# Annual Report 1999

# Project IP-4:

Improved Rice Germplasm for Latin America and the Caribbean

For Internal Circulation and Discussion Only

October 1999



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# **Annual Report 1999**

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

## Executive Summary

#### **OUTPUT 1. ENHANCED GENE POOLS**

#### Germplasm development

Improving rice germplasm for Latin American and the Caribbean by the IP-4 Project at CIAT is being accomplished through the increase of the genetic diversity and enhancement of gene pools for higher, more stable yields with adequate levels of resistance to pathogen and insect pests relevant to the region. CIAT breeding strategies focus on developing and improving populations. Such population development and enhancement aims to provide all our partners including national programs with sources of potential parents having specific traits or populations from which they may select and advance fixed lines for release as commercial cultivars.

Our collaborative project with CIRAD aims at: 1) Developing gene pools and populations using genetic male-sterility. 2) Enhancing basic germplasm for different rice ecosystems using recurrent selection. 3) Provide regional NARS with potential enhanced parents and/or lines with specific traits, and 4) Promote the use of population breeding by Latin American NARS. The main targeted traits in our population breeding for the upland savannas ecosystem are yield potential, tolerance to soil acidity, resistance to the blast pathogen, grain quality, and earlyness. Since 1995, we started to release basic and enhanced populations, as well as segregating lines to our LAC partners in Brazil (EMBRAPA Arroz y Feijao), Bolivia (CIAT Bolivia), Colombia (CORPOICA), Cuba (IIA), and Venezuela (Unellez). From our ongoing conventional breeding for the upland savanna ecosystem, several upland lines were released in 3 LAC and one in China.

Population Breeding for the Irrigated Rice Ecosystem focus on the following traits: a) for tropical: on yield potential, resistance to blast, resistance to the hoja blanca virus and its vector, grain quality and earlyness. Our partners are Colombia, Costa Rica, Cuba, El Salvador, Panama, and Venezuela. b) for subtropical conditions: yield potential, resistance to blast, grain quality, tolerance to cold, and earlyness. Our partners are: Argentina, Brazil, and Uruguay. c) for temperate conditions: tolerance tocold, grain quality, and earlyness. Our partners are: Argentina and Chile. Population enhancement and development of new site-specific populations has been achieved at each of our country partners. Line development exhibiting good variability is being reported auguring a promising future.

Conventional and recurrent selection has been used for the development of hillsides upland rice. During 1999, breeding populations with broad and narrow genetic base have been enhanced. Almost 2000 lines are being evaluated at 1700 masl. The production of best lines was twice as much as the controls used in some locations. New lines selected in trials conducted during 1999 will be proposed for inclusion in the participatory research project from CIAT. Lines and populations were distributed to partners during 1999.

A collaborative project aiming to characterize and use wild rice species as new gene sources to increase the yield potential and broaden the genetic base of cultivated rice was initiated in 1994. A backcrossing program associated with yield increments in segregating populations allowed the identification and association of molecular markers with yield. Several lines of the cross BG 90-2 / O. rulipogum surpassed the yield of the recurrent parent BG 90-2 in more than 1 t/ha, and two of them showed a yield advantage of more than 20%. Yield performance of these F5 lines is consistent with previous generations. Results are confirming putative presence of yield alleles in

the wild specie not present in the cultivated specie. Several lines selected from the cross between the cultivated variety Oryzica 3 and the same wild specie exhibited higher yield than the recurrent parent. Crosses between other commercial varieties and two more wild species are being evaluated and selected under field conditions.

# **OUTPUT 2. PHYSIOLOGICAL BASIS FOR RICE TRAITS UNDERSTOOD**

# Weed control enhanced by the use of new genotypes and practices

Preliminary studies at CIAT reported in 1998, suggested that it is possible to develop crop systems that reduce the weed E. colona competition during the earlier crop growth stages using varieties tolerant to submergence. Results obtained during 1999 showed that seedling age influence tolerance to submergence. Early flooding of rice fields, combined with cultivar tolerance to submergence, may be an effective practice to reduce E. colona competition. Flooding applied between 2-4 days after planting allowed less than 15 % germination of the weed while the tolerant cultivars had more than 60% emergence. Evaluating the effect of four different water layer depths applied 3 days after planting suggest that the water layer depth has no influence on rice nor E. colona emergence. Other experiments showed that emergence of E. colona seed placed at soil depths deeper than 2 cm are strongly inhibited by water layers equal to or higher than 5 cm. Evaluation of 48 elite rice genotypes for tolerance to submergence showed a range from 5 to 98% of emerged seedlings. Sixteen genotypes showing emergence haigher than 60% were considered as tolerant. One elite line exhibited higher values than the tolerant checks. Tolerance to submergence may also improve the rice seed stands by reducing the amount of non-emerged seed due to water management problems in lands having imperfect leveling. It was also found that it is possible to keep a rice crop free from weeds by using pregerminated seed and planting under water with rice cultivars tolerant to submergence

# OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED Blast

Understanding population structure and dynamics of the blast pathogen is critical for developing breeding strategies and identifying potential sources for durable blast resistance as well as for tagging resistance genes. Continuous characterization of the blast pathogen in our screening site show that the genetic structure of the blast pathogen in Colombia is simple. DNA fingerprinting determined by using MGR-586 AND Pot-2 rep PCR showed the same six genetic lineages. We have adopted now the PCR technique as it is simpler, more economic, and easier to adopt by our partners. Genetic lineage frequency change between years depending on the area planted with a rice cultivar in farmer's field, and on the virulence spectrum of the lineage. We have detected genetic structure and virulence changes within blast pathogen populations over time. A new lineage (SRL-6B) probably derived from lineage SRL-6, appeared after 1993. Greater gains in virulence observed in isolates were associated with changes in genetic structure. Rice cultivars may carry complementary resistance genes, which in combination confer resistance to all the targeted blast population. Breeders at CIAT are now aiming at combining genes exhibiting complementary resistance to the different lineages of the blast pathogen. Combinations of genes that in sum confer resistance to every lineage will confer resistance to the entire blast population. Complementary resistance sources within the Latin American rice germplasm of commercial cultivars have been identified for designing genetic crosses aiming at combining those complementary resistance genes. Gains in selection for blast resistance in F2 populations developed from crosses between progenitors exhibiting complementary resistance to different genetic lineages went from 29% prior to 1998 to 64% during that year.

The characterization and use of partial resistance to blast was initiated in 1998. We aim to identify and combine good levels of complete and partial resistance for the development of a more durable and stable blast resistance. A field methodology for evaluating partial resistance by inoculating specific strains of the pathogen was developed. It has been proved that part of the partial resistance observed in the field can be specific suggesting the need to inoculate different strains for each cycle of selection. The specific level of partial resistance of 85 varieties or progenitors from Latin America has been characterized. Best lines will be used as parents in genetic crosses.

Development of a selection strategy using recurrent selection for durable blast resistance is in progress. S3 populations were evaluated during 1999. The results show that in order to make a good selection for complete resistance, we should make trials in the field to take advantage of the natural variability of the fungus, and perform inoculations in the greenhouse using strains present in low frequency and exhibiting a wide spectrum of virulence. The results show a very important genetic progress for complete resistance to blast after the first recurrent selection cycle.

# Rice Hoja Blanca Virus and Tagosodes orizicolus

The main activities conducted on RHBV and its vector are directed towards lessening the losses caused by this complex and break the recurring cycle characteristic of this disease. The release of varieties with resistance to both RHBV and its vector are the outputs of a long term commitment between FLAR, NARS, NGOs, and CIAT. In Colombia, the variety Fedearroz 50 was released in 1998. This variety is resistant to this disease complex and exhibits a high yield. In Venezuela, a line developed from this project, and with resistance to the virus and the vector, is scheduled for release late in 1999. CIAT is still the only institution with the capacity to screen a large volume of materials for RHBV resistance. During this period more than 20000 lines were screened for resistance to the virus and 3000 for resistance to the insect. Efforts are being made to improve the methodology for evaluating the resistance to the insect. Intensive field trials to characterize RHBV resistance under high disease pressure yielded several elite lines with high resistance levels. Some of these lines have been included in regional trials in some countries being considered for their prompt release as commercial cultivars.

Characterization of the mechanisms of resistance to *T. orizicolus* in advanced lines and commercial varieties were initiated in 1999. The mechanisms under study include antixenosis, antibiosis, and preference. The cultivar Fedearroz 50 exhibited a lower settling preference and a lower number of eggs oviposited than the cultivars Oryzica Llanos 5 and IR-8. Emergence of nymphs was also higher in the latter two cultivars. Results observed help to explain the reaction of commercial cultivars to RHBV in farmer's fields.

Insecticide trials including entomopathogenic fungi to determine their effect on the insect *T. orizicolus* and its natural enemies were carried out during 1999. The most efficient insecticides for the control of the RHBV vector with less effect on the natural enemy populations were identified. Given the characteristics of the variety Fedearroz 50, the applications of chemical insecticides should be limited during the crop cycle, except when the pest occurs the first days after seedling emergence.

The monitoring of Colombia RHBV incidence and the analysis of viruliferous *T. orizicolus* population continued into the fourth year. Areas under a high risk for RHBV have been identified. The levels of RHBV are decreasing in those areas were a high percentage of vectors had previously been reported. With the increase use of RHBV resistant varieties, the trend towards decreased levels of RHBV is expected to continue. Farmer's perception of RHBV in Colombia indicates that the rice growers have a very realistic idea of problems with this disease. Knowing farmer's perception of the problems is essential for establishing a successful IPM program.

# Foreign genes as novel sources of resistance: Resistance of rice to rice hoja blanca virus and *Rhizoctonia solani*

One or two genes control the resistance to the RHBV. To ensure stable and durable resistance, additional sources need to be identified and incorporated into rice. Transgenic plants using the nucleoprotein mediated cross protection have been developed. These plants are showing stable RHBV resistance. The N transgene could be used to complement the natural resistance source to the virus. Besides RHBV, the fungus R. Solani causing sheath blight is increasing in importance in Latin America. All commercial varieties are susceptible and no sources of resistance to this fungus are known. Control depends on heavy applications of fungicides. In corporation of resistance for this disease by genetic engineering is an attractive approach. Work conducted in collaboration with other investigators is focused on the use of a pokeweed antiviral protein (PAP) which has a ribosome-inactivating ability and a potent antiviral activity against many plant viruses. Mutated versions of PAP gene also confer resistance to the fungal pathogen R. Solani. These results suggest the possibility of designing molecular strategies for incorporating dual antiviral and fungal resistance by introgressing mutant PAP gene(s) in transgenic rice plants. We are reporting the progress made in the last seven months. Agrobacterium mediated transformation of rice varieties is being optimized to incorporate the N (NS4) transgene and PAP genes for RHBV and Rhizoctonia resitance in indica rice.

## Rice stripe necrosis virus

Rice stripe necrosis virus (RSNV) and its vector *Polymyxa graminis* are niow fuly identified as the causal agent-vector of the "entorchamiento" (crinkling) disease in Colombia. Based on the results obtained in this study, this virus will soon be re-classified as a new member of the *Benyvirus* genus (previously classified as a member of the Furovirus group). One of the most important aspects of this project has been the renewed interest and awareness of the potential threat of RSNV's endemic situation in West Africa, where this virus was first reported.

A new purification method has been developed at CIAT for the isolation of RSNV. An efficient detection technique using antiserum for ELISA is making possible the detection of the virus in diseased rice plants. This antiserum and the ELISA protocol developed have been sent to Panama where the disease was reported recently. Molecular characterization of the virus is in progress. A high degree of homology with the beet necrotic yellow vein furovirus was found.

Conventional and hydroponic inoculation methods are being used to evaluate rice germplasm for resistance to RSNV. The virus can be transmitted up to 70% of test seedlings by these inoculation methods. Control practices including the effect of incorporating organic matter in sol infested by *Polymyxa graminis*, suggest that the decomposition of organic material has a negative effect in the colonization of rice roots and/or infection of rice seedlings. The disease is currently present in 22 municipalities in eight of the most important rice growing departments of Colombia. Predisposing factors for the development of the disease are being identified.

# **OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITIES ENHANCED FLAR and Economics**

Management of FLAR including the organization and direction of meetings of the administrative and technical committees are reported. Updating FLAR's rules and regulations was carried out according to recommendations. During 1999, FLAR received three new members: Argentina, Chile, and Nicaragua. An older member (Paraguay) withdrew its participation due to funding problems. Currently, FLAR has participants from 12 countries.

FLAR breeding activities have included several breeders' workshops and planting of germplasm for evaluation and selection. Germaplasm nurseries are distributed to partners. Field evaluations are being conducted under high pressure of rice hoja blanca virus, blast, helminthosporium, leaf scald, and grain discoloration. Resistant lines are then evaluated for grain quality traits such as white belly and gel temperature. Evaluation and use of germplasm by FLAR partners in different yield trials is reported. FLAR is also participating in the elaboration of breeding plans for its partners, analyzing farmer's questionnaires and cost of production data, as well as in surveys of crop production constraints. National plans for integrated crop management are being developed. FLAR also interacts in the organization and implementation of integrated crop management courses.

Economic studies are being conducted to update estimates of adoption in LAC of new rice varieties by area and production (1970-1999) in collaboration with IFPRI and the Impact Unit of CIAT. The data show that 59% of the rice area in Latin America is under modern semidwarf varieties and they account for 80% of rice production. In the irrigated sector, these varieties cover 88% of the area and account for 90% of the rice production. The Latin American Network of Rice Economists met during 1999 in Porto Alegre, Brazil, with 33 participants from six countries. A project on "commercial quality of rice in MERCOSUR" was developed. The project included studies on the economic impact of rice varieties in LAC showing that rice production coming from adoption of modern varieties will go from 88% in year 2000 to 94% in 2020. Adoption of the new varieties will permit an annual expansion of rice production at 2.1% instead of the 1.4-% annual growth without the project.

# Identification of improved upland rice genotypes for the Peruvian Selva. Strengthening the Peruvian Rice Program.

Local upland varieties lack some desirable agronomic and grain quality traits that limit their capacity to satisfy both farmers and market demands. To address this issue, upland varieties originated at CIAT were tested in farmer participatory trials. Most varieties were well accepted by farmers due to their greater lodging resistance, better grain milling and cooking quality than local varieties. Yield performance was about the same than local varieties but these checks are highly susceptible to lodging. Farmers are testing new introductions.

CIAT continued to carry out activities aimed to strengthening the Peruvian rice sector within the framework of the agreement with the Peruvian Ministry of Agriculture. Activities involved organization of courses, exchange and evaluation of germplasm and training of Peruvian researchers. Advanced lines introduced from CIAT are being field evaluated and are being tested for grain milling traits. Peruvian lines are being evaluated by CIAT for disease resistance, agronomic and grain quality traits. Effots must continue to identify or develop new rice varieties with resistance to the most important diseases along with desirable grain quality and agronomic traits.

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

## Introduction and overview

Global production of rice has outpaced population growth over the past three decades mainly due to important gains in productivity. Several indicators confirm these impressive results: higher per capita supplies, substantial yield increases and lower prices available to consumers. Increased productivity implies that millions of hectares were not disturbed (e.g. forests) to feed a rapidly growing population. Rice production in LAC over the 1990-98 period increased at an annual rate of 3.8%, the highest of all rice-growing regions in the world. This has been accomplished, to a large extent, through the continuous search for better varieties. Rice has become the most important source of calories and proteins for 20% of the population with the lowest incomes. Over the last 30 years, more than 300 rice varieties were released in Latin America and the Caribbean, mainly originating from crosses made at CIAT. As a result, large increases in yield and production occurred to the benefit of both consumers and producers.

CIAT's comparative advantage in rice research lies in addressing those constraints and opportunities, which are distinctive to LAC. We have evolved from the breeding and agronomy approach into a Rice Project with a focus on pre-breeding activities that complement the variety development and training activities of FLAR. Rice breeding at CIAT concentrates on the development of germplasm for irrigated (the main focus) and rainfed lowlands and for favored uplands of Latin America and the Caribbean. Research focuses on the following traits: yield improvement, durable resistance to blast, resistance to the rice hoja blanca virus and its vector, grain quality, iron toxicity tolerance, adaptation to acid soils, cold tolerance, and allelopathy and anaerobic vigor for better weed control. Research priorities are identified through economic studies in the region. The breeding methods used to broaden the genetic diversity of rice include pedigree selection, backcrossing, and recurrent selection. Molecular tools, mainly anther culture, molecular markers, DNA fingerprinting, and genetic transformation, are used to characterize pathogen variability; identify, tag, and transfer genes conferring resistance/tolerance to problems of regional importance. Research on population development for yield enhancement includes testing and crossing the IRRI new plant type and wild species of rice with high yielding commercial varieties. Thus, the CIAT Rice Project intends to develop and enhance gene pools and populations for welltargeted traits to be used as a source of potential parents by regional breeding programs.

Rice research activities at CIAT concentrate on four main outputs aiming at reducing the major constraints in LAC rice production: 1) enhance gene pools, 2) physiological basis for rice traits understood, 3) rice pests and genetics of resistance characterized, and 4) project priorities and research capacities enhanced.

FERNANDO CORREA

Phytopathologist and Project Leader

Ernando Cornea

# Research Highlights in 1999

## **OUTPUT 1. ENHANCED GENE POOLS**

- Two lines, CIRAD 410 and 411 were registered in the CIRAD Rice Catalogue.
- A new population (PCT-11) for the upland savannas was developed
- During last 2 years, 5 CIAT/CIRAD upland lines were released in 3 LAC countries, and one CIRAD line in China
- Basic populations for irrigated rice were developed and distributed to partners
- Partners using introduced populations and local varieties developed new site-specific populations
- Methodologies for populations with a broad and narrow genetic base have been developed
- Transgressive segregation for yield increase was observed in BC2F5 lines derived from the cross between BG 90-2 and Oryza rufipogum confirming observations in previous generations
- Positive traits in new plant type from IRI have been incorporated into CIAT's rice germplasm

# **OUTPUT 2. PHYSIOLOGICAL BASIS FOR RICE TRAITS UNDERSTOOD**

- Weed control enhanced by the use of rice genotypes with tolerance to submergence
- Rice varieties have been found to differ largely in their capacity to develop under submerged conditions
- Early flooding in tolerant to submergence cultivars can contribute significantly to reduce population of E. colona
- LAC rice germplasm contains an appreciable level of submergence tolerance that can be used in an integrated weed control management

# **OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED**

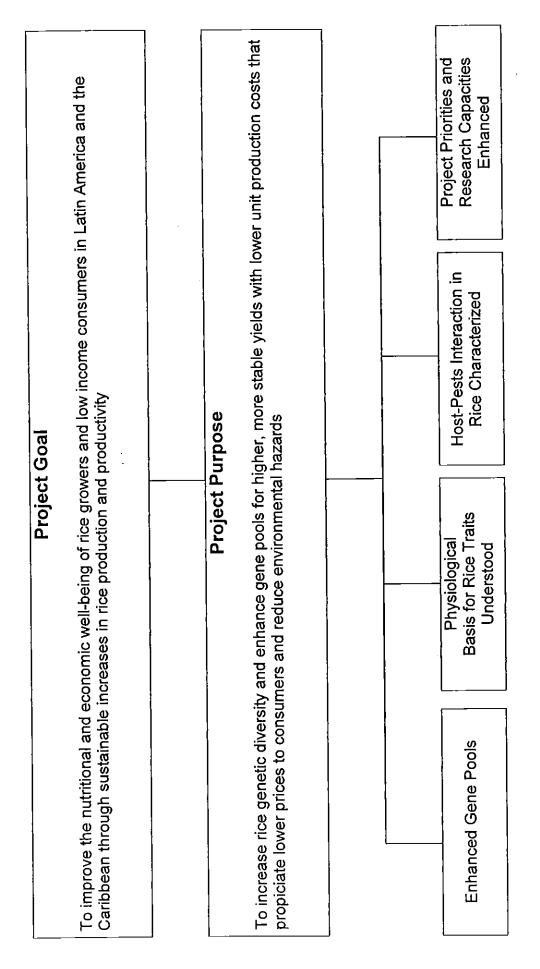
- Genetic structure and virulence changes in the blast pathogen leading to resistance breakdown have been detected over time
- Greater gains in virulence in the blast pathogen were associated with changes in genetic structure
- Rice lines exhibiting complete and complementary resistance to rice blast were identified in the field and greenhouse
- Gains in selection for blast resistance in F2 populations went from 29% prior to 1998 to 64% during that year
- A field methodology for evaluating partial resistance to rice blast was developed
- Partial resistance levels of 85 LAC rice cultivars has been characterized
- Important genetic progress for complete resistance to blast after the first recurrent selection cycle was obtained
- Rice cultivars with resistance to the RHBV and its vector are being released in several LAC countries
- Sources of resistance to RHBV and its vector were identified

- Epidemiological studies on RHBV continue helping to manage the disease in commercial rice fields
- Agrobacterium mediated transformation of rice varieties is being optimized to incorporate the N transgene and PAP genes for RHBV and Rhizoctonia resistance in indica rice
- The rice stripe necrosis virus and its vector have been isolated and physically, chemically and molecularly characterized

# **OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITIES ENHANCED**

- FLAR rules and regulations (estatutos) have been updated
- Three new members, Argentina, Chile and Nicaragua, joined FLAR during 1999
- FLAR members are reporting the first selections of rice lines derived from germplasm nurseries
  delivered in the past few years. Selected lines are being evaluated in yield trials
- Several LAC will release several selected lines as commercial cultivars in the coming months
- Several Integrated Crop Management courses were developed during 1999
- An analysis of variety adoption in collaboration with IFPRI is being conducted
- Yields in irrigated and lowland rainfed areas have increased substantially
- Improved upland genotypes have been identified for the Peruvian selva in collaboration with the Forest Margins Program
- The Peruvian Rice Program was strengthened through several activities during 1999

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



	Project Log-Frame	ame	
Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Germplasm of beans, cassava, tropical forages, rice and their wild relatives collected, conserved and enhanced and made accessible to NARS and other partners.	<ul> <li>A sufficient number of accessions (of beans, cassava and tropical forages) representing genetic diversity are conserved and managed ex-situ.</li> <li>Strategies and guidelines for in-situ management of biodiversity of beans, cassava and tropical forages have been developed and tested with users.</li> <li>Accessible germplasm of beans, cassava, tropical forages and rice meet NARS standards in terms of productivity, stability, agronomic traits and user needs.</li> <li>Techniques and relevant information for more efficient and reliable germplasm improvement are accessible to users.</li> </ul>	CIAT's germplasm bank inventories.      Partners technical reports.      Annual reports.	
Purpose To increase rice genetic diversity and enhance gene pools for higher more stable	Evaluations of yield potential (interspecific, wide alite choses and recurrent selection)	• Databases.	Stability (internal and external)
yields with lower unit production costs that propiciate lower prices to consumers and	<ul> <li>Continued use of improved germplasm by NARS.</li> </ul>	<ul> <li>Project, CIAT and NARS annual reports.</li> </ul>	<ul> <li>National policies favor adoption of new technology.</li> </ul>
reduce environmental hazards.	<ul> <li>Monitoring rice production practices and markets.</li> <li>IPM practices in place for stable production and cleaner environment.</li> <li>Rice lines selected with desired gene traits.</li> <li>Potential sources for high levels of biotic and abiotic stress resistance.</li> </ul>	<ul> <li>Publications.</li> <li>Promotional Activities (conferences, training, workshops, field days)</li> </ul>	
uts Enhanced Gene Pools. Physiological Basis for Rice Traits Understood.	<ul> <li>Pathogen/pest variation and source of resistance identified.</li> <li>IPM strategies.</li> </ul>	Project progress report for	Continued support from
Host-Pests Interaction in Rice Characterized		<ul> <li>Project progress report for 1998.</li> </ul>	יייייייייייייייייייייייייייייייייייייי
Project Priorities and Research Capacities Enhanced.	<ul> <li>rafmers' surveys.</li> </ul>	Publications.	<ul> <li>Weed scientist in place.</li> </ul>
		<ul> <li>Progress reports.</li> <li>Publications.</li> </ul>	Continued adequate funding.
		<ul> <li>Project progress and workshop reports</li> </ul>	<ul> <li>Kecommendations adopted by NAKS and implemented by farmers.</li> </ul>

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<ul> <li>Project progress report for 1998.</li> <li>Field visits and evaluations in testing sites.</li> <li>Breeding populations distributed to LAC.</li> </ul>	<ul> <li>Project progress report for 1998.</li> <li>Breeding populations in storage and field.</li> <li>Best lines and QTL'S</li> </ul>	<ul> <li>dentified.</li> <li>Project progress report for 1998.</li> <li>Breeding populations in storage and field.</li> </ul>	<ul> <li>Project progress report 1998.</li> <li>Double haploids in storage</li> <li>Project progress report for 1998.</li> <li>Publications.</li> </ul>
<ul> <li>Rice populations developed and improved (tolerance soil acidity, resistance to blast, RHBV, <i>T. orizicolus</i> (13); good grain quality; early maturity.</li> <li>Number of field trials planted and lines selected.</li> <li>Populations distributed to NARS for line development.</li> </ul>	<ul> <li>Populations developed (14); populations in process (12); populations yield tested/molecular characterized (4). Partners (WARDA, CIRAD, EMBRAPA, CORNELL).</li> </ul>	<ul> <li>Number of crosses made (433); tropical irrigated (226), temperate (155), upland (52).</li> <li>Number of selected lines.</li> <li>Double haploids: interspecific crosses (386), acceleration breeding populations (815),</li> </ul>	somaclones (3758-Venezuela; 4440-Colombia)  Screening methods for tolerance to submergence developed.  Number of genotypes with tolerance to submergence  Weed competitive varieties developed
r upland and ent selection and g. las upland rice an countries. nce through opulations ng populations to	hillsides upland rice. hillsides upland rice. and selection for useful rices with the aid of arkers. Improvement of al in interspecific crosses s and QTL analyses.	Evaluation of interspecific populations. Introgression of new plant type (NPT-IRRI) into LAC's gene pools. Use of anther and in vitro culture for enhancement of gene pools.	Weed control enhanced by the use of new genotypes and practices. Identification of rice cultivars with tolerance to submergence. Identification of rice cultivars exhibiting high weed competition. Characterization of useful traits in new plant type derived nonulations.
	ement for upland and grecurrent selection ar breeding. I savannas upland rice American countries. Amintenance through on of new populations of breeding populations.	vement for upland and ing recurrent selection ar lal breeding.  of savannas upland rice in American countries.  maintenance through tion.  n of new populations in of breeding populations ries.  al and recurrent selection in and selection for useful dices with the aid of markers. Improvement of thial in interspecific crossions and QTL analyses.	Rice improvement for upland and lowland using recurrent selection and conventional breeding.  Evaluation of savannas upland rice lines in Latin American countries.  Population maintenance through recombination.  Registration of new populations to LAC countries.  Conventional and recurrent selection breeding for hillsides upland rice.  Identification and selection for useful traits in wild rices with the aid of molecular markers. Improvement of yield potential in interspecific crosses by backcross and QTL analyses.  Evaluation of interspecific populations. Introgression of new plant type (NPT-IRRI) into LAC's gene pools.  Use of anther and in vitro culture for enhancement of gene pools.

		<del></del>	
Rice crosses and populations developed by breeders. Biotech. Unit identify molecular markers associated with resistance. Continue collaboration with FLAR. Continue adequate funding from Colombia and Rockefeller.	Continue support and adequate funding from CIAT, CIRAD, and FLAR. Continued funding from Colombia, Rockefeller, Colciencias.	Permission for field testing of transgenic plants is granted. Continued support and adequate funding.	Special funds continue. Recommendations adopted by farmers. Adequate funding and timely release of budget.
• • • •	<u> </u>	· • <u>-</u>	• • •
Collection of rice pathogens. Database of resistance sources Crosses made among resistance sources. F7 lines with stable blast resistance combining genes PI-1 and PI-2.	Rice genome map with blast resistance genes mapped. Rice progress report for 1998.	Rice progress report for 1998. Publications Rice progress report for 1998. Publication and diagnostic kit available. Resistant germplasm selected under artificial conditions.	Rice progress report for 1998.
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Virulence spectrum and genetic structure of rice pathogens. Molecular markers associated and number of resistance genes. Sources of complete, complementary and partial resistance.	Rice lines with diversified resistance to RHBV and <i>T. onzicolus</i> .  More effective colony management.  Crop management components developed.  Increases capacity of NARS to screen germplasm.	Transgenic lines with RHBV-viral genes (187) with reduced symptoms. Transgenes introgressed into commercial cultivars. Characterization of RSNV and vector finished. Different control strategies for RSNV are implemented.	Costs and coefficients of production. National breeding plans written. Number of scientists trained. Published reports of courses. FLAR publications. Budget.
• • •	• • • •	• • •	• • • • •
Monitoring changes in genetic structure and virulence diversity of the rice blast pathogen. Characterization and improving methods for developing durable disease resistance (complete/partial resistance) Genetics and dissection of blast	resistance genes using molecular markers. Development and characterization of resistance to RHBV and Tagosodes orizicolus. Develop and promote IPM strategies to control RHBV.	3.6. Control of RHBV through nucleoprotein mediated cross protection in transgenic rice.  3.7. Physical, chemical and molecular characterization of the rice stripe necrosis virus.  3.8. Development of RSNV diagnostic methods and gemplasm screening techniques to implement control measures.	Analysis of national rice samples in Colombia. Creation of a network of rice economics in Latin America (RECAL). FLAR breeding and crop management activities in Latin America and the Caribbean (training). Promotional and diffusion of activities and research impact. FLAR Interactions with CIRAD/CIAT/IRRI. Collaboration with Forest Margins project for the development of improved upland rice cultivars for the Peruvian Selva.
8. 9. 5. 8. 8. 9. 9. 9. 9. 9. 9. 9. 9. 9. 9. 9. 9. 9.	3.5.		7. 4. 4. 4. 4. 4. 4. 4. 6. 6. 4. 6. 6. 4. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6.

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#### **OUTPUT 1. ENHANCED GENE POOLS**

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

Marc Châtel, Yolima Ospina and Jaime Borrero

#### 1. Introduction

CIAT and CIRAD's breeding strategies focus on broadening the genetic base of rice for Latin America and the Caribbean (LAC).

The collaborative project aims at (1) developing gene pools and populations using genetic malesterility (2) enhancing basic germplasm for different rice ecosystems using recurrent selection, (3) provide regional NARS with potential enhanced parents and/or lines with specific traits, and (4) promoting the use of population breeding by LAC NARS.

This document presents the most relevant outputs of the project for the period Sept. '98 - Sept. '99.

# 2. Outputs of the project

# 2.1. Population Breeding for the Upland Savannas ecosystem

The activities are conducted in Colombia at two experimental stations. At La Libertad in the Llanos Orientales, during the cropping season, and off season at Ciat Palmira.

Collaborative activities are also developed with LAC partners were this ecosystem is present, in Brazil, Bolivia and Venezuela. The main targeted traits we are looking to enhance are Tolerance to Soil Acidity, Resistance to Blast, Grain Quality, and Earlyness.

#### 2.1.1. Line Development from basic and enhanced populations

From the basic populations and at each cycle of enhancement through recurrent selection, fertile plants are selected to develop promising lines or potential parents for our partners in LAC. A total of 1009 lines were evaluated.

- Advanced generations: 4 lines
- Generation S7: 468 lines (78 families)
- Generation S5: 210 lines (35 families)
- Generation S3: 48 lines (8 families)
- Generation S1: 279 lines

Two lines, CIRAD 410 and 411 were registered in the CIRAD Rice Catalogue.

#### 2.1.2. Yield Trials

In 1998, the INGER VIOAL Suelos Acidos made of 31 lines was dispatched to different LAC partners.

#### 2.1.3. Population Enhancement by recurrent selection

## Recurrent selection of the population PCT-4 using S2 evaluation and recombination.

Two cycles of selection, evaluation and recombination have been made. We star the third cycle. Genetic progress will be assessed in the coming years.

# Mass selection on both sexes for total blast resistance, and Hoja Blanca Virus (VHB)

Three populations (PCT-4, PCT-A, and PCT-5) were enhanced, and 4 cycles of selection recombination were completed.

# 2.1.4. Development of a new population for the Savannas

The population PCT-11 was developed by introduction of 18 upland lines into the population PCT-4. The first evaluation started in 1998 and the enhancement is planed from 1999 and beyond.

# 2.1.5. Use of upland population breeding and selected lines by LAC partners

Since 1995, we started to release basic and enhanced populations, as well as segregating lines to our LAC partners in Brazil (EMBRAPA Arroz y Feijão), Bolivia (CIAT Bolivia), Cuba (IIA), and Venezuela (UNELLEZ).

# 2.2. Conventional Breeding for the Upland Savanna Ecosystem

Since 1995, the CIAT rice project is phasing out the development of traditional breeding activities. But, lots of advanced lines are currently in the pipeline and can be useful for ours LAC partners. Also through the INGER Network or bilateral collaboration, CIRAD lines are available for possible direct release.

During the last 2 years, 5 CIAT/CIRAD and CIRAD upland lines were released in 3 LAC countries, and one CIRAD line in China.

#### Brazil

MARAVILHA (CT 6516-23-10-1-2-2)

CARISMA (CNA 8305, CT 11251-7-2-M-M)

VENEZA (CNA 8172, CT 11614-1-4-1-M)

Bolivia

JASAYE (IRAT 170)

Colombia

LINEA 30 (CIRAD 409, CT 11891-2-2-7-M)

China

**IRAT 359** 

# 2.3. Population Breeding for the Irrigated Rice ecosystem

The activities are conducted in Colombia at the experimental station of Palmira, CIAT's headquarters. Collaborative activities are also developed with LAC partners.

The partner's countries for the tropical ecosystem are: Colombia, Costa Rica, Cuba, El Salvador, Panama, and Venezuela. The main targeted traits we are looking for to enhance are: Yield Potential, Resistance to Blast, to the Hoja Blanca Virus and its vector (Tagosodes orizicolus), Grain Quality, and Earlyness.

For subtropical conditions we are working with: Argentina, Brazil (south) and Uruguay. The main targeted traits to enhance are: Yield Potential, Resistance to Blast, Grain Quality, Tolerance to Cold, Early maturity, and Hybrid Rice development (CIRAD-CA)

For temperate conditions our partners are Argentina (Buenos Aires Province) and Chile. We also maintain close collaboration with Europe. The main targeted traits to enhance are: Tolerance to Cold, Grain Quality, and Earlyness.

# 2.3.1. Basic Population Development and use by partners

Five basic populations for irrigated rice were developed (4 at CIAT and one in French Guyana). They are PCT-6, PCT-7, PCT-8, CPCT-9 and GPIRAT-10 respectively.

# 2.3.2. Germplasm characterization and line selection by LAC partners

Populations PCT-6 and PCT-7 are best adapted to the tropics. PCT-8 is best adapted to the subtropical conditions and GPIRAT-10 for temperate ones.

Fertile plants were selected in the best germplasm to develop fixed lines.

# 2.3.3. Development of new site-specific populations

- Argentina (Universities of Corrientes and La Plata) is developing 3 new populations, PARG-1, 2, and 3, from PCT-6, 7, and 8.
- Brazil (CIRAD-CA/G4I) is developing two populations, 00EP and 00NP, from the populations IRAT MANA, and PCT-6, respectively.
- Chile (INIA Quilamapu) has already developed 2 new populations, PQUI-1 and PQUI-1\CO, from the gene pool GPIRAT-10.
- Uruguay (INIA Treinta y Tres) is developing 3 populations, PURG-1, 2, and 3 from the gene pool GPIRAT-10.
- Venezuela (Fundación DANAC) is developing 2 new populations, PFD-1 and PFD-2, from the populations PCT-6 and 7, respectively.

# 2.3.4. Population Enhancement

In Colombia the populations PCT-6, 7, 8 and GPCT-9 are enhanced for resistance to the Hoja Blanca Virus. The enhanced population PCT-6HB best adapted to the tropics was remitted to Dr. M. Valès as basic germplasm for selection to durable resistance to blast.

In Brazil, the population PCT-6 was characterized and enhanced by mass selection of early male-sterile plants and recombination. The new population 00NP will be screened for male-sterile restoration, and General Combining Ability.

In Chile, the population PQUI-1 was selected in two different conditions (North and Southern sites of the rice-growing region). Two sub-populations were derived.

#### 2.3.5. Line development

Both from basic and enhanced populations, fertile plants were selected, and their progenies are evaluated. Great and good variability was found, what is auguring a promising future.

# 2.4 Training Activities and conference organization

# 2.4.1. International Courses

Brazil: Curso Internacional sobre melhoramento Genetico de Arroz, EMBRAPA Arroz e Feijao. Goiania-Goias, March 16-28, 1998

Belize: International Training Course in Germplasm Management, CRIDNet. Balmopan, October 26-30,1998

# 2.4.2. National Course

Paraguay: Primer Curso Avanzado sobre el Cultivo del Arroz de Riego APAI and FLAR. Itapua, March 24-26, 1998

**Venezuela:** Curso Nacional de Seleccion Recurrente en Arroz Fundación DANAC. San Felipe-Yaracuy, September 21-26, 1998

# 2.4.3. International Conference

The second International Conference on Rice Recurrent Selection Breeding was held in Brazil, 21 – 24 September 1999, and organized by CIAT, CIRAD, EMBRAPA and Fundación DANAC, with the support of FAO and FLAR.

All our partners from the different countries attended the conference and presented updated data on the use of population breeding. All the communications will be published by CIAT, EMBRAPA and Fundación Polar, and edited by Dr. Elcio Perpetuo Guimaraes.

The CIAT/CIRAD collaborative project presented 4 communications. Two on Upland presented by M. Chatel and Y. Ospina, and 2 on lowland presented by M. Chatel, Jaime Borrero and M. Triana

# 2.5. Publications and Conferences

Borrero, J; M. Chatel and M. Triana Espinel, 1998. MEJORAMIENTO POBLACIONAL DE ARROZ IRRIGADO PARA HOJA BLANCA. (1) Poster: RENAPA.Goiania-Goias.March 9-13. (2) Conference: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba. June 9-11.

Chatel, M; E.P Guimarães; J.Borrero; A.Moreno Berracol; L.C. Villega and C.A Quirós, 1998. EL ARROZ DE SECANO: UNA NUEVA OPCION DE CULTIVO PARA LA REGION ANDINA DE COLOMBIA. (1) Poster: RENAPA.Goiania-Goias.March 9-13. (2) Seminar presented at CIAT — Palmira. May 6. (3) Conference: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba. June 9-1. (4) Seminar presented at the Food Crops Research Institute, Yunnan Academy of Agricultural Sciences. Kunming, China. September 9-15.

Chatel, M; E. P. Guimarães; Y. Ospina and J. Borrero, 1998. NUEVAS POBLACIONES DE ARROZ DE SAVANAS PARA SELECCION RECURRENTE

(1) Poster: RENAPA.Goiania-Goias.March 9-13. (2) Poster: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba. June 9-11.

Chatel, Y; J. Borrero; Y. Ospina (CIRAD/CIAT); S. Hernaiz; R. Alvarado (INIA-Chile), and E. Graterol (DANAC-Venezuela), 1998. GERMPLASM WITH GENETIC MALE-STERILITY FOR RICE RECURRENT SELECCION BREEDING. Poster: International Symposium on Rice Germplasm Evaluation and Enhancement. Stuttgart, Arkansas.August 30-September 2.

Chatel, M; Y. Ospina; and J. Borrero, 1998. RECURRENT SELECTION BREEDING: USING GENE POOLS AND POPULATIONS WITH RECESSIVE MALE-STERILE GENE AND CONVENTIONAL BREEDING. Conference: Third International Upland Rice Breeders Workshop (IURBW) Goiania-Goias, March 10-12.

Chatel,M; Y. Ospina; and J. Borrero, 1998. COLLABORATIVE PROJECT BETWEEN CIRAD-CA, CIAT, AND FLAR.Rice Improvement: Recurrent Selection Breeding using Gene Pools and Populations with Recessive Male-Sterile Gene, and Conventional Breeding. Upland Savanna, Hillsides, and Lowland Rice.Annual Report, 1997. CIRAD/CIAT/FLAR publication.

Chatel, M; Y. Ospina; and J. Borrero, 1998. MEJORAMIENTO GENETICO DEL ARROZ: UTILIZACION DEL METODO DE SELECCION RECURRENTE

Conference: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba. June 9-11.

Chatel, M; Y. Ospina; and J. Borrero, 1998. RICE BREEDING USING RECURRENT SELECTION AND POPULATIONS WITH RECESSIVE MALE-STERILE GENE

Seminar presented at the Food Crops Research Institute, Yunnan Academy of Agricultural Sciences. Kunming, China. September 9-15, 1998.

Ospina, Y; M. Chatel and J. Borrero, 1998. MEJORAMIENTO POBLACIONAL DE ARROZ DE SABANAS PARA SUELOS ACIDOS. (1) Poster: RENAPA.Goiania-Goias.March 9-13. (2) Poster: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba. June 9-11.

Ospina, Y; M. Chatel; E. P. Guimarães and J. Borrero, 1998. MEJORAMIENTO POBLACIONAL DE ARROZ DE SABANAS PARA PRECOCIDAD. (1) Poster: RENAPA.Goiania-Goias.March 9-13. (2) Conference: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba. June 9-11.

Ospina,Y; M. Chatel; E. P. Guimarães and J. Borrero, 1998. MEJORAMIENTO POBLACIONAL DE ARROZ DE SABANAS PARA Pyricularia grisea Sacc.

(1) Poster: RENAPA.Goiania-Goias.March 9-13. (2) Poster: I Congreso Nacional de Arroz and I Encuentro Internacional de Arroz. La Habana-Cuba, June 9-11.

Taillebois, J (presentation made by M. Chatel), 1998. INVESTIGACION EN ARROZ EN AMERICA LATINA: PROYECTO ARROZ HIBRIDO. Conference: Taller Internacional: Semilla, Insumo Esencial en la Agricultura Moderna. Chiclayo-Perú. May 11-13.

# **OUTPUT 1. ENHANCED GENE POOLS**

# B. Hillsides Upland Rice, Conventional and Recurrent Selection

Michel Vales, Marc-Henri Châtel, Jaime Borrero

# Subproject: Collaborative Project in Rice between CIAT and CIRAD Executive summary

The Collaborative Program CIRAD-FOFIFA (FOFIFA is the NARS from Madagascar) has developed a new type of upland rice for hillsides. The first promising results obtained by Marc-Henri Châtel using this material in the hillsides of Colombia (International Conference on Rice of Altitude, Madagascar, April of 1996) lead to the development this routine research in Andean areas for the indigenous farmers. In August 1997 Michel Vales came from Madagascar to CIAT for reinforcing this activity.

We use recurrent selection to provide good material to the development of varieties. During 1999 A, we made one more time a genetic recombination in the population with broad genetic base PCT-13 made by Marc-Henri Châtel. We also made the last crossings to finish the constitution of a new population with narrow genetic base (new concept). This population was available in 1999 B.

In 1999A, at the substation of Popayán (1.700 m asl) we made evaluations and selections on 4301 introduced materials mainly from Madagascar, which were multiplied during the previous semester. At the moment, the material is at the heading stage.

In 1999A, collaboration with the Confronting Soil Degradation Project (PE2) of CIAT continued. In Pescador, we evaluated 24 varieties or fixed lines: 14 for confirmation of the good behavior during the precedent cycle and 10 new ones, but of the same genetic type. The production of the best line was the double of the check of best variety in the precedent years.

We were waiting for the results in 1999 of the selected lines in Popayan and for the evaluation in Pescador, to propose new materials for the participatory research project of CIAT. We were also waiting for financial support for the Colombian Institute for the Development of Science and Technology "Francisco José of Risaralda" (COLCIENCIAS) for a collaborative project CIRAD-CIAT-CIAO, The project was not funded.

In 1999 we distributed 48 F3 populations, and 80 progenitors and lines with cold tolerance to Argentina (University of Corrientes), Chile (INIA), China (Yunnan Academy of Agricultural Sciences, YAAS), France (CIRAD-Camargue) and Madagascar (CIRAD-FOFIFA Program of Hillsides).

#### Presentation

The traditional upland rice for the areas of the cold hillsides was not existing. But the CIRAD, in collaboration with the national investigation of Madagascar (FOFIFA), made this new type of rice and this cultivation has a great success in some countries (International Conference in Rice of Altitude in Madagascar in April of 1996). The first results obtained by Marc-Henri Châtel using this material in the hillsides of Colombia take to develop this routine in Andean areas for the indigenous farmer.

#### **Background information**

In 1993, Marc-Henri Châtel introduced upland lines developed by CIRAD-FOFIFA for the hillsides from Madagascar to Colombia and he increased the seeds. The new germplasm was distributed to

the CENICAFE and the Hillsides Project of the CIAT. In 1994, the evaluation of the lines began in the Cauca.

In 1995, the International Center of Organic Agriculture (CIAO) began evaluations at 1600 m asl. The first results were presented by Marc-Henri Châtel in the International Conference on the Rice of Altitude in Madagascar in April of 1996.

In 1998, we wrote a project that it was presented in June to the Colombian Institute COLCIENCIAS, for the CIAO.

For previous information see the annual reports of Marc-Henri Châtel, previously in position of this collaborative work. From 1998 Michel Vales has the position of this part of the Collaborative Project. The last 3 years he worked in the program of CIRAD-FOFIFA for the Rice of Altitude in Madagascar.

# **Activity 1. The Recurrent Selection: Population Development**

Michel Vales, Marc-Henri Châtel, Jaime Borrero

## **Objectives**

- The Recurrent selection is a very good method to provide better material to the creation of varieties, and to obtain better materials. For this raison Marc-Henri Châtel made a population with broad genetic base for the Andean hillsides of Colombia (1300-1600 m asl). In 1999 A, the purpose of the work was to make the genetic recombination once again in this population.
- The recurrent selection in population of broad genetic base is a long-term work. We form
  another population with narrow genetic base for the medium term. The work in populations F2
  is the short-term work.

#### **Material**

# Population with broad genetic base

In 1996 B, 11 lines, 6 of hillsides form the CIRAD-FOFIFA Program of Madagascar, 4 from the Project of Hillsides of the CIAT, and 1 line IRAT, was selected according to its previous evaluations in high altitudes for the precocity and the fertility.

Marc-Henri Châtel used a *japonica* upland population (PCT-4) very adapted as male-sterility source. Each line crossed at least with 4 male-sterile plants of the PCT-4.

During 1997 A, in the Experimental Station of Palmira (PES), each resulting F1 was cultivated individually, evaluated, and plants was selected. The seeds F2 of the selected F1 plants was taken in same proportions. Each mixture F2 was mixed with balanced proportions to constitute the new basic population, it is identified as PCT-13\0\0\0\0.

During 1997 B and 1998 A, in PES, the basic population was recombined a one more time. The result of these first two recombination cycles of the basic population will be identified as PCT-13\0\0\2.

During 1999-A the population MD1 (lowland) and MD2 (upland) from Madagascar was recombined to obtain new seeds.

# Population with narrow genetic base

The concept (see Output 3. B. Activity 2, Specific Germplasm Development) is to choose the minimum of progenitors that you/they grant with the main objectives of the selection and without consideration for their other characteristics:

Male-sterility source

PCT-13\0\0\1

# Progenitors for:

- Production

CT10069-27-3-1-4

- Grain quality

CT10069-27-3-1-4

- Cold tolerance

Inca

- Grain quality

Inca

IIICa

- Durable resistance to Blast

IRAT 13

- Durable resistance to Blast

Oryzica Ilano 5

- During 1998-A, the first cycle of crosses was done.

	Female			Male
Crosses	PCT-13\0\0\2	[ms]	1	CT10069-27-3-1-4
First	Male-sterile	[ms]	1	Inca
cycle 1998 A	plants	[ms]	1	IRAT 13
		[ms]	1	Oryzica llanos 5

#### Method and results

# • Population with broad genetic base

During 1998 A, the PCT-13 was recombined a one more time, harvesting male-sterile plants. The obtained basic population was identified as PCT-13\0\0\2.

## Population with narrow genetic base

During 1998 B, the second cycle of crosses was done.

	Female	}	Male
	CT10069-27-3-1-4	//	[ms] / Inca
Crosses	Inca	//	[ms] / IRAT 13
	IRAT 13	//	[ms] / Oryzica llanos 5
Secondcycle	Oryzica Ilanos 5	//	[ms] / CT10069-27-3-1-4
	[ms] / CT10069-27-3-1-4	//	IRAT 13
1998 B	[ms] / Inca	//	Oryzica Ilanos 5
	[ms] / IRAT 13	//	CT10069-27-3-1-4
	[ms] / Oryzica llanos 5	//	Inca

The final genetic participation of the population PCT-13\0\0\1 is 25% and for each one of the new progenitors it is 18.75%

## **Perspectives**

## Population with broad genetic base

With three recombinations the population PCT-13\0\0\3 is ready for the beginning of the recurrent selection. We will initiate this recurrent selection from the obtaining of enough resources.

# Population with narrow genetic base

With this method the participation of each one of the progenitors is high. Then a drastic selection in the population is possible without genetic derive, to obtain result for the medium term. This population is available for all the national programs working in rice of hillsides.

It was introduced new progenitors from Madagascar, in particular, resistant to the cold. With this material we will begin the formation of a new population with narrow base to replace in the future to the first one.

# Activity 2. Conventional selection and evaluation

Michel Vales, Marc-Henri Châtel, Jaime Borrero, Edmundo Barrios<sup>(1)</sup>, Juan-Guillermo Cobo<sup>(1)</sup>

(1) Confronting Soil Degradation Project of CIAT

# 2.1. Conventional selection and evaluation

#### Material

In 1999 A in the substation of Popayán (1.700 m asl) we made the selection in 4301 introduced materials, mainly of Madagascar, and multiplied during previous semesters:

- 8 varieties of the collection of the CIRAD-CA, potential progenitors for the grain type
- 48 populations ME (balanced bulk) F3
- 839 lines F2 and F3
- 3201 lines F4
- 104 varieties in 129 accessions
  - 2 traditional lowland varieties from Nepal
  - 2 traditional lowland varieties from Madagascar
  - 2 lowland varieties from the Program CIRAD-CA/FOFIFA of Madagascar
  - 1 traditional lowland varieties from China
  - 1 traditional lowland varieties from Japan
  - 16 traditional upland varieties from Japan
  - 9 of hillsides varieties from the Program CIRAD-CA/FOFIFA of Madagascar
  - 41 fixed hillsides lines from the Program CIRAD-CA/FOFIFA of Madagascar
  - 7 upland varieties from LAC and of Africa
- 76 populations F2 from 27 crosses

The 13 checks with different resistance levels to the cold are:

- 2 traditional lowland varieties from Nepal
- 1 traditional lowland varieties from Madagascar

- 1 traditional lowland varieties from Japan
- 1 traditional upland varieties from Japan
- 2 varieties of hillsides of the Program CIRAD-CA/FOFIFA of Madagascar
- 2 fixed hillsides lines from the Program CIRAD-CA/FOFIFA of Madagascar
  - 4 varieties upland varieties from LAC, and Africa

#### Methods

The trial was sowed in the substation CIAT in the Cauca department, in the Popayán municipality, vereda of Santa Rosa, property Santa Rosa, 1700 m asl, 2°25 N, 76°40 W. The climate of this station is characterized by 2000 mm of annual precipitation, a temperature average of 17°C, with a maximum of 21°C and a minimum of 13°C

Minus the populations F2 and MEF3 the whole material was sowed in 63 blocks of Federer of 80 materials: 67 varieties or lines and 13 randomized checks in each block.

#### Results

The trial was sowed the June 5<sup>th</sup> of 1999. At the date it is the flowering stage.

#### 2.2. Collaborative evaluation

#### **Materials**

In 1999-A collaboration with the Confronting Soil Degradation (PE2) of CIAT was followed. In Pescador we made a trial on 24 varieties or fixed lines: 14 for confirmation of the good behavior during the precedent cycle and 10 new ones, but of the same genetic type.

The 2 checks are Latsidahy / IRAT 351-1 and CIRAD 392, the best materials in the previous cycle.

#### Methods

The trial was sowed in the experimental place of the Hillsides Program of CIAT in the Cauca department, Pescador municipality, property San Isidro, 1500 m asl. The climate is characterized by 1800 mm of annual precipitation, a maximum temperature of 25°C and a minimum of 17°C. There is a rotation rice-bean.

You can analyze this experimentation as a trial with three repetitions, for search the more stable material, or as three trials, collection with two repeated checks, to look for the material which is the more adapted to each one of the three situations with very different soil.

#### Results

With the analysis of the experiment like complete blocs trial with 3 repetitions, the line PRA8-F169-6-3-2-6 from the CIRAD-FOFIFA program of Madagascar has a production greater than the double of the check one (Tab.1). The check was the previous best variety in the past years.

With the analysis of each repetition like a collection with repeated check the best lines o varieties are the following:

- Wetter soil (nearer a little wood)

PRA102-177 and CIRAD 393, then IRAT 352

- Richer organic soil (this accumulation is due to the erosion of the upper soil)

PRA58-291, then PRA8-F46-7-4-6 and PRA8-F169-6-3-2-6

- Poorer and dry soil (drought problem is this part)

Latsidahy / IRAT 351-1 (the check) and PRA102-177, then IRAT 379.

For other details see the annual report of the Dr. Edmundo Barrio (PE2).

Table 1.: Hillsides rice evaluation. Pescador 1999 A

	Block		_	111	Average	•
#	Name		g/10	m 2		
16	PRA8-FI69-6-3-2-6	3740.00	1282.67	1390.00	2137.56	а
19	PRA102-189	796.36	1153.33	1840.00	1263.23	b
13	LATSIDAHY / IRAT 351-1	1434.78	987.69	626.67	1016.38	b
6	IRAT 380	1440.00	509.33	1086.67	1012.00	b
9	CIRAD 393	488.00	1376.00	1100.00	988.00	b
5	IRAT 379	964.00	765.33	720.00	816.44	b
14	PRA29-F267-4-8-5-4	1488.00	864.00	0.00	784.00	b
23	FOFIFA 151 HT3	1100.00	0.00	940.00	680.00	b
18	PRA102-177	390.00	840.00	780.00	670.00	b
15	PRA8-F46-7-4-6	1288.00	585.00	0.00	624.33	b
8	CIRAD 392	0.00	940.00	822.86	587.62	b
2	IRAT 351	428.00	1217.27	0.00	548.42	b
10	CIRAD 394	762.67	832.00	0.00	531.56	b
20	PRA112-227	783.33	636.00	0.00	473.11	b
3	IRAT 352	368.00	696.00	0.00	354.67	b
21	PRA58-291	0.00	292.00	0.00	97.33	b
1	SHIN EI	0.00	0.00	0.00	0.00	b
4	IRAT 353	0.00	0.00	0.00	0.00	b
7	CIRAD 391	0.00	0.00	0.00	0.00	b
11	CIRAD 407	0.00	0.00	0.00	0.00	b
12	CIRAD 408	0.00	0.00	0.00	0.00	b
17	PRA122-139	0.00	0.00	0.00	0.00	b.
22	FOFIFA 151 HT1	0.00	0.00	0.00	0.00	b
24	CITE PLANTON	0.00	0.00	0.00	0.00	b
	e averages followed by a	General ave	rage		524.36	
	e letter are not differente	CV (%)			88.80	
(5%,	Test of Newman-Keuls)	ETM			268.77	

# 2.3. Germplasms delivery to identified partners

We give populations F3, progenitors and varieties with cold tolerance to:

- Argentina (University of Corrientes): 6 F3 and 19 varieties from Rumania
- Chile (INIA): 48 F3
- China (Yunnan Academy of Agricultural Sciences, YAAS): 80 varieties
- FRANCE (CIRAD-Camargue): 19 F3
- Madagascar (CIRAD-FOFIFA Hillsides Program): 48 F3.

## **OUTPUT 1. ENHANCED GENE POOLS**

# C. Advance and evaluation of inter-specific populations.

Carlos BRUZZONE, Jaime BORRERO and César MARTÍNEZ

CIAT started in 1994 a collaborative project aimed to characterize and use wild rice species as new gene sources to increase the yield potential and broaden the genetic base of cultivated rice (*Oryza sativa* L). With this goal, a strategy was designed based upon a backcrossing program for the development of near isogenic lines and the identification of molecular markers associated with yield increments in segregating populations. Martínez (Rice Project, Annual Report, 1998) has previously reported progresses made in the molecular characterization of these populations and the linkages observed between markers and yield. New developments on this regard are being reported elsewhere by the Biotechnology Unit.

We report here progresses that have been made on the yield evaluation and the generation advance of inter-specific populations developed by this project.

Yield trial of F<sub>2</sub>RC<sub>2</sub> lines derived from the cross BG 90-2 x O. barthii

Two hundred ninety six  $RC_2F_2$  lines derived from the cross BG 90-2 x O. barthii were evaluated in a yield trial, with two replications, under transplanting conditions, in Palmira. The planting was made last year, but most evaluations taken this year. Table 1 shows the main agronomic and grain quality characteristics from the ten more yielding lines of this trial.

No yield advantages were observed compared to the recurrent parent, BG 90-2, suggesting that *O. barthii* no possesses yield alleles different from those present in BG 90-2.

Yield trial of F<sub>4</sub>RC<sub>3</sub> lines from the cross Lemont x O. barthii

A yield evaluation of 55 F<sub>4</sub>RC<sub>3</sub> lines from the cross Lemont x *O. barthii* was made in a replicated trial, under transplanting conditions, in Palmira.

Most evaluated lines showed stronger plant vigor than recurrent parent Lemont. Table 2 shows the main agronomic and grains characteristics of the 10 more yielding lines of this trial. No statistically significant differences in grain yield were found between tested lines and Lemont.

Since Lemont is a variety adapted to direct seeding conditions in temperate climates, these lines may have a greater chance to show their potential under such conditions.

Yield trial of F<sub>5</sub>RC<sub>2</sub> lines derived from the cross BG90-2 x O, rufipogon

One hundred sixty four BC<sub>2</sub>F<sub>5</sub> lines and both parents of the cross BG 90-2 and *O. rufipogon* were tested in a four-replicated yield trial, under transplanting conditions in Palmira.

Most lines have growth duration period, plant height and plant type very similar to the recurrent parent, BG90-2. Some lines showed lesser white belly in the grain than BG 90-2. Segregation for grain color, with plants having black or striped long awned grains similar to *O. rufipogon*, was also observed.

Table 3 shows main agronomic and grain quality data from the twenty highest yielding lines. No statistical differences were found between these lines and the recurrent parent, BG 90-2, even though several lines surpassed such variety in more than 1.0 MT per ha., and two of them showed a yield advantage of more than 20%.

The yield performance of these F5 lines is consistent to that showed in previous generations, as can be observed in Table 4. Field observations recorded this year seem to confirm putative presence of yield alleles in *O. rufipogon* not present in BG 90-2. Most lines showing a yield advantage over BG 90-2 this year, also showed heavier grain weight. No other yield components were recorded this year.

## • Yield trials of F<sub>4</sub>RC<sub>2</sub> lines from the cross Oryzica 3 x O. rufipogon

Seven  $F_4RC_2$  lines derived from the cross Oryzica 3 x O. rufipogon selected in  $F_3$  for showing a desirable combination of agronomic traits, resistance to rice diseases (RHBV and P. grisea) and grain quality, were tested in a three-replicated yield trial, under transplanting conditions, in Palmira. Reaction to fungal diseases of these lines was also evaluated in Santa Rosa (Villavicencio, Meta). Table 5 shows main observations recorded in this trial. None of evaluated lines surpassed to Oryzica 3 in grain yield.

A set of 42 lines from this same population, selected on non-replicated plots yield data taken last year, was again tested in a three replicated yield trial in Palmira this year. Reaction to fungal diseases in Santa Rosa, was also tested.

As Table 6 shows, several lines outyielded Oryzica 3, although no significant differences were found. Even though this yield advantage was not as evident as in the case of lines derived from the cross BG 90-2 x O. rufipogon, however, it is apparent that these lines are higher yielders than Oryzica 3, and that this advantage was obtained through O. rufipogon.

## • BG90-2 / O. glaberrima BC₃F₁ population

One hundred thirty nine BC<sub>3</sub>F<sub>1</sub> families (CT16043 – CT16182) derived from the cross BG90-2 / O. glaberrima, having about 50 plants each one, were tested under transplanting conditions in Palmira.

Most families showed high sterility percentage, close to 100%. Best 49 plants from the 16 best families were selected for spikelet fertility (greater than 50%), medium to long grain, short to intermediate plant stature, short to intermediate growth duration, and erected tillers.

#### BG90-2 / O. glaberrima BC₂F₂ population

Five thousand BC<sub>2</sub>F<sub>2</sub> BG90-2 *I O. glaberrima* plants derived from a mass selection of the most fertile BC<sub>2</sub>F<sub>1</sub> plants made last year, were planted this year under both irrigated (in Palmira) and favored upland conditions (in Santa Rosa).

In CIAT-Palmira, this population showed wide variability in plant type and grain. The best 250 plants were selected based upon their plant type (semidwarf and erect tillers), grain fertility, grain type (medium to long grain), short maturation period and good tillering ability. Selection at the Santa Rosa location is in progress at this time.

## Caiapo / O. glaberrima BC₂F₁ population

One hundred two BC₂F₁ families (CT15940 – CT16042), having about 50 plants per family, were tested under transplanting conditions in Palmira.

The best 28 plants from the best 16 families were selected on the basis of grain fertility (greater than 50%), earliness, grain shape (medium to long) and plant type (semidwarf plant with erect

tillers). Since there was observed a high degree of sterility, a third backcross was made to the recurrent parent, Caiapó, using one or two plants from each of the 97 best families.

# Oryzica Llanos 5 / O. barthii BC₂F₁ population

We evaluated 48 BC<sub>2</sub>F<sub>1</sub> families (CT15059 – CT15107) derived from the cross Oryzica Llanos 5 / O. barthii (having about 50 plants each one), under transplanting conditions in Palmira. The best 62 plants from the best 24 families were then selected based upon seed set (higher than 50%), grain size, shape and color (medium to long, medium to slend and straw-colored grain) and plant type.

Given the very low seed set observed in the BC<sub>2</sub>F<sub>1</sub> families, a new round of backcrosses was carried out to the recurrent parent, O. Llanos 5, using a plant per each family, 45 crosses were made, producing about 60-70 seeds per backcross.

Table 1. Agronomic and grain quality traits of 11 BC₂F₂ lines from the cross BG 90-2 x O. barthii evaluated under transplanting conditions. CIAT, Palmira (Average of two replications).

	Grain Y	ield		1000-				
	MT/ha	Sign. 0.05	Days to	grain weight	1 White	² Grain	3	Amylose %
Pedigree		Tuckey	Flowe- ring	(g.)	belly	Length	GT°	
CT 14983-17	5.93	Α	100	29.5	3.4	L	1	30.8
CT 14983-31	5.86	AB	99	29.5	2.4	L	1	33.0
CT 15005-12	5.68	AB	104	31.0	2.8	L	i	31.2
CT 14983-16	5.61	AB	105	30.3	3.4	L	I	32.0
CT 14983-25	5.56	AB	99	27.0	2.6	L		32.0
CT 14983-24	5.48	AB	100	28.0	2.2	L	1	31.6
CT 14983-59	5.48	AB	100	28.0	1.0	Ļ	1	28.1
CT 15007-33	5.42	AB	102	25.8	3.6	L	ļ	32.5
CT 14988-44	5.41	AB	96	30.3	3.0	L	l	27.8
CT 14983-32	5.40	AB	106	30.5	2.8	L	ſ	31.1
Bg 90-2	5.74	AB	102	29.5	2.6	L	1	29.8
O. barthii	-	С	56	42.0	2.8	EL	1	29.8
	17.8			· -				

<sup>&</sup>lt;sup>1</sup> Scale 0-5; where 0 = free from grain chalkiness, 5 = 100% chalkiness.

 $<sup>^{2}</sup>L = 6.6 \text{ to } 7.5 \text{ cm, } EL = > 7.5 \text{ cm.}$ 

<sup>&</sup>lt;sup>3</sup> G T ° = gelatinization temperature; I = intermediate.

Table 2. Agronomic and grain quality traits of the 10 most yielding out of 55 BC₃F₄ lines from the cross Lemont x *O. barthii*, in comparison to the recurrent parent (Lemont) and other commercial varieties, under transplanting conditions. CIAT, Palmira (Average of three replications).

	Grain	Yield					
Pedigree	MT/h a	Sign. 0.05 Tuckey	Days to Flowe- ring	1 White belly	gT°	Amy- lose %	3 Grain length
CT 14954-14-3-M	4.37	BC	99	0.5	1	28.2	L
CT 14946-11-1-M	4.27	BCD	105	0.6	1	27.2	L,M
CT 14938-30-5-M	4.27	BCD	100	0.3	i	27.8	L
CT 14938-36-1-M	4.08	BCDE	95	0.5	1	28.0	L
CT 14938-16-M-M	4.05	BCDE	87	1.0	l l	28.6	L
CT 14946-65-3-M	3.91	BCDE	95	0.4	ı	28.2	L
CT 14936-19-M-M	3.88	BCDE	91	0.9	1	28.1	L
CT 14946-45-6-M	3.82	BCDE	101	0.3	I	26.4	L
CT 14937-24-M-M	3.81	BCDE	94	1.0	1	27.7	L,
CT 14938-67-1-M	3.80	BCDE	95	0.5	IA/I	27.5	Α
Lemont (recurrent p.)	3.69	CDE	88	1.3	1	28.5	L
Commercial checks							
PNA 1803-3-18-1-	5.31	Α	82	0.9	В	22.8	L
3-3	4.85	AB	90	0.5	Bi	23.3	L
Santa Ana-PH2	4.14	BCDE	83	8.0	l	27.2	L
Cypress INIA-Tacuarí	3.98	BCDE	74	0.5	IA/I	28.3	L
C.V. (%)	13.6						

<sup>&</sup>lt;sup>1</sup> Scale 0-5; where 0 = free from grain chalkiness, 5 = 100% chalkiness.

<sup>&</sup>lt;sup>2</sup> G T ° = gelatinization temperature; I = intermediate.

 $<sup>^{3}</sup>L = 6.6 \text{ to } 7.5 \text{ cm}, EL = > 7.5 \text{ cm}.$ 

Table 3. Agronomic and grain quality traits of the 20 most yielding out of 164 BC₂F₅ lines from the cross BG 90-2 x *O. rufipogon*, under transplanting conditions. CIAT, Palmira (Average of four replications).

	Grain Y	ield		1000-				
Pedigree	MT/ha	Sign. 0.05 Tuckey	Days to Flowe -ring	grain weight (g.)	White belly	<sup>2</sup> Grain Length	3 GT°	Amy lose %
CT 13956-29-M-3-M	7.38	Α	96	33.3	3.0	L		31.3
CT 13976-7-M-6-M	7.17	АВ	98	30.2	2.2	M	i	32.0
CT 13956-29-M-2M	7.03	AB	99	30.4	2.0	M	i	32.1
CT 13956-29-M-29-M	6.98	AB	98	33.5	3.4	L	1	28.9
CT 13959-3-M-30-M	6.88	АВ	98	31.5	2.0	L	i	31.6
CT 13956-29-M-14-M	6.87	AB	95	29.8	2.2	L	I	29.3
CT 13943-2-M-2-M	6.74	AB	101	30.2	3.0	L	i	32.0
CT 13958-13-M-26-M	6.72	ΑB	102	28.1	0.6	_ L	i	30.9
CT 13976-7-M-21-M	6.68	AB	103	32.2	2.8	L	i	30.8
CT 13959-3-M-10-M	6.65	АВ	99	31.1	1.8	L	1	32.0
CT 13956-29-M-4-M	6.64	AB	97	31.7	3.6	L	1	28.3
CT 13972-8-M-21-M	6.55	AB	100	30.1	1.8	М	i	30.8
CT 13946-26-M-15-M	6.55	AB	99	30.8	2.2	L	1	32.0
CT 13958-13-M-5-M	6.50	AB	101	26.0	1.6	L	1	30.4
CT 13956-29-M-1-M	6.50	AB	99	30.4	3.2	L	I	31.6
CT 13958-13-M-17-M	6.48	AB	101	33.1	2.8	L	1	30.5
CT 13972-8-M-5-M	6.47	AB	97	32.1	2.2	L	1	31.6
CT 13941-27-M-14-M	6.47	AB	104	28.5	8.0	L	1	32.0
CT 13958-13-M-11-M	6.46	AB	104	31.1	_	M	ì	30.6
CT 13972-8-M-17-M	6.45	AB	100	29.1	2.4	L	i	32.0
3G 90-2	5.86	AB	105	29.5	2.6	L	ı	29.8
O. rufipogon	3.80	С	90	26.7	1.4	М	1	28.5
CV (%)	10.9							

<sup>&</sup>lt;sup>1</sup> Scale 0-5; where 0 = free from grain chalkiness, 5 = 100% chalkiness.

 $<sup>^{2}</sup>L = 6.6 \text{ to } 7.5 \text{ cm, } EL = > 7.5 \text{ cm.}$ 

<sup>&</sup>lt;sup>3</sup> G T ° = gelatinization temperature; I = intermediate.

Table 4. Grain yield of the best  $BC_2F_5$  lines from the cross BG 90-2 x O. rufipogon, in comparison to their respective  $BC_2F_3$  y  $BC_2F_2$  ancestor lines.

Palmira, 1999 <sup>1</sup>		Palmira 19	Palmira 1997 <sup>2</sup>				
BC₂F₅	MT/ha	BC <sub>2</sub> F <sub>3</sub>	MT/ha	BC₂F₂	MT/ha		
CT 13941-27-M-14-M	6.47	CT 13941-27-M	7.29	CT 13941-27	6.93		
CT 13959-3-M-10-M	6.65	CT 13959-3-M	6.68	CT 13959-3	6.82		
CT 13959-3-M-30-M	6.88						
CT 13958-13-M-5-M	6.50	CT 13958-13-M	7.02	CT 13958-13	6.77		
CT 13958-13-M-11-M	6.46						
CT 13958-13-M-17-M	6.48						
CT 13958-13-M-26-M	6.72						
CT 13943-2-M-2-M	6.74	CT 13943-2-M	6.74	CT 13943-2	6.65		
CT 13956-29-M-1M	6.50	CT 13956-29	7.22	CT 13956-29	5.96		
CT 13956-29-M-2M	7.03						
CT 13956-29-M-3-M	7.38						
CT 13956-29-M-4-M	6.64						
CT 13956-29-M-14-M	6.87						
CT 13956-29-M-29-M	6.98						
CT 13946-26-M-15-M	6.55	CT 13946-26-M	7.36	CT 13946-26	5.95		
CT 13976-7-M-6-M	7.17	CT 13976-7-M	7.52	CT 13976-7	5.91		
CT 13976-7-M-21-M	6.68						
CT 13972-8-M-5-M	6.47	CT 13972-8-M	7.29	CT 13972-8	5.80		
CT 13972-8-M-17-M	6.45						
CT 13972-8-M-21-M	6.55						
BG 90-2	5.86		6.50		5.97		
O. rufipogon	3.80		5.00		2.20		

<sup>&</sup>lt;sup>1</sup> Average of four replications

<sup>&</sup>lt;sup>2</sup> Average of two replications

Table 5. Agronomic, grain quality traits and reaction to diseases of seven BC<sub>2</sub>F<sub>4</sub> lines from the cross Oryzica 3 x *O. rufipogon,* in comparison to the recurrent parent (Oryzica 3) and other commercial varieties, under transplanting conditions. CIAT, Palmira (Average of three replications).

				PA	PALMIRA 99A	V 99A				STA	STA ROSA 99	<u></u>
	Grai	Grain yield		-	. ~	;	m		, cion			
		Sign.	- Days	White		Amy- lose	Grain	Sogata	Blanca	Brown	Leaf	Neck
	MT/ Ha	0.05 Tukey	Flowe- ring	belly	GT°	% -	length		Virus	spot	blast	blast
CT 14546-6-M-M	4.93	AB	104	0.2	_	29.2		က	3	5	4	7
CT 14539-34-M-M	4.83	AB	108	0.2	_	27.0	_	က	Q	5	4	7
CT 14544-12-M-M	4.68	ω	104	0.2	_	29.3	_	7	ო	ო	ო	7
CT 14537-8-M-M	4.47	BC	108	0.2	_	29.6	_	7	2	ო	ო	7
CT 14537-6-M-M	4.45	BC	105	0.2	_	28.5	_	5	2	ო	4	7
CT 14545-5-M-M	4.25	BC	104	0.2	_	28.6	_	7	cy.	ო	ო	7
CT 14532-18-M-M	4.10	BC	106	0.2	_	29.0	_	ო	2	2	4	5
Oryzica-3 (recurrent)	5.05	AB	108	0.2	ω	27.0	١	വ	ო	ო	ო	_
Commercial varieties:												
ICTA Pazos	5.65	∢	110	0.2	_	30.4	_	ı	•			1
Fedearroz 50	4.45	BC	112	0.2	Ω	29.8	_	<del>-</del>	<b>-</b>	က	ო	က
Oryzica Llanos 5	4.39	BC	107	0.2	В	29.4	EL	7	5	,		
C.V. (%)	10.5											

Scale 0-5; where 0 = free from grain chalkiness, 5 = 100% chalkiness...

 $<sup>^2</sup>$  GT  $^{\circ}$  = gelatinization temperature; I = intermediate

 $<sup>^{3}</sup>L = 6.6 \text{ to 7.5 cm}, EL = > 7.5 \text{ cm}.$ 

Table 6. Agronomic, grain quality traits and reaction to diseases of the ten most yielding out of 42 BC<sub>2</sub>F<sub>4</sub> lines from the cross Oryzica 3 x *O. rufipogon*, evaluated in comparison to the recurrent parent (Oryzica 3) and other commercial varieties, under transplanting conditions. CIAT, Palmira (Average of three replications).

				PALI	PALMIRA 99A	99A			·	ST	STA ROSA 99	66 \
	Grain	Grain yield	Days	-	8	Amy-	es es		Hoja			
	MT/Ha	Sign. 0.05 Tukey	Flowe-	White belly	T.G	lose %	Grain length	Sogata	Blanca Virus	Brown spot	Leaf blast	Neck blast
CT 14545-5-M-1	6.11	4	107	0.4	-	30.4	_	3	5	က	3	7
CT 14537-6-M-3	60.9	∢	108	4.0	_	29.8	_	7	S)	_	4	2
CT 14534-25-M-M	6.05	∢	110	0.2	_	29.6	_	တ	5	5	4	7
CT 14534-35-M-M	6.04	∢	105	0.2	_	30.0	_	<b>о</b>	ო	က	ო	5
CT 14539-26-M-3	5.97	AB	110	9.0	_	28.6		1	7	က	ო	7
CT 14545-5-M-M	5.95	AB	106	0.3	_	28.4	L,M	7	5	ო	ო	7
CT 14539-34-M-M	5.87	AB	110	0.2	_	29.6	Ľ,M	ო	5	5	4	7
CT 14546-6-M-M	5.84	AB	106	4.0	_	30.7	<b>.</b>	5	5	2	4	7
CT 14556-2-M-M	5.83	AB	110	4.0	_	30.2	_	7	5	ო	ო	7
CT 14537-8-M-M	5.75	AB	110	0.2	_	28.5	_	7	2	ო	ო	7
Oryzica-3 (recurrent p.) Commercial checks:	5.45	AB	106	0.2	_	29.8	_	ო	м	ო	ო	7
ICTA Pazos	5.56	AB	110	0.2	_	31.0	EL,L			ι	ı	1
PNA 2002-HU1-2-EP1	5.12	AB	106	0.3	<u>B</u>	23.4	급	<b>o</b>	_		1	
Fedearroz 50	5.07	AB	112	0.2	۵.	31.4	_	ო	ო	က	ო	ო
Oryzica-1	4.44	АВ	<u>თ</u>	0.2	<b>∢</b> ₪	29.6	<b>-</b> 1	7	လ	7	4	7
C.V. (%)	13.6											
10-12-0	f f	12.11 - 41 - 12.22		70007								

<sup>&</sup>lt;sup>1</sup> Scale 0-5; where 0 = free from grain chalkiness, 5 = 100% chalkiness...

 $<sup>^2</sup>$  GT  $^\circ$  = gelatinization temperature;  $^1$  = intermediate.  $^3$ L = **6.6 to 7.5 cm, EL** = > **7.5 cm.** 

#### **OUTPUT 1. ENHANCED GENE POOLS**

# D. Introgression of new plant type (NPT-IRRI) genes into LAC's gene pools

Carlos BRUZZONE, James CARAVALÍ and Diana DELGADO (FLAR)

# 1. Advance, evaluation and selection of populations having a NPT parent

Two hundred ninety nine populations derived from crosses having a parent with NPT (introduced from IRRI) were tested this year. One hundred ninety five of them are aimed to tropical irrigated rice ecosystems from LAC, and 104 to temperate irrigated and upland rice.

A first group of 149 populations F1 were evaluated in Palmira for plant type and grain type having harvested 1,178 plants from 136 populations selected for grain fertility, semidwarf plant type and long grain. The F2 populations derived from those plants were later screened for their reaction to RHBV showing 351 of them a resistant reaction, 311 an intermediate reaction and 515 a susceptible reaction.

From this first group, 810 F2 families derived from 87 crosses having parents with complementary sources of resistance to all blast (*Pyricularia grisea*) lineages were tested in Santa Rosa, under a heavy pressure of *Helminthosporium*, *Pyricularia* and grain discoloration. Two hundred twenty five plants were harvested from 59 F2 families (derived from 32 crosses), based upon their grain type and healthiness. Twenty-five of these families showed a resistant reaction, 16 an intermediate reaction and 18 a susceptible reaction when screened for RHBV resistance. Plants (F3 lines) derived from families resistant to Hoja Blanca will be tested for their sogata tolerance and grain quality. These lines are best suited for tropical conditions, while those that showed susceptibility to Hoja Blanca may still be useful for temperate conditions.

From the same group of 149 populations, 368 F2 families derived from 49 crosses, whose parents have no resistance to all *P. grisea* lineages, are being evaluated for agronomic and grain quality traits at Palmira.

Two hundred thirty two plants were harvested from 41 populations, out of 46, selected for spikelet fertility, semidwarf plant type, and long grain. The F2 populations derived from them are being evaluated in Palmira for their agronomic and grain quality traits, and later on for their reaction to RHBV.

# 2. Fixation of irrigated temperate and upland rice populations through anther culture.

Anthers of 104 irrigated and upland rice crosses having a NPT parent were cultured in order to speed up the development of homozygous lines. So far about 1,000 green plants have been regenerated. Double haploid lines will be then tested for plant type, grain and milling quality. The best lines will be distributed to our partners through INGER for evaluation in LAC countries.

# 3. Evaluation of NPT lines recently introduced from IRRI

Forty-nine NPT lines introduced from IRRI this year were evaluated under heavy fungal disease pressure in Santa Rosa (Meta). Most of these lines showed poor seedling vigor and foliar reactions to *P. grisea* higher than 4 degree, and those few lines showing resistant leaf reaction to *P. grisea*, presented at panicle stage either susceptible reactions to *P. grisea* or grain discoloration (Table 1).

Table 1. Reaction to fungal diseases of NPT lines in Santa Rosa (Meta), 1999.

Response			Vi-gor	LBI	LBI	FL	Esc	Llus	NDI	
R6598-44-9-1   Iakaneshikußail Onliger	IR65564-44-2-3 <sup>1,2</sup>	Takaneshiki/Ball Ontier						Hm	ИВІ	GD.
R 65600-17-1-22		Takaneshiki/Bali Ontjer		3				_	-	-
R 65600-27-12-22   Shen Nung 89-366/Ketan Lumbu   5		Shen Nung 89-366/Genjah Wangkal						_	_	_
R 65600-38-1-2-1		Shen Nung 89-366/Ketan Lumbu			-			_		-
R65600-54-6-3   Shen Nung 89-366/Kelan Lumbu   5   3   4   108   3   -	IR 65600-38-1-2-1 <sup>1</sup>	Shen Nung 89-366/Ketan Lumbu							_	-
R 65600-77-4-2-1	IR 65600-54-6-3	Shen Nung 89-366/Ketan Lumbu			-			-	-	-
R 65600-127-62-33 Shen Nung 89-366/Kelan Lumbu 7 5 4 112		Shen Nung 89-366/Ketan Lumbu							-	7
R66600-129-1-1-2   Shen Nung 89-366/Ketan Lumbu   5	IR 65600-127-6-2-3 <sup>1</sup>	Shen Nung 89-366/Kelan Lumbu						3	3	,
R 66169-189-5-5-3   Shen Nung 89-366/Gmdil Kuning   5	IR 65600-129-1-1-2	Shen Nung 89-366/Ketan Lumbu						-	-	-
R66160-52-3-1	IR 66159-189-5-5-3	Shen Nung 89-366/Gundil Kuning					4	-	7	2
R 66160-52-3-2   Shen Nung 89-366   Jimbrug   5		Shen Nung 89-366/ Jimbrug								
R66160-121-4-1-1   Shen Nung 89-366/ Jimbrug	IR 66160-5-2-3-2 <sup>1,2</sup>	Shen Nung 89-366/ Jimbrug					-		′	3
R 66160-121-4-4-2   Shen Nung 89-366/ Jimbrug		Shen Nung 89-366/ Jimhrug		-					-	-
R 68160-121-4-5-3	IR 66160-121-4-4-2	Shen Nung 89-366/ Jimbrug		-					-	-
R 66738-118-1-2 <sup>12</sup>		Shen Nung 89-366/ Jimbrug	5						-	-
R 66750-6-2-1   Shen Nung 89-366/Sri Kuning   5	IR 66738-118-1-2 <sup>1,2</sup>	Shen Nung 89-366/ Sanoniana	5						-	-
R 67962-40-63-33   R 65600-1-2/Ketan Lombok   5		Shen Nung 89-366/Sri Kuning	5				-	1	5	3
R 67962-84-2-2	IR 67962-40-6-3-3	IR 65600-1-2/Ketan Lombok		-			-	-	-	-
R 67962-84-22-2		IR 65600-1-2/Ketan Lombok	5 5				-	-	-	_
R 6966-44-2-3-2   R 65600-1-2/Senkeu		IR 65600-1-2/Ketan Lombok						-	-	
R 67966-188-2-2-1		IR 65600-1-2/Senkeu					-	-	-	
R 68011-15-1.1   R 65602-44-1/Gundil Kuning   3			2					-	-	
R 68019-60-3-3-2   R 65603-22-2/Ketan Lumbu   5			ა ვ				_	-	-	9
R 68552-55-3-2		IR 65603-22-2/Ketan Lumbu	5		•		-	-	-	-
R 68552-100-1-2-2		IR 66154-52-2/Gundil Kuning	: :		-			-	-	-
R 69092-57-3		IR 66154-52-2/Gundil Kuning					•	-	-	-
R 69093-41-2-3-2   R 65600-1-2-3/Gundii Kuning   5		IR 65600-1-2-3/Gundil Kuning			-		-	-	-	-
R 69116-67-3-2-3		1R 65600-1-2-3/Gundil Kuning						-	-	-
R 69137-34-1-3-1		IR 65600-1-3-2/IR 65564-44-2-3					-	-	-	-
IR 69432-54-1-1-2-2		IR 65600-96-1-2/IR 64630-337-3			-		-	-	-	-
IR 69853-70-3-1-1		IR65564-44-5-2/Gundil Kng//IR65600 1 2 2			•			-	-	-
R 69923-3-1-3-2-3		IR 65598-112-2/ IR 65564-22.2 3					-			
IR 70479-45-2-3			_				•			
IR 70491-33-2-2	· · · · · · · · · · · · · · · · · · ·						-	7	-	
IR 70554-10-3-1-3		IR 65600-7-2-5-2/IR 66738-118-1-2			_			-	-	-
IR 70554-48-1-2		IR 66160-2-6-3-2/IR 66738-118-1-2			-	-	•	-	-	-
IR 70559-AC5		IR 66160-2-6-3-2/IR 66738 118 1 2				-	-	-	-	-
IR 71204-78-3-3		IR 66160-143-6-5-3/IP 66738 448 4 3					-	-	-	-
IR 71218-5-2-1		IR 66738-118-1-2/ID 66450 444 5 2 2	-				-		_	
IR 72926-AC1		IR 67966-44-2/IR 66729 449 4 2					-	7	5	9
IR 65564-22-3 Takaneshiki/Bali Ontjer 7 5 4 89 1 IR 65600-42-5-2¹ Shen Nung 89-366/Ketan Lumbu 5 3 4 114 3 IR 65600-87-2-2-3¹ Shen Nung 89-366/Ketan Lumbu 5 3 3 114 1 3 3 7 IR 65600-96-1-2-2¹ Shen Nung 89-366/Ketan Lumbu 5 3 4 114 1 IR 66158-38-3-2-1¹ Shen Nung 89-366/Ketan Lumbu 5 3 4 114 1 IR 66158-38-3-2-1¹ Shen Nung 89-366/Bali Ontjer 5 3 4 102 1 IR 68544-29-2-1-3-1-2 IR 66154-52-2/Gundil Kuning 5 4 4 92 1 IR 69132-17-2-2-2 IR 65600-96-1-2/Gundil Kuning 5 3 4 89 1 IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 7 4 3 92 1 5 9 7 Checks:  CICA 8		ID 60133 40 1 2/ID 67063 64 3 3					-	-	-	-
IR 65600-42-5-2¹ Shen Nung 89-366/Ketan Lumbu 5 3 4 114 3			_	-			-	-	-	-
IR 65600-87-2-2-3¹ Shen Nung 89-366/Ketan Lumbu 5 3 3 114 1 3 3 3 7 IR 65600-96-1-2-2¹.² Shen Nung 89-366/Ketan Lumbu 5 3 4 114 1 IR 66158-38-3-2-1¹.² Shen Nung 89-366/Bali Ontjer 5 3 4 102 1 IR 68544-29-2-1-3-1-2 IR 66154-52-2/Gundil Kuning 5 4 4 92 1 IR 69132-17-2-2-2 IR 65600-96-1-2/Gundil Kuning 5 3 4 89 1 IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 7 4 3 92 1 5 9 7 Checks:  CICA 8 3 5 7 CHYSVONI 1 5 5 102 1		Shen Mung 90 266/Koton Lumb			-		-	-	-	-
IR 65600-96-1-2-2 <sup>1,2</sup> Shen Nung 89-366/Ketan Lumbu 5 3 4 114 1 IR 66158-38-3-2-1 <sup>1,2</sup> Shen Nung 89-366/Bali Ontjer 5 3 4 102 1 IR 68544-29-2-1-3-1-2 IR 66154-52-2/Gundil Kuning 5 4 4 92 1 IR 69132-17-2-2-2 IR 65600-96-1-2/Gundil Kuning 5 3 4 89 1 IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 7 4 3 92 1 5 9 7 Checks:  CICA 8 3 5 7		Shen Nung 90 366/Katan Lumbu	_	_	-			-	-	-
IR 66158-38-3-2-1 <sup>1,2</sup> Shen Nung 89-366/Bali Ontjer 5 3 4 102 1 IR 68544-29-2-1-3-1-2 IR 66154-52-2/Gundil Kuning 5 4 4 92 1 IR 69132-17-2-2-2 IR 65600-96-1-2/Gundil Kuning 5 3 4 89 1 IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 7 4 3 92 1 5 9 7 Checks:  CICA 8 3 5 7	IP 65600 06 1 2 2 <sup>1,2</sup>	Shop Nung 99-300/Ketan Lumpu						3	3	7
IR 68544-29-2-1-3-1-2 IR 66154-52-2/Gundil Kuning IR 69132-17-2-2-2 IR 65600-96-1-2/Gundil Kuning IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 Checks:  CICA 8 ORYZICA 1 CEYSVONI 1 2 3 91 1	IR 66159.38.3.2.1 <sup>1,2</sup>	Shop Mung 90 266/Dali Oaliaa			•		-	-	-	-
IR 69132-17-2-2-2 IR 65600-96-1-2/Gundil Kuning 5 3 4 89 1 IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 7 4 3 92 1 5 9 7 Checks:  CICA 8 3 5 7		ID 66164 52 2/Oundil Kunin -			-			-	-	-
IR 69800-5-3-1-2 IR 66160-20-4-3-1/IR 65598-112-2 7 4 3 92 1 5 9 7  Checks:  CICA 8 3 5 7	IR 60132-17-2-2-	ID 65600 06 4 2/Constitutions			•		-	-	-	-
Checks:  CICA 8  ORYZICA 1  CEYSVONI  Fedearroz 50  CHACKS:  3 5 7		10 00000-90-1-2/Gunali Kuning			-			-	-	-
CICA 8  ORYZICA 1  CEYSVONI  Fedearroz 50  3 5 7		IN 00 100-20-4-3-1/IK 65598-112-2	7	4	3	92	1	5	9	7
ORYZICA 1					_					
CEYSVONI 1 2 3 91 1 Fedearroz 50 1 2 2 108 1						-	-	-	-	-
Fedearroz 50 1 2 2 108 1			1				-	-	-	-
<del></del>			1		3	91	1	-	-	-
			1	2	2	108	1	-	-	_

LBI = Leaf blast; FL = days to 50% flowering; Esc = Leaf scald, Hm = Helminthosporium;

NBI = Neck blast; GD = grain discoloration.

<sup>&</sup>lt;sup>1</sup>This line has been introduced from IRRI before.

<sup>&</sup>lt;sup>2</sup>This line has been used in crosses made in 1996.

# **OUTPUT 1. ENHANCED GENE POOLS**

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

- Use of anther culture to fix enhanced traits in backcrossed populations from rice X wild species hybrids.
- Four hundred and seventy lines from crosses and advanced backcross populations of Bg90/ O. rufipogon, Bg90/ O. glaberrima, and Progresso/ O. barthii were processed through anther culture from September 1998 to September 1999.
- About 100 plants per each line were planted in the field. Thirty plants per line were selected by plant type, and anthers of the selected individual plants were cultured in the laboratory.
- Total of 3,309 green plants were produced from Progressol O. barthii. The other crosses did not induced callus.
- Doubled haploids lines will be selected and evaluated for disease resistance and agronomic characteristics, and analyzed by molecular markers.
- The main objective of this activity is to determine if doubled haploids lines could be used to
  accelerate the introgression of QTL's associated with high yield potential from the wild species
  into the selected O. sativa varieties.

Collaborators: A. Mora (IP4), Z. Lentini (SB2, IP4), C. Martínez (SB2).

# 2. Use of anther culture (AC) to accelerate the development of breeding populations of FLAR

- Total of 54 crosses were cultured from July 1998 to December 1998. Total of 364 crosses are programmed to be processed on planting dates from Auguts to December 1999.
- Plants per each line were first selected based on plant type, earliness and grain length ( as
  predicted by the floret size), and the anthers from all the selected plants were cultured by cross.
- The 54 crosses yielded a total of 597 plants.
- R2 plants from the R1 doubled haploids plants generated from these crosses are being selected both at Palmira Station for plant and grain type, and at Santa Rosa Experimental Station for blast resistance.

Collaborators: A. Mora (IP4), Z.Lentini (SB2, IP4), J. D. Gonzalez (FLAR).

# 3. Somaclonal variation to increase genetic variability of advanced breeding lines.

- Somaclonal variation is being tested to determine if it is possible to improve some of the traits of selected advanced breeding lines.
- Previous work conducted in the rice project indicated the generation of somaclones with increased blast resistance.
- Traits of interest include one of the following: improve grain milling and cooking quality, rice hoja blanca resistance, sogata resistance or blast resistance.
- Eight advanced lines selected by the breeding program of Fonaiap (Venezuela) and Fedearroz (Colombia) were culture in vitro.
- Immature inflorescences (prior meiosis) were cultured to induced callus formation.

- Callus was either transfer onto regeneration medium to induce the differentiation of plants, or was subcultured on the same medium to increase the likehood of spontaneius mutation during the in vitro culture (somaclonal variation).
- Total of 3,309 somaclones for the Venezuelan National Plan of Rice leaded by Fundarroz with the participation of Fonaiap, Danac, and la Universidad de los Llanos were produced.
- 4,440 somaciones plants for Fedearroz, Colombia were generated.
- The somaclones plants are being grown in the field.
- The S1 seed (first self of the original somaclone, S0) will be harvested, and S1 plants evaluated for grain quality. Disease resistance will be evaluated on the S2.

Collaborators: A. Mora (IP4), Z.Lentini (SB2, IP4), Edgar Torres (Fonaiap, Venezuela), J. Holguín (Fedearroz, Colombia), D. Gonzalez (FLAR).

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# **OUTPUT 2. PHYSIOLOGICAL BASIS FOR RICE TRAITS UNDERSTOOD**

# 1. Weed control enhanced by the use of new genotypes and practices

Carlos BRUZZONE, Jaime FLORES and Jaime LOZANO

# A. Tolerance to submergence

Preliminary studies in CIAT, reported in 1998, suggested that it is possible to develop crop systems that reduce *E. colona* competition during the earlier crop growth stages using varieties tolerant to submergence. Dry seed plantings studies have shown that *E. colona* emergency was significantly reduced under given crop practices, under which FR 13A, a submergence tolerance rice variety, emerged satisfactorily.

A new set of trials were carried out this year in order to develop an screening method that allow us to identify rice genotypes tolerant to such submergence conditions under which *E. colona* growth may be restricted. We report here studies under both dry and pre-germinated plantings systems.

# A.1. Dry-seed planting

# A.1.1 Seedling age influence on tolerance to submergence

Results of a trial at two planting dates, September 98 and January 99, using a 13-cm. water layer during 20 days, starting from 4 different days after initial watering, indicate that early flooding of rice fields, combined to rice cultivars with tolerance to submergence, may be an effective practice to reduce *E. colona* competition.

On the first planting (September 98), *Echinochloa* emergence was significantly reduced when 20 days long floods where applied from the second and third DAP. Under the same conditions, the submergence tolerance cultivar FR 13A, reached 35% and 42% emergence, respectively. *E. colona* emergence increased to 57.5 and 52.5% when the water layer was applied from the third and fourth DAP, respectively, while FR13A showed 87.5 and 90%, respectively.

Emergences of IR 40931-26 and BR-IRGA 410 were slightly higher than *E. colona* when flooding was applied from the second and third day, but lower when it was applied from the fourth day. Cultivars Ta Hung Ku and IR 8 showed an intermediate emergence level under the conditions of this trial.

During the January 99 planting conditions were apparently more severe than the previous planting, and greater difference was observed among tolerant and susceptible genotypes. The critical point here was moved from the third to the fourth DAP. Under this last treatment, only 15% *E. colona* seedlings emerged, while tolerant cultivars, FR 13A and Ta Hung Ku, had 60% emergence. Ta Hung Hu, having enhanced elongation capacity under flooded conditions, reached 50 % emergence when the water layer was applied 3 DAP, while most genotypes had lesser than 10% emergence values. IR 8 performed as a susceptible cultivar reaching values similar to those from the very susceptible cultivars, IR 40931-26 and BR-IRGA 410 (Figure 1).

# A.1.2 Depth layer effect on tolerance to submergence

Studies done at two planting dates in Palmira, September 1998 and January 99, evaluating the effect of four different water layer depths (5, 10, 15 y 20 cm) applied 3 DAP, suggest that the water layer depth has no influence on rice nor *E. colona* emergence (Figure 2).

There was a significant interaction between genotypes and planting date. At both planting dates, FR 13A and Ta Hung Ku, showed higher emergence than all three *E. colona*, IR 40931-26 and BR-IRGA 410. On the other hand, IR 8 showed a tolerant reaction to submergence conditions prevalent at September planting, while showed a susceptible reaction at the January planting. Those observations suggest that stress during January planting was higher than during January

planting, probably due to differential water temperature. Plants performance under submergence conditions are subjected to both genetic and environmental factors. Among the latter is water temperature, which influences biologic activity, which, in turn, determines O<sub>2</sub> availability needed for the plants to emerge.

# A.1.3 E. colona tolerance to submergence under different water management practices.

Studies at three planting dates, using *E. colona* seeds, placed at five different soil depth and five different water layer depth, indicate that emergence of *E. colona* seed placed at soil depths deeper than 2 cm. are strongly inhibited by water layers equal to or higher than 5 cm.

There was observed a significant interaction between planting dates and seed placement.

During the first planting (September 1998), *E. colona* seed emergence placed at 5, 4 and 3 cm. soil depth was strongly inhibited (lesser than 5% emergence) by all studied water layers (0, 5, 10, 15 and 20 cm.). Layer "0" here means water saturated soil without a perceptible water layer. Only emerged 16% of seedlings derived from seeds placed at 2 cm., and 42 % on average 42.5% seedlings survived from seeds placed at 1 cm depth (Figure 3).

As Figure 3 shows, *E. colona* emergence was completely inhibited by all studied seed placements and water layer depths, during the second planting (November 13, 1998). Only without a water layer (saturated soil, water layer "0"), *Echinochloa* seeds had a chance to germinate and emerge, reaching the highest emergence rate when seeds were placed at 1 cm. soil depth (35%).

During the third planting (January 25, 1999), there was a 'water layer 0' treatment without saturated soil, which was watered only enough to allow germination of *E. colona* seeds. At water layer '0', *E. colona* emergence was very high at all studied seed depths, averaging 92.5%. Application of 2, 3, 4 and 5-cm water layers was equally effective in reducing *E. colona* emergence to very low levels. Seedling emergence from seeds placed at 1 cm. was significantly higher and varied from 22.5 to 77.5%.

# A.1.4 Screening rice cultivars for tolerance to early submergence

On the basis of the results reported above, 48 rice genotypes from the CIAT/FLAR working collection were evaluated for their tolerance to early submergence. They were planted in dry soil, inside concrete tanks, in 0.5 m. -rows, 200 seeds per row, 2.0 cm. deep, under a 10 cm. water layer, during 20 days, placed 3 days after watering. The percentage of surviving emerged seedlings was evaluated one week after the water layer was withdrawn. FR13A and Ta Hung Ku, as tolerant checks and BR-IRGA 410, as susceptible check were sown every fifteen entries. All entries were previously evaluated for their germination capability, in order to discard this potential error source. Most genotypes reported here had more than 90% in the germination test.

As Table 1 shows, the percentage of emergence registered by the tested genotypes range from 5% to 98% de emerged seedlings. Fifteen genotypes showing less than 40% of emergence may be considered as susceptibles, and those sixteen genotypes showing emergence percentages higher than 60%, as tolerants. It was identify an elite breeding line, CT 8008-3-5-8P-M-2P, having the highest emergence percentage, and showing slightly higher values than tolerant checks.

These results suggest that the screening method used was effective in screening out susceptibles genotypes from those with higher tolerance to early submergence. Aside from the real or apparent potential that tolerance to submergence may have as an additional strategy against some weeds, this trait is evidently advantageous for better rice stands. Tolerance to submergence may improve the rice seed stands by reducing the amount of non-emerged seed due to water management problems in lands having imperfect leveling.

# A.2 Pre-germinated seed planting

Results from two planting dates, using *E. colona* and five rice varieties, with their seed pregerminated, and planted under a 10 cm. water layer during 20 days, indicates that it is possible under this system to keep a rice crop free from weeds during the first growth stages (to 27 DAP) combining early flood with tolerant to submergence rice varieties.

At the first planting date, carried out in March 99, 70% of seeds from tolerant cultivar Ta Hung Ku survived at 27 DAP, after being submerged during 20 days. Under these same conditions, emergence of *E. colona* was completely inhibited. IR 8 and FR 13A, showed about 40% emergence, while cultivars IR 40931-26 and BR-IRGA 410, showed less than 25% emergence. Differential reactions of these cultivars are consistent to observations made in previous trials on early submergence in dry seed plantings.

All cultivars showed emergence levels higher than 80% at the second planting carried out in April 99, suggesting that the pressure then was lower than in the previous planting date. Nevertheless, the pressure was higher enough to impede the germination and emergence of *E. colona*.

These results suggest that tolerance to submergence, existent in some rice cultivars, may be combined with water management practices, such as early submergence, in order to reduce the population of some weeds, such as *E. colona*, during the earlier growth stages where rice is more susceptible to weed competition effects.

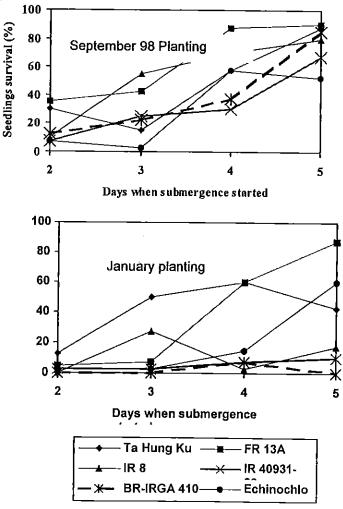
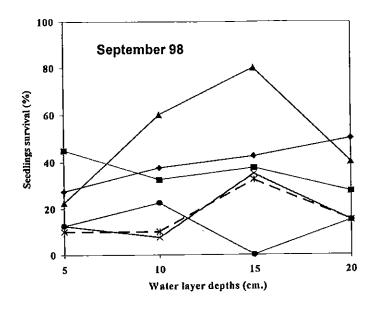


Figure 1. Survival of *E. colona* and five rice variteties seedings submerged for 20 days at different seedling ages. Results from two planting dates.



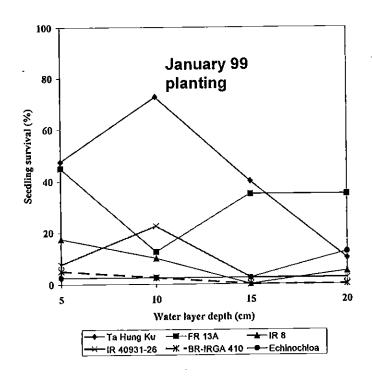


Figure 2. Seedling survival of *E. colona* and 5 rice varieties under five different water layer depths during 20 days from 3 DAP, at two planting dates

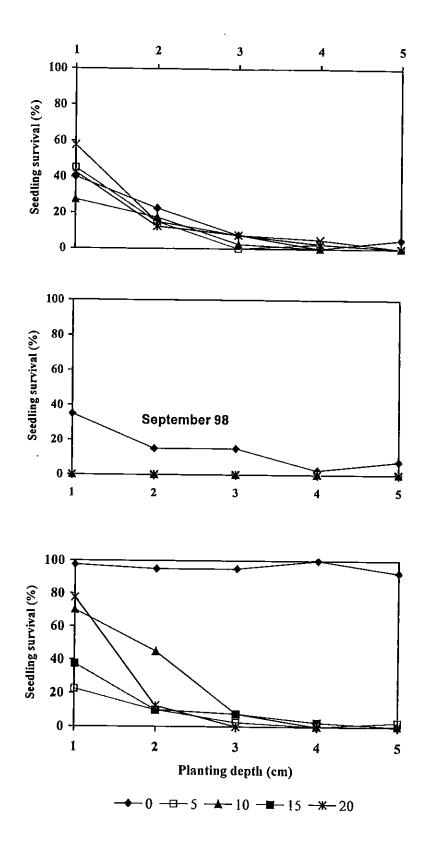


Figure 3. Seedling survival of E. colona at five water layer and fice planting depths. Results from three planting dates.

Figure 4. Emergence of *E. colona* and five rice varieties planted with pre-germinated seed, submerged 3 DAP, at two planting dates.

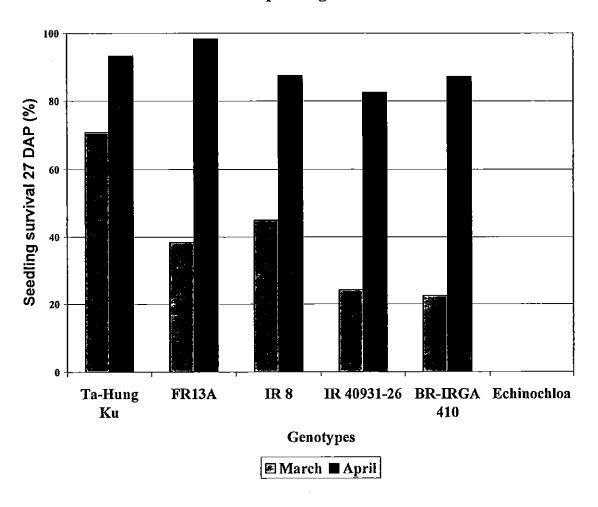
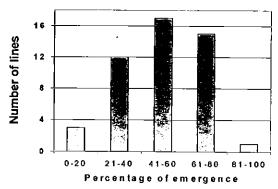


Table 1. Porcentage of emergence of rice genotypes screened for tolerance to early submergence in dry seed plantings.

	Germi- native			Germi-	%
Genotype		emergence	Genotype	native	emergence
	Test			Test	
CT 9807-3-5-1-2-M-2I-MI	89	5	CT 9868-3-2-2-3-2P-M	96	53
Selecta A-30	72	8	EPAGRI 109	100	53
CT 9506-12-10-1-1-M-2P-M	88	16	CT 6163-8-9-5-2-M-85-M	99	55
O. Caribe 8	96	21	CT 8238-6-13-1P1X	93	56
BR-IRGA 410	93	22	Fedearroz 50	98	57
IR 42	95	24	Oryzica Yacu 9	98	58
Selva Alta	100	24	Amistad 82	99	58
CT 11058-15-3-1T-1P-5P	92	26	CT 9748-13-2-1-M-M-1-1	99	60
CT 11623-13-M-5-2-3-1-M	94	27	CT 8008-3-5-1P-M	98	61
Oryzica 3	78	30	INIA – Tacuarí	97	61
CT 8455-1-13-1-M-2P	100	36	CT 11275-3-F4-8P-2	97	61
CICA 8	97	36	PsBRc 2	100	62
CT 8198-4-2-1P-1X	95	37	CT 11891-2-2-5-2-M-1-M	89	65
IRGA 417	97	38	CT 10825-1-2-1-3M	96	66
EPAGRI 108	98	39	INIAP 12	97	66
IRGA 411-1-6-1F-A	95	41	O. Llanos 5	97	67
Lemont	89	43	CNAx 5011-9-1-6-4B	99	71
P3050-F4-52	96	44	IR 50	98	71
IR 74	97	46	CT 11280-2-F4-12P-5	100	72
EMBRAPA 7 TAIM	98	49	CT 11782-2-F4-3-183	99	74
Capirona	92	50	CT 10310-15-3-2P-4-3	98	76
CT 8444-1-8-11P-1X	96	52	CT 10323-29-4-1-1-1T-2P	98	78
ICTA-Motagua	97	52	Caiapó	97	80
IRGA 659-1-2-2-2	98	52	CT 8008-3-5-8P-M-2P	95	98
Tolerant checks:		<u> </u>	Susceptible check:		
Ta Hung Ku			BR-IRGA 410	<del> </del>	
Mean	100		Mean	93	18.3
Range	100		Range	93	4 - 38
FR 13A					
Mean	95	74.8			
Range	95	59 - 84			

Frecuency distribution of emergence of rice lines



# **OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED**

#### A. Rice Blast

A.1. Monitoring changes in the genetic structure and virulence diversity of the rice blast pathogen and breeding for stable resistance

Fernando Correa-Victoria (1), Joe Tohme (2), Willy Roca (2), Fabio Escobar (1), Gerardo Gallego (2), Girlena Aricapa (1), Gustavo Prado (1), Norma Flor (1), Blanca Escorcia (1).

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#### INTRODUCTION

Rice blast, caused by *Pyricularia grisea* Sacc., the anamorph of *Magnaporthe grisea* (Hebert) Barr, is the most widespread and damaging disease of cultivated rice in both tropical and temperate latitudes. Control of the disease relies upon release of blast resistant cultivars, which normally are short-lived, rarely being effective for more than 1-3 years. The CIAT Rice Project has been breeding rice for blast resistance at a "hot spot" upland site, characterized by reliable and very high disease levels, as well as high pathogen diversity (Correa-Victoria and Zeigler, 1993). Stability of resistance selected at this site has been associated with selection for high levels of complete resistance, probably controlled by multigenic effects. In developing a breeding strategy for durable blast resistance, CIAT has been working on the population dynamics of the blast pathogen at its breeding site, identifying potential sources of durable blast resistance, and tagging of resistance genes to main pathotypes of different genetic lineages of the fungus. We have concluded that establishing a system for understanding pathogenic variability and its dynamics is essential for the identification and tagging of resistance genes and the development of feasible strategies to control blast with genetic resistance.

## **OBJECTIVES**

To develop breeding strategy for durable blast resistance based on continuous studies on the population dynamics of the blast pathogen, identification of potential sources of resistance, and tagging of resistance genes

# **MATERIAL AND METHODS**

MGR-586 (Levy et al. 1993), rep-PCR (George et al. 1998), and virulence spectrum on more than 40 rice cultivars has been used to examine the genetic diversity and pathotype characteristics over time of more than 1000 rice blast isolates collected from susceptible and resistant cultivars planted in commercial rice fields and a screening site in Colombia. Dissection of resistance genes of Oryzica Llanos 5, a highly resistant cultivar to all lineages of blast in Colombia, and their eventual transfer to susceptible commercial cultivars, is being developed by using gene tagging, the identification of resistance gene analogs (RGA) linked to resistance genes, and use of PCR based markers linked to known genes of resistance to specific lineages. Recombinant inbred lines from the cross between the highly blast susceptible cultivar Fanny and the highly resistant O. Llanos 5 are being used for the assays. PCR reactions were performed using degenerate primer combinations (Leister et al., 1996). The fragments found were sequenced and compared with the database of proteins in GenBank.

# **RESULTS AND DISCUSSION**

Continuous characterization of the blast pathogen in terms of race composition, compatibility with known resistance genes and frequency of virulent phenotypes show a very diverse pathogen with more than forty-five international races and virulence factors to all the international differentials and cultivars with at least 13 different genes for resistance. DNA fingerprinting determined by using MGR-586 (Levy et al, 1993) and Pot2 rep PCR (George et al, 1998) show that the genetic structure of the blast pathogen population in Colombia is relatively simple despite the great virulence observed. The pathogen race complexity has been simplified into six main genetic families or lineages, named SRL-1 to SRL-6. The mean genetic similarity between lineages was high, ranging from 92 to 98%. The mean similarity between lineages ranged from 37 to 85% (Levy et al, 1993). Genetic lineage frequencies change between years depending on the area planted with a rice cultivar in farmer's fields, and on the virulence spectrum of the lineage (Table 1). Frequency of lineages SRL-1 and SRL-3 has been near zero during the last three years due to the absence of susceptible cultivars in commercial fields and the narrow virulence spectrum exhibited on the Colombian commercial cultivars (Table 2). We have detected genetic structure and virulence changes within blast pathogen populations over time (Tables 2 and 3). A new lineage (SRL-6B) probably derived from SRL-6, appeared after 1993 (Table 3). While lineage SRL-1 was the only lineage recovered from cultivar Cica 9 in 1989, lineages SRL-2 and SRL-6B predominate now on this cultivar (Table 3). A summary of the combination of virulence assays and genetic lineages in the blast pathogen population at the screening site studied is shown in Table 2. Greater gains in virulence observed in isolates recovered from cultivar Cica 9 during the last few years (Table 3) were associated with changes in genetic structure (lineage SRL-6B) within existing genetic lineages (Tables 2 and 3). Monitoring gains in virulence is of practical use for identifying resistance donors that can be used in the event that resistance is defeated in the field by the new isolates. A high relationship between virulence spectrum and genetic lineage grouping is observed (data not shown) making it possible to design breeding strategies for the development of durable blast resistance. Characterization of the genetic lineage structure, together with the virulence spectrum and the virulence frequencies within the whole blast pathogen population, should provide a more reliable estimate of the durability of cultivar resistance than only consideration of virulence or lineage alone.

Although the six genetic families of the fungus share a high number of virulence genes, a high specific interaction between some avirulence/virulence factors in the pathogen and resistance genes in the host has been observed (Tables 2 and 5). A resistance gene such as Pi-1 or Pi-2 (Table 2) can be effective against all the isolates of an entire lineage yet is susceptible to most individuals of other lineages. This specific interaction is the basis for selecting the progenitors to be included in a breeding program aiming at obtaining more durable blast resistance. Cultivars may then have complementary resistance, which in combination could confer resistance to all the targeted blast population. The near isogenic lines C 101 A51 and C101 LAC, carrying the resistance genes Pi-1 and Pi-2, respectively, have complementary resistance which, in combination, confer resistance to all the blast population represented in Table 2.

Studies on the compatibility frequency reflecting the whole spectrum of virulence of the blast pathogen in Colombia was analyzed by inoculating representative isolates of each genetic lineage onto 201 Latin American rice cultivars (Table 4). Most cultivars were susceptible to genetic lineage SRL-6 (81%) followed by lineage SRL-5 (69%). Pattern of compatibility observed with lineages SRL-6 and SRL-5 should be a result of the narrow and similar genetic base present in the Latin American rice germplasm. Lineage SRL-6 has been the most frequent segment of the population over the years in Colombia. Breeders at CIAT are now aiming at combining genes exhibiting resistance to the different lineages of the blast pathogen. Combinations of genes that in sum confer resistance to every lineage will confer resistance to the entire blast population. This has been described as the "lineage exclusion hypothesis" (Zeigler et al, 1994). Identification of the

complementary resistance sources should be based on detecting those virulence factors whose combination in individual isolates within the pathogen population has a frequency near zero. Following such an approach, complementary resistance sources within the Latin American rice germplasm of commercial cultivars have been identified for designing genetic crosses aiming at combining those complementary genes (Table 5). The advantage in using these cultivars as progenitors is that they already carry other desired agronomic traits such as high yield, adaptation, plant and grain type, grain quality, etc. Crosses are being made and progenies tested under field conditions at the Santa Rosa screening site. Evaluation of segregating populations derived from these crosses has yielded rice lines exhibiting high levels of blast resistance under field conditions. Gains in selection for blast resistance in F2 populations developed from crosses between progenitors exhibiting complementary resistance to different genetic lineages of the blast pathogen in Colombia went from 29% prior to 1998 to 64% during that year (Table 6).

As a result of screening and selecting for blast resistance under high pathogen diversity and reliable disease pressure, the CIAT Rice Program identified a line that was released in 1989 as the cultivar Oryzica Llanos 5. This cultivar expresses broad resistance against all lineages of the pathogen (Table 2). Although some compatible isolates are recovered from the field occasionally, their virulence on Oryzica Llanos 5 is not stable under artificial inoculations and the resistance continues being durable and stable under commercial cultivation since the year of its release. Complementary resistance to all lineages of the pathogen is observed among the different parents used in the cross that gave origin to O. Llanos 5 (Correa and Martinez, 1994). Dissection of resistance genes using gene tagging and the identification of RGA linked to resistance genes in the cultivar Oryzica Llanos 5 have yielded the following results. We have placed 11 of the identified RGA as new markers on the rice map derived from the cross of O. Llanos 5 and Fanny. Many of them (7) map to chromosome 11 where Xa 21 as well as Pi-1, Pi-7, and Pi-18 are located, suggesting that we might be getting close to the blast resistance genes in O. Llanos 5. Another one map to chromosome 6 and one more to chromosome 7.

#### CONCLUSIONS

Development of Durable Rice Blast Resistance can be attained by:

- Characterization of the genetic structure, virulence spectrum and virulence frequencies
- Identification, recombination and release of resistance gene combinations that exclude relevant pathogen populations
- Gene mapping and genetic analysis through molecular techniques
- Field evaluation and selection of rice lines under high disease pressure and pathogen diversity (hot spot)

#### REFERENCES

Correa-Victoria, F.J., and Martinez, C. 1994. Genetic structure and virulence diversity of *Pyricularia grisea* in breeding for rice blast resistance, in: Induced Mutations and Molecular Techniques for Crop Improvement, IAEA, Vienna, Austria, pp. 133-145.

Correa-Victoria, F.J., and Zeigler, R.S. 1993. Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" site in eastern Colombia. Plant Disease 77:1029-1035.

George, M.L.C., Nelson, R.J., Zeigler, R.S., and Leung, H. 1998. Rapid population analysis of *Magnaporthe grisea*by using rep-PCR and endogenous repetitive DNA sequences. Phytopathology 88;223-229.

Leister, D., Ballvora, A., Salamini, F., and Gebhardt, C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genetics 14:421-429.

Levy, M., Correa-Victoria, F.J., Zeigler, R.S., Xu, S., and Hamer, J.E. 1993. Genetic diversity of the rice blast fungus in a disease nursery in Colombia. Phytopathology 83:1427-1433.

Zeigler, R.S., Tohme, J., Nelson, R.J., Levy, M., and Correa-Victoria, F.J. 1994. Lineage exclusion: a proposal for linking blast population analysis to resistance breeding, in Rice Blast Disease, R.S., Zeigler, S.A. Leong and P.S. Teng (eds.). CAB International, UK, pp. 267-292.

Table 1. Frequency of six genetic lineages of Pyricularia grisea

		FRECUENCY (%)	
GENETIC LINEAGE	1996	1997	1998
SRL-6	. 65	46	60
SRL-5	12	15	6
SRL-4	15	36	18
SRL-3	0	1	0
SRL-2	8	2	16
SRL-1	0	0	0
		v	U

Table 2. Virulence spectrum of six Colombian genetic lineages of *Pyricularia grisea* 

Cultivar			Ge	enetic Li	neage	<del>-</del>	
	SRL-1	SRL-2	SRL-3	SRL-4	SRL-5	SRL-6A	SRL-6B
Near Isogenic Line		<del></del>					
C101 A 51 (Pi-2)	+	+		+		+	+
C101 LAC (Pi-1)					+		
C101 PKT (Pi-4a)		+		+	+	+	+
C104 PKT (Pi-3)		+		+	+		+
C105 TTP (Pi-4b)		+		+	+	+	+
Commercial Cultivars							
Oryzica Llanos 5							
Oryzica 1		+		+		+	+
Oryzica Yacu 9		+				+	+
Linea 2		+				+	+
Cica 8					+		+
Colombia 1				+			+
Oryzica Caribe 8				+			
Cica 9	+	+					+
Fanny	+ ,	+	+	+	+	+	+

<sup>+ =</sup> Susceptible

Table 3. Genetic lineages recovered from cultivar CICA 9 (1989 - 1996)

LINEAGE			YEA	R OF CC	LLECTI	ON		
	89	90	91	92	93	94	95	96
				SOLATE	S (No.)			· -
SRL-6B SRL-2		7	7	10	7 3	3 7	3 6	3 7
SRL-1	10	3	3		_		3	•

Table 4. Compatibility frequency of six Colombian lineages of *Pyricularia grisea* on 201 Latin American rice cultivars

Genetic Lineage	Infected Cultivars (No.)	Compatibility Frequency (%)
SRL-3	12	6.0
SRL-4	34	16.9
SRL-2	52	25.9
SRL-1	61	30.3
SRL-5	138	68.7
SRL-6	162	80.6

Table 5. Latin American rice cultivars exhibiting complementary resistance to six Colombian lineages of *Pyricularia grisea* 

Cultivar	Origin			Geneti	c Lineage	<b>e</b>	
	•	SRL-1	SRL-2	SRL-3	SRL-4	SRL-5	SRL-6
	Brazil		-	-	+		
Rio Verde	Brazil				+		
O. Caribe 8	Colombia				+		
Sacia 2	Bolivia					+	
Amistad 82	Cuba					+	
IR 1529 ECIA	Cuba					+	
Perla	Cuba					+	
Juma 51	Dom. Republic					+	
Bamoa	Mexico					+	
Oryzica 3	Colombia						+
Capi 93	Honduras						+
PA-3	Peru						+
O. Yacu	Colombia		+				+
Line 2	Colombia		+				+
CICA 9	Colombia		+				+
Araure	Venezuela		+				+
Juma 61	Dom. Republic		. +				+
O. Llanos	Colombia				+		+
IR 65	Philippines				+		+
Cuyamel 3820	Honduras				+		+
lcta Crispo	Guatemala					+	+
Juma 62	Dom. Republic		+			+	
Fanny	France	+	+	+	+	+	+

<sup>+=</sup> Compatible reaction

Table 6. Gains in selection of F<sub>2</sub> rice populations using progenitors with complementary/ complete resistance to *Pyricularia grisea* 

YEARS	EVALUATED CROSSES (No)	SELECTED CROSSES (No)	%
1985 –98 (PROM.)	219	63	29
1998	338	215	64

# A.2. Effects of silicon fertilization on disease development and yields of rice in Colombia

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The savannas of Colombia contain soils (Oxisols) constrained by silicon (Si) deficiency. As upland rice production is expanding into this region, field experiments were conducted over two years on three representative soils to determine the extent to which Si deficiency may constraint rice yields and favor disease development. The experiments were complete factorials and included different levels of Si, Phosphorus and cultivars. Sources of Si tested included both calcium silicate and slag. Lime was applied to equalize lime value and Ca levels across treatments.

Silicon significantly reduced all observed rice diseases. Leaf blast severity and neck blast incidence were reduced from about 26% and 53% in non-amended plots to 15% in Si-amended plots. Leaf scald severity was reduced from 42% to 6% in Si-amended plots, while grain discoloration was reduced from 4.2 to 1.0 in Si-amended plots. Si application increased rice yields by about 40% on all three soils. A residual effect was also noted on both, reduction of disease development and yield increase. By amending these soils with Si, a very effective and potentially sustainable method for upland rice production and management of rice diseases appears available.

#### 1. INTRODUCTION

Silicon (Si) is one of the most abundant elements in soils as well as in the ash of plants, especially grasses and cereals (Elawad and Green, 1979). Si is present at much higher concentrations in rice tissue than the macronutrients Nitrogen (N), Potassium (K), and Phosphorus (P) under a typical irrigated rice crop (Yoshida, 1975). Despite its abundance and importance, Si has received far less attention than K, N, and P, and studies have been particularly lacking for upland rice environments,

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which may be at highest risk of Si deficiency (Winslow et al., 1997). According to Juo and Sanchez (1986), and Winslow et al. (1997), silicon deficiency, acidity and aluminum toxicity commonly occur together in tropical weathered upland soils characteristic of the Ultisol and Oxisol soil orders. One of the reason for the limited study of Si may be that it is not considered to be among the essential plant nutrients (Epstein, 1994).

The highly acidic infertile Oxisols, Ultisols, and tropic suborders of Inceptisols which occupy much of the landscape of the savannas of South America are dominated by the end-products of weathering: kaolinite and the oxides and hydrous oxides of iron and aluminun. Weathering has leached the bulk of mineral nutrients from these soils leaving a cation exchange complex in which Al is the governing cation. Since soil weathering is essentially a process of desilication (Friesen *et al.*, 1994; Savant *et al.*, 1997a), low levels of soluble silicon (Si) may also be expected in highly weathered soils.

The function and mechanism of Si in rice is not fully understood. However, inadequate levels may limit yields and increase crop susceptibility to diseases (Datnoff *et al.*, 1991, 1992, 1997; Savant *et al.*, 1997; Winslow 1992). Rice is a known Si accumulator and benefits from Si nutrition (Yoshida, 1975). In addition, rice response to Si applications has been shown on highly acidic soils in West Africa (Winslow, 1992). Consequently, there is a definitive need to consider Si as an agronomically essential element for increasing and/or sustaining rice production (Savant *et al.*, 1997a).

From several studies it appears that Si nutrition has direct and indirect beneficial effects on the rice growth largely due to different physiological roles (Takahashi and Miyake, 1990; Yoshida, 1975). Yield increases of 10% are common when Si is added and at times exceed 30% when blast disease is severe (Yoshida, 1981; Snyder et al., 1986; Datnoff et al., 1991, 1992). Numerous reports in the literature suggest that under intensive rice cultivation depletion of plant-available Si in soils could also be a possible soil-related factor for declining yields (Savant et al., 1997a,b). Si has been shown to suppress fungal diseases such as rice blast (Magnaporthe grisea, teleomorph; Pyricularia grisea, anamorph), brown spot (Cochliobolus miyabeanus, teleomorph; Bipolaris oryzae, anamorph), leaf scald (Gerlachia oryzae), sheath blight (Rhizoctonia solani), stem rot (Sclerotium oryzae), and a complex of fungal and bacterial pathogens that cause grain discoloration (Datnoff et al., 1991, 1992, 1997; Savant et al., 1997a; Seebold, 1998; Winslow, 1992) both under lowland and upland growing conditions. In field trials, Datnoff et al., (1991 and 1992) reported the reduction in blast ranging from 17% to 30% and in brown spot from 15% to 32% in rice grown on Histosols, and disease severity tended to be reduced with increasing tissue concentration of Si.

Upland rice is widely cultivated on the Brazilian Cerrados and production is rapidly expanding on the Colombian Llanos. However, this potential constraint of silicon deficiency has therefore gone unrecognized in this agroecosystem. Consequently, we conducted field experiments on three representative soils on the Llanos of Colombia during 1992 and 1993 to determine:

- (1) Whether upland rice responds to Si applications,
- (2) Whether disease development and subsequent rice yields can be influenced by Si,
- (3) Whether there are variety differences in responses to Si,
- (4) If there is an interaction of Si with phosphate which is adsorbed on similar mineral surfaces in these soils.

# 2. MATERIAL AND METHODS

Two sets of experiments with four replications were carried out. Experiment 1 evaluated the effect of Si on yields and disease incidence and severity on two rice cultivars at Santa Rosa, Meta Department in Colombia on piedmont terrain at the base of the eastern side of the Andes mountains. The soil is an Inceptisol with pH 4.7 and aluminum saturation of 18%. The crop is

dependent on direct rainfall as a water source. Si was applied as "slag" (a silicate-rich by-product of electric furnace phosphoric acid production: 22% Si, 33% Ca, 0.5% P, 0.1% K, 0.3% Mg, 0.5% Fe) at Si rates of 0, 1, 2 and 3 t/ha in 1992 three weeks before planting. In 1993, plots were split and 1 t/ha of Si as slag was applied to one-half of each plot. The rice cultivars Oryzica 1 and Oryzica Lanos 5, the former susceptible and the latter resistant to rice blast were sown each year.

Experiment 2 evaluated in 1993 the effect of Si and P on yields and disease incidence and severity on two rice cultivars at two acid upland research sites located on the eastern savanna plains (Llanos) of Colombia: Matazul (Oxisol, pH 4.7 aluminum saturation 79%) and La Florida (sandy Oxisol, pH 5.9, aluminum saturation 67%), Meta Department. Upland rice culture has been recently introduced to this area consisted of factorial combinations of three levels of Si (0, 250 and 500) kg/ha as wollastonite which is a native calcium metasilicate (CaSiO<sub>3</sub>: 24% Si, 34.5% Ca), two of Phosphorus (P) (25 and 50 kg/ha as triple superphosphate, TSP) and two rice cultivars (Oryzica Sabana 6 and IAC 165) of apparently contrasting tolerance to Si deficiency. Due to the basic properties of wollastonite, chemically equivalent amounts of calcite lime were applied to the 0 and 250 kg-Si/ha treatments to equalize the lime value and Ca levels across treatments. The total lime value applied to the experiment was equivalent to 2400 kg CaCO<sub>3</sub>/ha. All other nutrients were supplied at adequate levels. In addition to yield, grain quality factors such as coloration and milling quality, and Si tissue concentrations were also determined at harvest. Incidence and severity of diseases were also evaluated.

#### 3. RESULTS AND DISCUSSION

# 3.1 Disease response to Si application

Development of the rice diseases leaf blast (LB), neck blast (NB), leaf scald (LS), and grain discoloration (GD) were significantly (P ≤ 0.05) reduced by Si applications to upland rice in three different sites of the Colombian Llanos during 1992 and 1993. All diseases decreased with increasing Si rates, confirming previous reports (Datnoff *et al.*, 1991, 1992, 1997; Deren *et al.*, 1994; Seebold, 1998; Snyder *et al.*, 1986; Winslow, 1992). Leaf blast severity on the cultivar Oryzica 1 planted in 1992 at Santa Rosa, decreased over the control 62.0 and 69.0% at the lowest and highest Si rates, respectively (Table 1). Neck blast incidence was not significantly reduced at any Si rate during the same year and this observation needs further discussion. In 1993, leaf blast severity at the highest Si rate decreased 60.0 and 32.0% over the control for residual 1992 Si effects on the 1993 rice crop, and residual 1992 Si rate receiving 1 t/ha of Si in 1993, respectively (Table 1). Neck blast incidence at the highest Si rate decreased 58.0, and 64.0% over the control for residual 1992 Si effects on the 1993 crop, and residual 1992 Si rate receiving 1 t/ha of Si in 1993, respectively (Table 1).

Although the application of Si did not apparently suppressed neck blast on the 1992 rice crop, it is very important to note that the residual applications of Si were very effective in reducing significantly both leaf and neck blast in 1993. It is possible that in 1992 there was a high blast pressure during panicle formation. The experimental farm at Santa Rosa represents an area of unusually high inoculum pressure (Correa and Zeigler, 1993). Another possibility is that neck blast evaluations were performed too late in 1992, giving opportunity to the pathogen of growing on senescent tissue and therefore confounding a true infection. This latter affirmation seems to be supported by the fact that yields increased in 1992 with Si applications as will be shown in the following section. In addition, these yields and their differences with the respective controls were comparable to the residual effects on disease development and yields observed in 1993.

Other diseases such as leaf scald and grain discoloration were significantly reduced on the cultivar Oryzica Llanos 5 planted in the same years at Santa Rosa (Table 2). This cultivar was completely resistant to leaf and neck blast during those two years. Although applications of Si at the rate of 1t/ha were sufficient to influence blast development on Oryzica 1, it seems that more than 1 t/ha of

Si are needed to influence the development of leaf scald and grain discoloration on cultivar Oryzica Llanos 5 (Tables 1 and 2). Similar effects were observed on yields of the cultivar Oryzica 1 inboth years and at least in 1992 on cultivar Oryzica Llanos 5.

Leaf and neck blast, leaf scald, and grain discoloration were also significantly reduced with Si applications to the rice cultivars Oryzica Sabana 6 and IAC 165 planted in the Matazul and La Florida sites in 1993 (Tables 3 and 4). Reduction of disease development at these two sites in the presence of Si was equivalent with no significant differences between the two rates of Phosphorus used (25 and 50 kg/ha). Influence of Si on the reduction of disease development was higher at the Matazul and La Florida sites compared to Santa Rosa. Responses to silicon at the first two sites were observed at Si rates as low as 500 kg/ha compared to more than 1 t/ha used in Santa Rosa. While leaf scald development and grain discoloration were not reduced at Si rates less than 2 t/ha in Santa Rosa (Table 2), these diseases were significantly reduced at 500 kg/ha at La Florida (Table 4). Differences could have been however due to cultivar differences in Si response. Disease development at La Florida tended to be higher at the lower P rate in the absence of Si (Table 4). Neck blast incidence significantly decreased from a high of 48-55% in the control to as low as 12-21% with the addition of Si (Table 3) while leaf scald and grain discoloration significantly decreased from 28-42% to 6-9% and 4.0-4.5 to 1.0, respectively, with the addition of Si (Table 4), If disease reduction of the magnitude discussed here (17-79% reduction range) can be obtained with Si application in this region, it is worth to consider the economic, food security, and environmental benefits that an integrated nutrient management system including the element Si will have on the sustainability of rice production.

## 3.2 Yield response to Si application

Rice yields increased significantly ( $P \le 0.05$ ) with increasing Si rates, confirming previous reports (Datnoff *et al.*, 1991,1992; Deren *et al.*, 1994; Seebold, 1998). Responses to Si were observed up to a rate of 2 t/ha in 1992, and up to 1 t/ha applied fresh in 1993 at Santa Rosa for the cultivar Oryzica 1 grown on an Inceptisol on the Colombian piedmont (Table 5). Rice response to slag one year after application at Santa Rosa diminished only slightly in relation to response in the fresh application in the previous year. A supplemental application of 1 t/ha of Si to all treatments in the second year did not produce appreciably greater yields than the applications of one year earlier. Presumably this effect was due to the continuing dissolution of the slag amendment one-year after application. Water-soluble Si levels extracted from residual and freshly tested soils reflect a continuing ability of the slag application to maintain available Si at fixed concentrations over time, keeping a high relationship between available Si and total slag applied in the second year (data not shown). Yield responses were consistent with levels of available Si in soil (data not shown).

The greatest yield increases (76%) at the Santa Rosa site occurred in 1992 with Si applications to plots of 3 t/ha. Yields at the three Si rates used increased between 33 and 41% over the control for residual 1992 Si effects on the 1993 crop (Table 5). Yield increases at Santa Rosa in 1993 were at the level of 48% when 1 t/ha of Si was applied on untreated 1992 plots and up to 56% when the same amount of Si was applied to those plots receiving 3 t/ha of Si in 1992 (Table 5). Although there was not a significant effect of Si application on yield in 1992 on the blast resistant cultivar Oryzica Llanos 5, yield increases up to 100% over the control were observed for the residual effects of 1992 Si rates of 2 and 3 t/ha on the 1993 rice crop (Table 6). Similarly, yield increases of the same level were observed on the 1992 untreated and residual Si rates receiving 1 t/ha of Si in 1993 (Table 6). It is very important to note that the residual applications of Si were very effective in increasing the yield of both cultivars at the Santa Rosa site.

Yield responses to wollastonite, a mineral source of Si with solubility comparable to lime, were evident up to 500 kg-Si/ha, the highest rate applied on two more weathered soils (Oxisols) on the Colombian altillanura. Yields were significantly increased wit Si applications to plots of the rice

cultivars Oryzica Sabana 6 and IAC 165 planted in the Matazul and La Florida sites in 1993 (Tables 3 and 4). Yield increases at these two sites in the presence of Si tended to be higher at the highest rate of P (50kg/ha) used (Tables 3 and 4). Yields increased by 29-48% and 24-27% compared to the control in cultivars Oryzica Sabana 6 and IAC 165, respectively, with the addition of Si (Tables 3 and 4). Grain quality was also enhanced in the treatments that received 500kg of Si compared to the untreated control (Table 7). The percentage of unbroken white rice obtained after milling as well as the weight of 1000 grains were increased significantly at this Si level (Table 7). A better grain quality reflects an optimum grain filling probably due to the control of several rice diseases.

# 3.3 Cultivar differences in Si response

All diseases evaluated in these experiments at the three different sites seem to have a significant negative correlation with Si and yield as reported by Datnoff *et al.* (1991 and 1992). In addition, a positive correlation seems to exist between Si and yield. It is logical that reduced disease severities would be at least partially responsible for increased yield. However, Si in the absence of disease, as it is the case of the blast resistant cultivar Oryzica Llanos 5, may also increase yield solely as a plant nutrient. Increased yield is then probably a function of both reduced disease and more favorable plant nutrition (Datnoff *et al.*, 1991).

Although there were significant differences in absolute yields of the different rice lines sown in each of the experiments, the interaction of variety with Si rate was less clear. At Santa Rosa, Oryzica Llanos 5 produced both a higher yield and appeared to have a lower Si requirement compared to Oryzica 1. However, this cultivar yielded very poorly in 1993 when planted on the plots receiving no Si or only 1 t/ha of Si in 1992 and which did not receive any Si in 1993. In fact, the greatest yield increase (100%) observed in these experiments occurred for the residual effects of Si (1 and 2 t/ha) on the 1993 crop of cultivar Oryzica Llanos 5 (Table 6). Yields were only sustained in 1993 when this cultivar received and application of Si equivalent to 1 t/ha on the untreated or the residual 1992 plots (Table 6).

At Matazul, the same was apparent for the IAC 165 cultivar compared to Oryzica Sabana 6, although the interaction was not observed on the sandier Oxisol at La Florida (data not shown). The lower level of available Si at La Florida (2.2 ppm compared to 7.1 ppm) may explain this inconsistency since both cultivars responded up to the 500 kg-Si/ha rate. It is very interesting to note the highly response to Si in terms of both disease reduction and yield increase by the two cultivars planted in Matazul and La Florida. Cultivar IAC 165 responded much better than cultivar O. Llanos 5 in terms of disease reduction. Although leaf scald and grain discoloration pressure was higher at La Florida than at Santa Rosa, both diseases were significantly reduced on IAC 165 by the application of 500 kg/ha of Si (Table 4), while the same diseases were only reduced on cultivar Oryzica Llanos 5 with applications of 2 t/ha of Si (Table 2).

Different rice ecotypes appear to have evolved specialized adaptations to upland and lowland environments. The upland rice grown in South America are japonicas while indica ecotypes are grown in the lowlands of this region (Wilson *et al.*, 1997). The apparent adaptive advantage of japonica rice to the uplands compared to the indica rice may explain the different response to Si observed between the cultivars Oryzica Llanos 5 (indica) and IAC 165 (japonica). Winslow *et al.* (1997) present evidence suggesting that adaptation to Si deficiency could be considered as one possibility for the success of the tropical japonica ecotype on weathered upland soils. Adaptation to the uplands should have evolved after selection pressure into rice with more efficient Si uptake (Winslow, 1995; Winslow *et al.*, 1997). Considering the high levels of disease damage commonly observed when lowland-adapted indica cultivars are sown in tropical weathered upland soils, such an adaptation would even be necessary if the rice crop were to be successful in this agroecosystem (Winslow, 1992). Upland rice seems to be evolving to become more efficient at Si use, and less Si-dependent (Winslow, 1995).

# 3.4 Interaction of Si and Phosphorus

The efficacy of phosphorus has been reported to be enhanced when it is applied along with Si (Savant et al., 1997). There were not significant differences between the P rates for development of diseases when 500 kg/ha of Si were applied to the plots (Tables 3 and 4). Since silicate and phosphate are adsorbed by similar mechanisms on oxidic mineral surfaces in soils, large applications of Si can reduce P sorption and increase the availability and effectiveness of P fertilizer applications in soils. It is important therefore to be able to distinguish between a direct effect of Si on rice yields and an indirect effect in the soil through improved P nutrition. Such an effect would produce a negative interaction between Si and P on rice yields in that higher Si rates would result in reduced response to P applications. On the contrary, however, a positive interaction of Si and P was observed at both Matazul and La Florida sites (Tables 3 and 4). Rather than reducing the requirement for P fertilizer, response to TSP was greater at higher than at lower rates of Si. Evidently, these soils are so poor in both Si and P as to require substantial applications of both to achieve the full yield potential of the rice cultivars evaluated in these trials.

#### 4. CONCLUSIONS

These findings, on three diverse and representative soils of the upland savannas on the Colombian Llanos, suggest that Si deficiency is a major constraint, limiting rice yield potential and favoring disease development.

Results indicate that Si fertilization may be used to increase rice production of weathered tropical rice soils while enhancing integrated management of several important rice diseases in Colombia, thus reducing the reliance on frequent fungicide use.

A wider analysis is required to more fully define the extent and magnitude of this Si-deficiency constraint to rice production on highly weathered soils. Although rice germplasm is now available which is well adapted to the high levels of soluble aluminum in these soils, low levels of Si may limit their utility.

Among the limited selection of rice lines examined, there was some evidence of cultivar differences in response to and requirements for Si. Thus, there may be potential to address this problem through the development of more adapted germplasm.

Si applications to the soils considered in this study enhanced rice response to applied P fertilizer in that yield response to higher P rates were limited by inadequate levels of Si.

Si applications have an appreciable residual effectiveness as well as liming value, raising the possibility that applications need not be applied annually. However, the high rates required and the availability of this material in relation to the areas in which it is required raise questions of the economic viability of this type of intervention.

# **REFERENCES**

Correa-Victoria, F. J. and Zeigler, R. S. 1993. Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" breeding site in eastern Colombia. Plant Disease 77:1029-1035.

Datnoff, L. E., Snyder, G. H. and Deren, C.W. 1992. Influence of silicon fertilizer grades on blast and brown spot development and yields of rice. Plant Disease 76:1182-1184.

Datnoff, L. E. 1994. Influence of mineral nutrition of rice on disease development. In: "Rice Pest Science and Management" Teng, P.S., Heong, K.L., Moody, K., (eds.), pp. 89-100. International Rice Research Institute, Manila, Philippines.

Datnoff, L. E., Deren, C. W. and Snyder, G. H. 1997. Silicon fertilization for disease management of rice in Florida. Crop Prot. 16:525-531.

Datnoff, L. E., Raid, R. N., Snyder, G.H. and Jones, D.B. 1991. Effect of calcium silicate on blast and brown spot intensities and yields of rice. Plant Disease. 75:729-732.

Deren, C. W., Datnoff, L. E., Snyder, G.H. and Martin, G.G. 1994. Silicon concentration, disease response and yield components of rice genotypes grown on flooded organic Histosols. Crop Sci. 34:733-737.

Elawad, S. H. and Green Jr., V.E. 1979. Silicon and the rice plant environment: A review of recent research. I1 Riso 28:235-253.

Epstein, E. 1994. The anomaly of silicon in plant biology. Proc. Natl. Acad. U.S.A. 91:11-17

Friesen, D. K., Sanz, J. J. Correa, F. J, Winslow, M. D., Okada, K., Datnoff, L. E. and Snyder, G. H. 1994. Silicon deficiency of upland rice on highly weathered savanna soils in Colombia. I. Evidence of a major a yield constraint. IX Conferencia Internaciaonal de arroz para America Latina e para o Caribe, V Reuniao Nacional de Pesquisa de Arroz, Castro's Park Hotel. 21-25 Marzo. Goiania, Goias, Brasil.

Juo, A. S. R. and Sanchez, P. A. 1986. Soil nutritional aspects with a view to characterize upland rice environment. In: "Upland Rice Research", International Rice Research Institute, pp. 81-95 Los baños, Philippines.

Savant, N. K., Snyder, G. H. and Datnoff, L. E. 1997a. Silicon management and sustainable rice production. Adv. Agron. 58:151-199.

Savant, N K., Datnoff, L. E. and Snyder, G.H. 1997b. Depletion of plant-available silicon in soils: A possible cause of declining rice yields. Commun. Soil Sci. Plant Anal. 28(13&14):1245-1252.

Seebold, K. W. 1998. The influence of silicon fertilization on the development and control of blast, caused by *Magnaporthe grisea* (Hebert) Barr, in upland rice. Ph.D. Thesis, University of Florida, Gainesville, USA

Snyder, G. H., Jones, D. B. and Gascho, G. J. 1986. Silicon fertilization of rice on Everglades Histosols. Soil Sci. Soc. Amer. J. 50:1259-1263.

Takahashi, E., Ma, J. F. and Miyake, Y. 1990. The possibility of silicon as an essential element for higher plant. Comments Agric. Food Chem. 2:99-122.

Winslow, M.D. 1992. Silicon, disease resistance and yield of rice genotypes under upland cultural conditions. Crop Sci. 32: 1208-1213.

Winslow, M. D. 1995. Silicon: A new macronutrient deficiency in upland rice. CIAT working document No, 149. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Winslow, M. D., Okada, K. and Correa-Victoria, F. 1997. Silicon deficiency and the adaptation of tropical rice ecotypes. Plant and Soil 188;239-248.

Yoshida, S. 1981. Fundamentals of rice crop science. International Rice Research Institute, Los Baños, Philippines.

Yoshida S. 1975. Factors that limit the growth and yields of upland rice. In: Major Research in Upland Rice. Pp 46-71. International Rice Research Institute. Los Baños, Philippines.

Table 1. Influence of Silicon (Si) on blast development in rice cultivar Oryzica 1 at Santa Rosa, Colombia

<u>Silicon</u>	Leaf Blast (%)			Neck Blast (%)				
(Si) T/ha 1992	1992	1993 Residual 92	1993 Residual 92 +1t/ha Si	1992	1993 Residual 92	1993 Residual 92 +1t/ha Si		
0	55a	42 a	25 bc	65a	60a	47 b		
1	21 b	27 b	20 bcd	71a	41 bc	35 cd		
2	16 b	20bcd	20 bcd	57a	33 cd	25 de		
3	17 b	17 cd	17 cd	63a	25 de	17 e		

Table 2. Influence of Silicon (Si) on the development of leaf scald and grain discoloration in ricen cultivar Oryzica Llanos 5 at Santa Rosa, Colombia

Silicon		Grain Discoloration (1-9)				
(Si) t/ha	Leaf Scald (%)	1993	1993			
1992	1992	Residual 92	Residual 92 + 1t/ha Si			
0	23 a	3.5a	3.0 a			
1	22 a	3.0a	2.5 ab			
2	17 b	1.0 b	1.0 b			
3	19 b	1.0 b	1.0 b			

Table 3. Influence of Silicon (Si) and Phosphorus (P) on neck blast (NB) development and yield in rice cultivars Oryzica Sabana 6 and IAC 165 at Matazul, Colombia

Treatment			O. Sa	ıbana 6 —	IAC 165		
P Kg/ha	Si t/ha	Lime t/ha	NB (%)	Yield (t/ha)	NB (%)	Yield (t/ha)	
25	0	2.8	48a	2.3	30 a	2.7	
25	0.5	0	12 b	2.9	18 b	3.0	
50	0	2.8	55 a	2.3	29 a	2.9	
50	0.5	0	21 b	3.4	15 b	3.6	

Table 4. Influence of Silicon (Si) and Phosphorus (P) on disease development and yield in rice cultivars Oryzica Sabana 6 and IAC 165 at La Florida, Colombia

Treatment		O.Sabana 6		IAC165			
Р	Si	Lime	LB	Yield	LS	GD	Yield
Kg/ha	t/ha	t/ha	<u>(%)</u>	(t/ha)	(%)	(1-9)	(t/ha)
25	0	2.8	27a	2.0	42a	4.0a	2.6
25	0.5	0	18 b	2.4	9 с	1.0 b	3.5
50	0	2.8	15 b	2.4	28 b	4.5a	3.0
50	0.5	0	11 b	3.1	6 c	1.0 b	3.8

LB= Leaf Blast; LS= Leaf Scald; GD= Grain discoloration

Table 5. Influence of Silicon (Si) on yield of rice cultivar Oryzica 1 at Santa Rosa, Colombia

Silicon	Yield (t/ha)			
(Si) t/ha 1992	1992	1993 Residual 92	1993 Residual 92 + 1t/ha S	
0	2.1a	2.7 a	4.0 b	
1	3.2 ab	3.6 b	4.1 b	
2	3.6 b	3.7 b	3.9 b	
3	3.7 b	3.8 b	4.2 b	

Table 6. Influence of Silicon (Si) on yield of rice cultivar Oryzica Llanos 5 at Santa Rosa, Colombia

Silicon (Si) T/ha 1992	Yield (t/ha)			
	1992	1993 Residual 92	1993 Residual 92 +1t/ha Si	
0	4.1a	2.3a	4.7 b	
1	4.5a	2.2a	4.7 b	
2	5.1a	4.6 b	4.7 b	
3	5.0a	4.6 b	4.6 b	

Table 7. Influence of Silicon (Si) and Phosphorus (P) on two yield components

Treatment <sup>a</sup>	1000 grain weight (g)	Unbroken white grain after milling (%)
Si <sub>1</sub> P <sub>1</sub>	24.4	59.8
Si <sub>1</sub> P <sub>2</sub>	24.1	56.9
Si <sub>2</sub> P <sub>1</sub>	25.6	69.4

<sup>&</sup>lt;sup>a</sup>Si <sub>1</sub>= No Silicon; Si <sub>2</sub> = 500 kg Si/ha;  $P_1$  = 25 kg. P/ha;  $P_2$  = 50 kg. P/ha

# **OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED**

# B. Collaborative Project in Rice CIAT, CIRAD, and FLAR

# B.1. Knowledge of the Level of Partial Resistance to Blast of existing Germplasms Michel Vales, Jaime Borrero, Edgard Tulande, Joanna Dossman, Yolima Ospina

## **Executive summary**

For the last annual report we didn't have the complete analysis of the results of 1998 A. At the methodological level the conclusions are the following:

- Demonstration that is possible to control an inoculated strain in the field sufficiently. That allows
  evaluate the partial resistance.
- Demonstration that without the control of a compatible strain in the field you cannot evaluate the partial resistance
  - there is not significant difference among the materials
  - inside the materials that seem resistant there are susceptible lines
  - there is risk of making a wrong selection: discard resistant materials, and select susceptible materials.
- Prove that a part of the partial resistance in the field can be specific, with the demonstration of an interaction plant-parasite, with a ranking inversion. That demonstrates the necessity to change the strain for each selection cycle.

We have an idea of the specific level of partial resistance of 85 varieties or progenitors of Latin America.

#### Presentation

Blast (*Pyricularia oryzae*) it is the most important rice disease in the world and in particular in Latin America.

There are three not-excluding genetic strategies against rice Blast:

- The use of the monogenic, and specific complete resistance, with the two following strategies:
  - the accumulation of genes of complete resistance
  - the incompatibility of virulences.
- The use of a partial, polygenic, and general resistance.

For the first strategy the CIAT is using the knowledge of the lineages of *Pyricularia* to improve the sampling of the strains. But no strategy is without risk and we don't have all the necessary information on the lineages for the whole Latin America. For the recurrent selection we are making populations source of complete resistance to participate to the constitution of several other populations.

The second strategy is at the level of preliminary studies in the CIAT and in the CIRAD-CA of Montpellier. However we are already preparing its use in the recurrent selection with the constitution of appropriate populations.

Taking advantage of the experience of the CIRAD in the third strategy, we are proving this strategy now also in the CIAT. The probability of the durability of a resistance increases if it is general (knowledge and use of strains), polygenic (leads to the use of the recurrent selection) and partial (trial design, selection criterias).

The three strategies are not excluding, and they are associated in the breeding program:

- For the conventional selection (starting from F2 populations) the first step is the characterization of the level of partial resistance of the commercial varieties, and progenitors. He seconds step is to propose, and use, the appropriate methods to complete the existing breeding schema.
- For the recurrent selection the first step is the improvement of a population with broad genetic base. The second step is the constitution of populations with narrow genetic base, a new population type to complete the breeding program.

# **Background information**

Michel Vales arrived to CIAT in August of 1997 to reinforce the Collaborative Project CIRAD-CIAT-FLAR in rice Blast resistance. He has 15 years of experience on Blast. He worked during 9 years in Ivory Costa for resistance to rice Blast, the first program CIRAD in recurrent selection. This Program was collaboration CIRAD-IDESSA (Ivory Coast)-CNPAF (Brazil), and it includes the participation of Elsio Guimaraes, and Marc-Henri of Châtel.

# Activity 1. Blast strains identification for partial resistance studies

Michel Vales, Joanna Dossman, Jaime Borrero, Fernando Correa

# Objective

In the field, with a very variable pathogen population it is impossible to distinguish among complete and partial resistance, because both can reduce the number of lesions y/o the lesion size. To distinguish both mechanisms we have to work with a controlled virulent strain. A part of the partial resistance can be specific (cf. Activity 3.), so it is necessary to change of strain, in particular, to each selection cycle recurrent. In routine we should look adapted strains in Dr. Fernando Correa's collection.

# Material and methods

They are described in the precedent annual report.

Strains were identified for their virulence spectrum (see also Activity 4).

# **Perspectives**

Identified strains can be used to partial studies of resistance in greenhouse and in field. The observation of an interaction plant-parasite in the field for the partial resistance (cf. Activity 3) offers a study model of this partial resistance aspect.

# Activity 2. Identification of Partial Resistance to Blast in Commercial Varieties release in LAC

# Activity 3. Identification of the Partial Resistance to Blast in parents already used by **CIAT/FLAR Crossing Program**

Michel Vales, Jaime Borrero, Yolima Ospina, Edgard Tulande, Diana Delgado, James Gibbons, Myriam Cristina Duque, James Silva

Both activities were driven in same time.

# Methodological trials

# Objective

Field trail inoculation with a controlled strain is a necessity to study the partial resistance. For this reason we study the effectiveness of the methods of strain control in field.

#### Material and method

We take advantage of the evaluation trial to study the control of inoculated strain in field of Santa Rosa Experimental Station (EESR).

A barrier of plastic isolated the trials. A treatment was made fifty days after the sowing of the spreader with Hinosan (50 EEC (Edifenphos 50%) 750 p.c. ml / ha). It is necessary to clean it. The lines were sowed after the treatment. Ten days after the treatment the spreader was inoculated with dry leaves. Those leaves came from plants inoculated in greenhouse.

The susceptible variety Fanny was sowed to pick up spores of *Pyricularia*. The fungus isolates from the lesions can be use to study, and to identify the strains.

#### Results and discussion

Ten days after the treatment the new leaves of the spreader were clean, without lesion. The inoculation with dry leaves allowed the obtaining of a good epidemic pressure. So the control of inoculated strain seems good.

With the control of a strain differences for the partial resistance (area under the curve of the infected leaf area, ABCAFA) are significant what allows to make a selection (Fig.1). Without control of the strain there is not significant difference although the trial design (blocks of Federer), and the criteria (ABCAFA) are more effective than in the common selection experiments.

To insist we can see (Fig.2) that some materials that seem resistant without control of a strain are in fact susceptible. Without control of a strain, we can also see (Fig.3) the risk mistake with the discard of resistant materials and the selection of susceptible materials.

In the resistant materials we can see (Fig.4) a specific plant-parasite interaction for partial resistance (ABCAFA). This interaction is expressed in the strong way, with a ranking inversion.

In conclusion we can say that to make the selection for the partial resistance in the field there is an obligation to control an inoculated strain. There is an interaction plant-parasite, so we should change the strain for each selection cycle to try to eliminate the specific part of the partial resistance

# **Perspectives**

In Santa Rosa during the first semester, the conditions are very unfavorable for a control of an inoculated strain. Because the epidemic conditions are too favorable, and allow the development of an enormous inoculate outside of the isolated subtrials. So if the control was possible with these conditions we can propose this method for another area without restriction. We are also using already this method for the recurrent selection in the population PCT-6\HB.

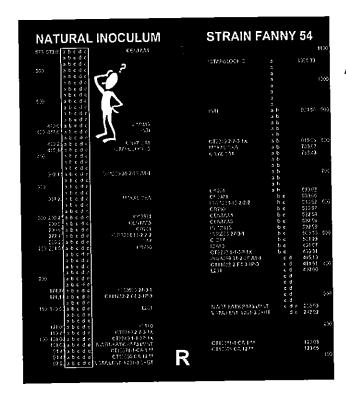


Figure 1

# AREA UNDER THE CURVE OF INFECTED LEAF AREA

Federer's design with incomplete blocs of 20 varieties and 5 checks

Values following by a same letter are no significantly different of the corresponding check (5%)

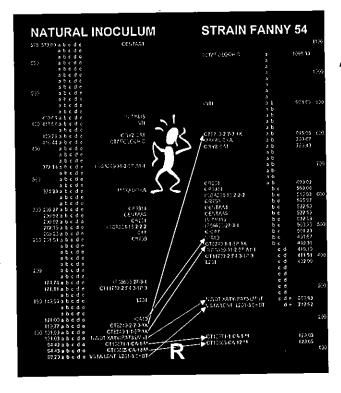


Figure 2

# AREA UNDER THE CURVE OF INFECTED LEAF AREA

Federer's design with incomplete blocs of 20 varieties and 5 checks

Values following by a same letter are no significantly different of the corresponding check (5%)

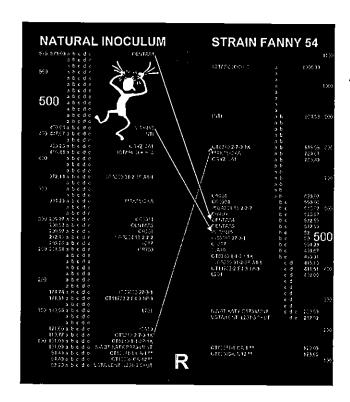
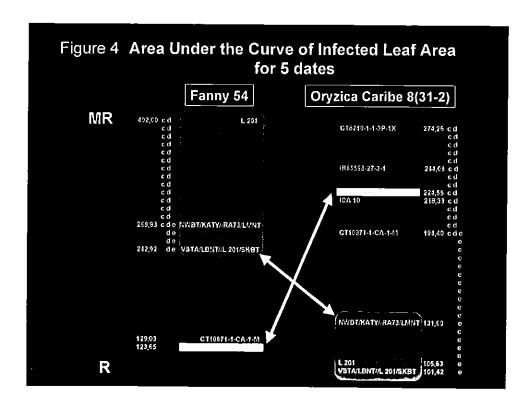


Figure 3

# AREA UNDER THE CURVE OF INFECTED LEAF AREA

Federer's design with incomplete blocs of 20 varieties and 5 checks

Values following by a same letter are no significantly different of the corresponding check (5%)



# Evaluation of the partial resistance

# Objective

The objective is to evaluate the partial resistance of varieties under conditions the nearest possible of the FLAR selection conditions of the rehearsals of.

# Material and method

Two trials were inoculated with a controlled strain. A barrier of plastic isolated those trials.

The first one was inoculated with the strain Fanny 54 (lineage SR 6). Oryzica 1 formed the spreader. This variety is specifically susceptible to the lineage SR 6. The second trial was inoculated with the strain Oryzica Caribbean 8 (31-2) (lineage SR 4). Oryzica Caribbean 8 formed the spreader. This variety is specifically susceptible to the lineage SR 4.

The other conditions were the FLAR trials ones. A third trial was managed with the FLAR conditions: spreader, inoculation, etc.

We use the incomplete blocks design of Federer. Twenty varieties and 5 checks formed each block. The 5 checks were ICA 10, Oryzica 1, INIAP 415, WC 360, and WC 362. For each variety and check: 2 lines, 26 cm behind the lines, 24 kg of seeds / ha.

In the first trial 76 varieties were studied, 72 in the second trial, and 112 in the third one.

#### Results

In the table 1 are the principal datas about the partial resistance level of 85 commercial varieties of LAC and/or progenitors of the FLAR.

The maximum coefficient of correlation between the ACAFA and the (number of necks with symptoms on 30 is 48%, in the case of Fanny 54 use. So the leaf Blast symptoms (ABCAFA) don't allow a prevision of the neck Blast symptoms (number of necks with symptoms on 30).

# Perspectives

The information on the partial resistance of the commercial varieties of LAC will be used to choose cultivars with less risk of production lost du to the apparition of virulent strains. The information on the partial resistance of the progenitors of FLAR will be used to choose parents with a good level of partial resistance.

Table 2: Field partial resistance of 85 varieties of LAC and/or FLAR progenitors RANKING ANALISIS OF ADJUSTED AVERAGES AREA UNDER THE CURVE OF LEAF INFECTED AREA (AUC, 5 dates of obervation) FOR LEAF BLAST

Strain FANNY 64	LLAI INI LOTED AREA	Strain ORYZICA CARIBE 8 (3)	
Variety or line	AUC	Variety or line	AUC
CICA4	1380.39	CT8249-2-7-3-1X	636.38 a b
CICAS	1360.21	INTI	547.24 a b
IR8	1305.71	T1 ORYZICA1	539.49 A A
PA-2	1281.51	ICTAPOLOCHIC	512.62 a b
BR-IRGA413	1248.73	CICA7	489.52 a b
ALTAMIRA9	1194.96 a	SELVAALTA	482.48 a b
ICTAPOLOCHIC	1056.33 a	IRGA284-18-2-2-2	480.01 a b
CR1821	998.78 a	ORYZICA1	478.60 a b
CICA9	993.90 a	T2 INIAP415	466.63 B B
BR-IRGA409	968.41 a b	ORYZICACARIBE	450.39 a b
ALTAMIRA10	904.58 a b	SELECTA3-20	445.84 a b
INTI	904.58 a b	IR50	445.63 a b
T1 ORYZICA1	832.76 A A	CR750	424.00 a b
CT8249-2-7-3-1X	815,95 a b	CT8008-16-31-3P-M	423.31 a b
CAPI-93	802.20 a b	CENTAA4	421,27 a b
MAKALIOKA	783.67 a b	CENTAA5	421.27 a b
ORYZICA1	758.43 a b	CT9882-16-4-2-3-4P-M	419.65 a `b
ICTAMOTAGUA	727.96 a b	CR8334	417.63 a b
LINEA2	727.96 a b	INIAP415	393.06 a b
CICA6	694.47 a b	CR201	366.67 a b c
BR-IRGA412	684.92 a b	IRGA369-31-2-3F-A1-1	340.10 b c
T2_INIAP415	650.37 B B	IRGA370-42-1-1F-C-1	340.10 b c
CR201	623.02 a b	IR65600-96-1-2-2	298.95 c d
BR-IRGA414	619.08 a b	CT11782-2-F4-3-1P-3	298.24 c d
BR-IRGA415	619.08 a b	MAKALIOKA	287.41 c d
IR22	601.58 a b	CT8240-1-1-3P-1X	274.25 c d
ANAYANSI	601.17 a b	82CAY21/LMNT//L202	268.09 c d
ELPASOL-144	601.17 a b	CIWINI	268.09 c d
BR-IRGA410	567.06 a b c	IR36	268.09 c d
EMBRAPA6CHUI	557.50 a b c	CT11691-17-F4-1-1P-2	268.09 c d
CR8334 IRGA284-18-2-2-2	553.06 b c	T3_ICA10	260.63 C C C C
CR750	546.92 b c 525.97 b c	INCA IR65598-27-3-1	255.13 c d
CENTAA4	522,59 b c	CR751	244.08 c d 242.90 c d
CENTAA5	522.59 b c	BLUEBELLE	234.60 c d
INIAP415	522.59 b c	CNAx5011-9-1-6-4-B	234.60 c d
ORYZICAYACU9	522.59 b c	INIAYERBAL	234.60 c d
IRGA416	520.34 b c	CT10865-CA-12-M	224.55 c d
CT11026-3-9-IT-2P-2P-2	508.36 b c	ICA10	218.33 c d
IR65598-27-3-1	508.36 b c	INIAP10	218.33 c d
CICA7	501.23 b c	T4_NWBT/KATY//RA73/LMNT	
EMPASC105	491.67 b c	CT10871-1-CA-1-M	194.40 cde
ICA10	491.67 b c	L202	160.90 d e
DAMARIS	489.48 b c	ARAURE4	151.81 d e
CT11691-17-F4-1-1P-2	471.59 b c	ICTAIZABAL	151.81 d e
CIMARRON	455.98 b c	IRAT216	148.59 d e
SELECTA3-20	443.60 b c	T5_VSTA/LBNT//L201-3/SKBT	134.94 E E E E E
CT8240-1-1-3P-1X	439.31 b c	NWBT/KATY//RA73/LMNT	131.60 e
IRGA369-31-2-3F-A1-1	415.10 c d	L201	105.63 е
CT11782-2-F4-3-1P-3	411.51 c d	CT6948-8-11P	101.42 e
INCA	411.51 c d	CT8198-4-2-6P-1X	101.42 e
L201	402.00 c d	VSTA/LBNT//L201-3/SKBT	<u>101.42</u> е
T3_ICA10	400.80 C C C C		
CAMPECHEA80	371.28 c d		
CR751	369.12 c d		
PALMAR	346.15 c d		
T4_NWBT/KATY//RA73/LMNT	289.77 D D D D D		
EMBRAPATTAIM	287.81 cde		
CIWINI	277.28 cde		
NWBT/KATY//RA73/LMNT	268.93 cde		
BR-IRGA411	255.95 d e		
VSTA/LBNT//L201-3/SKBT	242.92 de		
T5_VSTA/LBNT//L201-3/SKBT	221.70 E E E E E		
CR1113	196.47 d e		
CR5272 CT10871-1-CA-1-M	159.24 e 129.03		
CT10865-CA-12M	129.03 123.65		
OT 10003-CA-12191	143,00		

# Activity 4. Partial resistance of Potential Progenitors of the FLAR

Michel Vales, Jaime Borrero, Yolima Ospina, Edgard Tulande, Diana Delgado, James Gibbons.

# Objective

Some varieties have a durable partial resistance. The objective is to demonstrate the good level of its partial resistance under field conditions. The first step is the identification of virulent strains.

#### Material and methods

They were described in the last annual report.

#### Results

The strain Linea 2 (62) of the lineage ALL7 from Altillanura is virulent to the following rice:

- Silewah from Japan
- Progenies of the cross PRA29, from Madagascar
- Progreso
- Orizica Turipana 7
- Cuyamel 3820
- CT11685-7-F4-6-2P-1
- CT11696-9-F4-10-2P-1
- 63-83 (so normally for its mutant IRAT 13)
- IRAT 112
- Douradao
- Maravilha
- CT11891-2-2-7-M
- Azucena

Previously we had not virulent strain for this material.

# **Perspectives**

It is not possible to inoculate an altillanura (Andean area of Colombia) strain in field in Santa Rosa (in the llanos of Colombia). So for the future we need a place in altillanura for experimental trials.

# Activity 5. Study of the durability of the resistance of varieties (ATP-CIRAD)

Fernando Correa, Michel Vales and Edgard Tulande

# Objective

This trial is made with the financial support of a Programmed Thematic Action (French acronym ATP) of the CIRAD.

Some varieties have a durable resistance. The objective is to prove this durability under favorable conditions to the adaptation of the parasite in the EESR. To understand what happened we are

collecting strain during this experimentation. The strain analysis will be made in collaboration with the CIRAD-CA of Montpellier, France, Dr. Didier Tharreau.

### B.2. Selection strategies for the durable Blast resistance

Michel Vales, Jaime Borrero, Marc-Henri Châtel, Joanna Dosmann, Myriam Cristina Duke, Daniel Gonzales, Yolima Ospina, James Silva, Edgard Tullande,

### **Executive summary**

In 1998B began a selection with the segregated material of FLAR. The study of complete resistance in greenhouse showed that in field of EESR more than 20% of the plants S1 seem immune although they are without complete resistance to strains of this same area. That is due to the existence of strains with low frequency because they belong to lineages with low frequency y/o they have a wide spectrum of virulence. So the selection in greenhouse with such strains is justify to complete the field selection.

In 1999A in the S3 progenies, without complete resistance to a strain, from these plants S1, we are making an evaluation of the partial resistance in EESR. At the date the material is at the flowering stage. The FLAR will be able to use the data of this evaluation to select its best corresponding material.

In 1998B we made the first cycle of recurrent selection in the tropical lowland population PCT-6/HB for the partial resistance and other agronomic traits: production, precocity, quality of the grain, tolerance to Sogata (Tagosodes orizicolus), etc.

In 1998B we also made in the same population a selection cycle for the complete resistance. This work also allowed an evaluation of the genetic progress obtained after a selection cycle for the complete resistance and a genetic recombination. This progress is very important.

In 1999A we make a genetic recombination and selection for the complete resistance in the experimental station of Santa Rosa (EESR). At the date the material is at the flowering stage.

In 1999A we are proving in EESR the author's new method: the reciprocal recurrent selection plant-parasite. This method will already be used in the hybrid program of CIRAD for the Dr. J. Taillebois. At the date the material is at the flowering stage.

In 1998B in progenies of the best lines S1 of the FLAR we made the selection of plants without complete resistance to a strain. More than 20% of the S1 plants which was immune to Blast in EESR are in fact without complete resistance to at least a strain of EESR. This escape is more important for strains of lineages with low frequency, and for strains with a broad virulence spectrum (due to the contraselection of the useless virulences). The conclusion is that to make a good selection for the complete resistance we should make trials in the field to take advantage of the natural variability of the fungus, and inoculations in greenhouse to use strains with low frequency, in particular with wide virulence spectrum.

In 1999A we are evaluating the partial resistance in the field of EESR in S3 progenies of these better S1 lines of FLAR. At the date the material is at flowering stage. The datas should help to the FLAR selection.

### Presentation

See Output 3, B.

### **Background information**

See Output 3, B.

### Activity 1. Conventional selection

Michel Vales, Daniel Gonzalez, Joanna Dosmann, Edgar Tulande, Yolima Ospina.

### Objective

The objective is to reinforce the existing selection process of the FLAR with new complementary activities on partial resistance to Blast.

### Material and methods

The FLAR gives us progenies of its best S1 plants. These plants were immune in EESR with respect to Blast. The material comes from crossings with three parents.

In 1998 B we made the inoculation of these progenies to study their complete resistance spectrum. To do that we used 13 strains with broad virulence spectrum from the 7 lineages of llanos according to Fernando Corrrea's data. We also select plants without complete resistance with respect to a strain.

In 1999 A in EESR in an isolated parcel with *Panicum maximum*, we evaluated the partial resistance to Blast of the progenies of these selected plants. The design trial was the blocks of Federer, and it was inoculated with the elected strain.

### Results and discussion

More than 20% of the immune S1 plants in the field of EESR are in fact without complete resistance with respect to at least a strain of this same area (Tab.2). The low frequency of strains can explain this escape. Strains have a low frequency if they come from a lineage with low frequency, and/or if they have a broad virulence spectrum (Tab.3).

At the date we are waiting the result of the evaluation of the partial resistance in the field of EESR.

Table 2: Escape of S1 lines, evaluation in S2 progenies (Santa Rosa, Villavicencio, Meta)

	(OBINA Produ) Villarios			
Lineage	Strain	% of	escape	*
	Cica 9 (15)	0,00	0,00	
	Cica 9 (31-2)	0,00		1
SRL-2	Cica 9 (151-1)	0,00	1,05	
	O. Llano 5 (237-2)	1,05		
SRL-3	Metica 1 (33-20)	0,00	0,00	
	Metica 1 (33-18)	0,00		
SRL-4	O. Caribe 8 (31-2)	3,16	5,26	22,11
]	O. Caribe 8 (33-2)	4,21		
SRL-5	Isolinea 22 (3-1)		12,63	
	Isolinea 6 (7-1)	10,53		
SRL-6	Selecta 3-20 (1)	4,21	4,21	
	Fanny 54	0,00		
SRL-6b	Cica 9 (177-1)	4,21	4,21	
	to a Cd immuno pl	4 4		Iia bu C

<sup>\*</sup> In 95 S2 progenies from S1 immune plants; study on 20 plants by S2

Table 3: Escape of S1 lines, evaluation in S2 progenies (Santa Rosa, Villavicencio, Meta)

% of escape <sup>b</sup>					
ow frequency					
nce spectrum					
SRL-5 12,63°					
4,21 <sup>d</sup>					
ght frequency					
nce spectrum					
5,26°					
4,21°					
arrow virulence					
·					
0,00°					
1,05°					
0,00°					

- a Information on the lineages from Correa-Victoria et alii, 1998
- b Study on 95 S1 immune plant progenies; study on 20 plants by S2
- c Two strains
- d Only one strain

### **Perspectives**

The data on the complete resistance show clearly that there is a necessity to make the selection for complete resistance in greenhouse and in field. In greenhouse to use strains with natural low frequency, but the 13 used strains cannot represent the variability of the fungus population. In field to take advantage of the variability of the natural fungus population, but there is risk of escape to strains with low frequency. So both methods are complemented. The program of the FLAR already uses both methods and the recurrent selection also.

To limit the risk of escape, to increase the frequency of virulent strains with low frequency we are proving a new appropriate method, the selection recurrent plant-parasite (cf. 2.1.3.)

The data of the evaluation of the partial resistance of the materials will help to select in the FLAR corresponding families.

## Activity 2. Recurrent selection for complete and partial resistances

Michel Vales, Marc-Henri Châtel, Jaime Borrero, Edgar Tulande, Joanna Dosmann, Yolima Ospina, Mónica Triana, Myriam Cristina Duque, James Silva.

### 2.1. Use of existing germplasm

## 2.1.1 Tropical lowland population PCT-6\HB

### Material and methods

The selection schema was defined in 1997 (see previous annual report), and it has three part (Fig.5).

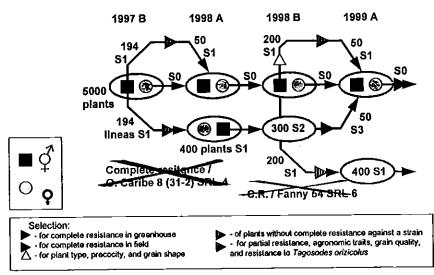


Figure 5: Recurrent population PCT-6\HB indica tropical lowland rice

- The selection for the complete resistance (exclusion of lineages): In 1998 B in 2500 male-fertile plant of the S0 population were selected 200 ones for plant and grain type, and precocity. Fifty

were selected in the 200 lines S1 for their spectrum of complete resistance to the 7 lineages (13 strains) in greenhouse. It was the second cycle of recurrent selection for the complete resistance

In 1999 A they are in phase of recombination in field (EESR) with a population's sample and the S3 material that comes from the selection for the partial resistance, and other agronomic traits, of 1999B. He/she takes advantage this recombination to select for the complete resistance in regarding the natural fungus population.

- The selection for partial resistance:

In 1997B 396 plants were selected in 190 S1 for their absence of complete resistance to a strain in greenhouse. They were multiplied in 1998 A. Not all the plants are male-fertile, so we have obtained 270 S2. In 1998 B these S2 was evaluated in EESR for the partial resistance and other agronomic traits. The trial was isolated with *Panicum maximum*, and it was inoculated with the previous used strain. Then an evaluation was made on the seeds S3 for the grain quality. It was also made in greenhouse an evaluation in S3 generation for the tolerance to *Sogata*.

In 1998B in 2500 male-fertile plant of the S0 population 200 individuals were selected for the plant and grain type, and the precocity. 400 plants of the 200 S1 were selected for their absence of complete resistance against a strain in greenhouse. In 1999 A we are multiplying this plants to obtain the S2 for the next cycle of recurrent selection for the partial resistance and other agronomic traits in field.

- The conservation of the gene of masculine sterility, and of a part of the variability:

In 1999 A we are making a cycle of recombination with a S0 sample for the maintenance of the male-sterility gene and of the variability, with the S1 and S3 corresponding to two other part of the schema.

### Results

We can say that the realization of this new selection schema for the complete and partial resistances, and for other agronomic traits, advances in a good way.

After the first cycle of recurrent selection for the complete resistance and a recombination we can observe (Tab.4) a very important genetic progress. The better results of the first cycle of recurrent selection for the partial resistance are:

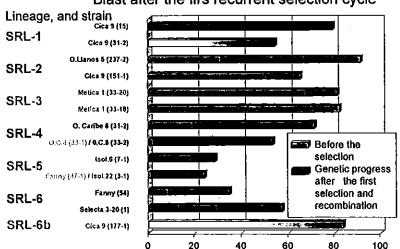


Table 4: Genetic progress for complete resistance to Blast after the firs recurrent selection cycle

Percentage of S1 with all the plants (20) with complete resistance

- With the applied methods and in this population there is possible to make the selection (Fig. 6)
- Although the correlation between Blast and production is not very strong (-50% with the leaf Blast, ABCAFA, and 48% with the neck Blast, number of necks attacked on 30) we can see that there is not productive and Blast susceptible material (Fig.7)
- The symptoms of leaf Blast, ABCAFA, cannot allow a prevision of the symptoms of neck Blast, number of necks attacked on 30, correlation coefficient of 45%.
- A good correlation between production (g/10m²) panicle weight (average) 81%, not between production and number of panicles, 32%. No significantly (negative) correlation between panicle weight and number by plant, so is already possible to select both traits.

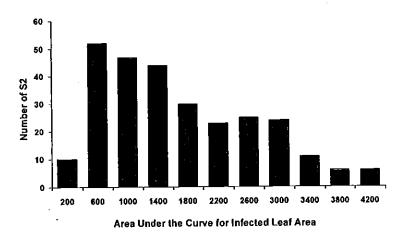


Figure 6: Partial resistance distribution of the S2 of the PCT-6\HB

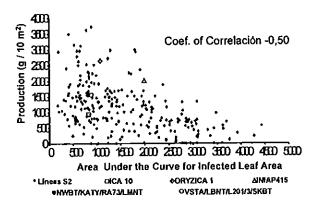


Figure 7: Distribution for production and partial resistance of the S2 of the PCT-6\HB

### 2.1.2. Tropical lowland population PARG-2 (Universidad de Corrientes y La Plata, Argentina)

Cause the limitation of the resources we do not follow this activity for the moment.

### 2.1.3. Reciprocal plant-parasite recurrent selection

### **Principle**

We have seen (cf. Activity 1) that some rice lines can escape in field to strains with low frequency. It is a risk in particular for the durability of the selected complete resistance. The objective is to increase the frequency of the virulent strains to improve the rice selection.

With recurrent selection the frequency of the useful resistance genes increases in each cycle, with regard to the fungus population. The increased resistance produces ago a selection pressure to the fungus population and favorites the virulent strains. If these strains had a low frequency, this frequency should change quickly and the escape risk decreases.

But this rice recurrent selection is just a small part of the breeding trials. So in the following cycle the change that happened in the fungus population is so diluted that it is not perceptible, and the escape risk stays.

To maintain the result of the selection in the fungus population it should be picked up strains at the final of each selection cycle to inoculate the following one.

### Material and methods

Practically we isolate progressively the rice recurrent population with a corn barrier. At the final of the cycle of recurrent selection we sow a susceptible variety is made inside the isolated parcel to obtain and to harvest leaves with symptoms. Then we can use these dried leaves to inoculate the spreader of the following cycle of recurrent selection.

In 1999 A in EESR we are beginning this reciprocal plant-parasite recurrent selection with the rice population PCT-6\HB and the natural population of *Pyricularia grisea* of Santa Rosa.

#### Results

At the date the population of rice is at the flowering stage.

We are using this method for the recurrent selection in the population PCT-6\HB.

This new method was proposed and immediately adopted for the hybrid program of CIRAD in the Brazil by Dr. James Taillebois.

## 2.2. Specific germoplasms development

# 2.2.1. Recurrent populations with narrow genetic base development with parents with complete and partial well-known resistances

Using the new personal concept of recurrent population with narrow genetic base (see previous annual report) the second cycle of crosses was made for the constitution of the following genetic germplasms:

### ◆ Temperate lowland rice

Final genetic participation

Male-sterility source, population

PCT-12

25%

Progenitors:

18,75% x 4

Yield

BR-IRGA 417

Grain quality BR-IRGA 417

Tolerance to the cold Inca
Grain quality Inca
Durable resistance to Blast IRAT 13

Durable resistance to Blast Oryzica llano 5

### ♦ Tropical lowland rice for Llanos

Male-sterility source, population: PCT-11 25%

Progenitors: 15% x 5

Yield CIRAD 409 (= Line 30)

Resistance to the *entochamiento*Grain quality
CIRAD 409
Precocity
CIRAD 409
Soil acidity tolerance
CIRAD 409
CIRAD 409
CIRAD 409
Durable resistance to Blast
IRAT 13

Durable resistance to Blast Oryzica llano 5

Resistance to *Hoja Blanca* FB0007
Resistance to *Tagosodes orizicolus* FB0007
Resistance to the *Entorchamiento* CT11026-3 -....

### ♦ Tropical lowland rice

Male-sterility source, population: PCT-6 25%

Progenitors: 18.75% x 4

Yield Oryzica Yacu 9
Grain quality Oryzica Yacu 9

Durable resistance to Blast IRAT 13

Durable resistance to Blast Oryzica Ilano 5

Resistance to *Hoja Blanca* FB0007
Resistance to Tagasodes FB0007

### • Upland rice for cold hillsides

Male-sterility source, population: PCT-13 25%

Progenitors: 18,75% x 4

Yield CT10069-27-3-1-4
Grain quality CT10069-27-3-1-4

Cold tolerance Inca
Grain quality Inca
Durable resistance to Blast IRAT 13
Durable resistance to Blast Oryzica Ilano 5

◆ Tropical upland rice

Male-sterility source, population: PCT-11 25%

Progenitors: 15% x 5

Yield CIRAD 403 Drought tolerance CIRAD 403 Grain quality

Soil acidity tolerance

Durable resistance to Blast

Durable resistance to Blast

Oryzica Ilano 5

All these populations are available in order to this year 1999.

# 2.2.2. Development of recurrent populations with numerous genes of complete resistance to complete the existing recurrent populations with broad genetic base

In 1999 we continued the crosses to constitute a recurrent population with all the identified resistance genes, another population with all the well-known progenitors of different complete resistance, and a last one with the resistance genes corresponding to the different models of incompatibility of virulence.

After the constitution of these populations we will have the possibility to associate some ones. Then it will be possible to introduce samples of this material in existing recurrent populations for their improvement.

### **Activity 3. Training**

Preparation and participation at the 2nd International Recurrent Selection Workshop (Brazil) An international workshop on the recurrent selection occurred in Sept '99, 21-24 in Goiania, Brazil. Preparation of the National Course of Recurrent Selection for the Argentina For more details see the annual report of Dr Marc-Henri Châtel.

### Activity 4. Preparation of a manual on Blast resistance selection

A didactic manual CIAT-CIRAD on selection for Blast resistance is in its final phase of writing.

To complete, a new document CIAT-CIRAD is already written and subjected to the CIRAD for publication financing. It is less didactic, but more detailed than the manual (3 chapters, 60 pages).

### Participation at meetings and conferences

- Participation at the CIAT-CIO Strategic Alliance Meeting, Montpellier-France, June 22-24,1999.
- Participation at the Secundo Taller Internacional de Selección Recurrente en Arroz, Goiania-Brasil, 21-24 septiembre 1999.

### Presentations in workshops and Conferences

VALES, M. J., BORRERO, J., OSPINA, Y., TULANDE E., GIBBONS, J., and GONZALES, D. 1999. Selection strategy for durable rice blast resistance. Pedigree selection for rice blast (*Magnaporthe grisea*) complete, and partial resistances. The 2<sup>nd</sup> Template Rice Conference. June 13-17, 1999. Sacramento, California.

VALES, M. J., CHATEL, M.-H., BORRERO, J., DELGADO, D., OSPINA, Y., TULANDE E., TRIANA, M., MENESES, R., KURI, V., DUQUE, M. C., and SILVA, J. 1999. Selection strategy for durable rice blast resistance. Recurrent selection for rice blast (*Magnaporthe grisea*) complete, and partial resistances. The 2<sup>nd</sup> Template Rice Conference. June 13-17, 1999. Sacramento, California.

VALES, M. J., CHATEL, M.-H., BORRERO, J., DELGADO, D., DUQUE, M. C., KURI, V., OSPINA, Y., TRIANA, M., TULANDE, E. 1999. Aplicación de la selección recurrente en arroz para la resistencia durable a la Piricularia y otros caracteres agronómicos. Il Taller Internacional de Selección Recurrente en Arroz. 21-24 septiembre 1999 – Goiania, Brasil: 13 p.

# OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

# C. Rice Hoja Blanca Virus and Tagosodes orizicolus

Lee Calvert and Rafael Meneses

**Objective:** The objective of this project is to mitigate losses due to rice hoja blanca virus and *T. orizicolus* and impact is measured as the prevention of losses.

# 1.1. Development of RHBV and T. orizicolus resistant rice.

# Output 1.1. The release of varieties with resistance to both RHBV and its vector.

In Colombia, the variety Fedearroz 50 was released in 1998. Although there are some problems with germination, susceptibility to *Rhizoctonia* and *Sarocladium*, the yield potential of Fedearroz 50 is excellent with farmers reporting yields of 6-10 tons per hectare. With the resistant to RHBV and the high yield potential, Fedearroz 50 is quickly being adopted. It is estimated 20% of the total rice growing area in Casanare, 20% in Huila, 35% in Meta, 40% in Tolima and 15% in Valle is planted with Fedearroz 50. Approximately 30% of the rice grown in Colombia is Fedearroz 50. The success is of Fedearroz 50 milestone in the control strategy for RHBV.

In Venezuela, a line now designated PN (plan national) 97004 is scheduled for release late in 1999. In the field trials at CIAT, this variety is even more resistant to RHBV than Fedearroz 50. These resistant varieties are the output of a long term commitment between the FLAR, NARS, NGOs, and CIAT to make breeding for RHBV and *T. orizicolus* a priority. These varieties are the fruits of these efforts and are allowing the farmers more options in the effort to eliminate the losses caused by RHBV.

# Activities 1.1.1.-1.1.3. Maintenance of *T. orizicolus* colonies and screening of rice germplasm for resistance to RHBV and the vector.

The screening of rice lines for resistance to RHBV is part of the breeding strategy that is used by CIAT, FLAR and many national programs. CIAT is still the only institution with the capacity to screen a large volume of materials for RHBV resistance. The evaluations are conducted twice a year in the field. Trials from the second semester of 98 and the first semester of 1999 are included in this report. During this period, more than of 20,000 lines were screened for resistance to RHBV (Table 1).

The lines that were evaluated for *T. orizicolus* were from CIAT, FLAR, FEDEARROZ and Colombia-CORPOICA, INGER, Costa Rica, Venezuela-NPAM, Surinam, Cuba-IIA, VIARC, and USA-LSU. The increasing number of resistant plants means that it is easier for the breeders to select for other traits (Table 2).

Table 1. Rice germplasm reaction to RHBV. (CIAT. II Sem. 1998, I Sem. 1999)

Source	No. of material	С	lassification	*
;	Evaluated	R	!	S
FLAR:				
VIOFLAR y VIOAL	232	133	15	84
F3- Trópico	9172	6572	1478	1122
F4- Trópico	3738	2570	710	458
Working Collection (WC)	479	121	23	335
IRRI	195	11	9	175
Countries:(Ecuador, USA, Surinam)	163	2	7	154
<u>SUBTOTAL</u>	13979	9409	2242	2328
CIAT- Pre-Breeding	2399	423	531	1445
CIRAD-CIAT	1014	885	126	3
FEDEARROZ- Colombia	3111	1640	921	550
FUNDARROZ- Venezuela	307	226	41	40
ICA- Colombia	142	73	6	63
		;	1	
TOTAL	20952	12656	3867	4429
PERCENTAGE	100	60.5	18.5	21.0

<sup>\*:</sup> R = Resistant (scale: 1-3); I = Intermediate (5); S = Susceptible (Standard Evaluation System for Rice. IRRI, 1996)

Table 2. Rice germplasm reaction to Tagosodes orizicolus. (CIAT, II Sem. 1998, I Sem. 1999)

Source	No. of material	C	Classification	*	
	Evaluated	R	ı	S	
FLAR:				,	
Working Collection (WC)	476	121	33	322	
Countries	144	110	5	29	
CIAT – Pre Breeding	827	380	67	380	
FEDEARROZ- Colombia	1149	602	80	467	
FUNDARROZ - Venezuela	94	49	6	39	
ICA – Colombia	117	25	3	89	
CIRAD-CIAT	299	102	52	145	
TOTAL	3106	1389	246	1471	
PERCENTAGE	100	44.7	8.0	47.3	

<sup>\* :</sup> R = Resistant (scale: 1-3); I = Intermediate (5); S = Susceptible (Standard Evaluation System for Rice. IRRI, 1996)

Table 3. Rice lines that were evaluated for resistance to *Tagosodes orizicolus* and rice hoja blanca virus.

	Year *	Total lines	R **	**	S **	Re- evaluation
T. orizicolus	1997	1404	42%	3. 5%	35%	19. 5%
T. orizicolus	1998	3235	63%	7%	28%	2%
T. orizicolus	1999	3106	45%	8%	47%	0%
RHBV	1997	12542	38%	11%	48%	3%
RHBV	1998	14769	47%	20%	33%	0%
RHBV	1999	20952	60%	19%	21%	0%

 <sup>=</sup> For the annual report the year is the second semester of the year before and the first semester of the stated year.

Since 1997, there has been more than a 200% increase in the number of lines evaluated for mechanical damage by *T. orizicolus*. This is during a time when the methods was being evaluated and the result has been increased organization to manage the trials in efficiently despite declining resources. This requires increased coordination with the major users including CIAT, FLAR, Fedearroz, and major national programs (Table 3).

In 1999, (second semester of 1998 y first semester of 1999) there has been an 167% increase in the lines evaluated for RHBV resistance as compared with 1998 (Table 3).

In both types of evaluation, the increased efficiency has resulted in a reduction of the number of lines that needed to be re-evaluated. Of some concern is the decrease in the number of lines that are being rated as intermediate. In the test for mechanical damage, less than 10% of the plants are rated as intermediate. In both tests nearly 50% of the materials are being rated as resistant. Much of the success is due to the emphasis by the breeders to produce lines with both resistance to the planthopper and the virus.

# 1.3.1.1. Influence of cage color and location on mechanical damage caused by *T. orizicolus* Introduction

Plant resistance is an ideal, environment-friendly method for controlling pests that is highly compatible with other control methods.

The development of resistant varieties has proved to be an effective method to control rice pests, especially *T. orizicolus* whose populations have decreased because of restricted feeding. Individuals are, accordingly, less vigorous and have shorter adult longevity.

The assessment of plants in the greenhouse to determine mechanical damage by *T. orizicolus* plays an important role in developing varieties resistant to the insect. Appropriate congruent

<sup>\*\*=</sup>R = Resistant; I = Intermediate; S = Susceptible

evaluation components should be used to ensure stability across evaluations.

Therefore a trial was carried out under greenhouse conditions at CIAT to determine the effect of cage mesh color and location within the greenhouse on the aggressiveness of *T. orizicolus*. This information will be used in future evaluations of rice lines for resistance to *T. orizicolus*.

### Materials and methods

An experiment was conducted during January-February 1999, using current methodology to evaluate mechanical damage caused by *T. orizicolus* (IR-8, susceptible check). A total of 111 rice materials from the rice improvement program were evaluated.

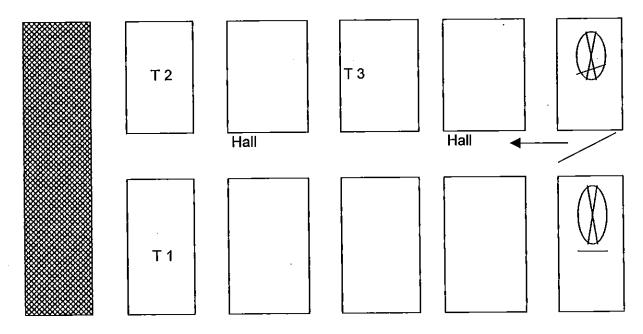
Treatments consisted of:

- T1: Cage with white mesh that is close to the greenhouse water filter.
- T2: Cage with yellow mesh that is close to the greenhouse water filter.
- T3: Cage with yellow mesh that is in the center of the greenhouse.

A hygrothermograph was placed in the center of each cage to determine differences in temperature and humidity.

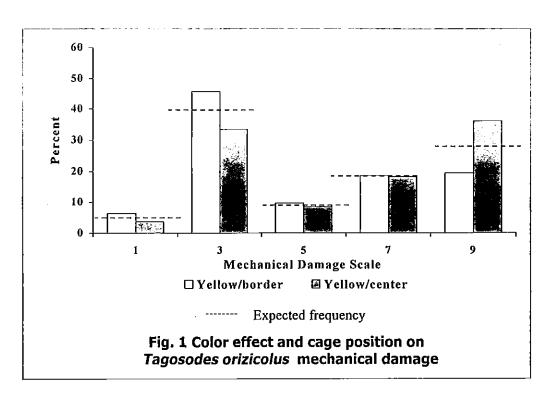
Following to the methodology to evaluate resistance, *T. orizicolus* adults were moved three times during the day. The nymphs were not moved because this can result levels of mortality and affect evaluation results.

The materials planted in the trays were distributed similarly in the cages to ensure the same arrangement. Cages were organized in the greenhouse as follows:



### Results

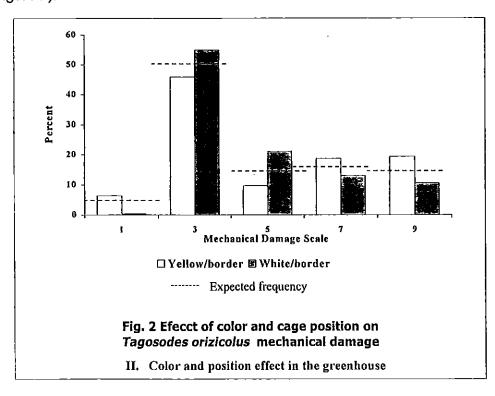
A higher percentage of rice lines in yellow-mesh cages placed in the center of the greenhouse (T3) were classified as 9 (highly susceptible) compared with those located toward the sides of the greenhouse (T1 and T2) (Figure 1).



The differences in the values 1 (highly resistant), 5 (intermediate), and 7 (susceptible) did not vary greatly between the two types of cages or in the form of planting the varieties.

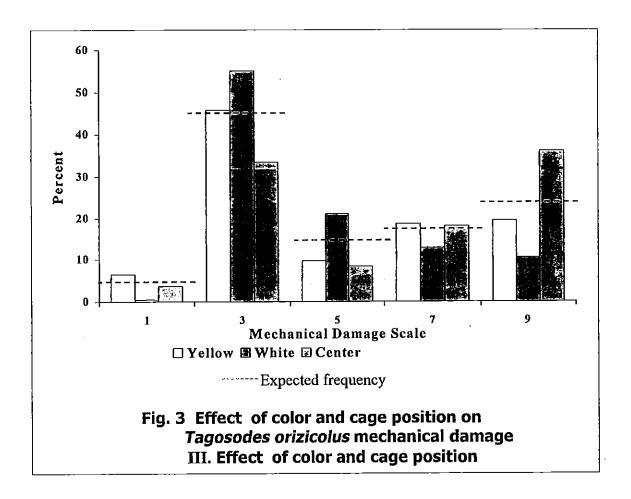
One factor that can influence the feeding reaction of *T. orizicolus* is the luminance. This affects the activity of the insects. The insects should remain longer on the screen in the cages closest to the water cooling system because this is the area of the greenhouse with the highest amount of light.

To determine the effect of color, a comparison was made of the cages with white and yellow screen that were next to the water cooling system. There were only minor differences in the values of mechanical damage, although the insects were slightly more aggressive in the cage with the yellow screen (Figure 2).



The only minor difference between results of the cages can be attributed to the way they were placed within the greenhouse, both at equal distances from the water filter, where light intensity was highest.

When all cages were analyzed together, a significant difference was observed between T3 and the other two treatments, mainly regarding insect aggressiveness. Insects in T3 were 39.03% more aggressive than in T1 and 25.61% more aggressive than in T2 (Figure 3).



A higher percentage of lines classified as resistant (3) and intermediate (5) were found in white-meshed cage compared with yellow-meshed cages.

There was a higher number of resistant lines (56) with a similar evaluation across all three treatments; no line was classified as highly resistant across all treatments (Table 4).

Table 4. Similar values from different classes

Lines with equal evaluation			
Class	Total		
Susceptible Intermediate Resistant	18 (15 with 9; 3 with 7) 2 56 (all with 3)		
TOTAL	76		

No large differences were observed in the temperature and relative humidity recorded in the cages.

#### Conclusions and recommendations

- 1. Marked differences were found in the aggressiveness of *T. orizicolus* in the different cages evaluated (mesh color and cage location).
- 2. A higher number of highly susceptible (9) lines were found in T3 compared with the T1 and T2.
- 3. Evaluations of mechanical damage by *T. orizicolus* should preferably be carried out in yellow cages placed in the center of the greenhouse to minimize the effect of external factors in the final evaluations.
- 4. The color of cage mesh should be standardized in the future to reduce the effect of color on evaluation of mechanical damage by *T. orizicolus*.

A trial, with three replicates, was also conducted to seek greater stability in the mechanical damage reaction of the susceptible check used in the evaluation of resistance to *T. orizicolus*. IR-8 from five different sources–CIAT, Cuba (1, 2, and 3) and IRRI–was used. Check plants from the source Cuba (2) showed greater stability to susceptibility. Therefore we recommend that this source be reproduced and used in all subsequent evaluations of germplasm for resistance to *T. orizicolus*.

## 1.4.2. Intensive field trials for to characterize RHBV resistance

This series of field experiments are designed to put two levels of inoculum pressure of RHBV on the test varieties in a randomized block design using 2 blocks each with 3 repetitions. The plots were 2M² and contain ten lines of rice per plot. In previous years the concentration has been on Colombian varieties and advance lines. This year the most popular commercial varieties in Venezuela were included in the trials. The varieties in the trials were Cimarrón, Cica 8, O. Caribe 8, O. Yacú 9, Selecta 3-20, Oryzica 1, FONAIAP 1, Palmar, Oryzica 3, O. Llanos 5, Araure 4, FEDEARROZ 50, CT10308, CT10192, CT10240 and CT10310. Only the four advanced lines had less than 20% infection in the block with the high level of inoculum pressure (Table 5). In the lower level of inoculum pressure Fedearroz and the four advance lines were below 20% of plants infected with RHBV. The line CT10310 had only 5% of the plants infected with RHBV. The best commercial variety was Fedearroz 50 with 30% of the plants infected with RHBV.

Four Venezuelan varieties were tested and the variety Cimarrón was highly susceptible and RHBV caused very high yield losses in Cimarrón. The high inoculum treatment had less than 1 tons per hectare compared with the control Cimarrón treatment that yielded 13 tons per hectare. This confirms that this variety is highly susceptible and that if a severe epidemic occurs that there is the potential for large yield losses.

This experiment is has been repeated several times and each time additional varieties are tested. In the next experiment commercial varieties from Peru will be included. As more commercial varieties are tested, this information is being incorporated into the control recommendations. Additionally each experiment includes advanced lines. Most of these lines have resistance that is better than the best commercial variety. This means that there is the potential to continue to improve the next generation of commercial lines. The next line scheduled for release in Venezuela is one with a very high level of RHBV and *T. orizicolus* resistance. As long as breeding for RHBV remains a priority, there is the potential to continue the release of enough high yield RHBV resistant lines and this should end the cycles of devastating RHBV epidemics.

Table 5. Evaluation of rice varieties and lines for hoja blanca virus during the second semester of 1998.

				Diseas	e pres	sure	-		
	High	level <sup>1</sup>		Low level <sup>1</sup>			Control		
Variety	RHBV	Yie	ld	RHBV	Yie	eld	RHBV	Yie	eld
·	% <sup>2</sup>	t/h	a	%	t/h	ıa	%	t/i	าล
Cimarrón	85.0	0.9	h <sup>3</sup>	69.3	4.2	h	2.8	13.0	ab
Cica 8	84.2	3.2	g	76.3	5.9	g	1.7	10.6	dc
O. Caribe 8 🔥	81.7	4.2	g	81.7	6.7	g	2.2	12.9	ab
O. Yacú 9	72.7	6.0	f	47.0	9.4	ef	0.6	12.8	ab
Selecta 3-20	72.0	7.8	de	39.6	10.9	cde	1.5	12.7	ab
Oryzica 1	69.1	7.0	df	34.6	11.0	cd	0.7	12.7	ab
Fonaiap 1	65.3	6.0	f	36.7	9.1	f	0.7	10.1	е
Palmar	60.1	8.4	cd	24.1	11.9	bc	0.6	13.2	, a
Oryzica 3	54.8	9.1	bc	27.2	11.6	cd	0.4	13.7	а
O. Llanos 5	52.5	9.3	bc	29.7	10.6	cde	1.0	10.8	de
Araure 4	50.2	8.2	cde	21.3	10.3	def	0.9	11.1	cde
Fedearroz 50	30.3	10.3	b	13.5	12.0	abc	0.5	11.5	bcde
CT10308	18.3	11.6	а	8.8	13.1	ab	1.0	13.4	а
CT10192	10.8	10.3	b	5.3	11.3	cd	0.3	11.5	bcde
CT10240	9.8	10.1	b	5.5	11.3	cd	0.1	11.7	bcd
CT10310	5.5	11.8	а	5.0	13.4	а	0.0	12.4	abc

<sup>&</sup>lt;sup>1</sup> The infestation was approximately 3, 1 y 0 insects/plant.

<sup>&</sup>lt;sup>2</sup> Visual scale transformed to percentage.

<sup>&</sup>lt;sup>3</sup> Averages with the same letter show no significant differences at the 5% level in using Duncan.

1.5. Comparison of field methods using natural pressure to determine varietal eactions to RHBV

### Introduction

In addition to the intensive field screening for RHBV, field testing using natural pressure was done. Most years this type of evaluation is not reliable because the incidence of RHBV is not high enough. Because of moderate levels of RHBV in Saldaña Tolima, the opportunity was used to compare methods of field evaluation.

#### **Materials and Methods**

Three methods of evaluation were used in this study. The RHBV visual scale rating each line using the standard evaluation scale of 0 a 9 (Standard scale method). The second method was using a square (0.25 x 0.25m), and counting all of the tillers and rating them as either healthy or infected and calculating the percentage of infected tillers (Square method). The third method is counting the total number of plants and those infected with RHBV in rows of 2 meters (Row method). The evaluations for RHBV were made at 40 and 60 days after the emergence of the crop. This experiment was at Saldaña, Tolima, in the FEDEARROZ experiment station Granja Las Lagunas. Twenty rice genotypes were evaluated for their reaction to RHBV. The experiment was done using a randomized block design using 2 blocks and 3 repetitions per block. The plots were 2M² and contain ten lines of rice per plot. The experiment was harvested by plots and the yield data was determined.

### Results

Using the results of the analysis of variance there were difference between the methods in rating varietal reactions to RHBV (Table 6). The number of active vectors of RHBV was determined to be 5.5% and the population of 14 planthoppers in 10 double passes of the entomological net in the Las Lagunas experiment station at the beginning of the experiment in October 1998.

1. The inoculum pressure was not as high as the field experiments at CIAT were known vectors are place in the field to ensure high inoculum pressure.. The incidence of RHBV in the susceptible control was: Square method 6.27, Rating scale (0-9); Rating scale (%) 2.60; 17.5%, and Rows (Plants/row) 4.85 (table 6). Using the standard Rating scale materials are classified as resistant with a rating between 1-3; intermediate with a rating of 4-6 and susceptible with 7-9. Since the susceptible control would be rated as intermediate in the experiment, the values must be considered to be relative and were analyzed for comparative difference. The lower RHBV pressure under natural conditions is one of the reasons that this type of evaluation is not recommended as the only evaluation for determining RHBV resistance.

Table 6. Comparisons of methodologies for their reaction to RHBV Saldaña 1998 B (Average of six replications).

Variety	Known reaction	Square <sup>1</sup> (%)	Scale <sup>2</sup> (0-9)	Scale <sup>3</sup> (%)	Row⁴	Yield. (t/ha)
FB0100-10-1-M-1	I	1.98 CDEF	0.98 FGHI	5.05 FG	1.83 DE	7.5 A
CT10240-10-1-2-1	R	0.87 EFG	0.61 GHI	3.16 GH	0.33 F	6.8 AB
CT10323-29-4-1-1	R	0.50 G	0.65 GHI	3.23 GH	0.28 F	6.2 ABC
CT9509-28-3-3P-M	R	0.64 FG	0.33 I	1.75 H	0.40 F	6.1 BC
TAILANDIA 1	S	3.54 ABCD	1.47 EFG	8.62 DEF	3.55 BC	5.8 BC
T10192-2-2T-1-1	1	0.66 G	0.51 HI	2.58 GH	0.48 F	5.7 BCD
ORYZICA 1	. 1	2.33 CDE	1.35 EFG	7.72 DEF	2.45 CD	5.7 BCD
CT1310-15-3-2P	R	1.34 EFG	0.54 HI	2.69 GH	0.73 EF	5.5 BCD
LINEA 2	S	2.25 CDEF	1.79 DEF	10.9 CDE	4.41 B	5.5 BCD
CT10308-27-3-3P	R	4.37 ABC	1.43 EFG	8.30 DEF	2.55 CD	5.3 CD
CT 9509-17-7-1P-1	R	0.17G	0.331	1.63 H	0.47 F	5.2 CD
LINEA 150	T	3.85 ABC	1.09	5.72 EFG	2.40 CD	5.1 CD
SELECTA 3-20	1	3.78 ABC	1.91 CDE	1.19 CD	4.18 B	5.0 CDE
CICA 8	S	6.27 AB	2.60 BC	17.5 BC	4.85 B	5.0 CDE
FEDEARROZ 50	R	1.32 DEFG	1.01 FGHI	5.40 FG	1.65 ED	5.0 CDE
O YACU 9	S	4.54 ABC	2.53 BCD	17.0 BC	3.63 BC	4.9 CDE
ORYZICA 3	1	2.62 BCDE	1.09	6.02 EFG	1.54 ED	4.9 CDE
O Llanos 5	R	7.78 A	3.65 A	26.8 A	7.22 A	4.3 DE
O CARIBE 8	S	6.94 A	3.28 AB	24.0 AB	5.66 AB	3.7 EF
COLOMBIA 1	R	2.57 CDEF	0.26	1.34 H	0.97 EF	2.6 F
SSE		0.14	8.62	0.14	0.71	19.66
GLE		40	40	40	40	38
CV		42.01	33.82	22.45	9.47	13.4

<sup>&</sup>lt;sup>1</sup> Number of disease tillers with RHBV/Total tillers. Arcoseno transformation

<sup>&</sup>lt;sup>2</sup> Visual scale (0-9) 0= 0%; 1= 1-10%; 3= 11-30%; 5= 31-50%; 7= 51-70%; 9= 71-100%.

<sup>&</sup>lt;sup>3</sup> Visual scale transformed to percentage. Arcoseno transformation

<sup>&</sup>lt;sup>4</sup> Number of plants with RHBV / row

- 1. The yields varied between 2.6 and 7.5 t/ha (Table 6) and were not correlated to the levels of RHBV. This is not surprising since the incidence of RHBV was relatively low. Also because this was a field experiment under natural inoculum pressure, there was not a "healthy" control of each variety. Healthy controls are needed to make accurate yield loss estimates.
- 2. The evaluation using the "Square" resulted in the following evaluation. Resistant: CT10240, CT10323, CT9509-28, CT10310, CT9509-17, Fedearroz 50; Intermediate: Oryzica 1, Oryzica 3, Línea 2, FB0100; Susceptible: Tailandia 1, Cica 8, Oryzica Yacú 9, Oryzica Caribe 8. Because of the lower inoculum pressure the were inconsistency between the known reactions and those found in this experiment. For example, CT10192 was classified as "R" and the known reactions is "I"; Línea 150 was rated "S" and the know reaction is "I" and Oryzica Llanos 5 was classified as "S" and the known reaction is "R". Even though the pressure is not so high, the result with O. Llanos 5 does fit with the field observations that it is one of the most susceptible varieties. This is because it has very good resistance to the virus but is highly susceptible to the planthopper. Natural infections even with moderate pressure do appear to complement the "controlled field experiments".
- 3. Using the rating system of "Rows" the evaluation was more similar to the known reactions. The following varieties were rated as Resistant: CT10240, CT9509-28, CT10323, CT10192, CT9509-17, CT10310, Colombia 1; Intermediate: Fedearroz 50, Oryzica 1, Línea 150, Oryzica 3, FB0100, CT10308 and Susceptible: Oryzica Caribe 8, Cica 8, Línea 2, Tailandia 1, Oryzica Yacú 9, Selecta 3-20 and O. Llanos 5. The variety Oryzica Llanos 5 was again classified as "S" and Fedearroz 50 was rated as "I". The line CT10308 was rated as "I", and Selecta 3-20 was rated "S". All of these varieties performed worst under the natural conditions than in the CIAT controlled experiments. Both Selecta 3-20 and Fedearroz 50 have proven themselves as resistant varieties under a wide range of growing conditions. Nevertheless, this new information should enable the development of varieties with even better field resistance to RHBV.

All of the correlations between the methods were significant (Tables 7 y 8) and indicate that results are consistent especially at 40 days after emergence. The method that is least reliable is the "Square".

Table 7. Correlation between methodologies to rate RHBV resistance in the field (40 days after sowing)

Method	RHBV Square	RHBV scale (0-9)	RHBV Scale (%)
RHBV scale (0-9)	0.70 **		
RHBV scale (%)	0.69**	0.99**	
RHBV row	0.73 **	0.92**	0.92**

<sup>\*.\*</sup> Significant at the 1% level

Table 8. Correlation between methodologies to rate RHBV resistance in the field (60 days after sowing)

Method	RHBV Square	RHBV scale (0-9)	RHBV scale (%)
RHBV scale (0-9)	0.62**		
RHBV scale (%)	0.61**	0.99**	
RHBV row	0.62**	0.89**	0.87**

<sup>\*.\*</sup> Significant at the 1% level

### Conclusions

- 1. O. Llanos 5 is classified as a susceptible variety and this corresponds with the farmers perception of this variety. This demonstrates the utility of different types of evaluations and confirms that RHBV resistant varieties must be resistant to both the vector and the virus.
- 2. The genotype CT10192-2-2T-1-1 classified as "I" was "R" in this test.
- 3. The methods of Scale, Rows and Square classified 70% of the varieties according to their known reaction. Those that appeared more susceptible in the field conditions should be considered as more susceptible and this adds a new dimension to the rating system.
- 4. Due to the low level of RHBV, the yield data was not useful.
- 5. The Row method was the most consistent with the expected results. Nevertheless despite some statistical problems in the Standard Scale method, it has proven reliable in the massive selection of lines and in more intensive evaluations. This is partially because it is the fastest method of evaluation. The Square is the most labor intensive and least reliable of the methods tested.

### Recommendations

This type of field evaluation under natural conditions and with moderate pressure evidently changes the emphasis on some of the components of resistance. Therefore this method is a valuable supplement to the current field evaluation done at CIAT. The major problem is finding a suitable site with sufficient level of natural population of active vectors of RHBV.

- 1.6. Studies of plant resistance to T. orizicolus.
- 1.6.1. Evaluation of mechanisms to *T. orizicolus* in advanced lines and commercial varieties.
- 1.6.1.1. Test of Antixenosis:

To determine the mechanism of resistance three types of experiments were designed. These included test for settling preference, oviposition preference and antibiosis. For the preference tests rice plants of he varieties IR 8 (susceptible check), Makalioka (antibiosis resistant check), Llanos 5 and Fedearroz 50 were transplanted in a circle using a random design. Approximately 200 female and 50 male adult *T. orizicolus* were place in the center of the circle of plants and released. The number of insects on each plant was counted at 24h, 48h, 72h, 96h, and 120. The number of eggs

was evaluated after 120h. The order of settling preference was IR8, Llanos 5, Fedearroz 50 and Makalioka (table 9). This same order of preference held for the number of eggs per variety (table 10).

Table 9. Settling preference of Tagosodes orizicolus using four commercial varieties of rice.

<u>-</u>	1	~	Time	( Hours)	_		
Variety	24	48	72	96	120	Mean	
,		Percentage of insects on each variety					
IR-8	52.15	53.23	53.14	51.74	46.89	51.43 a	
O.LL. 5	22.85	29.50	30.89	31.88	32.63	29.39 b	
FB- 50	13.91	10.16	9.28	10.76	13.02	11.42 c	
Makalioka	11.10	7.17	6.69	6.41	7.41	7.75 d	

Table 10. Number of eggs oviposited in a preferential manner on four varieties of rice.

Variety	Mean number of eggs
IR-8	344.0 201.20
Oryzica Llanos 5 Fedearroz 50	161.76
Makalioka	157.48

### 1.6.1.2. Tests of antibiosis:

### Oviposition after force feeding:

The oviposition was also determine for the four varieties but the planthopper was force fed on each variety. For the no choice oviposition experiment individual insects were placed on 30 plants of each variety at 20 days after planting. The plants were grown in acetate tubes and were infested with young adult pairs of planthoppers. The insects were transferred to new plants every five days until the death of the female. The leaves of the plants were evaluated for the number of eggs. The tendency was similar for preference with IR 8 having the greatest number of eggs followed by O. Llanos 5, Makalioka and Fedearroz 50 (table 11). Makalioka is reported to be antibiotic and was used as the control. It appears using the criteria of mean number of eggs oviposited after forced feeding that Fedearroz 50 is more inhibitory than Makalioka.

Table 11. Total mean number of eggs oviposited by *Tagosodes orizicolus* on four varieties of rice in force feeding experiments.

Variety>	Mean number of eggs oviposited		
IR - 8	1302		
Oryzica Llanos 5	1109		
Fedearroz 50	229		
Makalioka	539		

### **Emergence of nymphs:**

This experiment was conducted as a no choice or force feeding to determine the effect of monocropping on the mean number of eggs oviposited with the difference that the plants were kept for 15 days after the removal of the adult pair to assure the total eclotion. Again the same tendency occurred but the number of nymphs was much higher on IR-8 compared with O. Llanos 5 (table 12). The relation between total number of eggs and emergence nymphs in Makalioka and Fedearroz 50 are similar. In both cases, Fedearroz 50 had few eggs laid on it and the emergence of the nymphs was much less as compared to Makalioka. This data with field population on Fedearroz 50 indicate the variety probably is antibiotic. The stability of the inhibitory effect on the planthopper needs to be determined.

Table 12. Emergence of nymph of Tagosodes orizicolus after forced feeding on four varieties.

Mean number of accumulated nymphs		
4241 1352 172 629		

1.7. Insecticide trials to determine the effect of insecticides on the *Tagosodes orizicolus* and it's natural enemies.

### 1.7.1. Evaluation of insecticides in rice fields

The application of chemical insecticides is one of the most controversial phytosanitary activities. Insecticides have been and will continue to be indispensable in the control of plant pests. In many integrated pest management (IPM) programs, they play an important role and are very cost-effective. However, their use is also associated with many negative secondary factors. Therefore if chemical insecticides must be applied, farmers should use the most selective ones that cause the least disturbance to the rice agroecosystem.

Outbreaks of RHBV are cyclic therefore crop management must include measures aimed at preventing epidemics. For example, the disease incidence should be monitored in rice-growing areas and IPM applied (planting of resistant varieties, rational use of certain insecticides, and crop protection through natural enemies of the pest). The most selective insecticides should be identified because even resistant varieties are susceptible to the virus the first month of the crop.

One of the reasons why farmers apply insecticides without determining product selectivity or using appropriate application rates is because they are afraid of the risks involved in rice production. In Colombia where RHBV incidence is moderately high in susceptible varieties and there are virulent populations of the planthopper vector, insecticides are applied to control RHBV even when the farmer is growing rice varieties that are resistant to both the insect and to the virus.

The effectiveness of insecticides recommended to farmers should be determined so that farmers can be informed about those insecticides (both chemical and biological properties) that provide best pest control while preserving natural enemies of the pest. Those insecticides found most effective should be included in the IPM strategy.

Insecticide trials were conducted with the following objectives:

- 1. To evaluate the effectiveness of applying insecticides to control *T. orizicolus* and prevent losses due to RHBV.
- 2. To determine the effect of applying insecticides on the interaction of *T. orizicolus* with beneficial fauna, especially parasitoids and predators.

### Materials and methods

The experiments on the control, monitoring and levels of *T. orizicolus* were done in the 'Rojas' lot, located in the municipality of Jamundi (Valle del Cauca, Colombia) at an altitude of 1080 m above sea level. Average monthly precipitation in the area is 161 mm, with an average relative humidity of 75% and approximately165 hours of sunlight per month. Before insecticide applications, *T. orizicolus* populations in the area were relatively high (173 insects per ten double passes of entomological net) and the virulence of the insects significant (7.7% of the insects were active vectors of RHBV).

The variety Oryzica Caribe 8, which is susceptible to RVHB, was used in the experiment with ten insecticides and an untreated control. A randomized complete block design was used, with  $300m^2$  plots and three replicates per treatment. Populations of adults and nymphs of *T. orizicolus*; adult *T. cubanus*; and predators and parasitoids were evaluated before the insecticides were applied, 48 and 72 hours after their application, and then at 7-day intervals until 70 days after crop emergence. The insect predators monitored included spiders and coccinellids. The collections were made with an entomological net using 10 double passes per plot (10 dpn) and per treatment.

Pre-germinated seed of variety Oryzica Caribe 8 was planted. Fertilization, weed control, and harvest were done according by the farmer using his management practices.

Foliar insecticides and entomopathogenic fungi (Table 13) were applied at commercial rates, about 15 days after plant emergence, using a knapsack sprayer with a hollow cone nozzle, and at constant pressure.

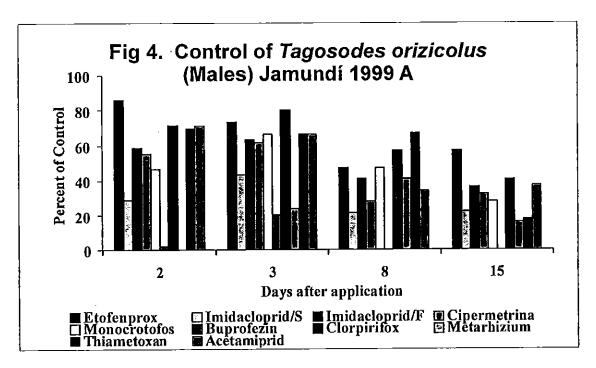
Table 13. Insecticides applied to Tagosodes orizicolus

Imidacloprid C	C-H-O Cloronicotynil	10 EW 350 SC	Trebon	700 ml/ha	Foliar
	_	350 SC	1		
	_		Confidor	100 ml/ha	Foliar
Imidacloprid C	Cloronicotynil	600 FS	Gaucho	50g/ia100kg	Seed
Cipermetrina P	Piretroide	200 EC	Insectrina	0.5 l/ha	Foliar
Monocrotofos C	Organofosforado	600 EC	Azodrin	1 l/ha	Foliar
Buprofezin I.	.C.S. <sup>1</sup>	25 SC	Oportunec	0.5 l/ha	Foliar
Clorpirifox	Organofosforado	4 EC	Vexter	1 l/ha	Foliar
Metarhizium B	Bioplaguicida	25 g	Destruxin	1x10 <sup>10</sup>	Foliar
Thiametoxan N	Nitroguanidina	25 WG	Actara	100 g/ha	Foliar
Acetamiprid C	Cloronicotinyl	20 SP	Rescate	150 g/ha	Foliar
Check without ch	nemical treatment	t	L		

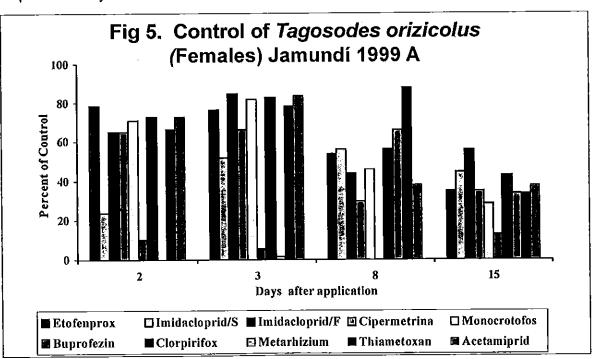
<sup>&</sup>lt;sup>1</sup> = I.C.S. = Inhibitor chitin synthesis.

### Results

A highly significant difference was found among treatments regarding the control of *T. orizicolus* males. The evaluations indicate that Thiametoxan is the most efficient insecticide for *T. orizicolus* males, followed by monocrotophos; etofenprox; cypermethrin; and imidacloprid (foliar) (Figure 4).



Eight days after applying the insecticides, a higher number of male insects were found in the buprofezin-treated plot than in the check, indicating a -145% increase in insect population. From the first evaluation, this insecticide showed very limited control of *T. orizicolus*. Fifteen days after applying the insecticides, only etofenprox showed over 50% control. These data indicate that the control performed by the insecticides tested decreased considerably after 15 days.



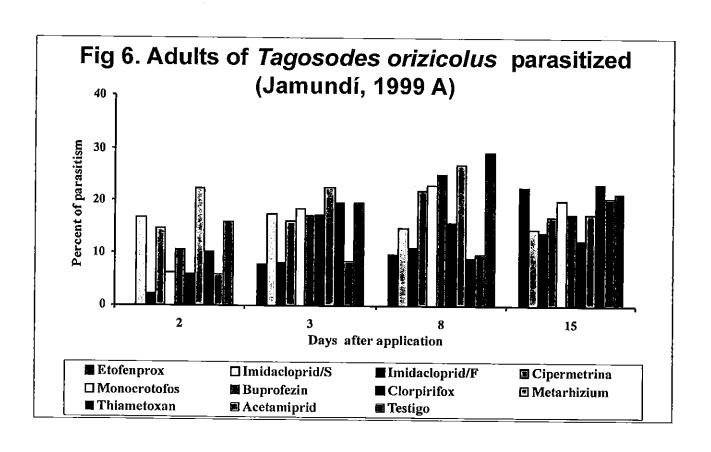
A highly significant difference was also recorded among treatments for female *T. orizicolus*. The lowest control of females was registered with *M. anisopliae*, and the highest with thiametoxan, as with males (Figure 5). Three days after application, imidacloprid (foliar), acetamiprid, monocrotophos, and chlorpyrifos presented over 80% of female insects.

The same trend in control was observed for both males and females of this insect. Buprofezin became ineffective 8 days after its application, and only imidacloprid (foliar) presented more than 50% control at 15 days.

The quick control of female *T. orizicolus* plays an important role in the integrated management of the rice planthopper-'Hoja Blanca' complex because females can transmit the virus transovarianly. Therefore products presenting good control of female insects should give the crop better protection, increasing yields and lowering production costs.

When analyzing the control of both female and male *T. orizicolus*, 60% control was obtained 2 days after applying the insecticides, with etofenprox presenting 82% control. The exceptions were imidacloprid (S), buprofezin, and *Metarhizium*, which presented poor pest control when compared with the untreated check. thiametoxan presented 83% control, 8 days after application.

Among the factors that most affect the reduction of *T. orizicolus* populations is the presence of natural enemies, both predators and parasitoids. That is why it is important to apply insecticides that do not affect natural enemies, while regulating insect pest populations. Given the characteristics of the biological insecticide *Metarhizium*, 22% parasitism was reached 2-3 days after application, although the control of adult *T. orizicolus* was low. This result is normal for this type of insecticide, which usually begin to affect the insects 4 days after application (Figure 6).



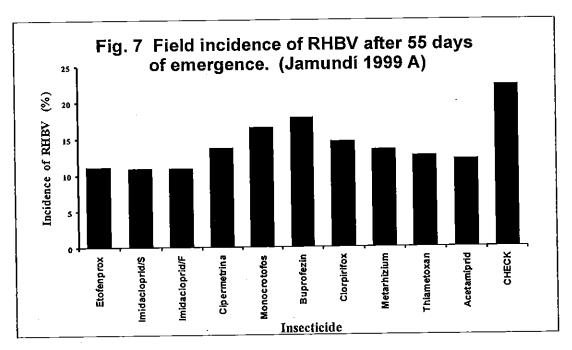
In this first evaluation, etofenprox showed more than 80% control of female and male *T. orizicolus* as well as 17% of parasitism, the highest values among chemical treatments tested and even higher than the untreated check.

Three days after insecticide application, the percentage of parasitism increased in most chemically-treated plots. Arachnids play an important role in reducing insects in rice fields. That's why it's so important that the insecticides applied to control pests affect these biological controls as little as possible.

In all evaluations in which the spider count was done during the crop cycle, populations of these arthropods did not differ significantly between insecticide treatments and the untreated check. Values fluctuated between 5 to 25 spiders per 10 double passes of the entomological net.

All treatments differed from the untreated check (23%), except Oportune (buprofezin), which did not control *T. orizicolus*. This difference, however, was not statistically significant, indicating that the incidence of RHBV can be reduced, depending on the percentage of virulence present in the rice lot and the timing of applications of chemical insecticides. Rice lots should therefore be monitored from the first days after plantlet emergence to determine, from the very onset of *T. orizicolus* infestation, the number of adults present in the lot. This way better IPM measures can be applied (Figure 7).

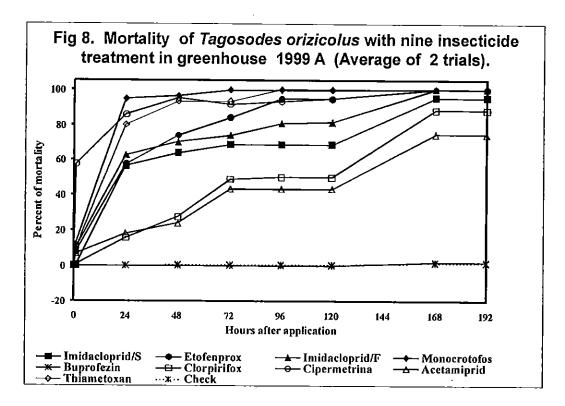
There was no significant difference in yields among treatments.



### Conclusions and recommendations

- 1. Thiametoxan and etofenprox presented better control of *T. orizicolus*, although all insecticides, except imidacloprid (S), buprofezin, and *Metarhizium*, showed more than 60% control of adult *T. orizicolus* three days after their application.
- 2. Given the characteristics of buprofezin, it should not be applied as soon as *T. orizicolus* appears because this infestation could be due to migration of adults from neighboring fields.

3. *Metarhizium* takes longer to control the pest. Therefore it should not be applied when a high percentage of virulence of *T. orizicolus* is detected, because the insect will transmit RHBV before the bio-insecticide can act.



### 1.7.2. Evaluating insecticides under greenhouse conditions

In the search for other IPM alternatives, pesticides with new, broad-spectrum active ingredients are being introduced as part of crop protection strategies. Because pesticide application currently plays a fundamental role in rural health and economics, they should also be environment-friendly and contribute to sustainable agriculture.

Adult *T. orizicolus* used were obtained from the colony reared at CIAT (source insects were from the Jamundi region). Pots 10 cm in diameter was used. 3 plants of rice variety Bluebonnet-50 were planted in each. The same insecticides used in the field trial in Jamundi ('Rojas' rice lot) were applied at the same rates, 25 days after germination.

Immediately after applying the insecticides, 20 adults (both female and male) of *T. orizicolus* were placed in each pot and covered with an acetate tube. Four replicates were done per treatment. Evaluation of adult mortality began 3 hours after insecticide application, and was repeated until insect mortality stabilized.

### Results

In the evaluation carried out 3 hours after of insecticide application, cypermethrin achieved 57.5% control of *T. orizicolus*, a characteristic of pyrethroid insecticides, reaching 95% control 129 hours after application (Figure 8).

Monocrotophos, thiametoxan, and cypermethrin achieved 80% control of adult *T. orizicolus* 24 hours after application. At the end of the evaluations, cypermethrin, imidacloprid (F), ethophenprox,

thiametoxan, and monocrotophos achieved 100% control.

Buprofezin, recommended basically against *T. orizicolus* nymphs, achieved 2% control of adults, a result that collaborates indications given for use of this chemical. Therefore it should not be applied during early infestations of adult *T. orizicolus* (Figure 5).

The calculation of LT-95 by this statistical method indicates that, for several insecticides that never reached 95% control, it is higher than the 192 hours that the evaluations lasted. In addition, it is very probable that these control values can be reached in view of the insect's biological characteristics and the nature of the chemical.

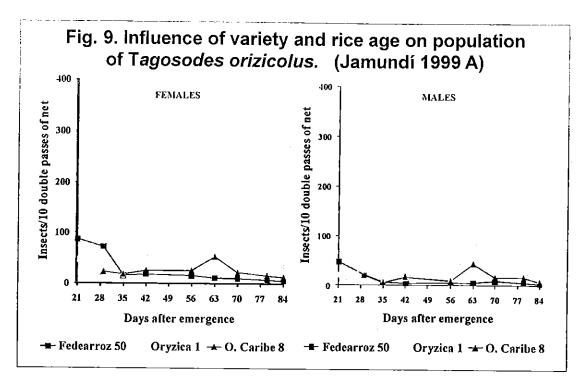
When the vector of RHBV (*T. orizicolus*) is infected by transovarically, it is capable to transmitting the disease from the beginning of its life cycle, and the time an insecticide takes to kill the pest plays an important role in the control of RHBV (Table 14). This should be taken into account when evaluating LT-95 in the cases of thiametoxan (58.3 hours) and monocrotophos (28.2 hours).

Although certain insecticides, mainly pyrethroids and organophosphorus compounds, only take only a short time to show high control values, they adversely affect beneficial insects and cause environmental pollution. Therefore their use should be limited to those occasions in which chemical control of *T. orizicolus* is needed.

Table 14. Influence of insecticide application on Tagosodes orizicolus lethal time

Insecticide	Lethal Time LT 50	(LT) (Hours) LT95	
Etophenprox	24.5	104.3	
lmidacloprid (S)	19.4	527.2 *	
Imidacloprid (F)	29.1	221.6 *	
Cipermetrina	18.4	162.4	
Monocrotofos	3.5	28.2	
Buprofezin	No control		
Clorpirifox	80.5	388.9 *	
Thiametoxan	11.1	58.3	
Acetamiprid	115.4	642.5 *	

<sup>\*</sup> During the evaluation of 192 hours never was reached 95 % of control. Hours are only theoretical data.



### Conclusions

- 1. Insecticides that best controlled adult *T. orizicolus* in this test were thiametoxan cypermethrin, imidacloprid (F), etofenprox, and monocrotophos.
- 2. Buprofezin did not control the adult insects; therefore it should not be applied to reduce *T. orizicolus* populations.

# 1.8. Population fluctuation in *Tagosodes orizicolus* in three rice varieties Introduction

The insect *T. orizicolus* is one of the constraints faced by rice crops in Colombia, causing mechanical damage to the crop and transmitting the 'hoja blanca' virus. Insect control is usually achieved by applying insecticides and, in most cases, varietal resistance is not taken into account. This study aims to quantify *T. orizicolus* populations and the pest's natural enemies in three rice varieties, planted in commercial fields, without insecticide application.

### Methodology

The trial was carried out on the Valparaiso farm, located in the rice-growing area of Jamundí (Valle del Cauca, Colombia) from February to June 1999. A total of 130 kg of pre-germinated seed of varieties FEDEARROZ 50, Oryzica 1, and Oryzica Caribe 8 was planted per hectare. Soil preparation, weed control, and fertilization were performed according to local technology. Insects were collected starting 21 days after planting and then at 8-day intervals until 84 days after planting. Three samples were taken per variety per visit; each sampling consisted of 10 double passes of entomological net.

Incidence of RHBV in each variety was determined 55 days after crop emergence.

Plots were left to mature until harvest and yields were determined. Data on insect populations, RHBV incidence, and percentage of parasitism were submitted to variance analysis and multiple comparison tests.

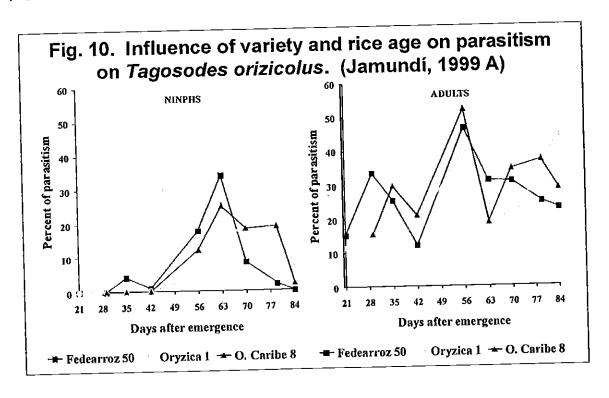
### Results

# 1. Population fluctuation

In the evaluation performed 21 days after crop emergence (DCE), the highest female and male population of *T. orizicolus* was found in the lot planted to variety FEDEARROZ 50. After that date, insect population declined considerably and, at 35 DCE, practically no adults were collected in this variety. In variety Oryzica 1, however, female and male pest populations were high throughout the crop cycle (Figure 9). Across all varieties, the highest female and male populations of *T. orizicolus* were found at 63 DCE. Once rice plants flowered, insect pest populations decreased considerably (Figure 9).

T. orizicolus populations found in variety Oryzica 1 differed significantly from those found in the other two varieties, reaching 350 females and 96 males per 10 double passes of the net. Variety FEDEARROZ 50 presented the lowest number of insects throughout the crop cycle. In general, the female population was three times higher than the male population. The high population of the rice planthopper recorded in Oryzica 1 indicates that the insect preferred this variety as compared to Oryzica Caribe 8 or FEDEARROZ 50. The high population could also be caused by the migration of T. orizicolus from adjacent lots.

Parasitism in adult *T. orizicolus* ranged from 11% to over 50% for the three varieties (Figure 4). Although variety FEDEARROZ 50 presented the lowest adult population of *T. orizicolus* at 28 DCE, it recorded 34% parasitism, reaching a maximum of 46% at the highest level of pest incidence. Contrary to FEDEARROZ 50, variety Oryzica 1 showed the lowest percentage of parasitism up to 77 DCE (from 11% to 33%). This result appears to be related to the low spider population recorded in these lots, which influenced the high population of *T. orizicolus* feeding on the variety. The same trend was observed for nymph parasitism in all three varieties. Insect parasitism surpassed 30% in FEDEARROZ 50, whereas the number of parasitized insects collected in Oryzica 1 was lower. (Figure 10)



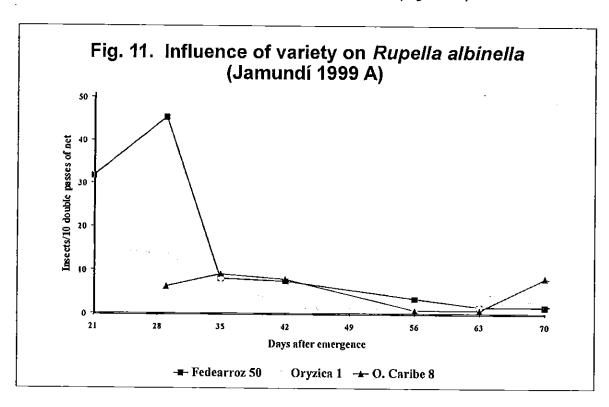
As indicated before, spiders play a decisive role in reducing insects in rice lots and an effort should be made to preserve them when applying different control measures, for example, broad-spectrum insecticides.

From the beginning of the evaluations up to 56 DCE, the lowest number of spiders was found in variety Oryzica 1, but this variety also presented one of the highest populations of T. orizicolus, indicating the close relationship between spider and pest populations.

The number of spiders collected per 10 double passes of the net ranged between 9, at the beginning of the evaluations, and more than 30 at 63 DCE, which coincided with the highest *T. orizicolus* population. Although the number of spiders collected in variety FEDEARROZ 50 was intermediate compared with the other two varieties, its spider population showed the greatest stability throughout the evaluation cycle (ranging between 9 and 19 individuals per 10 double passes of the net).

Although *Rupela albinella* (Cramer) (Lepidoptera:Pyralidae) can be found in rice throughout the entire crop cycle, it did not cause economic damage to the crop. Large numbers of adults are commonly observed on the plants, most of its eggs being parasitized. Thirty-one adult *R. albinella* were collected per 10 double passes of the net in variety FEDEARROZ 50, at 21 DCE; this number increased to 46 adults at 28 DCE.

These values are higher than those observed for the other two varieties, possibly because the insect prefers the much more intense green color of the leaves of FEDEARROZ 50. These values later decreased to 5 individuals/10 double passes of the net (Figure 11).



The reduction of adult *R. albinella* occurred in all test varieties because of the high percentage of parasitism of insect eggs, which increases because not all the rice lots received the application of chemical insecticides or because adults tend to fly towards other rice fields. The former is usually the case, especially when the natural enemies of the pest are protected thanks to adequate IPM.

### 2. Incidence of RHBV

Significant differences were found in the incidence of RHBV infected plants. Variety Oryzica Caribe 8 presented the highest incidence and the lowest yield, corroborating the variety's high susceptibility to RHBV. Variety FEDEARROZ 50 presented the lowest percentage of diseased plants and the highest yield, possible because of its resistance to RHBV. Oryzica 1's performance was intermediate (Table 15).

Table 15. Effect of RHBV in three rice varieties. (Jamundí, 1999 A)

Variety	T. orizicolus. Virulence (%) <sup>1</sup>	RHBV incidence	<i>T. orizicolus</i> en 10 dpn <sup>3</sup>	Yield (t/ha)⁴
Oryzica Caribe 8	7.7	16.5 a	21	4.5
Oryzica 1	6.6	7.6 b	27	5.0
Fedearroz 50	5.3	2.9 b	32	7.5
l	<u></u>	<u> </u>		<u> </u>

- 1 = ELISA Technique (90 insects/variety)
- 2 = Square Method (Average of 3 throws)
- 3 = T. orizicolus in 10 double passes of net
- 4 = Date obtained from farmers

Table 1 shows that, regardless of the fact that FEDEARROZ 50 showed the highest incidence of *T. orizicolus* at the beginning of the evaluations, pest population decreased considerably as the crop matured and the population of natural enemies increased. Therefore, regardless of the variety's genetic characteristics, the low incidence of insects also favors high crop yields.

# Conclusions and recommendations

- 1. Plants of variety FEDEARROZ 50 presented the lowest incidence (%) of RHBV and also the lowest level of active vectors of *T. orizicolus*.
- Given the characteristics of variety FEDEARROZ 50, the applications of chemical insecticides
  to control *T. orizicolus* should be limited during the crop cycle, except when the pest occurs the
  first days after seedling emergence.

#### 1.8. Epidemiology for RHBV

## 1.8.1. Surveys of RHBV incidence in rice and T. orizicolus.

The monitoring of Colombia for RHBV incidence and the analysis of viruliferous T. orizicolus population continued into the fourth year. Over 350 samples have been collected and analyzed The greatest change has been the increased incidence in the Department Valle. It is now at a high level of risk for RHBV. Other areas that are at high risk are in Villavieja Huila, Purificacion & Saldaña Tolima and Nechi. The levels of RHBV are decreasing in the northern areas of Tolima and throughout the Llanos. With the increase use of RHBV resistant varieties, the trend towards decreased levels of RHBV is expected to continue. (Table 16)

Table 16. Rating rice-growing region in Colombia for the risk of epidemics of RHBV.

Zone	RHBV risk level <sup>3</sup>	Zone	RHBV risk level
Llanos		Tolima	
Ariari	L	Saldaña	Н
V/cio	L	Espinal	М
Castilla	L	Purificación	Н
Yopal	L	Ibague	L
		Venadillo	. L
<u>Huila</u>		Lérida	L
C. Alegre	L	Alvarado	L
Villavieja	Н		<u> </u>
Palermo	М	Caribe húmedo	
Tello	L	Nechi	Н
<u>Valle</u>		Caribe seco	
Jamundí	Н	Cúcuta	L
		V/upar	L

<sup>&</sup>lt;sup>1</sup>L = Low; M = Medium; H = High

### 1.8.2. Farmers' perceptions of rice hoja blanca virus (RHBV) in Colombia

We conducted a survey in five rice areas to investigate farmers' perceptions of RHBV (Table 2). One hundred forty-seven farmers, randomly chosen from the five areas were survey using a questionnaire between February and June of 1999. Farmers regarded sogata as a serious problem in Tolima, Huila and Valle and as minor problem in Meta and Casanare. The part on RHBV (Table 2) indicates that the rice growers have a very realistic idea of problems with RHBV.

Table 17. Farmer survey of RHBV incidence and losses in 1999.

Class	Incidence of RHBV					Losses due to RHBV				
(%)	Cas. 1	Meta	Tol. 2	Huila	Valle	Cas. 1	Met a	Tol. 2	Huila	Valle
0-10	89.6	90.0	69.3	81.9	69.3	88.4	100. 0	72.7	89.8	63.6
10-20	10.4	10.0	1902	1509	30.7	11.6	0.0	27.3	10.2	27.3
20-40	0.0	0.0	11.5	0.0	0.0	0.0	0.0	0.0	0.0	9.1
>40	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0
l		<u> </u>	<u> </u>		<u> </u>	<u> </u>		<u> </u>	<u> </u>	

Cas.1 = Casanare.

Tol.2 = Tolima

Their reports on incidence correspond well with our monitoring of incidence and active vectors. Most farmers indicated that the incidence of RHBV was between 0-10 % (Table 1), however the disease during the last year hit very hard Cimarrón and Oryzica Llanos 5 in Tolima; and Oryzica Caribe 8 and O. Llanos 5 in Valle. They estimate that the RHBV incidence and damage are about equal with most farmers estimating losses of less than 10 %. Farmers pest management strategies were based on the use of insecticides and resistant varieties. In areas at risk for outbreaks, most farmers switched from susceptible to intermediate and resistant varieties.

This monitoring is 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 farmers perception of the problems are essential to a successful IPM programs and these surveys will become a standard part of the monitoring for RHBV.

# 1.8.3. Survey on Insect Pest Management in Rice in Colombia Introduction

A survey was carried out from February to June 1999 in five rice-growing areas of Colombia to identify farmers' management practices of phytophagous insects and their understanding of the Sogata-"hoja blanca" complex.

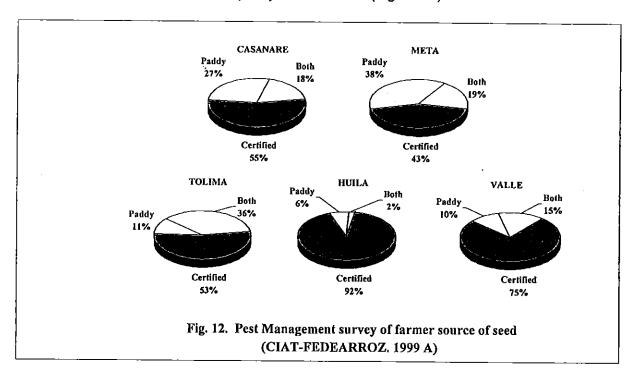
#### Methods

The survey included 147 rice growers (75% agricultural engineers and 25% farmers) of both irrigated and upland areas of the departments of Casanare (Yopal, Aguazul, Nunchia, Tauramena, Tame, Mani); Huila (Palermo, Campo Alegre, Aipe, Villavieja, Yaguara, Tesalia, Rivera); Meta (Villavicencio, Acacia, Puerto López, Cabuyaro); southern Tolima (Espinal, Guamo, Saldaña, Purificación, Doima); and Valle del Cauca (Jamundí, Santander de Quilichao, Villarica). Detailed information was gathered on the farmers' understanding of agronomic practices, insect pests, and the rice planthopper-'hoja blanca' complex and on their use of insecticides. Survey results were put into a database and averages and frequencies calculated using the SAS Statistical Program.

#### Results

#### a. Agronomic practices

Of the farmers surveyed, 72% use certified seed and 28% paddy seed. Worth mentioning is that 91% of the farmers from Huila use quality certified seed (Figure 12).



Among the five most cultivated varieties, farmers reported the following five (percentage in parentheses): Oryzica 1 (36%), Oryzica Caribe 8 (19%), Fedearroz 50 (13%), Oryzica Llanos 5 (7.5%), and Oryzica 3 (6.1%).

The survey revealed that 70.5% of the farmers destroy the ration after harvest and 29.5% do not. The eradication of the rations is known to be a very effective phytosanitary practice that help manage the rice planthopper-'hoja blanca' complex as well as other pests and diseases. A total of 57% of the farmers follow the rice-rice cropping pattern; 22% rice-fallow, and 21% rice-other crop. Rotating rice with other crops should be encouraged because it not only enhances pest management but also improves soil properties.

In the areas surveyed, 85% of the farmers reported that they use a planting density between 150 and 250 kg/ha, 5% use less than 150 kg/ha, and 10% use 300 or more kg/ha.

#### b. Insect pest management practices

Of the farmers surveyed, 81% applied insecticides to control the pests, 5% used biological control, 4% did not apply any control measure, and the rest used other methods. The average number of applications per crop cycle was 2. Eighty four percent of the respondents reported that insecticides eliminate between 75% and 100% of the pests in their rice fields. This observation is visual because pest surveys are generally not done either before or after insecticide application.

When farmers were asked about the most important pests in their area, the respondents from Casanare answered: rice stem borers (35%), rice stink bugs (19%), white grubs (16%), rice leaf-folders (12%), rice armyworms (7.6%), rice whorl maggots (3.8%), and rice planthoppers (3.8%). The respondents from Huila replied: rice planthoppers (33%), rice stem borers (32%), rice armyworms (10%), *Blissus* and mole crickets (7.5%), and rice stink bugs and rice whorl maggots (5%). The respondents from Meta reported: rice stem borers (53%), rice stink bugs and rice armyworms (13%), rice leaf-folders, mole crickets, and mites (7%). The respondents from southern Tolima reported rice planthoppers (64%), rice whorl maggots (15%), rice stink bugs (14%), and rice armyworms (7.1%); and the respondents from Valle del Cauca reported rice planthoppers (50%) and rice whorl maggots (50%).

Respondents from southern Tolima, Huila, and Valle del Cauca classified the rice planthopper as the most important pest; of these, 86% apply a pesticide once to control the pest, whereas the remaining 14% make 2-3 applications. In Casanare and Meta, farmers reported rice stem borers to be the main pest; 85% applied a pesticide once to control the pest, whereas the remaining 15% made from 2 to 4 applications.

Most applications were made during the first 50 days of the crop cycle (Table 18), except in the case of rice stink bugs, where pesticides are applied between 60-90 days.

Table 18.	Time of pest	control for rice
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Age (days)	Plant hopper	Age (days)	Rice Stink bugs	Age (days)	Stem borer	Age (days)	Whorl maggot	Age (days)	Rice Army worm
< 15	27 <sup>1</sup>	<50	21	<25	18	< 15	42	<20	70
16-25	40	51-70	10	26-50	64	16-30	44	>20	30
26-50	30	71-90	60	>50	18	>30	14		
> 50	3	>90	9					<u> </u>	

<sup>1 =</sup> Percent of farmers

#### c. Use of insecticides

The farmers surveyed reported that organophosphorus compounds and pyrethroids were the most commonly used insecticides to control the rice whorl maggot, rice stink bugs, and rice leaf-folders. Etofenprox and organophosphorus compounds were used to control the rice planthopper; organophosphorus compounds, pyrethroids, and etofenprox for the rice armyworm; organophosphorus compounds and carbamate insecticides for the rice stem borer; pyrethroids and carbamate insecticides for white grubs; and fipronil, pyrethroids, and carbamate insecticides for mole crickets (Table 19). Some farmers applied insecticides on more than one occasion in different

stages of the crop and for the same pest. In general, the most commonly used insecticides in the 5 study areas were organophosphate compounds (39%), pyrethroids (18%), carbamate insecticides 14%, chitin synthesis inhibitors (4%), biological insecticides (4.2%), phenyl-pyrazoles (6.7%), etofenprox (12.7%), nitroguanidines (0.7%), and growth regulators (0.7%).

Table 19. Insecticides used to control rice pest.

Class	Pest							
	RWM	RPH	RAW	RSB	SB	RLF	WG	MC
O. fosforados	46 <sup>1</sup>	30	28	72	58	60	13	4
Piretroides	25	14	23	- 10	2	10	37	26
Carbamatos	12	12	3	2	18	5	38	22
Inhibidores	2	2	16	2	2	10		
Reg. Crec.		5						
I. Biológicos			3	7	15	<b>-</b>		
Fipronil							6	48
Etofenprox	15	30	22	7	5	15	6	
lmidacloprid		7						

<sup>&</sup>lt;sup>1</sup> = Percent of farmers.

RWM = Rice whorl maggot

RSB = Rice stink bug

WG = White grub

RPH = Rice plant hopper (sogata) RAW = Rice army worm

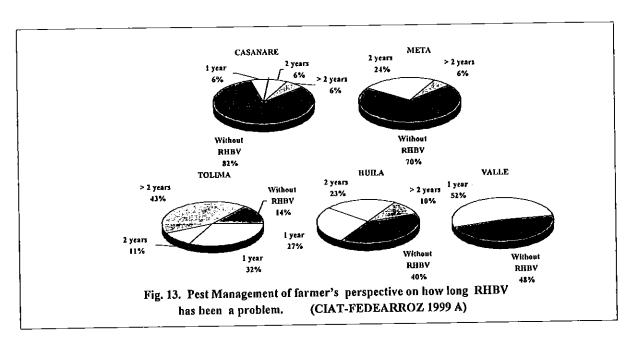
SB = Stem borer

MC = Mole Cricket

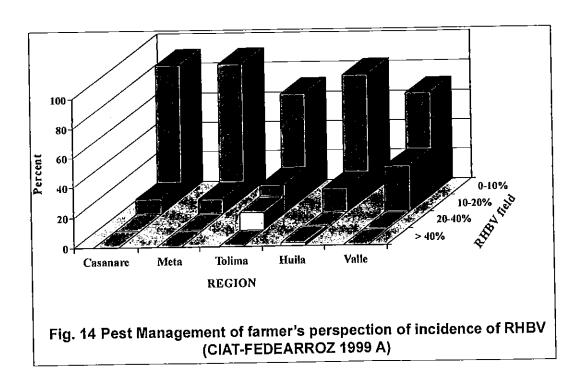
RLF = Rice leaf folder

## d. Rice planthopper-'hoja blanca' complex

Farmers in Tolima, Huila, and Valle del Cauca consider the rice planthopper to be a serious problem because it transmits the RHBV; farmers in Meta and Casanare give this pest little importance (Figure 13).



Most respondents indicated that RHBV incidence ranged between 0%-10%; however, in areas such as Valle del Cauca, Huila and Tolima, disease incidence reached 40% (Figure 14).



Of the respondents, 80% estimated that grain losses due to RHBV were under 10%; however, some farmers of Tolima, Huila, and Valle del Cauca claim to have suffered losses up to 20%.

#### Discussion

This study has shown that, at present, the application of insecticides is an important pest control practice in rice in Colombia.

Farmers understand the problems caused by RHBV, and the incidence values reported by farmers coincide with RHBV monitoring carried out in Colombia. Disease control strategies are based on the use of resistant varieties and on the application of selective insecticides first 15 days after crop emergence. In areas with RHBV problems, farmers have replaced susceptible varieties with intermediate or resistant varieties. Farmers' perceptions of the problems caused by pests must be taken into account if IPM is to be successfully implemented in rice crops.

#### **Training**

The following meetings were with either farmers or agronomist on IPM control of the planthopper *T. orizicolus* and RHBV.

Date	Local	Conference	Theme
Octuber/98	Jamundi	L. Reyes	IPM Sogata-RHBV
November/98	Cúcuta	M. Triana	IPM Sogata-RHBV
November/98	Tolima	M. Triana	IPM Sogata-RHBV
February /99	lbagué/Tolima	L. Reyes	IPM Sogata-RHBV
March/99	Neiva/Huila	L. Reyes	IPM Sogata-RHBV
March/99	Bogotá	L. Reyes	IPM Sogata-RHBV
April/99	Yopal/Casanare	L. Reyes	IPM Sogata-RHBV
April/99	La Habana/ Cuba	R. Meneses	IPM Rice
May/99	Villavicencio/Meta	L. Reyes	IPM Sogata-RHBV
June/99	Jamundí	L. Reyes	IPM Sogata-RHBV

### **Training on Integrate Pest Management**

Country	Date	Participants
BOLIVIA	7 al 10 Dec. 1998	91 (62% Farmers,
Santa Cruz de la Sierra		14% Technicians)
NICARAGUA		
- Malacatoya	26-27 Apr/99	41 (41% Farmers,,
		42% Technicians)
- Sébaco	28-29 " " "	72 (35% Farmers,,
		21% Technicians)
PERÚ <sup>1</sup>		ļ
- Nueva Cajamarca	11-13 May/99	72 (11% Farmers,,
-		46% Technicians)
- Chiclayo	17-19 " " "	119 (6% Farmers,,
-		40% Technicians)

<sup>1 =</sup> L. Calvert, R, Meneses y Mónica Triana.

### Other training

Date 1999	Institution	Theme
3 May/27Jun	Brígida Borges. DANAC- Venezuela	Manejo T. orizicolus – VHB
13-19/July	René Guzmán. CIAT- Bolivia	Manejo T. orizicolus – VHB
23 Agost	Orlando Palacios y Fernando Montero INIA – Perú	Manejo T. orizicolus – VHB

#### Participation in scientific meetings

Date	Name	Meeting	Activity
26-27 May/99	Rafael Meneses	l Congreso Arrocero Internacional. David. Chiriquí, Panamá	Speaker
26 –29 July/99 21 al 24 de September/99	Luis A. Reyes Mónica Triana	XXVI Congreso de la Sociedad Colombiana de Entomología. Bogotá Il Taller de Selección Recurrente en Arroz. Goiania, Brasil.	Speaker Speaker

#### Publications:

Monitoreo del vector del virus de la hoja blanca del arroz (RHBV) en diferentes zonas de Colombia. A. Velasco, L. Reyes, C. Pardey; L. Calvert. Congreso Nacional de Fitopatologia Mayo 1998.

Manejo del Complejo "Sogata-Virus de la Hoja Blanca" en el Cultivo del Arroz. L. Calvert; L. Reyes; Plegable divulgativo, CIAT, Corpoica, Fedearroz. 1999. Distribución para Colombia.

Manejo del Complejo "Sogata-Virus de la Hoja Blanca" en el Cultivo del Arroz. L. Calvert; L. Reyes; Plegable divulgativo, CIAT, Corpoica, Flar, Fedearroz. 1999. Distribución países miembros del FLAR.

Avanza campaña nacional para controlar virus de hoja blanca. CORREO Fedearroz, Abril 1999, No 100, p.p.1,2.

El Progreso en el Mejoramiento de Arroz a través de esfuerzo conjuntos en América Latina: Una mirada a las evaluaciones del Virus de la Hoja Blanca y de su vector, la sogata. Mónica Triana. Foro Arrocero Latinoamericano 5(1):18-21, 1999.

Guía para el Trabajo de Campo en el Manejo Integrado de Plagas del Arroz. IIA-CIAT-FLAR.

R. Meneses, A. Gutiérrez, A. García, G. Antigua, J. Gómez, F. Correa. CIAT, 3a. Edic. revisada y ampliada. Diciembre 1998. 55 p.

Manejo de "Sogata-Virus de la Hoja Blanca" en el cultivo del arroz. Poster. L. Calvert, L. Reyes, Maribel Cruz y Mónica Triana. CIAT, 1999.

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# **OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED**

Lee Calvert and Zaida Lentini

# D. Control of Rice Hoja Blanca Virus through Nucleoprotein Mediated Cross Protection in Transgenic Rice

#### Introduction

Rice hoja blanca virus (RHBV) is present and is cyclic problem throughout the Caribbean region, Central and Northern Latin America. Although the distribution of varieties that are resistant to both the vector and the virus is growing, most countries still do not have these varieties. Consequently there have been severe outbreaks of RHVB in Peru and other countries. Also the conventional resistance to RHBV comes from a single source and it does not confer complete resistance. When varieties such as O. Llanos 5 and Fedearroz 50 are inoculated with high RHBV pressure during the first 25 days after planting a significant level of infection occurs. Therefore, additional sources of RHBV are needed to ensure stable and durable resistance and protect the crop from early attacks of RHBV. This is being accomplished by transforming rice with novel genes that confer resistance to RHBV, and incorporating these genes into Latin American commercial varieties or into genotypes to be used as parents in breeding programs.

Evaluation of the inheritance of the N gene and resistance to RHBV in crosses with three convention genotypes.

Four promising T5 trangenic rice lines designated A3-49-60-4-5, A3-49-60-4-13, A3-49-60-12-3 & A3-101-18-19 were selected using the criteria of presence of the N protein and resistance to RHBV. These lines were crossed with CT8008 (RHBV susceptible), Oryzica 1 (intermediate resistance) and Fedearroz 50 (highly resistant). The progeny of these crosses were tested last year for their reaction to RHBV and for agronomic characteristics (see IP-4 Annual Report 1998). A study was conducted to determine the stability of the inheritance of the N-gene. The progeny were analyzed using either PCR or nested PCR techniques to determine if the N protein was present. A total of four repetitions (10 plants per replication) were inoculated at either 10 days or 20 days after planting with RHBV.

Out of 217 plants, the N gene was detected in 138 and 79 appear to contain no N gene (table 1). This 2:1 ratio was not expected in the F1 generation. Since the N protein gene is not present in the non-transgenic parent, a possible explanation is the elimination of the gene because of incompatibility or recombination. Of the 138 plants with the N gene, 105 were rated as resistant and 33 as susceptible to RHBV. If only the plants that were tested using the nested PCR technique are included, the N-gene was detected in 118 plants and was not detected in 43 plants. While it appears that the nested PCR is more sensitive in detecting the N-gene, the 3:1 ratio is different than expected for an F1 population and the same there appears to be either incompatibility or recombination occurring. The F2 populations will be tested for both their reactions to RHBV, and to the stability of the gene. It is expected that each generation should become more stable as the trait is fixed in the populations.

Table 1. Evaluation of the inheritance of the nucleoprotein gene of RHBV virus in crosses with different rice genotypes.

Cross	Presence of N-protein	Absence of N-protein
	gene product <sup>1</sup>	gene product
A3-49-101-18 x CT	5	14
A3-49-101-18 x FB	8	9
A3-49-101-18 x Ory	7	13
A3-49-60-12-3 x CT	8	6
A3-49-60-12 <b>-</b> 3 x FB	13	6
А3-49-60-12-3 х Огу	16	2
A3-49-4-13 x CT	15	5
A3-49-4-13 x FB	15	5
A3-49-4-13 x Ory	13	2
A3-49-4-5 x CT	<b>11</b> ·	9
A3-49-4-5 x FB	12	8
А3-49-4-5 х Огу	15	0
Total	138	79

<sup>&</sup>lt;sup>1</sup> The A3-49-101-18 crosses were tested using PCR. All other crosses were tested using a nested PCR assay.

# The effect of different ages of inoculation on transgenic rice plants containing the RHBV N gene

In order to obtain a better idea of the degree of resistance, one transgenic line A3-49-60-4-13-2 was inoculated with RHBV at 15 and 30 days after planting and compared with non-inoculated controls. To simulate high inoculum pressure 4 nymphs from a planthopper colony that was determined to have more than 70% active vectors were placed on each plant for 5 days. Cica 8 was the susceptible control. There were two repetitions with 50 plants in each treatment.

The evaluation of the plants included an estimate of the leaf area with RHBV symptoms, severity of symptoms and vigor of the plants. The plant were evaluated at periodic intervals until 70 days after the inoculation. At 96 days after inoculation a final evaluation was made.

The effect of age was the most significant effect. Both the transgenic and the control Cica 8 plant were much less affected by the 30 day treatment as compared to the 15 day treatment (Figure 1). At both times of infestation, the tendency was that the transgenic plants performed as a group better than the Cica 8 control. Despite this, the level of resistance to RHBV was not a high as in previous experiments and the difference were not as large as expected. In some cases such as foliar area affected at the 15 day treatment, there was no significant difference between the control and transgenic plants. The transgenic plants did not perform as well as the non-inoculated controls. In this experiment, the line A3-49-60-4-13-2 was not resistant to RHBV at 15 days of age and the general level was not has high as desired. This is evidence that stable resistance of a high level still has not been achieved. It was concluded that many more lines should be screened in preliminary trials and only the best should be used in the larger trials. This highlights the limitations of doing all the select for resistance in the greenhouse and the need for field experiments.

## Cloning and sequencing of the N-protein gene from transgenic plants

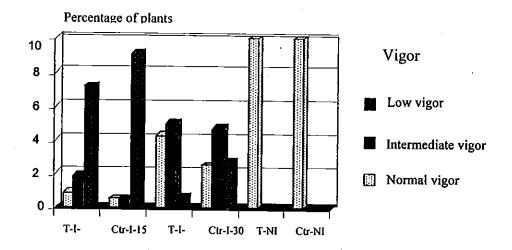
During four generations (T4), the N-protein gene was evidently inherited in a stable manner. In order to confirm the integrity of the N-protein gene, primers were used to amplify the transgenic copy of the gene. This was cloned into a bacterial plasmid and sequenced. A fragment of 435 bases was sequenced and it had more than 99% homology with the N-gene that was inserted into the plant. A second N-protein transgene was cloned from the T5 generation. It was a 213 base fragment and was 100% homologous with the original cDNA clone. This is evidence of the stable inheritance of the N-protein gene during five generations.

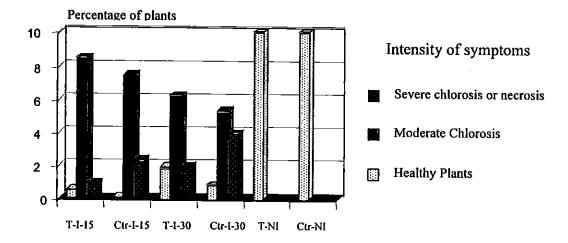
#### Conclusions

After several years of greenhouse experiments, the number of materials that need to be analyzed far exceed the capacity of the bio-safety greenhouse. Colombia did pass the regulations to permit field experiments with transgenic plants and an application to test the RHBV resistant transgenic rice was submitted. Pending approval, RHBV resistance experiments should soon be in the field. Despite the limitations of space, good progress is being made and materials from five generations have been tested. The N-protein gene is being inherited and in many lines there is an adequate level of resistance. The are still questions about the long-term stability of the N-protein gene. Can this gene be fixed in the population and continue to maintain a high level of resistance? Another pending question is the exact mechanism of resistance. It is proposed that gene silencing is the mechanism. Gene silencing places a minimal burden on the plant, because little or no foreign proteins or RNAs are produced in the plant. If stable, this should be an ideal type of resistance. The other question that will take both field and greenhouse experiments to determine concerns the complementary of conventional and transgenic resistance. All these experiments are progressing and will determine if these plants become a new source of RHBV resistance that is incorporated into breeding programs.

#### Collaborators

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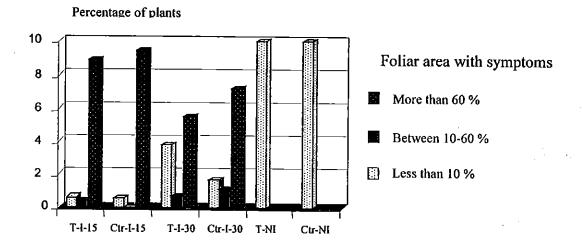


Figure 1. A comparison of one line of transgenic rice inoculated at 15 and and 30 days after planting. The T: transgenic; C: Cica 8; I: Inoculated; NI: non-inoculated; followed by the date of the inoculation.

#### **OUTPUT 3. RICE PEST AND GENETICS OF RESISTANCE CARACTERIZED**

E. Foreign genes as novel sources of resistance: Resistance of Rice to rice hoja blanca virus (RHBV), and *Rhizoctonia solani*.

Zaida Lentini and Lee Calvert

#### Background

Last year, results from research conducted by our research group suggested that the resistance conferred by the N transgene towards RHBV disease is expressed independently of the genotype background. The N transgene could be used to complement the natural resistance source to the virus, when crossing selected transgenic lines with diverse genotypes carrying the breeding resistance gene(s) since it is expressed on plants at 10-day-old. Significant progress has been attained, however, immunity at this early age has not been recovered yet in the hybrids containing the RHBV-N transgene and the breeding resistance source. The resistance conferred by the N gene is characterized by a significant delay in the progression and severity of disease respect to inoculated non-transgenic controls. Besides RHBV, Rhizoctonia solani (sheath blight) is already causing important rice yield losses in the Southern cone of South America and increaseing spreads had been reported in Colombia, Mexico and Venezuela. All rice varieties are susceptible and there is not known source of stable genetic resistance in rice. Biological control of this disease has not been successful either. At present, the control depends on heavy use of fungicides (Dr. Fernando Correa, CIAT Rice Pathologist, Cali, Colombia, personal communication). Therefore, incorporation of resistance for this disease by genetic engineering is an attractive approach. Work conducted by another principal investigators in this project (Nilgun Tumer, Rutgers University, USA) showed that a pokeweed antiviral protein (PAP), a 29-kDa protein isolated from Phytolacca americana, has a ribosome-inactivating ability and a potent antiviral activity against many plant and animal viruses, including HIV (Tumer et al., 1997). Interesting enough, mutated versions of PAP gene also confer resistance to fungal infection (Zoubenko et al., 1997). Homozygous progeny of transgenic tobacco plants expressing these PAP genes displayed resistance to the fungal pathogen Rhizoctonia solani. These results suggest the possibility of designing molecular strategies for incorporating dual antiviral and fungal resistance by introgressing mutant PAP gene(s) in transgenic rice plants. Here we report the progress made during the seven months when this project was initiated.

#### **Materials and Methods**

Indica rice varieties Cica 8 (control for transformation efficiency), Palmar, Cimarrón, and PNA004 will be used as targets. Palmar and Cimarrón shows high and moderate tolerance to sheath blight, and PNA004 carries the Colombian 1 source of resistance to RHBV. Agrobacterium mediated transformation of these varieties is being optimized (see work on this annual report). To generate new point mutations in the PAP gene, a quick change site directed mutagenesis kit from Startagene was used. Genes constructs carrying various mutant versions of the PAP gene, or the RHBV NS4 (non-structural RHBV RNA4) gene in sense or antisense directions are being placed in vectors driven by 35S CaMV promoter or maize ubiquitin promoter, and using hygromicin resistance as gene selection.

#### Results

Eight new PAP mutations were generated directed to change aminoacid composition in the PAP protein (Table 1). These new mutated genes were placed into yeast vectors, and transformed into yeast to check for no toxicity. The non-toxic mutated genes will be transformed into tobacco first to check the gene expression before using them for rice transformation.

Table 1.- New mutations generated in PAP gene.

	Mutation	AA Change	Name in Plant Vector	Name in Yeast Vector
1.	PAPI Del	I4M	NT296	NT299
2.	PAPI Del	T18M	NT298	NT300
3.	PAPI Del	I13M	NT317	NT311
4.	PAPI Del	V8M	NT319	NT312
5.	PAPI Del	Y16M	NT	NT
6.	PAPI Point	Y16A	NT	NT
7.	PAPI Point	Y16S	NŤ	NT
8.	PAPI Point	Y16Phe	NT	NT

Two mutated versions of PAP (I and II) already tested for no toxicity in turfgrass (another monocot species) will be used as the first approach to transform rice (Table 2). These genes driven by the ubiquitin promoter were placed in the plasmid vectors pWBVec8, pWB10a, and pBGXiHGFP kindly supplied by Dr. Peter Waterhouse (CSIRO, Australia). These plasmid had been used succesfully by Waterhouse to trasnform rice via Agrobacterium. They contain a hpt gene with a CAT-1 intron for increased expression of hygromycin resistance and selection in rice, a gus-intron-gene, or a gfp (green fish fluorescent) gene, respectively, to aid the recovery of transgenic plants.

Table 2. Description of PAP constructs for Plant Transformation generated.

	GEN	Promoter	Vector/orientation	Other genes	Transf	Name
1	PAPId	Maize Ubiq.	NT168	Amp	В	NT178
2	PAPId	Maize Ubiq.	NT294	Spec,GFP,Hy gi	Α	NT301
3	PAPId	Maize Ubiq.	PWBVec10a	Spec,GUS,Hy gi	Α	NT303
4	PAPId	Maize Ubiq.	PWBVec8	Spec,Hygi	Α	NT306
5	PAPII	Maize Ubiq.	NT168	Amp	В	RTT126
6	PAPII	Maize Ubiq.	NT294	Spec,GFP,Hy gi	Α	NT302
7	PAPII	Maize Ubiq.	PWBVec10a	Spec,GUS,Hy gi	Α	NT304
8	PAPII	Maize Ubiq.	PWBVec8	Spec,Hygi	A	NT305

The RHBV NS4 gene in sense and anti-sense orientation driven by the 35S CaMV promoter were placed into the plasmid pCAMBIA 1301 carrying the gus-intron and hygromycin resistance gene (Table 3). The NS4 gene in both directions driven by the unbiquitin promoter is ready to be moved into vectors carrying the hygromycin-cat 1 intron gene from Watehouse laboratory.

Table 3.-Description of RHBV-NS4 constructs generated in CIAT

	GEN	Promoter	Vector/orientation	Other genes	Name
1	NS4	35S	PC1300/sense	Hyg,Kan	plC001
2	NS4	35S	PC1300/asense	Hyg,Kan	plC003
3	NS4	35S	PC1301/sense	Hyg,Kan,GUSi	plC002
4	NS4	35S	PC1301/asense	Hyg,Kan,GUSi	pIC004
5	NS4	Maize Ubiq.	NT168/sense	Amp	plC005
6	NS4	Maize Ubiq.	NT168/asense	Amp	pIC006
7	NS4	Maize Ubiq.	PWBVec8/sense	Spec,Hygi	pIC007
8	NS4	Maize Ubiq.	PWBVec8/asense	Spec,Hygi	pIC008
9	NS4	35S	PWBVec8/sense	Spec,Hygi	pIC009
10	NS4	358	PWBVec8/asense	Spec,Hygi	pIC010

#### **Future Plans**

- Optimize the genetic transformation mediated by *Agrobacterium* of the genotypes selected using the gene constructs carrying the gus-intron gene.
- Generate transgenic rice carrying the different versions of PAP or NS4.
- Evaluate the gene-integration by Southern, and gene expression by Northern and Western of the transgenic plants evaluated.
- Characterized transgenic plants for stable expression and inheritance of the transgenes.
- Study the level and stability of the RHBV resistance confer by PAP alone or in combination with the RHBV-viral trasngenes and/or the Colombia 1 breeding resistance source.
- Evaluate spectrum of Rhizoctonia resistance encoded by PAP gene.

#### Collaborators

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#### References

Nilgun T.E., Hwang, D.J., and Bonness, M. 1997. C-terminal deletion mutant of pokeweed antiviral protein inhibits viral infection but does not depurinate host ribosomes. PNAS 94: 3866-3871.

Zoubenko, O. et al., 1997. Plant resistance to fungal infection induced by non-toxic pokeweed antiviral protein mutants. Nature Biotechnology 15: 992-996.

# AGROBACTERIUM MEDIATED GENETIC TRANSFORMATION OF INDICA and JAPONICA RICE

#### BACKGROUND

Last year, we reported the preliminary results on the optimization of Agrobacterium mediated transformation of local rice varieties. It included *indica* rices (CICA 8, IR-72, INIAP 12) adapted to irrigated (flooded) conditions, and *japonica* rices (CT 6241, CT 10069, and Lastisday-Fofifa) adapted to acid soils (savanna) and highland (hillside) environments. Protocols established for rice were reviewed (Hiei *et al.*, 1994; Aldemita,1996; and Toki, 1997) and the necessary modifications for optimization using the selected breeding genotypes were introduced. The studies conducted suggested that the selection of the explant source and preculture conditions of the target tissue are major key factors for *Agrobacterium* mediated transformation of rice. Embryogenic scutellum derived callus showed higher transient GUS expression than immature embryos. Higher number of hygromycin resistant (Hyg') callus was recovered by preculturing the callus three days before co-cultivation with the Agrobacterium. Between 60% and 100% of these Hyg' callus showed stable GUS expression (GUS<sup>+</sup>) 90 days after infection. Following we report the efficiency on plant regeneration after selection on 50 mg/l hygromycin, the analysis on Southern blot of the T0 plants, and the inheritance of GUS<sup>+</sup> and Hyg' in T1 plants. Furthermore, preliminary results on three other indica genotypes (Palmar, Cimarrón, PNA004) using the established protocol are also described.

#### **MATERIALS AND METHODS**

The three protocols developed for rice (Hiei et al., 1994 and 1997; Aldemita, 1996; and Toki, 1997) were tested. Preliminary results showed differences between the three protocols established for rice transformation. Following are described the modifications introduced into Aldemita and Hodges (1996)'s protocol, which gave the highest response for the genotypes tested so far. Instead of using N6 as recommended by Aldemita and Hodges (1996), scutellum derived callus was induced and sub-cultured for 0 to 3 days on NBA medium (Li te al., 1993) containing proline and NAA prior the co-cultivation (preculture) with the bacteria. The the callus was co-cultivated with A.tumefaciens strain LBA4404 (pTOK233) or AGL1 (pCAMBIA 1301) in NBA-AS medium containing 100µM acetosyringone for 3 or 5-6 days. Casamino acids and kinetin were omitted in the co-cultivation and selection media, and 20µl 100µM acetosyringone were added 2 hours prior the co-cultivation and onto the co-cultivated callus to reactivate further the vir genes. After cocultivation, the agro-infected callus were washed with N6 salts (Chu et al., 1975) containing carbenicillin (250 mg/L), cefotaxime (100 mg/L), and hygromycin (50 mg/L) to kill the bacteria. The callus were then transferred onto the selection medium A INBA containing carbenicillin (250 mg/L). cefotaxime (100 mg/L), and hygromycin (30 mg/L)] for three weeks. The healthy looking callus were sub-cultivated onto medium B [NBA containing carbenicillin (250 mg/L), cefotaxime (100 mg/L), and hygromycin (50 mg/L)]

for other three weeks. Following the transgenic calli were first transferred onto a proliferation medium (LS with 0.5 mg/l 2,4-D and 50 mg/L hygromycin) for 3 weeks and then to a regeneration medium (MS with NAA 1 mg/l, kinetin 4 mg/l).

#### **RESULTS AND DISCUSSION**

A total of 306 Hyg<sup>r</sup> callus derived from co-cultivation with LBA 4404 (pTOK233) were transferred onto regeneration medium containing 50 mg/l hygromycin. Between 20% to 68% of the callus regenerated plants. Total of 86 T0 plants were transferred to the greenhouse, and 80% to 96% of these plants were GUS<sup>+</sup>. Southern blot and PCR analysis confirmed the integration of both GUS<sup>+</sup> and Hyg<sup>r</sup> genes in 50% to 100% of the regenerated plants. These results suggest a transformation

efficiency of 7% to 18%. T1 seeds from plants showing integration of both transgenes were harvested and analyzed for germination on medium containing 50 mg/l hygromycin and GUS expression. For Cica 8 (indica), 73% of the T1 lines derived from independent T0 transgenic lines inherited GUS expression; whereas for CT 6241 and CT10069 (japonica), 100% of the T1 lines were GUS<sup>+</sup> (Table 1 and 2). Segregation for GUS expression at the T1 generation indicated a Mendelian segregation of 3:1 or 1:0 ratio for GUS<sup>+</sup>:GUS<sup>-</sup> suggesting the presence of one (3:1) or more active GUS locus. Similar results were obtained for the inheritance of the Hyg<sup>r</sup> gene indicating either a 3:1 (eight T1 lines) or 1:0 ratios (four T1 lines).

Table 1. Gus expression in T1 generation derived from Cica 8 plants transformed with LBA 4404 (pTOK233).

T0 line	Seeds	Seedlings	GUS⁺	GUS <sup>-</sup>
5	25	25	25	0
7	25	25	25	0
12	27	25	25	0
18	25	25	0	25
21	28	23	23	0
22	28	25	25	0
25	26	25	25	0
26	28	26	26	0
27	28	27	0	27
28	27	27	0	27
30	28	27	27	0
34	28	27	26	1
38	28	27	27	0
40	27	26	0	26
41	27	26	26	0
Cica-8 control	26	26	0	26

Two lines showed a significant lower number of Hyg<sup>r</sup> plants than expected suggesting a 1:1 ratio (Table 3). Of the 14 T1 lines analyzed showing resistance to hygromycin, 21% did not cosegregated for GUS expression, indicating gene silencing or sorting out of the GUS gene throughout meiosis. Preliminary results on transient gus expression on recalcitrant rice genotypes, such the indica varieties Palmar, Cimarón, and PNA004, using the *Agrobacterium* strain AGL1 (pCAMBIA 1301) suggest that the protocol establish for LBA 4404(pTOK233) can be used with minor modifications. These include increasing the days of agroinfection from 3 to 6 days. Selection of these callus on hygromycin containing medium is in progress.

Table 2. Gus expression in T1 generation derived from CT 6241 and CT 10069 plants transformed with LBA 4404 (pTOK233).

Genotype	T0 line	GUS <sup>†</sup>	GUS.
CT10069	3	25	0
	5	21	4
	6	25	0
	7	20	5
_	8	23	0
	9	24	0
	11	25	0
-	12	24	5
	13	24	5
	14	23	2
<del></del>	15	21	4
	17	24	1
	18	24	1
•	1	20	5
	5	19	6
	6	16	7
-	11	23	2
	15	15	10
CT6241	16	17	8
	17	23	2
	18	19	6
	19	19	6
	20	25	0
-	21	20	5
	22	21	4
-	24	24	1
	25	17	8

Table 3. Co-segregation for hygromycin resistance and GUS expression in T1 generation derived from Cica 8 plants transformed with LBA 4404 (pTOK233).

T0 line	Seeds	Seedlings Hyg <sup>r</sup> : Hyg <sup>s</sup>	Seedlings Hyg <sup>r</sup> tested for Gus expression	GUS⁺	GUS <sup>-</sup>
5	25	23:2	20	17	3
7	24	23:1	6	6	0
12	26	21:5	6	6	0
18	22 25	16:6	6	0	6
21	25	22:3	6	6	0
22 25	24	21:3	6	6	0
25	22	18:4	6	6	0
26	27	16:11	6	6	0
27	22	16:6	6	0	6
28	23	17:6	6	1	5
30	23	17:6	6	6	0
34	24	19:5	6	6	0
40	26	14:12	6	0	6
41	25	18:7	6	6	0
Cica-8	22	0:22	0	0	0

#### **Future Plans**

- Complete phenotypic and genetic inheritance analysis of T1 lines.
- Comparison of transformation efficiency between LBA 4404 and AGL1 strains.
- Comparison of efficiency between Agrobacterium mediated transformation and particle bombardment.
- Use of *Agrobacterium* transformation to incoprorate NS4 and PAP genes for RHBV and *Rhizoctonia* resistance in indica rice.

Collaborators: Eddie Tabares (SB2), Luis Orlando Duque (SB2), Luisa Fernanda Fory (IP4), Faustina Giraldo (SB2), María Angélica Santana (Instituto de Estudios Avanzados, IDEA, Caracas, Venezuela), and Zaida Lentini (SB2).

#### References:

Aldemita R.R., T.K. Hodges T.K. (1996). *Agrobacterium tumefaciens*-mediated transformation of *Japonica* and *Indica* rice varieties. Planta 199:612-617.

Hiei, Y., S. Ohta, T. Komari and T. Kumashiro. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J. 6:271-282

Hiei, Y., T. Komari and T. Kubo. 1997. Transformation of rice mediated by *Agrobacterium tumefaciens*. Plant Mol. Biol. 35: 205-218.

Li L., R. Qu, A. de Kochko, C. Fauquet, and R.N. Beachy. 1993. An improved rice transforamtion system using biolistic method. Plant Cell Rep. 12: 250-255.

Toki, Seiichi. 1997. Rapid and efficient *Agrobacterium*-mediated transformation in rice. Plant Mol. Biol. Rep. 15(1)

#### OUTPUT 3. Rice pests and genetics of resistance characterized

rancisco Morales

#### F. Rice Stripe Necrosis Virus

#### Activity 3.1. Characterization of rice stripe necrosis virus

Rice stripe necrosis virus (RSNV) and its fungus vector *Polymyxa graminis* are now fully identified as the causal agent-vector of the "entorchamiento" (crinkling) disease in Colombia. This virus/vector characterization study has been conducted with the support of the Department for International Development (DFID) of the United Kingdom.

Based on the results obtained in this study, RSNV will soon be re-classified as a new member of the *Benyvirus* genus (previously classified as a member of the Furovirus group). We still do not know the implications of this re-classification, but it should help to focus the research on this new group of viruses that include two other members that infect sugar beets. The fungus vector of RSNV in Colombia, has been finally characterized as *Polymyxa graminis* at Rothamsted, U.K. by Dr. Elaine Ward.

One of the more important aspects of this project, has been the renewed interest and awareness of the potential threat of RSNV's endemic situation in West Africa, where this virus was first reported. Prior to this project, RSNV had disappeared from the memory of the pathologists working in that part of Africa, although it was still present there. According to Dr. David Johnson of WARDA, RSNV has been observed in Senegal, Guinea, Sierra Leone, Ivory Coast, Ghana, Togo and Nigeria.

According to WARDA, this disease is not considered an immediate threat to rice production in West Africa, due to the artesanal rice production systems that predominate in that part of the Tropics. Also, the existence of RSNV-resistant cultivated rice species in Africa, such as *Oriza glaberrima*, might have contributed to maintain the incidence of RSNV low. However, RSNV has significantly affected some mechanized rice crops in Ivory Coast and Sierra Leone, forcing the withdrawal of susceptible upland rice varieties. This observation clearly demonstrates that RSNV does have the potential to cause significant yield losses in West Africa, should the mechanization of the crop increase in the near future, as expected.

In Latin America, rice production is relatively more mechanized in comparison with Africa. Last year, we stated that the probability for rapid dissemination of RSNV in the main rice-growing areas of Latin America, is high. This year, we can announce that RSNV and its vector, *P. graminis*, have already emerged in Panamá. The National Agricultural Research Program (IDIAP) and the Panamanian quarantine authorities (Ministerio de Desarrollo Agropecuario) have requested CIAT's help in diagnosing the problem and suggesting feasible disease control measures. The Virology Research Unit of CIAT has provided antisera and virus detection protocols to Panamanian virologists and pathologists, to assess the extent of the dissemination of RSNV in Panama.

#### 3.1.2. Physical and chemical characterization of RSNV

Last year, we reported on a new purification method developed at CIAT for the isolation of RSNV, which allowed us to isolated the virus in sufficient quantity to pursue the molecular characterization of the virus and develop efficient virus detection techniques. The antiserum obtained, was successfully fractionated for the implementation of the ELISA technique. This technique is making possible the detection of the virus in diseased rice plants, including late infections when the virus is in low concentration. As mentioned before, this antiserum and the ELISA protocol developed, have already been sent to Panama, to study the epidemiology of RSNV in that country.

#### 3.1.3. Molecular characterization of RSNV and its fungus vector

The molecular characterization of RSNV was initiated last year. Previous attempts to design degenerate primers for amplification of random and target genomic segments of RSNV and other related furoviruses, did not yield satisfactory results. Following the purification of RSNV, the isolation of the viral RNA was accomplished for cloning experiments. cDNA clones were generated from the viral RNA using random primers. One of the clones identified, approximately 2,200 bases in length, was subcloned for sequencing. So far, 1,750 bases have been sequenced and compared to other sequences of plant viruses available in the GenBank. The RSNV clone was shown to have the highest degree of homology with a segment of RNA 1 of *Beet necrotic yellow vein furovirus*, recently re-classified as the Type member of the *Benyvirus* genus. These results agree with previous observations, suggesting that RSNV was similar to BNYVV. This characterization work will continue despite the termination of the project this year.

#### 3.2.1. Development of efficient germplasm screening methods

So far, we have implemented conventional and hydroponic inoculation methods to evaluate rice germplasm for resistance to RSNV. In the case of the hydroponic method, the critical doses to be applied to the nutrient solution, is the concentration of cystosori. In the case of trays (5x33x28 cm), 100 g of infected rice roots are applied to one liter of nutrient solution. The second method of inoculation involves the use of dried root powder prepared from systemically RSNV-infected rice roots. The powder contains the resting structures of the RSNV vector, *Polymyxa graminis*, which give rise to zoospores once the cystosori encounter free water in the medium. The powder is placed in the sand medium together with the rice seed of the genotypes to be tested. This method has been shown to be adequate to transmit RSNV up to 70% of test seedlings in the experimental conditions described above.

#### 3.2.2. Testing of RSNV control practices

The biology of the fungus vector, *Polymyxa graminis*, is being studied under controlled glasshouse conditions at Rothamstead, England, by Dr. Michael J. Adams. In a recent investigation conducted with Indian isolates of *Polymyxa graminis*, the cystosori germination, development of plasmodia, zoosporangia and cystosori were optimal at 27-30° C. In contrast, the same parameters determined for *Polymyxa graminis* isolates from temperate countries, had an optimal development at 15-18° C (Legreve *et al.*, 1998. European J. Plant Path. 104:195). This observation might explain the difficulties experienced in Great Britain in establishing viable cultures of the Colombian isolates of the fungus vector.

Continuing experiments at CIAT, on the effect of incorporating organic matter in soil infested by *Polymyxa graminis*, suggest that the decomposition of organic material, has a negative effect in the colonization of rice roots and/or infection of rice seedlings. We are monitoring the development of disease under zero tillage conditions.

Preliminary results obtained in the department of Tolima, where the legume *Crotalaria* sp.was incorporated as green manure to the soil, also show a reduction in the incidence of RSNV (Ing. Alvaro Salive, FEDEARROZ).

### 3.2.3. Ecology of RSNV and its vector Polymyxa graminis

The "entorchamiento" (crinkling) disease of rice is currently present in 22 municipalities, in eight of the most important rice-growing departments of Colombia: Meta, Casanare, Tolima, Huila, Antioquia, Cundinamarca, Valle and Cordoba.

One of the critical factors determining the incidence of the RSNV, seems to be the effect of the environment on the fungus vector. For massive virus/vector infection to take place, alternate periods of water stress and free water are necessary in the rhizosphere of susceptible plants. These wet and dry conditions, induce the release of zoospores of plasmodiophorid fungi. Thus, it is not surprising that most RSNV outbreaks are associated with periods of water stress (drought) before planting time. A look at the weather conditions preceding the first RSNV outbreak in the Eastern Plains of Colombia, in 1991 and 1992, showed that the first quarter of both years had relatively low rainfall values (172 and 170 mm accumulated rainfall for the months January-March) when compared to the total precipitation (332 mm) for the same period in 1990.

Another predisposing factor in the emergence and dissemination of RSNV, is soil texture: the lighter the soil, the higher the incidence of RSNV. Irrigation, has also been observed to contribute to the rapid dissemination of RSNV, creating a disease gradient that follows a path similar to the flow of water in the field. Finally, the movement of soil particles contaminated with the fungus vector of RSNV, is also associated with the use of agricultural machinery and contaminated seed.

#### 3.2.3. Technology transfer

Efforts are being undertaken to disseminate more information on RSNV to the affected rice-growing areas of West Africa. Up till now, however, it has been difficult to coordinate a meeting with the pathologist of WARDA, in order to produce a brochure in French, similar to the one produced for Latin America. A recent publication (Hoja Técnica) was published in the MIP Journal of CATIE, which has ample distribution in Central America.

#### **Publications**

Morales, F.J. 1998. La necrosis rayada del arroz: una nueva enfermedad viral en America. Hoja Técnica No. 27. . Revista *Manejo Integrado de Plagas*. CATIE, Costa Rica.

Morales, F.J., Ward, E., Castaño, M., Arroyave, J., and Adams, M.J. 1999. Emergence and partial characterization of rice stripe necrosis virus and its fungus vector in South America. European Journal of Plant Pathology. in press.

### **OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITY ENHANCED**

#### A. FLAR and Economics

Luis R. Sanint

#### 4.1 Manage FLAR

### 4.1.1 Organize and direct meetings of the administrative and technical committees

Administrative Committe. Two meetings wee held since the last annual report. The seventh meeting of this corporation took place in November 2 and 3, 1998 in San José, Costa Rica. This year, the eight meeting had its deliberations in Montevideo, Uruguay.

Technical Committees. This year, FLAR separated its technical meetings into to Subcommittees, one for each of the main ecoregions: the tropical and the temperate. The main objectives of these meetings are:

- to review the recent research activities of FLAR
- to revise priorities
- to plan future activities for the year in the area of breeding, crop management, training and other relevant actions in response to partners needs and demands.

The first meetings of 1999 coincided with breeders' workshops, to evaluate and select material and to discuss results from the nurseries.

The tropical committee met at CIAT in February 22-26. All partners from the region were present, except Guatemala. They are: Colombia, Costa Rica, Cuba, Nicaragua, Panamá and Venezuela. Besides them, CIAT, FLAR and CIRAD also participated in the meetings. The group emphasized the high priority of the work in blast and grain quality (both cooking and milling) as well as the need to break the yield ceiling. Support in crop management was also identified as a high priority. This year, the workshop of August in Santa Rosa was cancelled, due to the security situation in Colombia. The workshop in Palmira focused on F3 lines that were evaluated and selected by participants from the tropics.

The temperate subcommittee met twice. The first reunion was in Itajaí, on March 2-4. The workshop included materials from the VIOFLAR as well as elite lines from Santa Catarina that were evaluated and selected by participants from Brazil, Argentina, Uruguay, Bolivia, Chile, CIRAD and FLAR. In this group, the top priorities were reconfirmed to be grain quality, blast and higher yields, where they expect that FLAR should make the biggest contribution. Crop management is also a key activity where FLAR should offer support. A redefinition of germplasm flows was recommended: now, the F2 lines will be split into two sets. One set will be evaluated in Santa Rosa (Colombia) and the other set will be evaluated in Rio Grande do Sul. EPAGRI expressed much concern about blast outbreaks in the CT8008 varieties, which occupy almost 100% of the area in Santa Catarina. A mission from FLAR and CIAT should make a field visit soon to evaluate the problem and suggest specific action. Coinciding with the meeting, a breeders workshop was held at the EPAGRI station.

The second meeting of this committee took place in July 1-2 in Uruguaiana. There was a report by some breeders from the region that participated in the Second Cold Temperate Irrigated Rice meetings held at Davis. The Third meetings will be held in Montevideo and will be jointly organized by INIA-ACA and by FLAR, in the year 2003. In terms of germplasm flows, the committee decided that the F2 populations that will be evaluated in Santa Rosa (Colombia) will also be evaluated for pyricularia at IRGA and EPAGRI-SC. The F3 populations will be planted at IRGA, where breeders from member countries will select their own materials. The F5 populations will be sent to all partners for evaluation of straight head ("vaneo."). The lines from the VIOFLAR and VIOAL,

evaluated and selected by breeders at the Santa Catarina workshop are now ready for distribution. Breeders that require FLAR to make crosses will send Takazi Ishiy the list.

The next meetings of the technical subcommittees of FLAR will take place in February, 2000.

- 4.1.2 Update FLAR's rules and regulations (Estatutos). Following instructions from the Administrative Committee, the rules and regulations were modified. A major change has to do with fee payments. Due dates were set as follows: 20% in January; another 40% in may; the final 40% in October of each year. Failure to meet deadlines causes a penalty of 10% on the due balance and the member looses its rights until payments are normalized. With respect to Intellectual Property Rights both subcommittees revised the three chapters on the subject and made their own suggestions and comments. All FLAR dispatches to nonmembers are now accompanied by a Material Transfer Agreement.
- 4.1.3 Seek new members and expand participation in current member countries. During 1999, FLAR received three new members (annual fee in parenthesis): Argentina (\$52,500), Chile (\$15,000) and Nicaragua (\$22,500). An older member withdrew its participation due to funding problems: Paraguay (\$15,000). Currently, FLAR has participants from 12 countries that contribute US\$472,500 in annual fees. IRRI and CIAT contribute US\$100,000 more for a total annual income in fees of US\$572,500.

Peru: during 1999, FLAR participated in two courses in that country, to promote the participation of this country in our group. Conversations are under way but the high atomization of the rice sector constitutes an impediment to achieve consensus at the country level.

Ecuador has also shown interest. FLAR participated in a meeting in July, where the issue of their participation was raised. There has not been further contacts.

In May, a new Central American Rice Federation was created with participation of all countries of the region. Given that two of them (Honduras and Salvador) are not members of FLAR they have been invited by their colleagues to join. Little progress was achieved in this regard. Another potential partner. EMBRAPA, from Brazil, has declined any participation in FLAR.

- 4.1.4 Ensure adequate flow of funds and level of reserves. There has been a delay in the 1998 fee payment of \$140,000 by IRGA (from Brazil), the largest contributing partner. In a budget of about \$500,000 this is a sizable loss of liquidity. Provisions were taken to maintain the team of collaborators intact, while cutting back on operational expenses to avoid incurring in deficit spending. The delay is a reflection of the instability conferred by the continuous changes in leadership at IRGA (5 presidents in the five years that have been participating in FLAR). The past president did not pay and the incoming one (since January 1999) argued that he could respond for 1999, but not for 1998. Currently, FLAR has a schedule for the payment of the debt from IRGA and our level of reserves for 1999 was set at \$120,000, up from \$35,000 a year ago. This will isolate fee delays. On top of that, the new rules approved by all members (see 4.1.2 above), became more strict as a result of this negative experience.
- 4.1.5 Personnel management, supervision, recruiting. While the core of the team maintains its critical mass and its capacity to execute the agreed agendas, there have been several changes in personnel.

Departures: The main breeder in Palmira, Dr. James W. Gibbons left FLAR in December 1998 to join the University of Arkansas. The assistant to the breeder in Palmira, Ing. Agr. Daniel I. González resigned effective Sept. 30, 1999. He was hired by AgreEvo.

Arrivals: the breeder position in Palmira was filled by a part time consultant (Dr. Peter Jennings, former leader of the Rice Program at CIAT). In addition, a permanent breeder was appointed for the Southern Cone: Dr. Takazi Ishiy, from EPAGRI, who is under a joint agreement EPAGRI-IRGA-FLAR. He is posted at the rice research station of IRGA, near Porto Alegre. Dr. Edward Pulver (also a former CIAT Rice Program staff member) was hired as a consultant to assist members with integrated rice management aspects. Ing. Agr. Marco A. Oliveira (former technical director from IRGA) was appointed as a part-time deputy director for administration in the Southern Cone. An assistant for pathology, Ing. Agr. Diana Delgado, was hired to work at the Santa Rosa Station.

FLAR is still recruiting a scientist to fill the vacant of the main breeder in Palmira. The requirements of the post are rather strict in terms of language and knowledge of the region, which makes the selection rather difficult. The current arrangement (a high level consultant) is agreeable to everyone and allows us to buy time into the recruiting process.

#### 4.2 Breeding at FLAR

#### 4.2.1 Plan, organize, direct breeding and selection activities at Palmira

#### 1 INGER-LAC

From its creation in 1995, FLAR became the sponsor of INGER-LAC (the International Network for Germplasm Evaluation of Rice - Latin America and the Caribbean). INGER concentrates its efforts in the characterization, selection, multiplication and distribution of rice germoplasm and its distribution to all national programs of the region (members and nonmembers of FLAR). This network has 3 main sources of germplasm as indicated in Table 1. CIAT, as a source of material, plays an increasingly smaller role. This trend, coupled with the fact that about 40% of the varieties released in Latin America and the Caribbean in the past 30 years came from CIAT made crosses, indicates that those countries that have been basically depending upon these materials and that have not yet joined FLAR will have to resort to make their own crosses. The Caribbean countries have their own network (CRIDNet) that includes participation from CIAT, IRRI and CARDI. The main problem will be faced by Latin American nonmembers: Honduras, Salvador, Peru, Ecuador and Mexico. It is hoped that these countries will be able to overcome their current institutional difficulties to join FLAR.

Table 2 shows the number of sets for the 1999 nurseries distributed by request to different countries from the region, all of them members of FLAR, with the exception of Ecuador and Peru.

To conform the nurseries for next year (2000), we only have pre-selected 30 lines from the 1999 nurseries from the INGER-Global of IRRI, 6 entries provided by Ecuador, 3 lines from the USA and 5 lines from CIAT. This number of nominations is too low, if we compared it with the 3 previous years (Table 3). When we put these lines through evaluations of quality, sogata and Hoja Blanca Virus, there will be little germoplasm available to conform future INGER-LAC public nurseries.

#### 2. FLAR

#### a) Germoplasm Workshop

The VIOFLAR 1998-99 for the temperate zone was conformed to a total of 132 lines: 30 came from national programs, 26 were developed through anther culture and 76 come form the first set of F5

lines developed as FLAR crosses, FL. Table 4 shows the countries and number of distributed sets. Most lines from the cross FL144 (CT8008-16-31-3P-M//CT9682-2-M-14-1-3P-M1/CT11008-12-3-1M-4P-4) excelled by its good plant type, good stem and vigor, as well as good grain aspect. VIOFLAR-Trópico, 1999 was formed with 68 lines F5 originating from the first group of FLAR crosses, FL (Table 5).

#### b) Breeders workshop

Tropical material. This workshop took place in Palmira, during the week of February 22-25. Participants from tropical FLAR members included Colombia (FEDEARROZ), Cuba, Nicaragua, Panama, Venezuela, CIAT and FLAR. The main objective was to obtain a greater participation from breeders of partner countries in the evaluation and selection of the materials generated by FLAR for the tropics. Selection was done from 4422 F3 lines (from 137 different crossings) previously selected by its good quality and tolerance to the Hoja Blanca Virus. A total of 3738 lines F4 were selected (109 crossings). During the first semester of this year, this seed was planted in Santa Rosa for disease evaluation and in Palmira for its multiplication. It was also evaluated for Hoja Blanca Virus and amylose content.

Temperate material. Selection from 1456 lines F3, originating from 69 crosses for the tempered zones was done by breeders from Brazil and Argentina in Palmira. From this selection, seed from 1719 lines F4 (63 crossings) was obtained. This seed was also planted during the first semester of 1999 in Santa Rosa (for disease evaluation) and simultaneously in Palmira, where its amylose content was determined.

Breeders from temperate countries in FLAR agreed to send F3 seed selected by Argentina in Palmira for evaluations in that country to straight head. Some 265 F3 lines were dispatched in July to the FLAR representative in Argentina, Dr Juan Carlos Ruffini.

#### c) Plantings of germoplasma in Santa Rosa and Palmira, 1999A

Table 6 shows the materials planted during the first semester in Santa Rosa (evaluations to diseases) and Palmira (multiplication, evaluation to Hoja Blanca Virus, tagosodes and quality). Selection and harvest of the materials were done in Santa Rosa this past August with the participation of breeders from FEDEARROZ, Bolivia, Guatemala and Venezuela, as well as CIAT and FLAR. A summary of this activity follows next:

#### **Tropical Germoplasm**

Population F4: out of 3738 F4 lines planted, and according to the results of evaluations in Palmira, 31,2% were discarded due to Hoja Blanca Virus (1168 lines) and 3.6% due to low amylose content (136 lines). In Santa Rosa, 2043 lines were discarded (54.7%), mainly because of blast, helmintosporiosis (which had a severe incidence this year), grain stain (manchado) and by some agronomic characteristics like vigor and yield potential. Based on these evaluations, plus some evaluations at Palmira, a total of 386 lines F5 (10,3% of the total) derived from 61 different crosses (56% of the total) were preselected, which will then be subject to quality evaluations (gelatinization temperature, white belly and milling) as well as Hoja Blanca Virus. The lines that pass these evaluations will be evaluated to sogata, to thus elaborate the VIOFLAR-Trópico for 2000.

In addition to these evaluations, breeders from member countries selected their own materials (FEDEARROZ-Colombia, 363 lines; Venezuela, 101; Bolivia, 79; and Guatemala, 140). Of these lines, it was agreed that those with low quality will be discarded and the rest will be included in this year's VIOFLAR that will be dispatched to members of this region.

Among the materials that were found to exhibit intermediate amylose content and intermediate temperature of gelatinization, a set will be sent to to be sent the United States to corroborate these characteristics. The objective is to identify progenitors with these characteristics.

F2 Population: From 4508 populations F2, obtained from 343 crosses, 9,8% of the populations were selected (440 of the total) corresponding to 43% of the crosses (147 of the total). These selection percentages are some 50% lower than those of last year. The reduction is mainly due to higher pressure for blast, helmintosporiosis and of grain stain (manchado). Harvesting of the 440 selected populations resulted in 3340 plants (F3 seed). All this material will be evaluated to white belly, gel temperature, Hoja Blanca Virus. Also, each of the lines will be planted in five meter rows in Monteria to measure lodging and white belly, mainly, and in Palmira to multiply the F4 seed for plantings in Santa Rosa next year.

In addition, Venezuela will also receive the F3 lines for evaluation under their own conditions (1025 F3 lines).

#### Germoplasm for the Temperate Zone

In 1998, 267 crosses were programmed; in October 1998, 104 of them were planted (and 39 were processed by anther culture) and 44 were selected. As a result, we have 372 F2 populations which were evaluated this year in Santa Rosa. The response to anther culture was high (77% or 30 crosses). The best response in terms of R1 green plants generated corresponded to (FL01951, FL01988, FL02027, FL02034, FL02041). This material is now ready for selection and harvest. The remaining 163 crosses (of the 267 programmed) will also be processed through anther culture and then they will be subject to evaluation and selection to form the R2 that will be planted in Santa Rosa next year.

F4 Population: Out of 1719 F4 lines and according to observations made in Palmira and Santa Rosa, a total of 192 F5 lines were preselected (11.2%) coming from 40 different crosses (63,5% of the total). These lines will be subjected to evaluations for white belly and gel temperature to conform the VIOFLAR-Templado, 1999-2000. From this group, 34 lines were selected to reconfirm, in the US (Arkansas), their grain quality characteristics for intermediate amylose and gel temperature and include them as progenitors in our working collection.

R2 Anther Culture: out of 234 R2 lines originated through anther culture, 26 lines were preselected (11.1%); they will be evaluated for grain quality, so as to include them among the VIOFLAR-Templado lines.

F2 Population: out of the 372 F2 populations for the temperate zone, represented in 44 different crosses, 29,3% were selected (109 populations) corresponding to 68,2% of the crosses (30). Harvesting of these 109 populations resulted in 433 plants (F3 seed).

As agreed in the July meeting of the temperate technical subcommittee (see 4.1.1 above) this segregating material will be sent for plantings at IRGA by the FLAR breeder stationed there. These materials will also be evaluated in Palmira only for grain quality.

#### d) F1 plantings in Palmira

A total of 398 F1 crosses for the tropical zone (208 programmed by Venezuela and 190 by FEDEARROZ-Colombia), were planted in April for their evaluation to the Hoja Blanca Virus. Some 25% of the 398 crosses were discarded and the rest were transplanted. In addition, 22% have been discarded for grain type, sterility, plant height and cycle. Therefore, some 200 crosses of F2 seed will be kept for plantings at Santa Rosa next year.

In October, 163 crosses F1 crosses for the temperate zone will be planted in 4 different groups, depending on their response to anther culture (See table 7). Thus, F2 and R2 seed should be available by May of next year. A decision is still pending on whether to plant this material in Santa Rosa or send it directly to IRGA.

#### e) New crosses

About 170 triple crosses, programmed with Venezuelan breeders, were made upon their request. Seed from some 70 of these crosses will be available to be planted next moth for Hoja Blanca Virus evaluation. The resistant lines will be transplanted and the resulting F2 seed will be planted at Santa Rosa. Seed from the remaining crosses will be planted next year for Hoja Blanca evaluation.

The Guatemalan breeder reviewed the progress in the crosses that this country has ordered, during his visit to Santa Rosa in August; up to now, a total of 48 triple crossings were made, but the seed will not be ready for the coming Hoja Blanca evaluation of October 1999. We should be able to obtain F2 seed by March. This seed will be simultaneously evaluated to Hoja Blanca in Palmira and planted in Santa Rosa in April, 2000 for disease reaction.

Seed of 6 simple crosses from the 3 ancestors of the CT8008 line (which originated 9 varieties released in the region after 1995) is already available. In January, we will be programming the triple crosses. The objective is to replicate these highly successful crosses, given that the released varieties originated by the line are now exhibiting blast susceptibility, to correct the problem and look for higher yield potential.

F4 material and a line of our Working Collection (WC) bank showed a high incidence of "mancha ojival" (*Dresslera gigantea*). Susceptible and resistant lines were identified to carry a program of backcrosses. In addition, the variety Cica 7 (susceptible to this disease in previous years) will be used.

The plantings of the progenitors of EPAGRI 108 and 109 (which have shown incidence of blast) were done to make several backcrosses with Oryzica llanos 5 and Fedearroz 50 (highly resistant to the fungus) to incorporate resistance to blast into them.

A program for 116 triple crossings for the tempered zone, requested by member countries in that region, is under way. All of them use progenitors from our WC bank. Seedings for simple crosses we will be done in October to make seedings for the triple crosses in March and thus to obtain F1 seed F1 by September 2000.

#### f) Other activities

Germplasm Bank: discarding old lines from the cold storage room is under way to make room for seed storage of blast spreaders for Santa Rosa. A single working collection bank, the BCF (Banco CIAT-FLAR), groups ACC entries, commercial varieties, WC-Secano and WC-Riego. Currently, this bank has 1705 entries.

Characterization of the BCF: All the entries in this bank will be sent to Itajal for evaluation to Iron Toxicity and also to Montería to evaluate lodging and white belly, mainly. Similarly, renovation of ACC entries (acronym for the older accessions in the bank) is under way. Most important, all missing scores on main breeding objectives (like Hoja Blanca Virus, tagosodes, diseases and grain quality) are been filled to complete the database.

Black root in Venezuela: 73 lines of BCF were dispatched (WC-Riego); all of them showed tolerance to straight head when evaluated in Corrientes, Argentina. The hypothesis is that this problem is associated with the black root problem existing in Portuguesa, Venezuela.

Identification of new Progenitors to improve grain quality. To improve grain quality is the overwhelming objective for all FLAR members, as US rice has penetrated the markets; it becomes an imperative for both importers (to compete in the internal market with foreign rice) and exporters (to compete for new markets). Specifically, what is needed is to obtain varieties with intermediate amylose content and intermediate gel temperature, similar to the preferred US varieties in the world market. The F4 lines with GT = Intermediate, will be reconfirmed through the traditional method for amylose content. Also, data for intermediate readings in both amylose and GT from 18 lines obtained from VIOAL 1997 and 1999, and 24 entries from BCF (WC-Riego) are being reconfirmed. Then, these lines will be sent to the US for further reconfirmation of these traits. Finally, we hope to identify suitable progenitors for new crosses, envisioning higher competitive tropical and temperate materials in terms of quality, yield and resistance to main constraints in the region.

Methodologies to evaluate Hoja Blanca Virus and *Tagosodes oryzicolus*: trials on optimal plant ages for inoculations with the Hoja Blanca Virus are being conducted. Similarly, the methodology for evaluation to the mechanical damage caused by *Tagosodes oryzicolus* is being revised again, as we have been obtaining inconsistent results.

#### Germoplasm use by INGER collaborators and FLAR partners

VIOAL-S.ACIDO (acid soils), 1998: In INTA-Aguilares, Argentina, the cooperators reported the selection of 5 lines, which will be advanced to yield trials in this cropping season. Some of these lines are: CIRAD 409, CIRAD 410 AND CIRAD 411.

VIOFLAR - Trópico, 1999: Of the 68 lines included in this nursery, 16 of them were selected in Venezuela for preliminary yield trials.

VIOFLAR-Templado (temperate), 1998,1999: Bolivia reported 26 lines that will included in the next cropping season. Bolivia needs also tropical material; their breeder came to Palmira and Santa Rosa, where he had the opportunity to select his own lines this year.

VIOFLAR-Templado, 1998-1999, planted in Cachoeirinha (IRGA station in south Brazil). Most of the lines from the FL144 cross showed good behavior; this line also excelled in Santa Catarina. Lines 29 and 31 from the cross CT13503 appear to be promising, and, specially, line no. 116, obtained from anther culture FL00208-CA-3-M (CT8008-16-31-3P-M/CT10865-CA-12-M//IRGA 234-21-5-6-1); it shows good plant type (good vigor, good panicle exertion, good grain and good yield potential).

Varietal releases: In Costa Rica, lines CT10825-1-2-1-1-1 and the CT8665-1-1-1P-4, are candidates for release this year. It was notified to them that the second line was found to be susceptible to Hoja Blanca Virus in our last evaluation.

In Venezuela, line CT10310-15-3-2P-4-3 will be released in January 2000. A susceptible line to Hoja Blanca Virus is also being considered for release by FONAIAP (CT 10)

In Bolivia, the variety PANACU, selected from the VIOAL, of Cuban origin (Perla), will be released. Guatemala sent five promising materials and two checks to be evaluated for grain quality, Hoja Blanca Virus and Sogata. The list of materials include IG 2428, IG 2447, IG 2451, IG 2473, IG 2488, ICTA PAZOS, ICTA COLOMGUÁ.

#### Rice resistant to herbicides (glyfosate)

In a study conducted in 1995, Didlay et al, from Arkansas, identified 26 rice lines as being tolerant to Glyfosate and Sulfosate. Among them, Colombia 2 and Colombia 3 were included. From that information, FLAR will plant a preliminary trial in October 1999 using Oryzica 1 as a check and

glyfosate as the herbicide. The objective is to identify resistance genes occurring naturally and to transfer them into new varieties by using the progenitors in our crosses.

Multiplication of varieties in Palmira for disease evaluations in Santa Rosa. Table 8 shows the volumes of material harvested in Palmira to be used in trials next year in Santa Rosa, our disease hot spot for breeding.

Lab quality activity. Table 9 summarizes the number of samples processed this year in our lab. The joint acquisition by CIAT and FLAR of a NIR machine made possible to increase the samplings for amylose in a sizable manner.

#### 4.2.2 Participate in the elaboration of breeding plans for partners of FLAR

A planning meeting of FUNDARROZ was also accompanied by FLAR and CIAT. It took place in July 13-17. The main conclusion there is that FLAR will send F3 lines to Venezuela, instead of F5 lines; many lines are being selected in Colombia that do not behave well in Venezuela. Likewise, many of the discarded lines may be suitable for their conditions. This change reflects an upward trend from partners, that are prepared to receive earlier generations, reliving FLAR from an important burden in routine field work. The other important topic discussed is related to the declining yields problem and the actions that are being taken to correct the problem. FLAR sent a consultant (Edward Pulver) in April and FUNDARROZ already drafted a plan based on his recommendations.

#### 4.2.3 Coordinate and control collaboration between CIRAD, FLAR and CIAT

FLAR includes in its trials and breeding activities many lines from this partner institutions. In addition, they have participated in training activities jointly.

# 4.2.4 Coordinate and control collaboration between IRGA, EPAGRI and FLAR in Rice Experiment Station of Cachoeirinha (Rio Grande do Sul, Brazil)

A permanent breeder was appointed in February to fulfill requests the five countries from the region that are now members of FLAR: Argentina, Brazil, Uruguay, Chile and Bolivia.

# 4.2.5 Organize and implement three Breeders workshops: Palmira (February), Itajaí (March), Santa Rosa (August)

The three activities took place, but the August meeting in Santa Rosa had a reduced level of participation (Guatemala, Bolivia, Colombia and Venezuela) due to security problems in Colombia. See point 4.1.1 above

#### 4.3 Crop Management

#### 4.3.1 Analyze farmers questionnaires and cost of production data

FLAR is collaborating as an external reviewer to the project on Cost of rice production, conducted by Miguel Padrón, from VOM Corporation and commissioned by Fundarroz.

# 4.3.2 Collaborate in surveys of crop production constraints

FLAR collaborated with Fedearroz in the elaboration of the questionnaire for the Second National Rice Census which has been under way during the second semester of 1999.

# 4.3.3 Participate in elaboration of national plans for integrated crop management

Ed Pulver, from FLAR, visited Venezuela and Santa Catarina, to help in the formulation of crop management plans.

Venezuela. The base document of the plan is complete. Particular activities related to resolving the black root syndrome (high priority), soil fertility (secondary priority) and weed control (secondary priority) have been completed and added to Annexes of the main document. Pending are secondary projects on sogata management and planting density. Also, student thesis projects require further attention and should be added as Annexes to the project as students and project supervisors are identified.

Santa Catarina, Brasil. EPAGRI has requested FLAR to provide assistance for strengthening their rice breeding and also review crop management. The purpose of the support was:

- examine current activities and provide recommendations in the area of crop management,
- review the organizational arrangements of rice research and technology transfer and
- advise on other technical issues requested by EPAGRI staff.

Several meetings were held with Director of the Itajai Research Station and the Director of Research. The purpose of the meetings was to convince research administrative staff on the need to conduct on-farm research in collaboration with extension staff. Key production zones that could be used for pilot work will be selected. In these zones, the extension staff would work in collaboration with rice research personnel on farmers' fields. Rice would be a priority crop for the extension service in the pilot areas and other duties of the extension service would be decreased. The compromise should permit the rice program to focus research-extension activities in areas where average yields are far below the potential and state average. It can be expanded over time based upon the success in the pilot areas.

It is pertinent that the research program addresses the environmental concerns from rice production. This can be a major problem if current feelings that rice production is contributing to environmental degradation is permitted to grow without sound technical information. The weed scientists have initiated activities to provide recommendations that could prevent water-applied herbicides from entering water sources that empty into beaches near tourist areas. However, it doubtful that these studies, although they are technically sound, will have much of an impact on quieting environmentalists. Modeling studies can be conducted in a matter of weeks or months as opposed to in-depth studies on each compound. Information on the type of studies proposed will be provided to EPAGRI research staff.

The low use of quality seeds is a major problem. The seed production industry is highly organized under the umbrella of Associacao Catarinense de Produtores de Sementes De Arroz. We meet the secretary of the organization – Ing. Ronaldir Knoblauch, also a member of EPAGRI's staff. We proposed that the association initiate activities to stimulate the use of certified seeds via demonstration plots since such an approach was highly successful in the Juriti cooperative. The seed producers will be the beneficiaries of increased seed use and it is only reasonable for them to undertake activities to stimulate the use of their product. This suggestion was presented to the

seed producers and it appears they will assist in the financing of field activities demonstrating the advantages of certified seed. The fieldwork will be conducted in collaboration with EPAGRI's rice research staff. It is reasonable to assume that much of this work should be conducted in areas of low yields, i.e., pilot zones for the research-extension integrated project.

The problem associated with damages from stinkbugs appears to be serious. Numerous millers complained about the reduction in grain quality due to stinkbug damages. Controlling stinkbugs presents a challenge in SC since fields are small and the damage occurs during maturation. Applying insecticides to control the insects is very difficult, since fields are too small for aerial applications but too large for manual applications. Additionally, the potential environmental risks associated with aerial applications may prohibit widespread aerial applications. Stinkbugs are attracted to rice due to the presence of a food source and aggregate due to both female and male mediated pheromones. This simple biological principle can be used to attract stinkbugs to trap crops, either planted on the canals or the edges of the rice fields. Once the insects aggregate, directed insecticide sprays on the trap crop can destroy them. This only requires less than 5% of the area being treated. Trap cropping has been successfully employed in other crops, i.e., soybeans, where stinkbugs are a serious problems. Potential candidates for traps are beans (cowpeas, commom bean, and soybeans) since stinkbugs may prefer succulent beans to rice grain. Also, early maturing rice can be employed as a trap. More information on trap cropping for the control of stinkbugs will be provided to EPAGRI research staff.

# 4.3.4 Organize and implement Integrated Crop Management courses on demand (Panama, Peru, others)

Bolivia: a one week course on ICM for rice was organized by CONARROZ. FLAR contributed with scientists from the US, Cuba, Colombia, Paraguay and Brazil. A total of 91 people attended.

Perú: FLAR participated in two courses organized by CIAT, INIA and NGOs from Peru, one in Selva and one in Costa. We sponsored the participation of Dr. Meneses (from Cuba), E. García (from Colombia) and L. Sanint (FLAR). Attendance was quite high in both one-week courses (over 80 participants in each course).

Panama: the first rice congress was cosponsored with APACH. Over 500 people attended the two-day conference on rice management.

#### 4.4 Rice Economics

# 4.4.1 Update estimates of adoption in LAC of new rice varieties by area and production, 1970-99 (with IFPRI and with Impact Unit of CIAT)

During 1999, this activity is being conducted with the Impact Unit at CIAT. In summary, the data shows that 59% of the rice area in Latin America is under MSVs and they account for 80% of rice production. In the irrigated sector, MSVs cover some 88% of the area and account for 90% of the rice production in that ecosystem.

# 4.4.2 Coordinate meetings and activities of the Latin American Network of Rice Economists

The network met during 1999 in march 4-5, in Porto Alegre, Brazil. There were 33 participants from six countries: BRASIL: IRGA (Instituto Riograndense del Arroz); COLOMBIA: FEDEARROZ (Federación Nacional de Arroceros); PANAMA: APACH (Asociación de Productores de Arroz y Granos Básicos de Chiriquí); URUGUAY: ACA (Asociación Cultivadores de Arroz); VENEZUELA: FUNDARROZ (Fundación Nacional del Arroz); ARGENTINA (CIALA). CIRAD was also present.

The Uruguayan Rice Producers Association (ACA) was appointed as coordinator of the network for this year, replacing CIRAD. A project on rice quality was to be presented to FONTAGRO through PROCISUR.

#### 4.4.3 Participate in study of the rice industry chain in Venezuela

This study is at the preliminary stages. Little progress was achieved during 1999, pending approval from donor agency (FUNDARROZ). The cost study (see 4.3.1 above) is the initial stage of this project.

#### 4.4.4 Monitor world rice markets

As a service to FLAR members, FLAR publishes twice a year a summary of world rice conditions in its Foro Arrocero.

# 4.4.5 Seek participation from private sector in project on "Commercial quality of rice in MERCOSUR", submitted to FONTAGRO

The project was submitted to this donor in June. Participating institutions are:

- Instituto Nacional de Tecnología Agropecuaria (INTA) Argentina
- Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Brazil
- Instituto de Investigaciones Agropecuarias (INIA) Chile
- Dirección de Investigación Agrícola (DIA) Paraguay
- Instituto Nacional de Investigación Agropecuaria (INIA) Uruguay
- Centre de Coopèration International en Recherche Agronomique pour le Dèveloppement (CIRAD) – Francia
- Fondo Lationamericano de Arroz de Riego (FLAR) Colombia
- Centro Internacional de Agricultura Tropical (CIAT) Colombia
- Instituto Rio Grandense do Arroz (IRGA) Brasil
- Asociación de Productores de Arroz de Itapúa (APAI) Paraguay
- Fundación CIEPE Venezuela

The project had an intermediate ranking and does not qualify for funding from FONTAGRO. Other donors will be sought.

Economic Impact. An important aspect of the project has to do with the economic impact, as calculated ex-ante. Economic surpluses associated with the new technology generated by this research were measured using MODEXC, a partial equilibrium model developed at CIAT (details appear at the site http://www.cgiar.org/ciat/impacto). Basic parameters for these calculations appear in Table 10. Countries that will benefit from the impact are: Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay, Colombia and Venezuela.

The objective of this project is to facilitate the obtaining of new varieties with higher grain quality and lower unit costs of production. Thus, the supply curve shifts outwards. In addition to that, the demand curve is also shifted as consumers (both in local and external markets) find the new rice more appealing to their tastes; and new products based on agroindustrial uses of rice will also be in the market.

The model used two different scenarios: a conservative and a more optimistic one. We will report here the conservative scenario.

Results show that rice production coming from adoption of modern varieties in these countries will go from 88% in 2000 to 94% in 2020. The rest of the area in traditional varieties corresponds to upland rice in Brazil. Adoption of the new varieties will permit an annual expansion of rice production at 2.1% instead of the 1.4% annual growth without the project. The region will be a net importer of rice during the six first years of the project to become an exporter afterwards.

The annualized flow of benefits (discounted at an annual rate of 5%) to society will be US\$185.4 million; of those, US\$105.0 will be accrued by producers and US\$85.4 million will go to consumers. Under the assumption of closed economies, the total annual benefits are similar (US\$184.1 million) but the main beneficiaries will be the consumers (US\$124.2 million).

#### 4.4.6 Obtain data of investment in rice research in LAC during the 1990's (with IFPRI)

A questionnaire was dispatched to twelve countries but the rate of response has been low (25% or three respondents). The counterpart in the study from IFPRI has been visiting several countries to collect data directly from the research agencies involved in rice activities.

#### 4.4.7 Additional activities not included in plan.

Two concept notes were prepared for funding through FAO:

1. Yield gap among farmers in (selected) members: a FLAR project.

The concept note on this project will be sent to FAO Rome to identify a donor and then it will be sent to their regional office in Santiago.

#### 2. E-mail Conference on Crop Management: a FAO-FLAR project

Potential participants are being identified among FLAR members. The conference should run for two months; it has four different topics --or sessions-- of two weeks each. The budget runs around \$20,000. The conference will serve to strengthen the Latin American Network of Rice Economists (see 4.4.2 above).

Table 1. Sources of VIOAL-Irrigated and Favored Upland, 1996, 1997, 1999, INGER-LAC

Source	1996	1997	1999
	No. lines (%)	No. lines (%)	No. lines (%)
CIAT	140 60.0	71 56.3	20 15.4
INGER-Global	36 15.5	14 11.2	48 36.9
NARS	57 24.5	41 32.5	62 47.7
TOTAL	233 100.0	126 100.0	130 100.0

Table 2. Number of distributed sets of VIOAL, 1999. INGER-LAC.

Country	No. of distributed sets		
	VIOAL	VIOAL-Acid soils	
Argentina		1 .	
Bolivia		1	
Brazil	1		
Colombia	1	1	
Cuba	1		
Ecuador	1		
Guatemala	3	1	
Nicaragua	4		
Panama	3	1	
Peru	2		
Venezuela	2		
TOTAL	18	5	

VIOAL= 130 lines VIOAL-Suelo Acido (acid soils)= 28 lines

Table 3. Contributions from National Programs (advanced lines) to INGER-LAC nurseries, 1996 to 1999

Country/Institution	No. Lines				
	VIOAL, 1996	VIOAL, 1997	VIOAL, 1999		
Argentine					
Argentina: INTA					
Gallo	3	<u></u>	 5		
Brazil:			3		
IRGA	19				
Itajai			9		
Colombia:					
CIAT,Fedearroz, Corpoica	1				
Semillano	1				
Fedearroz			1		
Costa Rica			9		
Guatemala/ICTA	10	h-sri	2		
Guyana			3		
Nicaragua			1		
Panama/CEIAT	2				
Peru/INIAA	1	7			
Dominican Republic/CEDIA	~-		9		
Surinam/SNRI	2	1.	8		
Philippines/Phil Rice		7			
Thailand		2	11		
USA		1	4		
TOTAL	57	41	62		

Table 4. Number of VIOFLAR sets, 1998-1999 sent to the Temperate Zone, distributed in Oct. '98.

Country	No. Sets	
	Distributed	
Brazil:		
IRGA	4	
ITAJAI	2	
Bolivia	2	
Cuba	1	
Paraguay	1	
Uruguay	2	
TOTAL	12	

VIOFLAR-Temperate = 132 lines.

Table 5. Number of VIOFLAR sets, 1999 for the Tropical Zone Distributed in January-March, 1999. FLAR.

Country	No. sets Distributed	
Colombia	1	
Costa Rica	2	
Guatemala	2	
Nicaragua	2	
Venezuela	2	
Panama	1	·
TOTAL	10	·

VIOFLAR-Tropics = 68 lines.

Table 6. Rice Germoplasm planted in Villavicencio and Palmira 1999 A.

Type of material	Villavicencio	Palmira	Hoja Blanca	Tagosodes
-Commercial Varieties	240	240		
-Countries: Ecuador	7	7	7	7
USA	6	6	6	6
Venezuela	94		94	94
-R2 (Anther culture)	234	234		
-F4 Lines:				
Topics	3738	3738	3738	
Temperate	1719	1719		
-VIOFLAR, 1998-1999:				
Tropics	72			
Temperate .	136		·,	
-VIOAL, 1999	136			
-Germplasm Bank (WC)	479		479	479
-F2 Populations:				
Tropics	4508			
Temperate	372			. <b></b>
- INGER-IRRI Nurseries, 1999:				
IIRON	120	120	120	
IRLON	75	75	75	
IRCTN	68			
- Other (Quality)	17	17	17	17
Total Lines	12021	6156	4536	603

Note: All F4 lines were evaluated for amylose content.

Table 7. Plantings of F1 triple crosses - temperate irrigated rice- to be processed by anther culture, 1999FLAR, 1999. Palmira.

Code	No. Crosses	No. Plants	Date	Observaciones
FL02580 - FL02642 Except 9 crosses (*)	54	15 each	Aug. 2	To test response
FL02643 - FL02696	54	15 each	Aug. 16	To test response
FL02697 – FL02742 Plus 9 crosses	55	15 each	Aug. 30	To test response
Several	38	25 each	Sep. 13	Harvest panicles of best plants
FL02580 - FL02627 Except 7 crosses	41	Variable	Oct. 1	Harvest panicles of best plants responding to AC
FL02628 - FL02670 Except 2 crosses	41	Variable	Oct. 25	Harvest panicles of best plants responding to AC
FL02671 - FL02711	41	Variable	Nov. 15	Harvest panicles of best plants responding to AC
FL02712 – FL02742 Plus 9 crosses	40	Variable	Dec. 6	Harvest panicles of best plants responding to AC

<sup>(\*) =</sup> Planting of seed from these crosses had to be delayed until the third round.

Table 8. Inventory of varieties to be sent to Santa Rosa in March, 2000.

	Quantity	Quantity in Kgs				
VARIETY	1998 Harvest	1999 Harvest				
FANNY	-	*				
CICA 8	-	2150	43			
CICA 9	700	_	14			
METICA 1	1000	-	20			
LINEA 2	-	300	6			
ORYZICA 1		600	12			
O. CARIBE 8	-	250	5			
SELECTA 3-20	-	350	7			
O. LLANOS 5	250	-	5			
TOTAL	1950	3650	112			

<sup>\* 60</sup> kg bags to be harvested in Dec. 1999 in Palmira.

Table 9. Samples analyzed by the quality lab during Jan. - Sept. 1999.

Institution	Type of ANALYSIS	Type of MATERIAL	No. SAMPLES
FEDEARROZ	COMPLET	F4 - F5 Y F6	2507
PAISES	COMPLET	LINEAS Y VARIEDADES	427
ICA	COMPLET	LINEAS AVANZADAS	246
CIAT	GT and WB	F2 FAMILIES	2523
FLAR	AMYILOSE	F4-TROPIC & TEMPERATE	5457
FLAR	COMPLET	VIOAL-1999	143
FLAR *	COMPLET	F5 TROPIC & TEMPERATE	1208
FLAR *	GT and WB	F3 TEMPERATE	3444
TOTAL			15955

COMPLET: This analysis includes gel temperature, white belly and % amylose

<sup>\*</sup> These samples are being analyzed at the time the report was written (Sep. 1999).

Table 10. Basic parameters used to run MODEXC

Parameter	Unit	Value
Period for analysis	Year	2000-21
Internal rate of discount for benefits of the project (annual)	%	5
Initial production of paddy rice for countries considered, 1986-97 and projections for 2000	Millon. T	16.2
Initial price of paddy rice, average for several countries, year 2000	   US\$/T	190
International cif price (paddy rice equivalent)	US\$/T	188
Minimum supply price (paddy)	US\$/T	140
Price elasticity of demand (estimated from data for Brazil and Colombia)		-0.3
Supply elasticity of rice (Ernstberger, 1988)		0.6
Autonomous demand shift (w/o the project)		
Automorphism I I I I I I I I I I I I I I I I I I I	%	1.6
Autonomous supply shift (w/o the project)	<u></u> %	1.1
Demand shift associated with the project	%	0.3
K-shift (pivotal divergent), conservative scenario of adoption (88 a 94%)		1.25
K-shift (pivotal divergent), ceiling scenario (adoption 88 a 99%)		1.50
Lag-time for research and diffusion of technology	Year	4

# 4.5 Publications

4.5.1 Edit and publish Foro Arrocero Latinoamericano

Two annual issues have been published since the creation of FLAR in 1995. This bulletin constitutes a diffusion mechanism to inform the general public about FLAR's activities, priorities and points of view on topics related to research, training, commercialization, policies, etc related to the rice sector.

- 4.5.1.1 Write editorials and articles
- 4.5.1.2 Seek contributions from colleagues. Edit and produce two issues per year
- 4.5.2 Co-Edit Arroz en las Américas

This publication was not produced in 1999

4.5.3 Submit articles to publications from partners (FEDEARROZ, ACA, etc.)

Articles were reproduced in several rice magazines, as follows:

Revista Arroz, ACA, Uruguay (March 1999): El arroz de la Globalización, pp. 10-19 Revista Induarroz, Colombia, March 1999: La competitividad del arroz: una visión integral, pp. 47-56

4.5.4 Submit article to refereed journal

An article presented a the Poverty Workshop in Costa Rica will be considered for publication in that Journal.

4.5.5 Write annual reports for FLAR and for CIAT and Committee Acts

Acts for the FLAR committee meetings were written and signed by witnesses. CIAT financial office gets a copy as proof of funding commitments by partners.

- 4.5.6 Present papers in Workshops, Conferences, Seminars, etc.
- Chile: May 10. Presentation on FLAR at the ceremony of acceptance of Chile as member.
- Nicaragua: May 24. Presentation on FLAR at the ceremony of acceptance of Nicaragua as
- Panamá: May 26. Presentation on rice research in Latin America, at the First International Rice Congress in David.
- Costa Rica: Sep. 24. Presentation of a paper coauthored with Libardo Rivas ("Tecnologías basadas en el mejoramiento de germoplasma que benefician al consumidor: el caso de ganadería vacuna y Arroz en América Latina y el Caribe") at the Poverty Workshop organized by CIAT.
- Argentina: Oct. 8. Presentation of a paper at the V Congress of the Rice Milling Industry of Argentina, entitled "La investigación de arroz en América Latina: evolución, retos y oportunidades"

your

# **OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITIES ENHANCED**

# B.1. Identification of improved upland rice genotypes for the Peruvian Selva. Collaboration to Forest Margins Project.

Carlos BRUZZONE, Sam FUJISAKA, John AVILÉS (CIAT-Pucallpa), Ricardo LABARTA (CIAT-Pucallpa) and Isaias GONZÁLEZ (UNU-Pucallpa).

Local upland rice varieties lack some desirable agronomic and grain quality traits that limit their capacity to satisfy both farmers and market demands. To address this issue, genetic material was introduced to the area for testing purposes, in different steps of development: CIAT advanced upland rice lines, and improved varieties, released in different Latin American countries.

Twelve advanced lines locally selected last year on the basis of agronomic performance and grain milling traits are being tested this year in replicated trials in collaboration with CIAT-Pucallpa, Universidad Nacional de Ucayali (UNU), and Instituto Nacional de Investigación Agraria (INIA). The aim is to identify outstanding lines better suited to farmer demands than local varieties.

Four upland varieties originated at CIAT: Oryzica Sabana 6 (Colombia), Oryzica Sabana 10 (Colombia), CIRAD 409 (Colombia) and Progresso (Brazil), and a CIRAD variety, IRAT 146 (Ivory Coast), introduced and seed multiplied in 1998, were tested in farmer participatory trials.

Table 1. Yield (t/ha) of introduced rice varieties tested in farmer plots in comparison with local varieties.

		intro	duced var	ieties		Local varieties			
Tested	CIRAD	IRAT	IRAT Sabana		Progre-	Chanca-	Aguja	Caro-	
plots	409	146	10	6	<b>S</b> SO	Вапсо		Lino	
Average	2.75	2.55	2.76	2.34	1.63	2.90			
S <sup>2</sup>	0.35	0.25	0.82	0.90	1.21	0.45			
N	3	4	4	3	2	4			
Average	3.15	2.73	2.60	2.30	2.69		2.17		
S <sup>2</sup>	0.39	0.87	2.19	0.23	0.97		0.35		
N	4	3	3	5	4		6		
Average	2.33	2.13	2.36	2.03	2.34			2.43	
S <sup>2</sup>	0.84	0.94	0.83	1.11	1.24			0.66	
N	3	3	3	3	3			3	
Overall:									
Average	2.78	2.48	2.59	2.24	2.34	2.90	2.17	2.43	
S <sup>2</sup>	0.52	0.55	0.97	0.51	1.01	0.45	0.35	0.66	
N	10	10	10	11	9	4	6	3	

Source: Avilés, J.1999 (CIAT-Pucalipa).

Most varieties were well accepted by farmers due to their greater lodging resistance, better grain milling and cooking quality than local varieties. Yield performance was about the same than local varieties Chancabanco and Carolino, and greater than local variety Aguja. Table 1 shows preliminary results from some of the plots tested last season.

Seeds of a second set of varieties introduced this year was locally multiplied by our collaborators at UNU and is being tested in farmer participatory trials by CIAT-Pucallpa farmer participatory team. These varieties are Canastra, Primavera, Confiança, and Maravilha (released in Brasil), Oryzica Turipana 7 (from Colombia), ICTA Izabal (Guatemala) and CT 11253-6-M-M.

# B.2. Strengthening the Peruvian Rice Program

Carlos Bruzzone

#### 1. General activities

CIAT continued to carry out activities aimed to strengthening the Peruvian rice sector within the framework of the agreement with the Peruvian Ministry of Agriculture.

Drs. Calvert and Bruzzone visited Alto Mayo (Rioja, region San Martín) rice growing areas to observe the characteristics of a severe RHBV epidemic occurring in that region. Future activities were coordinated with local institutions, such as Fundación para el Desarrollo Agrario del Alto Mayo and Proyecto Especial Alto Mayo (PEAM).

In order to make a brief diagnosis of the coastal region, Bruzzone and Mark Bell (IRRI) visited rice-growing areas of Lambayeque, La Libertad and Piura, accompanied by M. Arca (Research Director, INIA), F. Montero and O. Palacios (INIA rice breeders). A plan establishing responsibilities for planned activities for each one of the international centers involved was prepared. In general terms, CIAT committed itself to continue the support for INIA breeding efforts and to organize defined technology transfer and training activities.

Other activities involved organization of courses, exchange and evaluation of germplasm and training of Peruvian researchers.

# 2. Coordination and support of breeding activities

CIAT continued the close coordination of breeding activities with both Peruvian breeders from the Costa (temperate irrigated) and the Higher Selva (tropical irrigated).

This year INIA oficially resumed its rice research activities in the Peruvian Coast after a five-year long withdraw. Fortunatedly, INIA did not have to start from zero, and now can take advantage of the genetic material developed by a four-year effort carried out by a small informal network of institutions and private companies in the Coast. This genetic material, composed by 3,285  $F_5$ , 738  $F_4$ , 462  $F_3$ , and 1,759  $F_2$  lines, was planted and evaluated this year in Vista Florida Exp. Station.

Advanced lines introduced from IRRI and CIAT were also field evaluated this year, and are being tested for grain milling traits.

A new variety, named PITIPO (PNA 1562-44-10-1-4-3-ph10), with higher yield potential and greater cold tolerance than VIFLOR, the most planted variety in the Coast, is ready to be released by the Peruvian government.

INIA advanced lines and Peruvian varieties were evaluated this year in CIAT for disease resistance, agronomic and grain quality traits (Tables 1 and 2). One thousand seven hundred eighty six F4 lines from El Porvenir Experimental Station (INIA-Tarapoto) are in the process of being evaluated for their reaction to RHBV and grain quality traits.

A new promising variety (PNA 2002-HU4-2-EP1-1-1P) with good grain quality (long, slender, translucent grains and good cooking traits), resistant to RHBV, and good yielding potential has been identified and their seed multiplied in El Porvenir. Even though this new variety is susceptible to the blast lineage SRL-4, overpass CAPIRONA, the current leading variety, in having resistance to a greater number of blast lineages. The release of this new variety will temporarily reduce the pressure on Capirona, which is planted in more than the 50% of the irrigated rice tropical area. Efforts must continue to identify or develop new rice varieties with resistance to the most important diseases along with desirable grain quality and agronomic traits.

# 2.1 Development of genetic material for Perú in CIAT

One thousand two hundred two plants were selected from 117 triple crosses made last year. Derived F2 families are being evaluated for plant, panicle and grain type, under transplanting conditions in Palmira. Simultaneously, they are being screened for RHBV, sogata and grain quality traits (white belly and temperature of gelatinization).

Six hundred twenty of these F2 lines were derived from 63 triple crosses aimed to temperate conditions (Peruvian Coast), and 582 F2 lines were derived from 54 triple crosses intended for tropical conditions (Higher Selva). Best lines will be evaluated for their disease reaction in Santa Rosa next year.

# 2.2 Fixing temperate irrigated families through anther culture

Aiming to accelerate the production of homozygous lines, the F1 of 16 triple crosses having at least one highly responsive parent were processed through anther culture. A total of 772 green R1 plants have been produced and are in the process of being transferred to the screen house and then to the field (Table 3). R2 seed from truly dihaploid plants will be harvested by December.

## 3. Training activities

Two IPM courses on rice were organized in collaboration with Peruvian local institutions. CIAT/FLAR scientists Lee Calvert, Luis Sanint, Rafael Meneses, Elfas García, Mónica Triana, Gustavo Prado and Carlos Bruzzone, participated as speakers on these courses.

The first course was held, May 11-13, at Nueva Cajamarca (San Martín region), and was organized in collaboration with Proyecto Especial Alto Mayo (PEAM) and Fundación para el Desarrollo Agrario del Alto Mayo (FUNDAAM), having 71 attendees among farmers and professionals.

The second course was held, May 17-19, at Chiclayo, in the Peruvian Coast, in collaboration with Comité Departamental de Semillas de Lambayeque, with the assistance of about 120 persons.

Two Peruvian rice researchers from the INIA Rice Program: Fernando Montero (EE Vista Florida) and Orlando Palacios (EE El Porvenir), had a 20-days training period in Colombia, getting acquainted of the screening and selection methods carried out by CIAT/FLAR breeding programs, at Palmira and Santa Rosa (Villavicencio).

Table 1. Grain quality of Peruvian varieties in Palmira, Colombia.

		Gelatini	zation		
Variety	White	Tempera	ature	Amylose	•
-	Belly <sup>1</sup>	Dispers	ion² Class³	<del>%</del>	Class <sup>3</sup>
Selva Alta	0.2	5.0	Í	31.6	Α
Capirona	0.2	5.0	ŀ	30.9	A
IR 43	0.2	7.0	В	24.9	I
Porvenir 95	0.2	2.0	Α	21.7	В .
Santa Ana	0.4	6.6	B/I	22.8	В
Uquihua	0.4	7.0	В	31.0	Α
Costa Norte	0.6	7.0	В	30.1	Α
INIA-14	0.6	5.0	1	29.6	Α
Oro	0.6	7.0	В	31.5	Α
Pítipo	0.6	5.4	I/B	22.5	В
San Antonio	0.8	3.2	A/I	31.0	Α
Taymi	8.0	7.0	В	31.5	Α
Urpi	1.0	7.0	В	31.8	Α
Viflor	1.2	5.0	1	21.9	В
Linea 415	- 2.0	7.0	В	27.2	I
PNA 314-F4-149-1	2.6	5.0	1	30.9	Α
PA 3	3.6	7.0	В	31.3	Α
BG 90-2	3.8	5.0	I	31.2	Α
International varietie	es:				
INIA-Tacuarí (Uruguay)	8.0	4.0	I	26.9	I
Lemont (USA)	1.0	5.0	1	26.4	1
Oryzica-1 (Colombia)	0.2	7.0	В	30.8	Α

<sup>&</sup>lt;sup>1</sup> Scale from 0 to 5. <sup>2</sup> Degree of alkaline dispersion; 1 to 7 scale. <sup>3</sup> A = high; I = intermediate; B = Low

Table 2. Plant vigor and reaction to fungal diseases in Sta. Rosa and reaction to RHBV in Palmira of Peruvian promising lines in comparison with commercial varieties. CIAT, 1999.

	·	L	eaf bla	st					
Genotypes	Vigor	35 DAP	50 DAP	62 DAP	Neck Blast	Leaf Scald	Brow nSpot	Grain Discolo- ration	VHB
CT 10321-7-2-2P-1-2	1	3	3	3	1	1	1	3	7
CT 10321-7-2-3P-2-1	1	2	2	3	1	3	1	3	
CT 10175-3-10-1-1P-1-3	1	5	- 5	5	7	3	7	3	5
CT 10310-5-1M-YA1-EP1	3	2	3	2	1	3	1	1	1
CT 12127-1-2-EP1-1	3	3	3	5	5	1	1	3	7
CT 11922-2-2-EP1-1	1	2	2	2	1	3	1	1	-
CT 12158-1-1-EP1	3	2	2	3	1	3	1	1	-
Peruvian varieties:									
Uquihua	1	2	2	3	3	5	7	5	1
INIA 14	1	4	5	4	5	3	3	7	9
Capirona	3	2	4	5	1	3	5	3	7
IR 43	1	1	2	2	1	5	3	9	7
International varieties:									
Fedearroz 50	1	1	2	2	1	3	3	1	3
Ceysvoni	5	2	3	2	3	1	3	1	-
Oryzica 1	1	5	5	5	7	3	7	3	7
CICA 8	3	6	7	8	_	-	<i>.</i> -	-	,

Table 3. Number of R1 green plants regenerated through the anther culture of the F1 of crosses intended for the Peruvian temperate Coast.

Nº Or- der	CIAT Nº	Feminine Parent	Masculine Parent	R1 Green plants Nº
1	CT 15652	INIA-Yerbal/	ECIA 213-F4-J153/	125
	}	PUSA 169	RHS 376-57-CX-2CX-3CX-OZA	
2	CT 15656	H13-4-1-2-1/	CT 7948-AM-14-3-1/	86
		CT 8008-AM-8-2-1	CT 9038-5-5C-8C-3C-1C-M	<u> </u> 
3	CT 15657	H13-4-1-2-1/	CT 7948-AM-14-3-1/	72
		CT 8008-AM-8-2-1	CT 9852-3-2-1-2-1P	
4	CT 15659	H13-4-1-2-1/	INIA-Tacuarí	190
!		CT 8008-AM-8-2-1		}
5	CT 15660	H13-4-1-2-1/	INIA-Yerbal/	75
		PNA 1377-F4-85-3	PUSA 169	
6	CT 15664	H13-4-1-2-1/	CT 7948-8-4-1P-2X	53
		PNA 1376-F4-42-2		
7	CT 15666	H13-4-1-2-1/	CT 10310-15-3-2P-4-3	10
		PNA-1376-F4-42-2	•	
8	CT 15667	H13-4-1-2-1/	CNAx 5011-9-1-6-4-B	17
		PNA 1376-F4-42-2		•
9	CT 15668	H13-4-1-2-1/	CT 11275-3-F4-8P-2	35
		PNA 1376-F4-42-2		
10	CT 15671	RHS 376-57-CX-2CX-3CX-OZA	CT 11275-3-F4-8P-2	18
		PUSA 169		
11	CT 15672	CT 7948-AM-14-3-1/	CT 7948-8-4-1P-2X	0
		C109Cu84		
12	CT 15673	CT 7948-AM-14-3-1/	CNAx 5011-9-1-6-4-B	13
		C109Cu84		
13	CT 15675	CT 7948-AM-14-3-1/	Selva Alta	0
		C109Cu84		
14	CT 15676	ECIA 213-F4-J153/	EPAGRI 109	0
		RHS 376-57-CX-2CX-3CX-OZA		
15	CT 15680	ECIA 213-F4-J153/	CT 7948-AM-14-3-1/	63
		RHS 376-57-CX-2CX-3CX-OZA	CT 9038-5-5C-8C-3C-1C-M	
16	CT 15670	RHS 376-57-CX-2CX-3CX-OZA/	Costa Norte/	28
		CT 6142-F2-RH-3-4-3	L29-30	
			.l <u>.,.</u>	

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#### ANNEX 1. PRINCIPAL AND SUPPORT STAFF

#### PRINCIPAL STAFF

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James Gibbons, Plant Breeder, FLAR\*
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