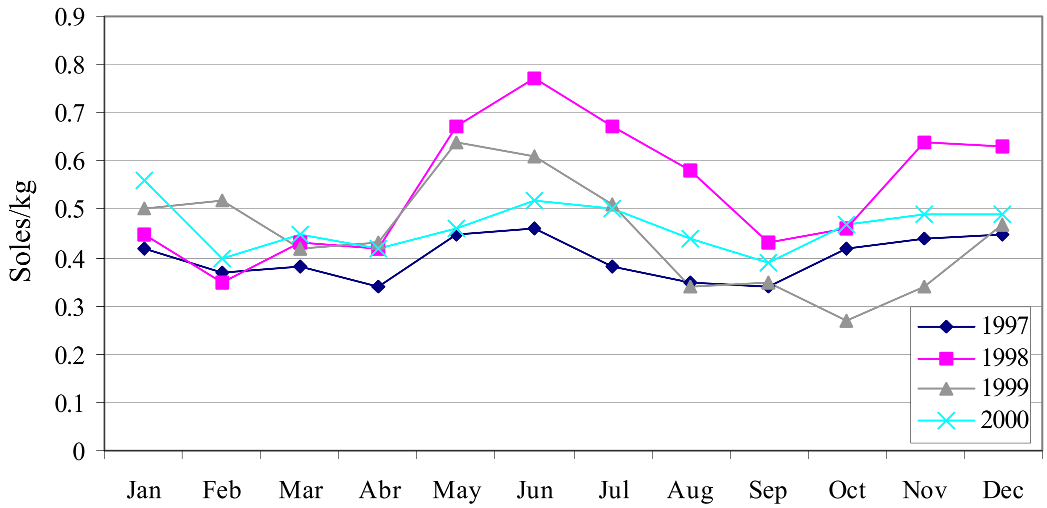


**Figure 3. Monthly price of rice in Pucallpa market 1997-2000. (Source: Ministry of Agriculture, 2000)**

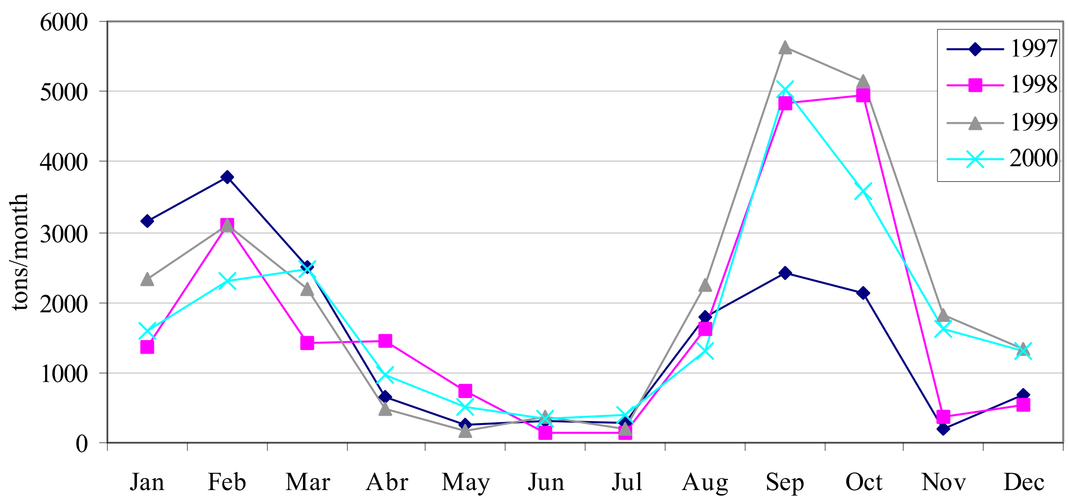


**Figure 4. Monthly rice production in Ucayali 1997-2000. (Source: Ministry of Agriculture, 2000)**

## Rice in the National Context

Throughout Peru, rice production and consumption increases yearly. Rice production for 2000 was 1.11 million tons (milled). But production is not stable. While the 2000 level represents a 47% increase over 1991, 70,000 fewer tons were produced in comparison with the previous year (Figure 5). The majority of Peru's rice production is irrigated (paddy rice) in the departments of Lambayeque and Piura in the north and Arequipa in the south (USFAS, 2000).

In the major production zones, rice quality and yields vary greatly. Average yields are 6.3 t $ha^{-1}$ but some farmers produce as high as 14 t $ha^{-1}$ (USFAS, 2000). Smallholder farmers produce the majority of the rice supply. Thus, chemical input levels are highly dependent on changing prices, national economic conditions and credit policies.

Annual rice consumption is estimated at 46 kilograms per capita. Rice is sold in traditional markets by weight from 50 kg sacks. In recent years, however, Peru has seen a rapid expansion of supermarkets chains. As a result, consumers increasingly purchase prepackaged one-kilogram bags of rice, which now total 13 percent of all rice sales. Higher quality rice, especially imported, is also marketed in this manner (USFAS, 2000).

Currently, Peru has few support programs for the agricultural sector. The government purchases limited quantities from local producers to support social food programs. Production supports, however, are more elaborate. After years of government neglect, rice production was encouraged in 1998 with input credit and rotating fund programs. This policy resulted in a bumper crop in 1999 that was disastrous to farmers: prices plummeted and caused many to lose money. A flooded market, large carry-over stocks and no possibility of exporting (other than what is smuggled into Ecuador through the northern border) created added difficulties for a short-term recovery. In many areas, farmers were not able to repay loans; and in more extreme cases they lost their lands to creditors. The May through September harvest period of the major rice producing regions affected prices even in Pucallpa markets. From August to November 1999, prices declined to their lowest levels during the 1997-2000 period (Figure 3).

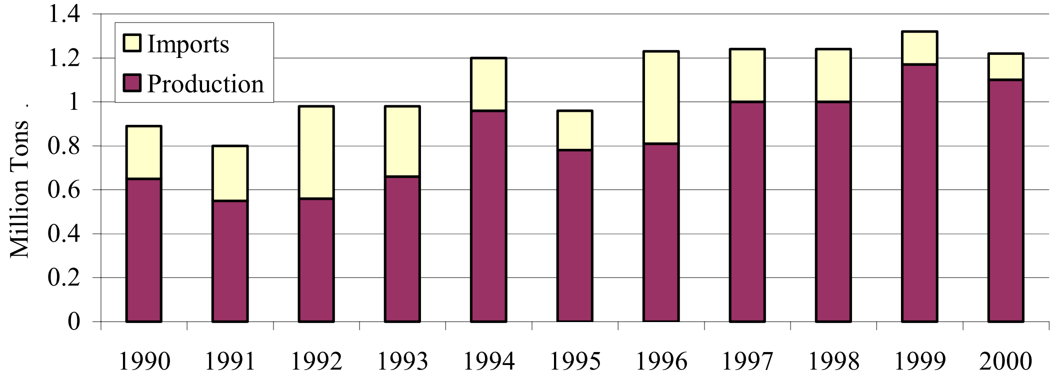


**Figure 5. Peruvian Rice Production and Imports 1990-2000 (Source: USFAS, 2000)**

The United States and Uruguay have shared the market of rice imports into Peru since the Peruvian government implemented a ban on Asian rice, ostensibly based on phytosanitary concerns. Rice imports are assessed a 25 percent import duty, an 18 percent value-added tax, and a variable levy, which depends on international prices (USFAS, 2000).

## CIAT Rice Research in Pucallpa

The CIAT Systems for Smallholder project initiated a participatory research project with new rice varieties in 1998. The decision to conduct rice research was based upon a diagnosis of the Pucallpa site where farmers prioritized rice as an important crop requiring scientific inquiry (Fujisaka et al. 1999). Rice research followed a tradition of CIAT efforts in the forest margins. Both the pastures and cassava projects conducted research in the same region. Other CGIAR centers, ICRAF and CIFOR, also work in the area with many activities pertaining to the Alternatives to Slash-and-Burn system-wide program of the CGIAR. This section is divided into two parts. The first describes the rice trial work and reviews the major results. The second section presents results of a farm survey (n=60), which focused on rice production and variety adoption, conducted in July 2003.

## Rice Trials

Farmers wanted to solve problems associated with their upland rice. The research objective (in work led by Fujisaka and his team) was to increase farmer participation and to gradually, and in step-wise fashion, widen their scope of such participation in the research process. Scientists first worked with farmers interested in testing new rice varieties. Seed was provided for small plot trials. Researchers helped farmers in the use of scientific methods such as replications, controls, crop cut sampling, moisture correction of yields, and yield calculations. Farmers selected varieties and trial layouts, and managed all trial operations. During the five planting seasons of what turned out to be a two-year project, field visits and field days provided continual feedback among farmers and researchers.

Farmers tested the five introduced rices: CT-11253-6-1-M-M (a CIAT line), Porvenir 95, INIA 14, Ucayali 91, Capirona, and Uquihua along with their "traditional" varieties, Carolino (released in the 1940s, 125 cm height, 120 day duration, low N response), Chancabanco (from CIAT, released in 1984, 90-100 days, 110-140 cm, low N response) and Aguja Blanca (135 days, 125 cm) in the sowings starting in early November 1997 and continuing until early February 1998. Yields of the introduced varieties were not always superior to yields of farmers' varieties because the rice materials also included lowland varieties that were introduced to farmers as such. An early project goal was to start the participatory testing, albeit with an understanding by farmers that the obtaining of seed of more appropriate upland rices was in process. Yields of the new varieties on farmers' fields were satisfactory (mean 0.91 t/ha); but again not superior to farmers' own varieties (0.94 t/ha) in Table 4. Although plantings suffered heavily from el Niño related drought, farmers were not discouraged. Some farmers adopted trial varieties for use on their farms.

**Table 4. Rice Varieties and Yields of 1997 – 1998 Campaign (Source: Fujisaka and Labarta Field Data, March 1998)**

| Variety | Average yield (t/ha) | Standard Deviation | Number of participating farmers |
|---|---|---|---|
| **New** | | | |
| CT-11253-6-1-M-M | 1.15 | 0.5 | 8 |
| Porvenir-95 | 1.23 | 0.5 | 11 |
| INIA-14 | 1.07 | 0.4 | 10 |
| Ucayali-91 | 0.76 | 0.5 | 8 |
| Capirona | 0.64 | 0.3 | 18 |
| Uquihua | 0.59 | 0.3 | 11 |
| Average of means | 0.91 | 0.5 | |
| **Traditional** | | | |
| Carolino | 1.08 | 0.4 | 5 |
| Chancabanco* | 0.90 | 0.8 | 6 |
| Aguja Blanca | 0.83 | 0.4 | 7 |
| Average of means | 0.94 | 0.4 | |

* Calculated at 14% humidity of rice grain
** Early-maturing variety

During the next planting period (September through October 1998), farmers tested the introduced varieties CIRAD 409 (upland rice adapted to acid soils from IRAT and CIAT lines developed by CIAT/CIRAD, 90 days), IRAT 146 (upland rice cross of IRAT 13 from the Ivory Coast and Dourado Precoce from Brazil), Sabana 6 (CT7244-9-2-1-52-1; upland rice adapted to acid soils released in Colombia in 1991), Sabana 10 (CT6196-33-11-1-3-M released in Colombia in 1995), and Progresso (upland rice based on CIAT crosses released in Brazil; all parenthetical rice descriptors from Chatel 2003, personal communication).

Mean yields were slightly higher than of traditional varieties, 2.06 t/ha in period 4 and 1.9 t/h (Table 5). Farmers continued to adopt selected introduced varieties on their own fields.

**Table 5. Rice Varieties and Yields of 1998 – 1999 Campaign (Source: Fujisaka and Labarta Field Data, March 1999)**

| Variety | Average yield (t/ha) | Standard Deviation | Number of participating farmers |
|---|---|---|---|
| **New** | | | |
| O. Sabana 10 | 2.18 | 0.78 | 29 |
| CIRAD 409** | 2.13 | 0.92 | 27 |
| IRAT 146** | 2.11 | 0.66 | 26 |
| Progresso | 1.93 | 0.91 | 26 |
| O. Sabana 6 | 1.89 | 0.73 | 22 |
| Average of means | 2.06 | 0.80 | |
| *Traditional* | | | |
| Carolino | 2.70 | 0.47 | 4 |
| Chancabanco** | 2.40 | 0.66 | 9 |
| Aguja Blanca | 1.54 | 0.66 | 21 |
| Others | 1.85 | 0.49 | 2 |
| Average of means | 1.90 | 0.77 | |

* Calculated at 14% humidity of rice grain
** Early-maturing variety

The "traditional" Chancabanco had the highest yields during the entire project horizon (Figure 6). Chancabanco or "Bank Breaker" was introduced in the 1980s of CIAT parentage and has been widely adopted. It acquired the name from local lore. At that time, the agrarian bank was obligated to purchase agricultural harvests. An unexpectedly large harvest of Chancabanco broke the bank by straining the government capacity to make the payments to farmers.

While Chancabanco may have high yields, grain quality is inferior. A high percentage of grains break during milling, thereby reducing its market value and cooking quality. Three of the "new" introduced varieties, Sabana 10, CT and CIRAD 409 had higher yields than the other traditional varieties. Most of the new varieties had higher grain and culinary quality.

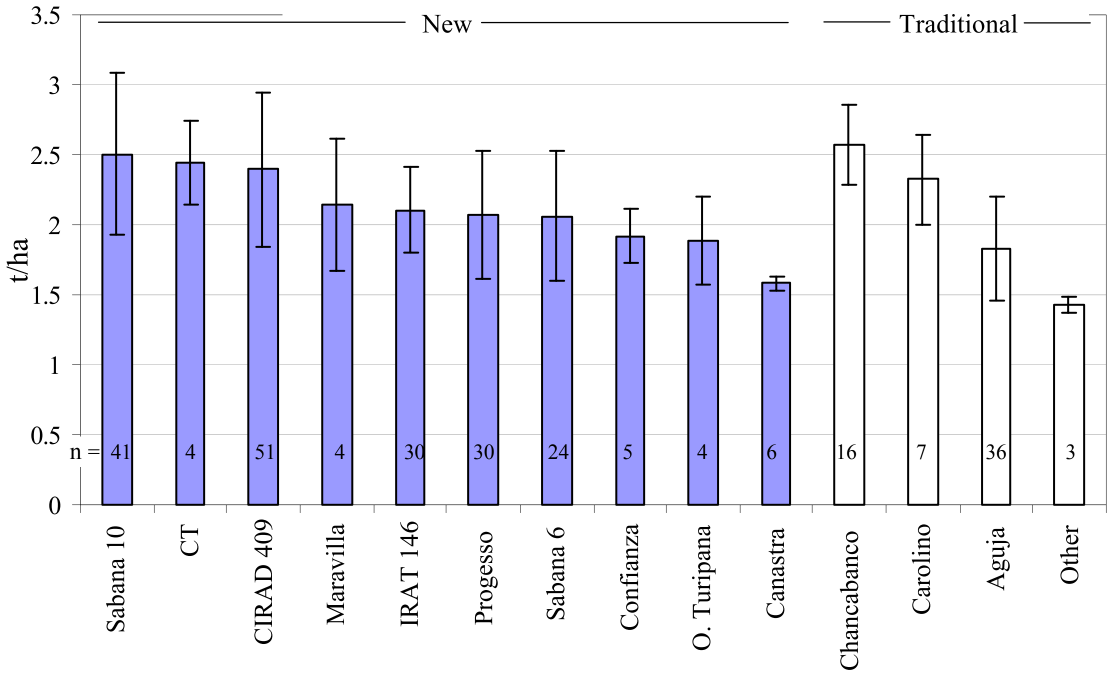


**Figure 6. New and Traditional Varieties, Average Mean and Standard Deviation of Yield (Source: Fujisaka and Labarta Field Data 1999, 2000)**

Yield is not the only important criteria for rice production. Farmers are interested in many other characteristics. During a field day consultation with farmers in April 1999, the following preferences of varieties were mentioned (Table 6).

**Table 6. Preferred Traits of Rice Varieties**

| Variety | Early maturity | Pest and disease resistance | Large panicle | Grain quality | Milling quality | Cooking quality | No lodging | Threshing ease | Not itchy | High tillering |
|---|---|---|---|---|---|---|---|---|---|---|
| Cirad 409 | x | x | | x | x | | x | | | |
| Sabana 10 | | x | x | | | | | | | x |
| Maravilla | x | x | x | x | | x | | | | |
| Chancabanco | x | | | | | | | | x | |
| Aguja | | | | x | x | x | | x | x | |
| Carolino | | | | | x | | | x | x | |

## Rice Variety Adoption

In July 2003, researchers returned to interview 60 farmers about farm management and rice varieties planted. The five communities visited represent the diversity of slash-and-burn farm types, which are categorized into established farmers and pioneers. The two types of sites can be found on the Pucallpa map in Figure 1**.** Average age of established farmers is slightly higher than that of pioneers (Table 7). They also arrived at the farm a few years earlier. These estimates do not necessarily reflect the establishment year of the farm since migration is common to the region. Average farm size of pioneers is more than 50% larger than established farms. Variation about the mean of these summary statistics is quite high reflecting the inherent heterogeneity of the farms.

| **Established (n=28)** | **Pioneer (n=32)** |
|---|---|
| Nueva Dinamarca | 9 de Febrero |
| Sarita Colonia | Sarita Rosa |
| Curinamá | |

**Table 7. Summary Statistics of Pucallpa Farmers and Farms**

| | Age (Male) | Arrival Year | Farm Size (ha) |
|---|---|---|---|
| Established | | | |
| Average | 44.0 | 1994.8 | 22.2 |
| Standard Deviation | 12.3 | 4.8 | 15.0 |
| Max | 78 | 2001 | 60 |
| Min | 26 | 1980 | 2.5 |
| Pioneer | | | |
| Average | 41.5 | 1996.7 | 34.1 |
| Standard Deviation | 12.5 | 4.8 | 14.9 |
| Max | 74 | 2003 | 84 |
| Min | 21 | 1980 | 4 |

Pioneer farmers tend to plant more rice area than established farmers on average (Figure 7). While pioneers also cultivate larger areas of maize, plantain, and beans, established farmers have greater extensions of cassava, pasture and fruits. This difference in crop mixtures reflects a common evolution of slash-and-burn systems (Fujisaka and White, 1998). Cassava production can withstand lower fertility soils. And longer-term investments in pastures (cattle) and fruit trees are being realized. Again, the large standard deviations about the mean estimate reflect the diversity of agricultural systems.

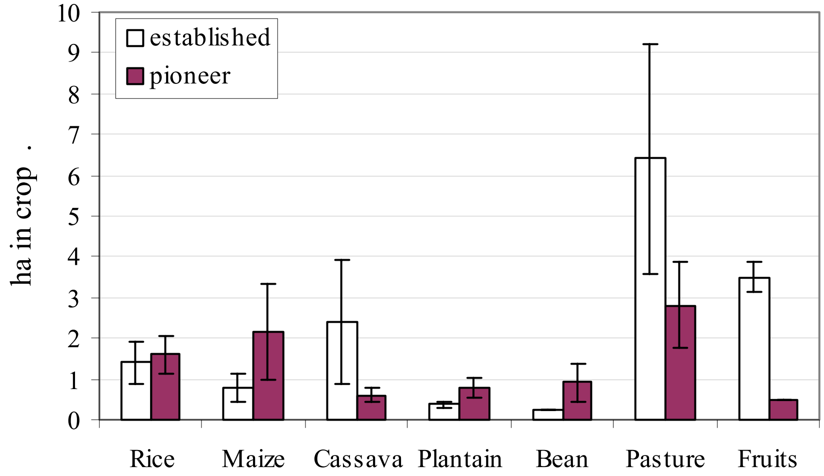


**Figure 2. Cultivated Areas of Selected Crops, Established and Pioneer Farmers**

Pucallpa farmers plant an array of rice varieties (Figure 8). Only two of them are new, CIRAD 409 and Maravilla. The use of the varieties varies, as does the average yield. Farmers planted traditional varieties in 80 instances versus 28 with new varieties. Aguja Blanca was the variety most often sown, followed by Chancabanco, both traditional. The new varieties demonstrated relatively high average yields (~2.3 t $ha^{-1}$). Of the traditional varieties, Chancabanco produced higher yields on average (~2.0 t $ha^{-1}$), approximately 0.3 t $ha^{-1}$ more than either the Aguja Blanca or Carolino.

Farmers often plant different rice varieties according to distinct objectives. Varieties produced for household consumption have higher grain quality but lower yield. Farmers consider the traditional Aguja Blanca and new CIRAD 409 and Maravilla varieties as high quality. Market-oriented rices typically have traits of higher yield but of lower grain quality. Chancabanco, for instance, produces a higher proportion of broken grains. Since high quality rice rarely receives a price premium from buyers and mills, most farmers sell the lower quality rice.

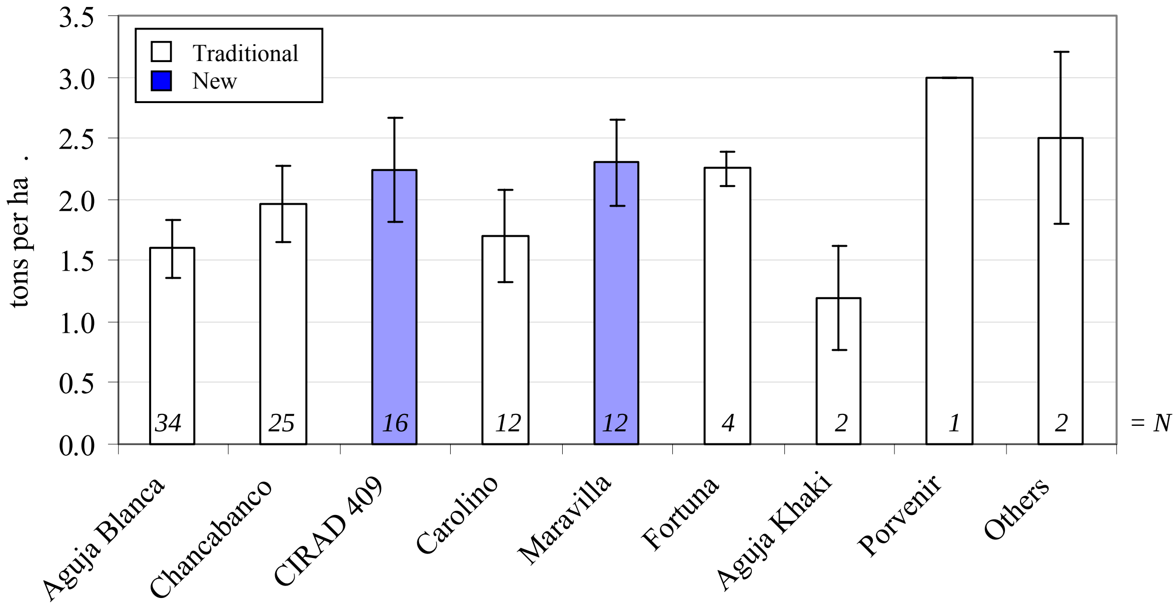


**Figure 3. Average Yields of Traditional and New Rice Varieties Planted in the Pucallpa Region**

Differences in average rice yield are apparent between established and pioneer farms. Where both types of farms planted a variety, established farms produced slightly higher yields with Aguja Blanca (1.7:1.6), Chancabanco (2.1:1.9) and CIRAD 409 (2.3:2.0). These results run counter to the conventional wisdom that the best rice harvests occur in newly-deforested areas. The adaptation of the early maturity rices to low soil fertility and their ability to compete with weeds, enables higher rice yields in fallows. Only with Carolino did pioneer farmers produce slightly higher yields (1.8:1.6). The relatively small sample size of the study points to a need for further research to better strengthen statistical significance of analysis results.

Pioneer farmers on average harvest approximately 150 kg more rice than established farmers (Figure 9). Despite slightly lower yields, the larger areas dedicated to rice made this possible. Pioneer farmers sold more rice than established farmers and only consumed about 50 kg less. Harvests from other crops may permit pioneers to sell more of their production. Also, rice is a relatively easy crop to transport and sell in markets, thereby causing a relatively large proportion to be sold. (Again, the precision of these mean estimates is not high; therefore inference must be made with caution.)

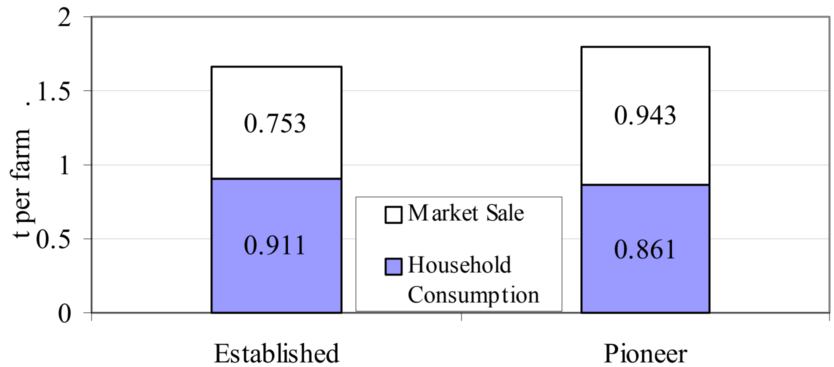


**Figure 4. Market Sale and Household Consumption of Rice by Pioneer and Established Farmers**

The sale of rice per season is worth approximately US$150 to the pioneer family and US$120 to the established farmers. The use of new varieties enables farmers, with 20 ha, to earn an additional US$55 per year (White et al. 2003a). This represents about 7% of total farm earnings. The benefit from the early maturity characteristic of new varieties often surpasses the financial benefit realized with higher yields. Early maturing rices have many direct benefits to farmers:

1. They typically require less labor input (i.e. one less weeding).
2. The harvest can occur when the household labor supply is not as scarce.
3. Production can be marketed earlier when market prices are higher.
4. Farmers can cultivate rice two times within the year.
5. Rapid establishment enables the plant to more adequately compete against invasive weeds.
6. Shorter time duration in the field reduces the probability of production losses due to disease, pests and drought.

Early maturing rices also have important beneficial environmental and social impacts that are more subtle and indirect:

1. Farmers can more successfully plant in fallows rather than on only plots of newly-converted forest. Fallow land can used more intensively, thereby potentially reducing the need to deforest.
2. A lessened labor requirement enables farmers to increase rice production or diversify into other activities. Smallholder livelihoods improve with greater and more stable production.
3. These environmental and social benefits are likely to reduce the need for settlers to migrate to new areas.
4. As with the introduction of any technology, the need to carefully consider tradeoffs is likely. While such technology impacts upon the forest can be estimated in a shorter-term, on-farm context, the wider and longer-term implications of technology improvements are difficult to foresee clearly (Angelsen and Kaimowitz, White et al. 2001).

Benefits of rice production also accrue to areas outside the Pucallpa region. The production of lower quality rice enables many poor citizens in the Andes to purchase rice at a lower cost. The lesser quality grain of Chancabanco, for example, has a lower price. Thus, a smaller proportion of a household budget can be spent on food. The Ucayali department exports 40% of rice produced to other departments of Peru.

## Discussion

Despite the financial, environmental and social benefits, relatively few farmers plant new rice varieties. Widespread adoption is relatively slow for a number of reasons. Farmers share new varieties only with close neighbors and family relations. At times new varieties are kept a secret in order to perpetuate personal advantage such as early sale and higher market prices. In addition, various institutional and organizational shortcomings limit the adoption process.

## National Agricultural Research Strategy

A formal dissemination strategy of new germplasm no longer exists in the region. Government staffing changes and budget cutbacks during the past four years have hampered a consistent research and development strategy for rice and other crops. The national agricultural research institute (INIA) is still in the process of undergoing drastic change. Organizational restructuring continues with a second year of priority-setting exercises. All research stations across the nation have been affected. One of the most notable positive differences is an increase in scientists' salaries, which were woefully inadequate. Researchers now earn respectable wages. Funds to finance the increase came, in part, from layoffs of what were seen as less-necessary field technicians and staff. But despite these salary improvements, operational funds remain scarce.

A decentralization process of government responsibilities and budgets from Lima to the departments continues. Instead of monies being apportioned from the capital, decisions are being made more locally. Now the Ministry of Agriculture office in Ucayali must address its budget cutback and shortfall by requesting funds from the departmental government.

Research remains divorced from extension. While INIA has the mandate of research and technology transfer, the extension staff remains under the Ministry of Agriculture. This structural problem manifests itself in many ways. For example, in the Ucayali department, the Ministry of Agriculture is a separate building, blocks away from the INIA station.

Farmers generally lack access to improved germplasm. Many agricultural extensionists employed during the Fujimori administration (1990-2001) have been dismissed. Some non-government development efforts do provide technical assistance in agriculture. Crops such as pineapple, oil palm, and cotton receive NGO and sometime private industry assistance.

**CIAT Strategy**

Like INIA, CIAT has also experienced organizational turbulence. In 1999, human resources were redirected to other geographic regions. Thus continuity of the participatory rice research project was broken when responsibility transferred among different scientists.

**Potential Geographic Scope of Impact**

The early maturing varieties developed by CIAT and partners can benefit many smallholder farmers in other regions of South America. A climate model identifies other areas in which the new varieties are like to thrive (Figure 10). Darker regions reflect a higher probability of similar climate conditions to those found Pucallpa. Climate variables assessed are monthly rainfall patterns and totals along with high and low daily temperatures (Jones, 2003). Large areas of western Brazil and north-eastern Bolivia have very similar climate conditions. Small areas in Mexico, Colombia and Venezuela are also analogous. Other spatial models can be calibrated to estimate how soil characteristics may affect the performance of different rice varieties. These, bio-physical models can be used in conjunction to more accurately estimate potential impact of upland rice varieties.

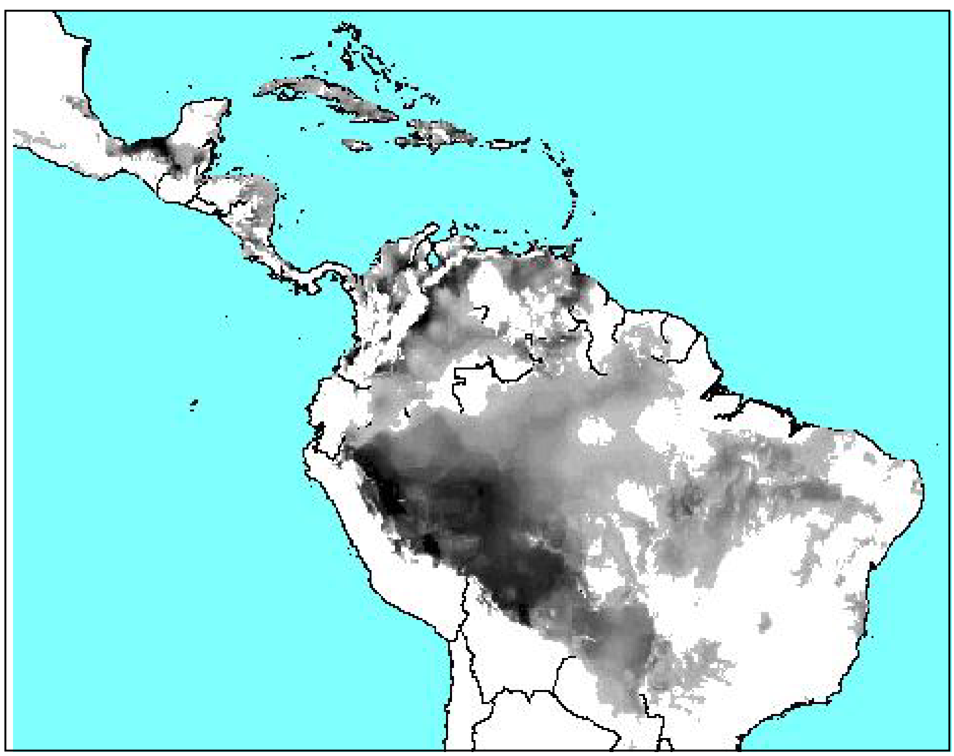

**Figure 5. Climate Similarity Map: Regions with Similar Rainfall and Temperature Condition to Pucallpa (Source: Jones 2003)**

## Conclusion

Field interviews conducted as a follow up to the participatory research initiated in the mid-1990s by CIAT and NARS (INIA) scientists reveal that farmers continue to plant two of the modern varieties. Some nine upland rice varieties are planted in the region. Average yield of traditional (i.e. older) varieties is 1.8 t $ha^{-1}$ while modern varieties produced 2.2 t $ha^{-1}$, a 20% increase.

The improvement of any one crop will not lead to amazing income increases, especially in diversified production systems. Nevertheless, the advances provided by new varieties to smallholder farmers in Pucallpa are significant. Higher and more reliable yields lead to greater household earnings and economic security. Only with continued research can marginalized farmers remain somewhat competitive as irrigated production elsewhere in the country and the region becomes more technified.

Continued research to develop a range of modern rice technologies is an important poverty alleviation strategy. The diverse bio-physical and socio-economic conditions that smallholder farmers face illustrate the need for a choice between numerous rice varieties. Careful diagnosis of the agricultural systems with farmer participation increases the probability of developing and identifying successful varieties.

A technology-only strategy is incomplete and insufficient to improve earning from rice production (Albeit one of the main initial research goals was to enable farmers to become better researchers). Concurrent improvements to farm management, institutions and organizations will increase the probability of success. Improved access to seed and storage are vital for more resource-poor farmers to realize benefits. Merchants and middlemen currently take advantage of seasonal price instabilities. Household silos for rice (and other crops such as maize) storage and micro-rice mills, as advanced in Asia, may be inexpensive investments that enable farmers to realize better product prices.

Information and organizations can foster more equitable marketing. Farmer organizations can enable producers to negotiate more fair prices with intermediaries and mills. Radio programs can provide crucial production and marketing information to reach more distant and difficult-to-access farmers.

Rice research to benefit the poor is a public good and, therefore, requires public investments. If national agriculture research institutes do not receive adequate funding for operations (e.g. variety testing and seed multiplication) and extension efforts, then NGOs will need to fill such roles.

The dire financial situation of the Peruvian government will likely not change in the short- or medium-term. Alternative and low-cost tactics to disseminate varieties and to ensure that farmers receive fair market prices are required. Improved coordination with other government institutions (e.g. health and education) can facilitate dissemination of modern varieties to more communities in a cost-effective manner.

**Annex**

**Personnel**
Douglas White, Agricultural and Environmental Economist
Efrain Leguía, Agronomist
José Sánchez, Agronomist
Sam Fujisaka, Agricultural Anthropologist

**Pucallpa Scientists Interviewed**
Javier Soto, Proyect PRA
Walter Navalte, Station Leader INIA
Angel Salazar, Agroforestry Program Leader INIA
Manuel Vázquez, Economist INIA
Fulvio Hidalgo, Rice Agronomist INIA
José Sánchez, Agronomist Consultant

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## OUTPUT 3. ENHANCING REGIONAL RICE RESEARCH CAPACITIES AND PRIORITIZING NEEDS WITH EMPHASIS ON THE SMALL FARMERS

### 3D. Collaborators, Training and Information

- **Collaborators**

**Africa and Madagascar**
Gabriel Aluko, Ph.D. Student, WARDA/Lousiana State University
Gunther Hahn, WARDA

**Argentina**
Marta Lucrecia Bonell, INTA, Concepción del Uruguay,
Carina Cáceres, INTA, Concepción del Uruguay
Fabiana Consolo, FIBA, Mar del Plata
Enrique Figueroa, INTA, Mercedes,
Alberto Livore, INTA, Concepción del Uruguay
Maria Antonia Marassi, University of Corrientes
Juan Eduardo Marassi, University of La Plata
Marta Nicosia, University of Tucumán
Graciela Salerno, FIBA, Mar del Plata
Alfonso Vidal, University of La Plata
Alberto Villegas, University of Tucumán

**Austria**
M. Maluszyinski, IAEA, Vienna
Shu, Qinsyao, IAEA, Vienna

**Bolivia**
Victor Hugo Callaú, CIAT Santa Cruz-Bolivia
Jorge René Guzmán, CIAT Santa Cruz de la Sierra
Roger Taboada, Plant Breeder, CIAT Santa Cruz de la Sierra
Juana Viruez, CIAT Santa Cruz de la Sierra

**Brazil**
Richard Baya, EPAGRI, Santa Catarina
Péricles de Carvalho Neves, EMBRAPA, Rice and Beans Center
Marcelo Gravina de Moraes, Universidad Federal do Rio Grande do Sul
Joao Maciel, IRGA, Rio Grande do Sul
Orlando Peixoto de Morais, EMBRAPA-CNPAF Arroz e Feijão
Anne. S. Prabhu, EMBRAPA, Rice and Beans Center
Héctor Ramírez, IRGA, Rio Grande do Sul
Beatriz da Silveira Pinheiro, EMBRAPA, Rice and Beans Center

**Chile**
Roberto Alvarado, INIA Quilamapu-Chillán
Dalma Castillo, INIA Quilamapu-Chillán
Santiago Hernaiz, INIA Quilamapu-Chillán

**China**
Tao Dayun, Food Crop Research Institute of the Yunnan Academy of Agricultura Sciences
Lee Kai Mian, Chinese Academy of Tropical Agricultural Sciences
Victor Kuo, Misión Técnica China-Taiwan

**Colombia**
Diego Aristizábal, CORPOICA, La Libertad, Meta
Hernando Delgado, CORPOICA, La Libertad, Meta
Edgar Corredor, FEDEARROZ, CIAT, Palmira
Alberto Dávalos, FEDEARROZ, Villavicencio, MetaHarold Bastidas, FEDEARROZ, Villavicencio, Meta
Miguel Diago, FEDEARROZ, Santafé de Bogotá
Patricia Guzmán, FEDEARROZ, Ibagué, Tolima
Argemiro Moreno, CENICAFÉ, Manizales, Caldas
Javier Osorio, Universidad del Tolima
Néstor Ramos, Semillano Ltda, Villavicencio, Meta
Luis Antonio Reyes, FEDEARROZ, Saldaña, Tolima
Manuel Rosero, COPROSEM, Ibagué, Tolima
Juan Sierra, FEDEARROZ, Montería, Córdoba
Roberto Simmonds, Cultivos y Semillas El Aceituno Ltda, Ibagué, Tolima

**Costa Rica**
Randolph Campos, INTA
Ronald Castillo Fonseca, National Rice Corporation
José Gerardo Cháves Alfaro, Technological Institute of Costa Rica, National Rice Corporation
Ana Mercedes Espinosa, Universidad de Costa Rica

**Cuba**
Luis Alemán, Instituto de Investigaciones del Arroz (IIA)
Rubén Alfonso, Instituto de Investigaciones del Arroz (IIA La Habana)
Jorge Fuentes, CEADEN
Nelson González, Director UAIA, Ministerio de Agricultura
Alfredo Gutiérrez, Viceministro de Agricultura, Ministerio de Agricultura (q.e.p.d.)*
Jorge Hernández, Instituto de Investigaciones del Arroz (IIA)

**Dominican Republic**
Angel Adames, Instituto Dominicano de Investigaciones Agrícolas y Forestales (IDIAF)
Luis Arias, IDIAF
Vinicio Castillo, IDIAF

Freddy Contreras, IDIAF
Guillermina Gerónimo, IDIAF
César Moquete, IDIAF
J.R. Santana, IDIAF

**France**
Guy Clément, CIRAD
Alain Ghesquiere, IRD
Emmanuel Guiderdoni, CIRAD
Marc Henry Lebrun, AVENTIS-Bayer
Didier Tharreau, CIRAD

**Germany**
Andreas Springer-Heinze, GTZ

**Honduras**
Napoleón Reyes, Rice Breeder DICTA
Elizabeth Santacreo, Leader Training DICTA

**Japan**
Tokio Imbe, Director, Department of Rice Research, National Institute of Crop Science
Kensuke Okada, Crop Physiologist, Japan International Research Center for Agricultural Sciences (JIRCAS)

**Korea**
Seong-Kook Han, Plant Pathologist, Crop Environment and Biotechnology Division, National Crop Experiment Station (NCES)
Hung-Goo Hwang, Director Rice Genetic and Breeding Division, (NCES)
Huhn-Pal Moon, Genetics, (NCES)

**Nicaragua**
Lázaro Narváez Rojas, Plant Breeder, Rice Program INTA
Marlon Ortega, INTA

**Peru**
Carlos Bruzzone, INIA
Fernando Montero, INIA
Orlando Palacios, INIA
Douglas White, CIAT Pucallpa
A. Vigil, INIA

**Philippines**
Swapan Datta, Molecular Biologist, IRRI
Brar Darshan, Plant Breeder, IRRI
Glenn Gregorio, Plant Breeder, IRRI

Gurdev S. Khush, Plant Breeder, IRRI
Hei Leung, Plant Pathologist, IRRI
David Mackill, Plant Breeder, IRRI
Tom Mew, Bacteriologist, IRRI
Casiana Vera Cruz, Plant Pathologist, IRRI
Parminder Virk, Plant Breeder, IRRI

**Italy**
Elcio Perpétuo Guimarães, FAO, Rome

**Suriname**
Terry R. Tjoe-Awie, Plant Breeder, Anne van Dijk'Rice Research Centre Nickerie (ADRON)

**United Kingdom**
Tim Chancellor, NRI

**United States**
Albert Fischer, Davis, California University
James W. Gibbons, University of Arkansas
Scot Hulbert, Kansas State University
Jershon López, Kansas State University
Anna McClung, Texas A&M University
Susan R. McCouch, Cornell University
James Orad, Louisiana State University
Robert S. Zeigler, Kansas State University

**Uruguay**
Stella Avila, INIA, Treinta y Tres
Gisela Beldarrain, INIA, Treinta y Tres
Pedro Blanco, INIA, Treinta y Tres
Fernando Pérez de Vida, INIA Treinta y Tres (currently in the U.S)

**Venezuela**
Fernando Ajmad, Fundación DANAC
Ramiro de la Cruz, Fundación DANAC
Carlos Gamboa, Fundación DANAC
Eduardo Graterol, Fundación DANAC (currently in the U.S.)
Yorman Jayaro, Fundación DANAC
Gelis Torrealba, Inia

**FLAR**
Luis Eduardo Berrío, Research Associate
Maribel Cruz, Research Assistant
Peter R. Jennings, Consultant

Edward Pulver, Consultant
Edgar A. Torres, Research Fellow
Luis Sanint, Executive Director

* Passed away

## Training

- **Thesis**

1. Silvio James Carabalí. Master Thesis: Genetic gains in rice grain quality obtained through several cycles of recurrent selection. Universidad Nacional de Colombia Seccional Palmira. Febrero 2000-Diciembre 2003. Presidente de Tesis Dr. César Martínez.
2. Johanna Paola Dossman. Master Thesis: Evaluación del progreso genético obtenido por un ciclo de selección recurrente en la población PCT-6 para la resistencia durable a *Pyricularia grisea* Sacc. y otros caracteres agronómicos. Universidad Nacional de Colombia Seccional Palmira. Enero 2001-Diciembre 2003. Presidente de Tesis Dr. Michel Valès.
3. Fabio Escobar Rioja. Master thesis: Caracterización de la estructura genética del patógeno del arroz *Rhizoctonia solani*. Universidad Nacional de Colombia Seccional Palmira. Enero 1999-Diciembre 2003. Presidente de Tesis Dr. Fernando Correa.
4. Castilla Luis Armando. PhD thesis. Biological Nitrogen Fixation in Rice. Universidad Nacional de Colombia, Seccional Palmira. June-Dec. 2003. Presidente de Tesis Dr. César P. Martínez.
5. Claudia Patricia Flóres. Ph.D. Thesis. Desarrollo y uso de la tranformación genética de *Brachiaria decumbens* stapf en fitomejoramiento. Universidad Nacional de Colombia Seccional Palmira. 2003. Presidente de Tesis Dra. Zaida Lentini.
6. Andrea María Garavito E. Master thesis: Evaluación del mecanismo de resistencia viral de una planta transgénica de arroz. Universidad de los Andes de Bogotá. Julio 2001-Enero 2003. Presidente de Tesis Dr. Lee Calvert.
7. Rosana Pineda. Master thesis. Evaluación de Flujos de Genes en Arroz con Marcadores Microsatélites. Universidad Nacional de Colombia Seccional Medellín. Septiembre 2002 a Diciembre 2003. Presidente de Tesis Dra. Zaida Lentini.
8. Gustavo Adolfo Prado Patiño. Master thesis. Estudio de la herencia de la resistencia de las líneas isogénicas (C101 LAC y C101 A51) y uso de marcadores moleculares asociados a la resistencia. Universidad Nacional de Colombia Seccional Palmira. Enero 2001-Diciembre 2003. Presidente de Tesis Dr. Fernando Correa.
9. Manuel Quintero. Undergraduate thesis. Ajuste del sistema RITA® para la inducción de callo embriogénico y regeneración de plantas a partir del cultivo de anteras de arroz. Universidad Nacional de Colombia Seccional Palmira. Noviembre 2001 a Octubre 2003. Presidente de Tesis Dra. Zaida Lentini.
10. Hernando Ramírez. Ph.D thesis. Incorporación de resistencia al cogollero *Scrobipalpuloides absoluta* y al pasador del fruto *Neolucinodes elegantalis.*

Universidad Nacional de Colombia Seccional Palmira. Octubre 1997 a Septiembre 2002. Presidente de Tesis Dra. Zaida Lentini.

11. Paola Ruíz. Undergraduate thesis. Caracterización fenotípica y genotípica de los diferentes tipos de arroz rojo (*Oriza sativa*) del Departamento del Tolima: Ecotipos de granos largos y delgados, con o sin arista. Universidad Javeriana de Bogotá. Julio 2001-Octubre 2002. Presidente de Tesis Dra. Zaida Lentini.
12. Sandra Milena Salazar Erazo. Master thesis. "Evaluación de resistencia parcial a Pyricularia, *Magnaporthe grisea* del arroz (*Oryza sativa*) en descendencias del cruzamiento IR64/Azucena e identificación de QTLs". Universidad Nacional de Colombia Seccional Palmira. Enero del 2002 a octubre del 2003. Presidente de Tesis Dr. Michel Valès.
13. Vanesa Segovia Buchelli. Master thesis. Optimización de la regeneración de lulo (*solanum quitoense*), orientada a la transformación genética de plantas. Universidad Internacional de Andalucía-España. 2002-2003. Presidente de Tesis Dra. Zaida Lentini.
14. Mónica Triana. Master thesis. Molecular markers associated with resistance to *Tagosodes oryzicolus* M. Universidad Nacional de Colombia Seccional Palmira. Febrero 2001-Junio 20034. Presidente de Tesis Dr. César Martínez.
15. José Alejandro Vargas (FEDEARROZ). Master thesis. Evaluation of two breeding schemes in breeding for yield potential. Universidad Nacional de Colombia Seccional Palmira. Febrero 2001-Septiembre 2003. Presidente de Tesis Dr. César Martínez
16. Johanna Patricia Villamizar Ruíz. Undergraduate thesis. Identificación de cambios genéticos en el hongo *Pyricularia grisea* Sacc. asociados a la pérdida de resistencia en líneas/variedades de arroz *Oryza sativa*. Universidad del Valle. Mayo 2001-Octubre 2003. Presidente de Tesis Dr. Fernando Correa.

- **Workshops**

1. Curso de Manejo Integrado del Cultivo del Arroz. Villavicencio, 12 al 14 Noviembre del 2002.
2. Biosafety Workshop for the Ministry of Environment: Z. Lentini, P. Chavarriaga, J. Tohme. Coordination of Workshop on Agriculture Biosafety for the Colombian Ministry of Environment. November, 19-20, 2002. CIAT, Cali, Colombia. A total of 25 participants**.**
3. Curso sobre Manejo Integrado del Arroz. Febrero 7 al 14 de 2003. Chiclayo y Bagua, Perú.
4. 2 $^{nd}$. National Upland Rice Workshop for Small-farmers. CIAT-Santa Cruz, Bolivia, February 16-23, 2003.
5. 2$^{nd}$. National Upland Rice Workshop for Small-farmers. CIAT-Santa Cruz, DISAPA (JICA) and CIAT/CIRAD Project. February 19-21, 2003. Yapacani, Bolivia.
6. Châtel, M., Ospina, Y. The CIRAD/CIAT Rice Project. Upland Rice Breeding for Latin America and the Caribbean: Conventional and Advances Methods. Rice National Workshop. CIAT-Santa Cruz y DISAPA.. February 20, 2003. Yapacani -

Bolivia.

7. Monitoring field-work at DANAC Foundation. San Felipe and Calabozo-Venezuela. February 20-27, 2003.
8. Marassi, M., Marassi, J.E., Châtel, M.; Ospina, Y. Advances in Populational Rice Breeding in Argentina. 3rd. International Temperate Rice Conference. March 10-13, 2003. Punta del Este-Uruguay.
9. Châtel, M. CIRAD, one of the Institutions of the Scientific Park "Agronatura". 4 th Board Meeting of the Scientific Park "Agronatura". April 10, 2003. CIAT Palmira-Colombia.
10. Biosafety Workshop for Journalists: J. Tohme, P. Chavarriaga, Z. Lentini,. Coordination of Workshop on Agriculture Biosafety for Journalists. April, 2003. CIAT, Cali, Colombia. A total of 15 participants.
11. Annual conference of PPCMCA in La Ceiba, Honduras (April 28-30).
12. Châtel, M., Guimarães, E.P. "Doing Research Together" Contribution and Impact of the CIRAD Rice Genetic Resources in Latin America. CIAT Intern Seminar. May 7, 2003. CIAT HQ-Palmira.
13. Châtel, M. 6 th. CIO-CIAT Strategic Alliance Meeting. The CIRAD/CIAT Project: Rice Improvement using Crossbreeding and Composite Population - Outputs 2001-2003. CIO-CIAT Meeting. May 26-28, 2003. Montpellier-France.
14. Annual meeting of the Mesoamerican PPB Network in Veracruz, Mexico (June 24-26, 2003).
15. Asociación Colombiana de Fitopatología, ASCOLFI. Congreso Anual. Junio 25-27 de 2003. Armenia, Colombia
16. XXX Congreso de SOCOLEN. 17 al 19 de Julio 2003. Cali.
17. American Phytopathological Society, APS, Annual Meeting. August 9-13, 2003. Charlotte, NC, USA.
18. Ospina, Y., Châtel, M., Rodríguez, F., Lozano, V.H. Upland Rice Composite Population Breeding for the Savannas Ecosystem. 3rd. International Upland Rice Seminar-Workshop. August 19-22, 2003. Villavicencio-Colombia.
19. 3rd. Conference on Rice Composite Population Breeding. DANAC Foundation, CIRAD/CIAT and FAO. October 13-14, 2003. Maracay-Venezuela.
20. 1st. Venezolan Congress of Plant Breeding and Biotechnology. Central University of Venezuela, DANAC Foundation, CIRAD/CIAT and FAO. October 15-17, 2003. Maracay-Venezuela.
21. DANAC Foundation: Monitoring Tour. Venezuela, October 18-22, 2003.
22. International Biosafety Workshop: Organized by Agrobio, Canada. October 2003. Cartagena, Colombia. Z. Lentini Lecturer. A total of 50 participants.
23. Curso sobre Manejo Integrado del Cultivo del Arroz. Noviembre 11-13 de 2003. Yopal, Casanare, Colombia.

- **Formation and Training**

1. José Martínez Teruel. Ecole Nationale d'Ingenieurs en Techniques Agricoles -ENITA- Clermont-Ferrand, France 15 days at CIAT HQ-Palmira and 5 Months

(May-October, 2002) at Fundación DANAC, Venezuela. "Efecto de la arquitectura de planta (cerrada o abierta) sobre las caracteristicas agronómicas y los componentes de rendimiento de 5 líneas de arroz y dos testigos". Effect of plant architecture (Open or Erect) on different agronomic characteristics and yield components in 5 rice lines and 2 checks. Supervisor Dr.M. Châtel.

2. Stéphane Bauguil. Ecole Européenne Supérieure: Tutelle des Universites de Savoie, Jean Moulin Lyon III, et du lycée agricole de Poisy-Annecy. 4 Months (April-July, 2003) at CIAT Santa Cruz, Bolivia. "Estudio de los elementos de la cadena productiva del Arroz en el Departamento de Santa Cruz de la Sierra-Bolivia". Study of the elements of the Rice production chain of the State of Santa Cruz de la Sierra, Bolivia. Supervisor Dr.M. Châtel.
3. Juana Viruez. CIAT Santa Cruz, Bolivia. Upland rice composite population breeding and evaluation of segregating lines. August 11-22, 2003. CIAT Villavicencio-Colombia. Supervisor Dr.M. Châtel.
4. Yorman Jayaro. DANAC Foundation, Venezuela. Upland rice composite population breeding and evaluation of segregating lines. August 11-28, 2003. CIAT Villavicencio and HQ-Palmira-Colombia. Supervisor Dr.M. Châtel.
5. Dr. Lázaro Narváez, INTA, Programa de Arroz de Nicaragua. Entrenamiento en Evaluación de Arroz en invernadero y campo por su resistencia a Pyricularia. 9- 21 de Junio de 2003 en CIAT Palmira y en la Estación Experimental de Santa Rosa. Supervisor Dr.F. Correa.
6. Marlon Ortega, INTA, Programa de Arroz de Nicaragua. Entrenamiento en Evaluación de Arroz en invernadero y campo por su resistencia a Pyricularia. 9- 21 de Junio de 2003 en CIAT Palmira y en la Estación Experimental de Santa Rosa. Supervisor Dr.F. Correa.
7. Dr. Jorge Fuentes, CEADEN, Cuba. Identificación de marcadores moleculares asociados a genes de resistencia de *Pyricularia grisea.* Agosto de 2003 a Febrero 2004. Supervisor Dr.F. Correa..
8. Adriano Alejandro Carrera. B.Sc. Military Polytechnic School. Quito, Ecuador. Training on tissue culture, anther culture, and genetic transformation. Supervisor Dra.Z. Lentini.
9. Carlos Hidalgo. B.Sc. Military Polytechnic School. Quito, Ecuador. Training on issue culture, anther culture, and genetic transformation. Supervisor Dra.Z.Lentini.
10. Cesar A. Vera. B.Sc. Military Polytechnic School. Quito, Ecuador. Training on tissue culture, anther culture, and genetic transformation. Supervisor Dra.Z. Lentini.
11. Ángela Mina. B.Sc. Del Valle University. Training on microsatellites analysis of rice and their use for gene flow analysis into wild/ weedy relatives. Supervisor Dra.Z. Lentini.
12. Paola Olaya. B.Sc. Del Valle University. Training on microsatellites analysis of rice and their use for gene flow analysis into wild/weedy relatives. Supervisor Dra.Z. Lentini.
13. Margarita Pineda. B.Sc. National University of Colombia, Palmira. Training on tissue culture. Supervisor Dra.Z. Lentini.
14. Jhon Alex Cambindo. Specialization as technician in agronomy. Six months. Instituto Técnico Agropecuario (ITA). Buga, Valle del Cauca. Supervisor Dr.M.

Lorieux.

15. Sandra Lorena Acosta Lopez. Database management and large data set organization. Two months. SYSTEM PLUS. Palmira, Valle del Cauca. Supervisor Dr.M. Lorieux.
16. Training of the INTA rice scientists in conventional and participatory breeding, with a particularly focus on resistance to blast, with the participation of the Colombia Rice Project team. Supervisor Dr.G. Trouche.
17. Course on on-farm experimentation for NITLAPAN technicians. Supervisor Dr.G. Trouche.
18. Workshop on PR process and PVS methodology applied on rice with INTA regional scientists in Posoltega. Supervisor Dr.G. Trouche.
19. Course on rice production in high hillsides conditions for Yorito farmers. Supervisor Dr.G. Trouche.
20. Supervision of four student works. Supervisor Dr.G. Trouche.

## Information

- **Refereed Publications**


1. Châtel, M., Guimarães, E.P. International Partnership for rice improvement in Latin America: CIRAD, a Case study. Brazilian Society of Plant Breeding. Crop Breeding and Applied Biotechnology (CBAB - 165) v.2, n.4. p. 639-648, 2002. Received: September 04, 2002. Accepted: October 08, 2002.
2. Correa-Victoria, F.J., Tharreau, D., Martínez, C., Valès, M., Escobar, F., Prado, G., y Aricapa, G. 2003. Combinaciones de genes en arroz para el desarrollo de resistencia durable a *Pyricularia grisea* en Colombia. Fitopatología Colombiana 26(2): 47-54.
3. Coulibaly, I., J. Louarn, M. Lorieux, A. Charrier, S. Hamon, M. Noirot. 2003. Pollen viability restoration in a *Coffea canephora* P. and *C. heterocalyx* Stoffelen backcross. QTL identification for marker-assisted selection. Theoretical and Applied Genetics 106: 311-316.
4. Coulibaly, I., B. Revol, M. Noirot, V. Poncet, M. Lorieux, C. Carasco-Lacombe, J. Minier, M. Dufourand P. Hamon. 2003. AFLP and SSR polymorphism in a coffea interspecific backcross progeny [(*C. heterocalyx/C. canephora*)/*C. canephora*]. Theoretical and Applied Genetics 107:1148-1155.
5. Fuentes, J.L., Correa-Victoria, F.J., Escobar, F., Mora, L., Duque, M.C., Deus, J.E., Cornide, M.T. 2003. Genetic Diversity Analysis of the Rice Blast Pathogen Population at two Locations in Cuba. Biotecnología Aplicada 20:14-19.
6. K. Langar, M. Lorieux, E. Desmarais, Y. Griveau, L. Gentzbittel, A. Bervillé. 2003. Combined mapping of DALP and AFLP markers in cultivated sunflower using $F_9$ recombinant inbred lines. Theoretical and Applied Genetics 106:1068-1074.
7. Lentini Z., Lozano I., Tabares E., Fory L., Domínguez J., Cuervo M., Calvert L. 2003. Expression and inheritance of hypersensitive resistance to rice hoja blanca virus mediated by the viral nucleocapsid protein gene in transgenic rice. Theoretical and Applied Genetics 106: 1018-1026.
8. M. Lorieux, G. Reversat, S. X. García Díaz, C. Denance, N. Jouvenet, Y. Orieux, N.

Bourger, A. Pando-Bahuon, A. Ghesquière. 2003. Linkage mapping of *Hsa-1*$^{Og}$, a resistance gene of African rice to the cyst nematode, *Heterodera sacchari.* Theoretical and Applied Genetics 107: 691-696.
9. C. Sallaud, M. Lorieux, E. Roumen, D. Tharreau, R. Berruyer, P. Svestasrani, O. Garsmeur, A. Ghesquière, J.L. Nottéghem. 2003. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theoretical and Applied Genetics 106:794-803.
10. Seebold, K.W., Datnoff, L.E., Correa-Victoria, F.J., Kucharek, T.A., and Snyder, G.H. 2003. Effects of Silicon and Fungicide on the Control of Leaf and Neck Blast in Upland Rice. Plant Disease (Accepted for Publication in August 25, 2003).
11. Thomson M.J., Tai T.H., McClung A.C., Hinga M.H., Lobos K.B., Xu Y., Martínez C., McCouch S.R. 2003. Mapping quantitative trait loci for yield components, and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* Jefferson. Theoretical and Applied Genetics 107:479-493.
12. Thomson M.J., Tai T.H., McClung A.C., Hinga M.H., Lobos K.B., Xu Y., Martínez C., McCouch S.R. 2002. Mapping quantitative trait loci for yield components, and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* Jefferson. Submitted to Theoretical and Applied Genetics.

- **Book Chapters**

1. Calvert Lee A., Hiroki Koganezawa, Denis Fargette, G. Konate. 2003. Major Virus Diseases of Rice in "Virus Diseases of Rice in "Virus and Virus-Like Diseases of Major Crops in Developing Countries". Editors: Gad Loebenstein and George Thottappilly Klower Academic Publishers, the Netherlands. (In press).

2-7. Châtel M. 2003.
Chapter 1 . Explorando los recursos genéticos del arroz a través del mejoramiento poblacional. Guimarães, E.P., Châtel, M.
Chapter 7. Exploración de los recursos genéticos del arroz en Argentina a través del mejoramiento poblacional. Marassi, M.A., Marassi, J.E., Châtel, M., Ospina, Y.
Chapter 8. Mejoramiento de poblaciones de arroz para clima templado en Chile.
Hernaiz, S., Alvarado, J.R., Castillo, D., Châtel, M., Ospina, Y.
Chapter 11. Las bases para el uso del mejoramiento poblacional del arroz en Cuba. Polanco, R., Châtel, M., Guimarães, E.P.
Chapter 13. Mejoramiento poblacional y obtención de líneas de arroz para el ecosistema de sabana: Proyecto CIAT/CIRAD. Châtel, M., Ospina,Y., Rodríguez.F., Lozano, V.H.
Chapter 16. Efectos de la selección y de las recombinaciones en una población de arroz de secano. Ospina, Y., Guimarães, E.P., Châtel, M., Duque, M.C.
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- **IP-4 Web Site – María Nelly Medina**

This Web site contains relevant information on activities carried out by our rice scientists, as well as information on events related to the rice sector.

The manual "Methodologies to Raise and Evaluate *Tagosodes orizicolus* (Muir)" gives guidelines for evaluation of rice lines for their resistance to direct damage caused by *T. orizicolus* as well as to infection by Rice hoja Blanca Virus.

The parentage of CIAT crosses is available and contains data on the crosses made at CIAT (CT) since 1984. It also contains the history of crosses made since 1957 by the collaborative project ICA-CIAT (P). This database will be updated periodically.

The list of the CIAT-ION nurseries will be available on the CIAT web site soon. These nurseries are made up of breeding lines that potentially could lead to varieties or used as parental lines for crossing. These nurseries are available on request. Seeds will be made available upon request and after signing a Material Transfer Agreement (ATM). Contact: César P. Martinez <c.p.martinez@cgiar.org>.

The CIAT rice web site has a link to the official web site of "2004: International Rice Year" http://www.fao.org/rice2004. This is the site with the information of the events throughout the world that are being done to celebrate the International Year of Rice.

We hope this information to be useful for those interested in the rice sector.

http://www.ciat.cgiar.org/riceweb/index1.htm
http://www.ciat.cgiar.org/riceweb/esp/inicio.htm

## ANNEX 1. PRINCIPAL AND SUPPORT STAFF

### Principal Staff

Lee Calvert, *Virologist and Project Leader*
Marc Châtel, *Plant Breeder, CIRAD-CA*
Fernando Correa, *Plant Pathologist*
Zaida Lentini, *Plant Breeder*
Mathias Lorieux *Molecular Biologist*
César P. Martínez, *Plant Breeder*
Rafael Meneses, *Entomologist, I.I.A., Cuba*
Gilles Trouche, *Plant Breeder, CIRAD-CA*
Michel Valès, *Plant Pathologist, CIRAD-CA* *
Carlos Bruzzone, *Consultant Peru*
Douglas White, *Consultant*

### Support Staff

- **Associates and Assistants**

Jaime Borrero, *Genetics*
Diana Delgado, *Genetics (VVC)* *
Myriam Cristina Duque, *Biometry*
Joanna Paola Dossman, *Pathology, CIRAD-CA* *
Fabio Escobar, *Biotechnology/Pathology*
Iván Lozano, *Virology*
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Alejandro Vargas, FEDEARROZ, Colombia *

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Elizabeth Hurtado, *Leader*

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Felix Acosta, *Genetics*
Tomás Agrono, *Genetics*
María Girlena Aricapa, *Pathology*
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Jaime Gallego, *Genetics*
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Alexander Ríos, *Genetics*
Francisco Rodríguez, *Genetics (VVC)*
Luis H. Rosero, *Pathology*
Sory H. Sánchez, *Genetics*
Pedro Nel Vélez, *Genetics*
Daniel Zambrano, *Pathology* *

* Left during 2003

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